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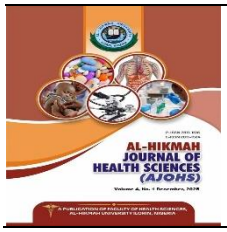

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Original Article

Sleep Deprivation Aggravates Placental Oxidative Status and Alters Haematological Variables in Pregnant Wistar Rats

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ABSTRACT

Background: Prenatal sleep deprivation is associated with increased risk for preterm delivery and complications, partly due to a burst in oxidative stress from increased placental mitochondrial activity and establishment of intervillous circulation. Studies linking this relationship are limited. Hence, we evaluated how maternal sleep deprivation affects placental stress and haematology in Wistar rats.

Methods: Thirty pregnant Wistar rats (180g-200g) were assigned to six groups (n=5): control and sleep-deprived (SD) subgroups across three Gestational periods/days (GD1-7, GD8-14, and GD15-21). Sleep deprivation was induced by the multiple platform technique (20h/day for 7 days). The dams were euthanized on GD7, 14, and 21, respectively, after which the placentas were excised for biochemical studies and blood was collected for haematological analysis.

Results: Haemoglobin level decreased significantly at GD15-21, while WBC increased at GD8-14 and GD15-2, with concomitant increase in platelets at GD15-21 in the SD group. Lymphocytes increased at GD1-7, whereas monocytes exhibited trimester-specific variation in SD rats, decreasing at GD1-7 and increasing at GD15-21. Eosinophils decreased at GD15-21 in the SD. Placental SOD activity decreased at GD1-7, while MDA levels increased at GD1-7 and GD15-21 in SD, with no significant difference in catalase concentrations.

Conclusion: Maternal sleep deprivation worsens oxidative stress and impairs haematological functions. This may adversely affect the mother and fetal health, with the potential to epigenetically programme the fetus for increased susceptibility to chronic diseases later in life.

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Introduction

Sleep is a biological phenomenon observed across most species and is necessary for proper physical, immunological, and mental performance. According to the WHO (2020), sleep health has become a global health concern.

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Sleep disorders in pregnant women have not drawn equal attention in clinical practice despite pregnancy being a physiological condition characterized by deep anatomic, metabolic, hormonal, and psychological changes (Smyka, *et al.,* 2020). In fact, sleep deprivation and poor sleep quality are highly prevalent during pregnancy, with meta-analyses reporting that over 75% of women are affected (Sedov *et al.*, 2018). Sleep deprivation during pregnancy can aggravate oxidative stress, disrupt mitochondrial function, and alter hematological parameters, which are critical for maternal and fetal health (Pizzino *et al.*, 2017). In animal models, sleep deprivation has been reported to cause adverse alterations in haematological parameters and the generation of reactive oxygen species (ROS), leading to oxidative stress (Wang *et al.*, 2021). Sleep deprivation worsens this oxidative burden through increased neuronal energy demand, which elevates oxygen consumption and mitochondrial ROS generation, as well as depletion of key antioxidant defenses, like glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and total antioxidant capacity, while raising markers of lipid peroxidation such as malondialdehyde (MDA) (Villafuerte *et al.*, 2015; Gao *et al.*,2019). Collectively, this imbalance can promote oocyte and embryonic dysfunction via apoptotic pathways, thereby compromising reproductive cell viability and developmental competence (Lateef *et al.*, 2020; Wang *et al.*, 2021).

Multiple studies have consistently demonstrated that a lack of sleep can have deleterious impacts on blood-related factors and the body's ability to clot. Liu and his colleagues (2009) observed fluctuations in lymphocyte, eosinophil, and neutrophil counts, as well as changes in coagulation factors. They also reported that sleep deprivation (SD) can increase white blood cell and neutrophil counts, as well as reduce prothrombin time and activate the partial thromboplastin time, indicating a state of hypercoagulability.

Previous literature on sleep deprivation (SD) during pregnancy regarding hematological variables and placental oxidative status is unclear, contradictory, or inconclusive, and several of these studies do not account for trimester-specific effects or changes in placental cytoarchitecture. Therefore, we investigated the impact of maternal sleep deprivation on placental oxidative status and hematological parameters at different gestational ages in Wistar rats.

Materials and Methods:

Animals

Thirty adult female Wistar rats weighing 170–180 g was obtained from the Central animal facility of the College of medicine, University of Ibadan and group-housed in a well-ventilated cages in the Central Animal House of the College of Medicine, University of Ibadan, under conditions of controlled lighting (lights on 7:00–19:00 h) and temperature (24 ± 1C). Food and water were provided *ad libitum*.

Mating

Animals in their proestrous phase were exposed to mature male breeders overnight (2 females: 1 male), and the presence of spermatozoa in the vaginal smear the next morning was regarded as evidence of pregnancy and was considered gestation day (GD) 1.

Experimental design and Sleep Deprivation Protocol

The pregnant rats were then randomly assigned either to the sleep deprivation group or the control group (n=5) for GD1-7, GD8-14 and GD 15-21. Sleep deprivation was induced using the multiple platform method, as described in previous literature (Pardo *et al.*, 2016). The multiple platforms, consisting of 10 narrow, circular platforms (7 cm in diameter and 10 cm in height), were placed inside a glass tank (110 × 50 × 60 cm) filled with water to within 1 cm of the upper border of the platforms. In the control group, tanks had the same characteristics and dimensions as the tanks used for SD; however, instead of water, the floor was covered with sawdust. Animals in both groups had to climb to a platform to access food and water hung on the lid. Pregnant rats were submitted to sleep deprivation for 20 h per day (14:00–10:00 h) for 7 days. The animal was allowed to sleep for four hours (from 10:00 hour to 14:00 hour) each day to enable partial compensation for the sleep loss. The time interval 10:00 hour – 14:00 hour was chosen because that is when paradoxical sleep is at its highest in rats and slow wave homeostatic pressure is generated (Machado *et al*; 2004 and 2005).

Placenta Removal and Blood Collection

On GD 7, 14, and 21, all pregnant rats were euthanized, and fetuses and placentas were removed by caesarean section under sodium thiopentone anaesthesia (40 mg/kg) administered intraperitoneally (Kurwale *et al.*, 2015). Blood was collected through cardiac puncture.

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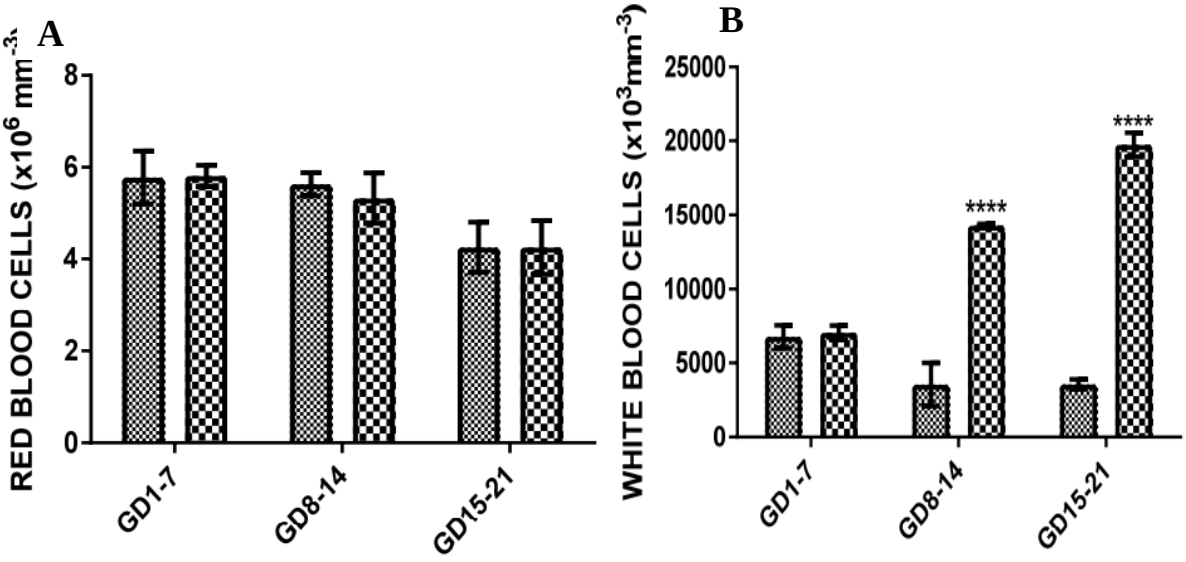
Placental Processing
Two placentas per dam were homogenised in 100 mM ice-cold phosphate-buffered (pH 7.4) solution, four times their weight, and the homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C for the determination of oxidative status.

Determination of Hematological Parameters
Blood samples were analyzed using an automated hematology analyzer (Mindray BC-5000 Auto 5-Diff Hematology Analyzer model) to determine haematological parameters.

Biochemical assays
Assessment of oxidative stress biomarkers was carried out using commercially available ELISA kits according to the manufacturer's manual: Rat MDA (EU2577), Rat SOD1 (ER0332), Rat CAT (ER0264

Data Analysis
Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to compare the test SD and the control groups. The results were expressed as mean ± standard error. GraphPad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA) was used for statistical analysis, and p-values < 0.05 were considered statistically significant.

Results
Effects of Maternal Sleep Deprivation on the formed elements of Blood
The effects of SD on red blood cells (RBC), white blood cells, platelets, hemoglobin concentration, and packed cell volume were determined at GD1-7, GD8-14, and GD15-21. RBC counts were not statistically significant at any trimester in the sleep-deprived group compared with their respective controls. However, there was a trend toward a decrease at GD8-14 (Fig. 1A). Conversely, at GD8-14 and GD15-21, white blood cell counts significantly increased ($P<0.0001$) in the SD group compared with the control (Fig. 1B). A statistically significant increase (****$P<0.0001$) in platelet count was observed at GD15-21 in the SD group, whereas at GD1-7 and GD8-14, it remained unchanged (Fig. 1C). In contrast, the hemoglobin concentration of the sleep-deprived group significantly decreased ($P<0.05$) at GD15-21 compared with the control, and no observable difference was found at GD1-7 and GD8-15 (Fig. 1D). No significance difference was found in the Packed cell volume (PCV) at all the trimesters compared to their respective control.



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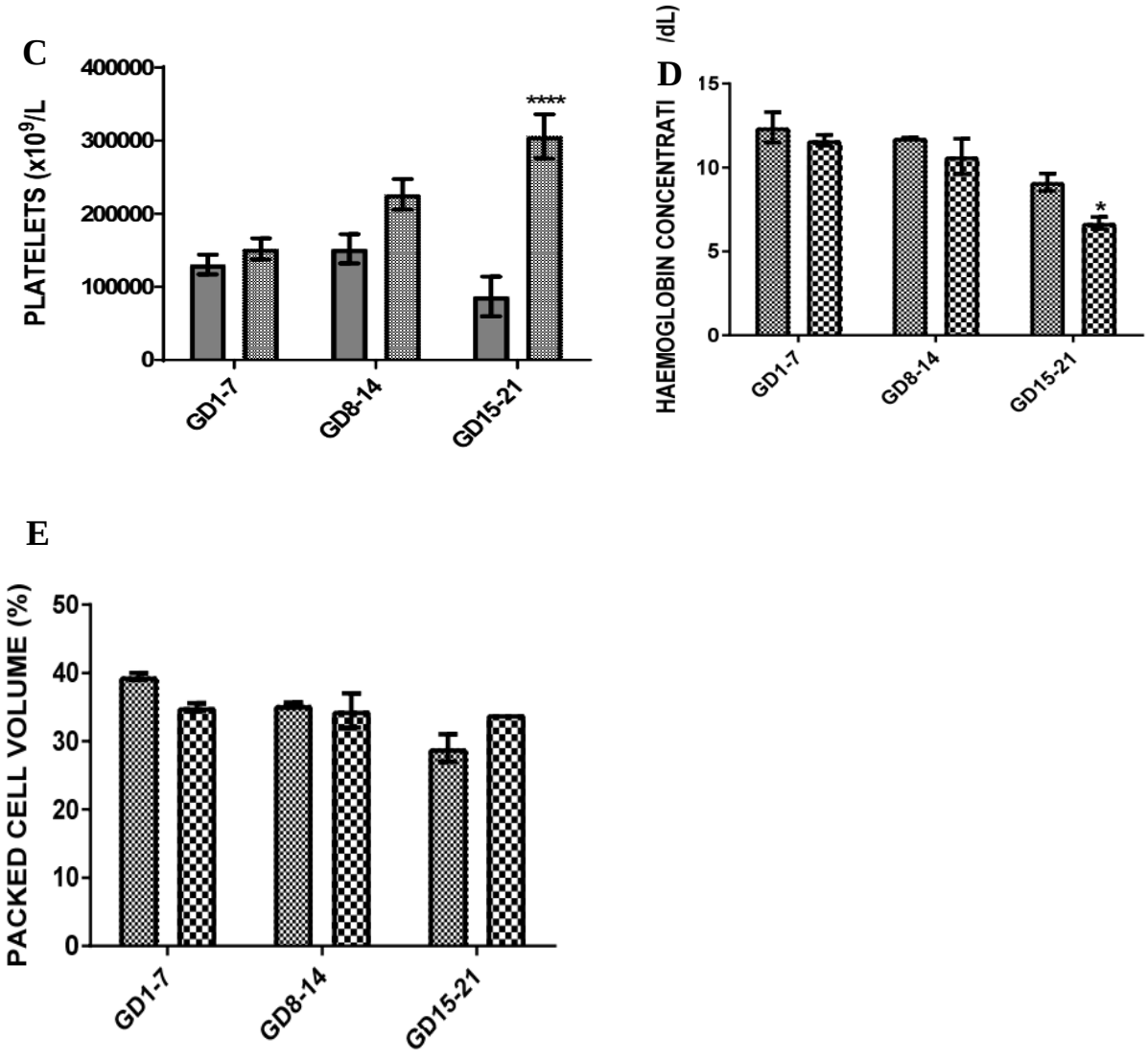

Figure 1 (A-E): Shows the results of the effects of maternal sleep deprivation on red blood cell count, white blood cell count, platelet count, Haemoglobin concentration and packed cell volume. All data were expressed as Mean ± SEM, n=4. * show statistically significant difference, P < 0.05. GD: Gestation day

Sleep Deprivation in pregnant Wistar alters white blood Cell Differentials

There was no statistically significant difference observed at all the GDs on the neutrophils count of).

the SD group compared with their respective control (fig. 2A). However, Lymphocyte count showed a significant increase (P<0.05) at GD1-7 in the SD group when compared with the control (fig 2B). Conversely, there was a significant decrease (p<0.05) in the percentage of monocytes following sleep deprivation at GD1-7 compared with the control, this decrease was reversed at GD15-21 (fig 2 C). Although the eosinophil count was unaltered at GD 1-7 and GD 8-14, but, decreases significantly (p<0.05) at GD 15-21 compared to the control group (fig 2D

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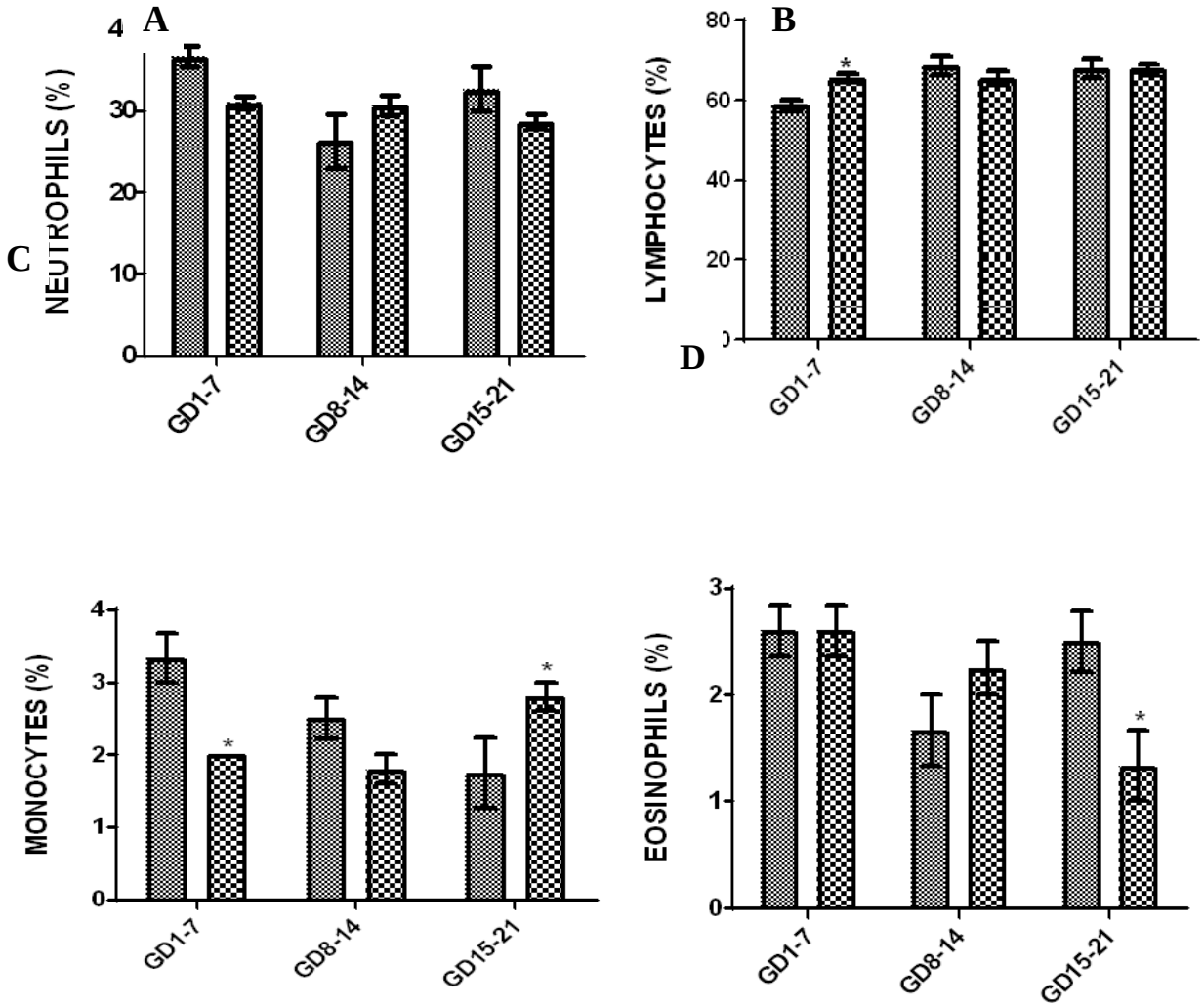

Figure 2: Effect of sleep deprivation on (A) Neutrophils (B) Lymphocytes (C) Monocytes and (D) Eosinophils count in pregnant Wistar rats. *P< 0.05 vs control (n=5). All data were expressed as Mean ± SEM, n=4. * show statistically significant different, P < 0.05. GD: Gestation Day.

Sleep deprivation Deteriorate Oxidative stress Defense during Pregnancy

MDA activities in the placenta of the SD group increase (P<0.05) significantly at GD1-7 and GD 15-21 when compared with control. However, no significant difference was observed at GD8-14 compared with the control (fig 3A). Placental SOD concentration decreased significantly (p<0.05) at GD 8-15 and GD 15-21 in the SD, while no observable difference was found at the GD1-7 and GD8-15 (fig. 3B). Interestingly, catalase levels remained unchanged throughout pregnancy (fig 3C).

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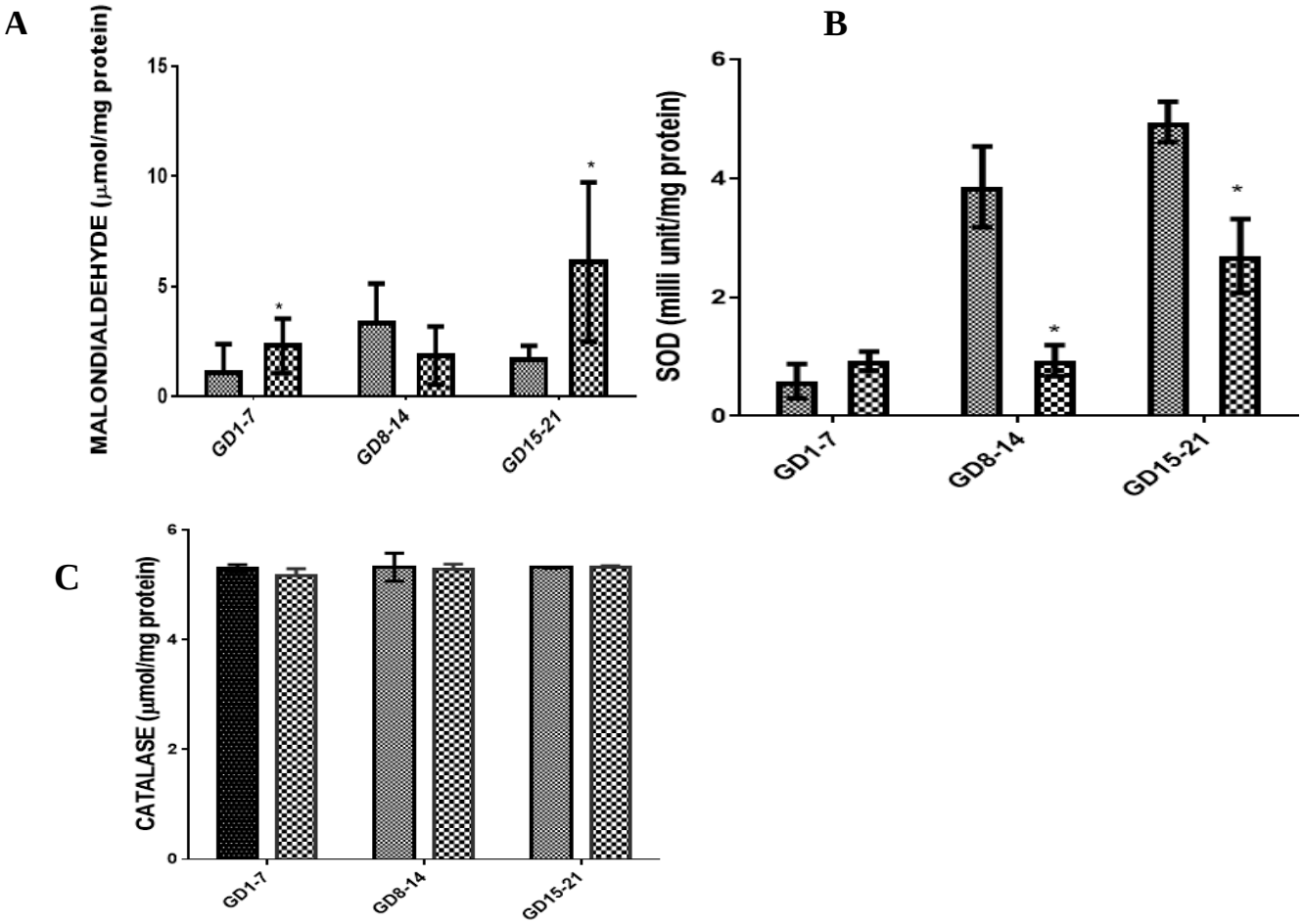

Figure 3: Effect of sleep deprivation on placental (A) MDA) (B) SOD (C) Catalase concentration in pregnant Wistar rats. *P< 0.05 vs control (n=5). All data were expressed as Mean ± SEM, n=4. * show statistically significant different, P < 0.05. GD: Gestation Day

Discussion

This study was designed to evaluate haematological indices, placental and serum oxidative status in pregnant Wistar rats as they affect maturation and development of offspring, as well as maternal and fetal outcomes. Several studies have examined the consequences of stress during pregnancy on the development of the offspring (Lamichhane *et al.*, 2020; Caparros-Gonzalez *et al.*, 2021; Wei *et al.*, 2021). However, studies that directly analyze the effects of sleep deprivation at different gestational ages during pregnancy are limited. Hematological profile is measured globally to assess general health, because it is a reliable indicator and is a simple, fast, and cost-effective test (Wu *et al.*, 2018). The progressive decline in Haemoglobin concentration as observed from the first to the third trimester in this study may be due to an increased demand for iron as pregnancy progresses. These findings corroborate the study of (2019), who also reported a progressive decline in Hb concentration from the first to third trimester, and these findings may be suggestive of pathological anemia occurring in the sleep-deprived pregnant group. RBC was found to show no significant difference across all trimesters in the sleep-deprived groups compared with their respective controls, suggesting that sleep deprivation does not have a significant effect on RBC changes during Pregnancy. Similarly, PCV was also not significantly different at all the trimesters of the sleep-deprived pregnant rats, although Anurag and his colleagues (2020) earlier reported a drop in PCV in the first and second trimesters.

The white blood cell counts and differential white blood cell counts reflect the systemic status of an animal in relation to its response and adjustment to injurious agents, stress, and/or deprivation; the indices are of value in

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confirming or eliminating a tentative diagnosis, in making a prognosis, and guiding therapy (Chan *et al.*, 2022). In this study, WBC increased slightly at GD1-7 but significantly at the second and third trimesters (i.e., GD8-14 and GD15-21) in sleep-deprived pregnant rats compared with their respective controls. This is in accordance with the findings of Akinbami et al. (2013) and (2018), who reported an increase in the WBC count from the first to the third trimester during pregnancy. The physiological relevance of this finding may be suggestive that more white blood cells are produced in response to stress to provide a powerful defense against the invasive behavior of the blastocyst in uterine tissue, and to mechanisms that promote rejection of the fetal allograft. Contrary to the studies of Akinsegun *et al.*, (2013), who reported an increase in neutrophils, which may represent a response to stress due to redistribution of the WBCs between the marginal and circulating pools, our studies reveal otherwise. The lack of increase in neutrophils observed in this study, despite the stress responses, might be one of the placenta's adaptive mechanisms employed in utero to prevent immunologic rejection of the conceptus. Lymphocytes were significantly decreased at GD1-7, whereas no significant difference was observed at GD8-14 and GD15-21. This increase indicates that the maternal body recognizes stress during the critical period of pregnancy, prior to the formation of the placenta and the establishment of adaptive mechanisms during intrauterine programming. Another important finding of this study is that the percentage of eosinophils in the first and second trimesters remains unchanged, but decreases significantly in sleep-deprived rats at the third trimester (GD 15-21). Monocytes are the largest cells among the leukocytes. Like neutrophils, monocytes are motile and phagocytic in nature. These cells wander freely through all tissues of the body and play an important role in the body's defense. In this study, sleep-deprived rats showed a significant decrease in monocytes at GD 1-7, which may be suggestive for a reduction in the formation of colony-forming blastocyst at the early stage of pregnancy. At GD15-21, monocyte numbers increase, suggesting the action of IL-1 in inflammatory responses occasioned by the blastocyst's invasion under stressful conditions.

Normal pregnancy is characterized by an increase in platelet aggregation and a decrease in the number of circulating platelets with gestation. Increased platelet consumption in the uteroplacental circulation has been proposed as the explanation for the reduction in circulating platelets (Reese *et al.*, 2018). However, we observed an increased platelet count at GD15-21 in the sleep-deprived group.

The elevated MDA level observed at GD1-7 and GD14-21 in the sleep-deprived rats is in accordance with the report of the study of Soundravally *et al.*, (2015). SOD activities significantly decreased at GD8-14 in this study. This is in line with the study of Fetoui et al. (2009), who reported a decrease in SOD activities in undernourished pups, suggesting the possibility of oxidative stress. Notably, Catalase activity was not altered in this study, suggesting that compensatory mechanisms are activated during the 4 hours of sleep provided to the sleep-deprived rats each day. SOD and MDA were both significant in determining placental oxidative status. Oxidative stress associated with pregnancy complications may be a contributing factor in the postnatal consequences of the neonate.

Conclusion

The evidence that maternal sleep deprivation has damaging effects on pregnancy and fetal outcome has been shown in this study. It is therefore concluded that maternal sleep deprivation at certain critical periods of pregnancy has detrimental effects on placental structure and hematological variables and aggravation in the level of oxidative stress. This could contribute to the global burden of diseases.

Recommendation

Further research into the mechanistic basis of sleep-deprivation-induced oxidative stress is recommended, which could help in the discovery of intervention measures.

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Conflict of Interest

None

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References

Ahola, A. J., Lassenius, M. I., Forsblom, C., Harjutsalo, V., Lehto, M., & Groop, P-H (2017). Dietary patterns reflecting healthy food choices are associated with lower serum LPS activity. Scientific Reports. 7:6511.

Akinbami, A. A., Ajibola, S. O., Rabiu, K. A., Adewunmi, A. A., Dosunmu, A. O., Adediran, A., Osunkalu, V. O., Osikomaiya, B. I., & Ismail, K. A. (2013). Hematological profile of normal pregnant women in Lagos, Nigeria. International Journal of Women's Health, 5, 227–232. https://doi.org/10.2147/IJWH.S42110

Anurag, A., Jha, P. K., & Kumar, A. (2020). Differential white blood cell count in COVID-19: A cross-sectional study of 148 patients. Diabetes & Metabolic Syndrome, 14(6), 2099–2102. https://doi.org/10.1016/j.dsx.2020.10.029

Caparros-Gonzalez, R. A., Torre-Luque, A. de la, Romero-Gonzalez, B., Quesada-Soto, J. M., Alderdice, F., & Peralta-Ramírez, M. I. (2021). Stress During Pregnancy and the Development of Diseases in the Offspring: A Systematic-Review and Meta-Analysis. Midwifery, 97, 102939. https://doi.org/10.1016/j.midw.2021.102939

Chan, S. H. T., Yu, T., Zhang, Z., Chang, L.-Y., Guo, C., Bo, Y., Tam, T., Lau, A. K. H., & Lao, X. Q. (2022). Total and differential white blood cell count and cause-specific mortality in 436 750 Taiwanese adults. Nutrition, Metabolism, and Cardiovascular Diseases: NMCD, 32(4), 937–947. https://doi.org/10.1016/j.numecd.2021.11.004

Cherian, S. B., Bairy, K. L., & Rao, M. S. (2015). Effect of Prenatal Stress on Birth weight, Postnatal Weight gain and Developmental Milestones in Wistar Rats. International Journal of Advanced Research, 3(9).

Churchill, D., Nair, M., Stanworth, S. J., & Knight, M. (2019). The change in haemoglobin concentration between the first and third trimesters of pregnancy: A population study. BMC Pregnancy and Childbirth, 19(1), 359. https://doi.org/10.1186/s12884-019-2495-0

Gao T, Wang Z, Dong Y, Cao J, Lin R, Wang X, et al. (2019). Role of melatonin in sleep deprivation-induced intestinal barrier dysfunction in mice. J Pineal Res. 67(1):e12574.

Gebreweld, A., Bekele, D., & Tsegaye, A. (2018). Hematological profile of pregnant women at St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. BMC Hematology, 18(1), 15. https://doi.org/10.1186/s12878-018-0111-6

Kurwale NS, Suri V, Srivastava A, Suri A, Mohantis S, Yadav S, Sharma MC, Sharkar C., (2015). Role of bone marrow pleuripotent stem cells in peripheral nerve repair in adult rats: morphometric evaluation. J. Neurosci. Rural pract. 6:152-159

Lamichhane, N., Olsen, N. J., Mortensen, E. L., Obel, C., Heitmann, B. L., & Händel, M. N. (2020). Associations between maternal stress during pregnancy and offspring obesity risk later in life-A systematic literature review. Obesity Reviews: An Official Journal of the International Association for the Study of Obesity, 21(2), e12951. https://doi.org/10.1111/obr.12951

Lateef OM, Akintubosun MO (2020). Sleep and Reproductive Health. J Circadian Rhythms.18:1.

Liu, H., Wang, G., Luan, G. and Liu, Q. (2009). Effects of sleep and sleep deprivation on blood cell count and hemostasis parameters in healthy humans. Journal of Thrombosis and Thrombolysis. 28 (1):46–49

Machado, R.B., Hipolide, D.C., Beneditto-silva, A.A., Tufik, S. (2004). Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. Brain Res;1004:45-51.

Machado, R.B., Suchecki, D., and Tufik, S (2005). Sleep homeostasis in rats assessed by a long-term intermittent paradoxical sleep deprivation protocol. Behav Brain Res. 160: 356–364.

Pardo, G. V. E., Goularte, J. F., Hoefel, A. L., de Castro, A. L., Kucharski, L. C., da Rosa Araujo, A. S., & Lucion, A. B. (2016). Effects of sleep restriction during pregnancy on the mother and fetuses in rats. Physiology & Behavior, 155, 66-76.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, & Bitto A, (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev*, 2017 8416763.

Reese, J. A., Peck, J. D., Deschamps, D. R., McIntosh, J. J., Knudtson, E. J., Terrell, D. R., Vesely, S. K., & George, J. N. (2018). Platelet Counts during Pregnancy. The New England Journal of Medicine, 379(1), 32–43. https://doi.org/10.1056/NEJMoa1802897

P-ISSN: 2811-1516

E-ISSN: 2811-1524

Sedov ID, Cameron EE, Madigan S, Tomfohr-Madsen LM. Sleep quality during pregnancy: A meta-analysis (2018). Sleep Med Rev. 38:168-176. doi: 10.1016/j.smrv.2017.06.005.

Smyka, M., Kosińska-Kaczyńska, K., Sochacki-Wójcicka, N., Zgliczyńska, M., & Wielgoś, M. (2020). Sleep Problems in Pregnancy—A Cross-Sectional Study in over 7000 Pregnant Women in Poland. International Journal of Environmental Research and Public Health, 17(15), 5306. https://doi.org/10.3390/ijerph17155306

Villafuerte G, Miguel-Puga A, Murillo-Rodríguez E, Machado S, Arias-Carrión O. (2015). Sleep Deprivation and Oxidative Stress in Animal Models: A Systematic Review. Oxid Med Cell Longev. (2):234952.

Wang X, Wang Z, Cao J, Dong Y, Chen Y. Melatonin Alleviates Acute Sleep Deprivation-Induced Memory Loss in Mice by Suppressing Hippocampal Ferroptosis (2021). Front Pharmacol. 12:708645. pmid:34335271

Wei, Q., Shi, H., Ma, X., Shi, Y., Zhang, Y., & Wang, L. (2021). The impact of maternal stress on offspring birth weight and the mediating effect of dietary patterns: The Shanghai Maternal-Child Pairs Cohort study. Journal of Affective Disorders, 278, 643–649. https://doi.org/10.1016/j.jad.2020.09.077

World Health Organization (WHO). (2022). *"Mental health and psychosocial considerations during the COVID-19 outbreak."* (Annex: Considerations for pregnant and postpartum women). WHO Reference Number: WHO/2019-nCoV/MentalHealth/2020.1.WHO/2019-nCoV/MentalHealth/2020.1

Wu, M., Zhou, L., Zhu, D., Lai, T., Chen, Z., & Shen, H. (2018). Hematological indices as simple, inexpensive and practical severity markers of obstructive sleep apnea syndrome: A meta-analysis. Journal of Thoracic Disease, 10(12), 6509–6521. https://doi.org/10.21037/jtd.2018.10.105