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Original Article

Effect of lauric acid on some of the hematological parameters and indices in male Wistar rats exposed to sleep deprivation

[1]Avidime O.M., [2]Suleiman I. & [2]Oduneye F.M.

ABSTRACT

Background and aim: Sleep deprivation is a prevalent issue affecting a substantial portion of the population, leading to numerous adverse health outcomes including alterations in blood parameters, increased inflammation, dyslipidemia, and oxidative stress. Sleep deprivation is highly prevalent in the modern world, possibly reaching epidemic proportions. Lauric acid is the most abundant medium- chain fatty acid of coconut oil, which has been reportedly used for various health benefits. This study aimed at assessing the effect of Lauric acid on some hematological parameters and their indices in sleep-deprived male Wistar rats.

Methodology: Sixteen male Wistar rats were assigned into 4 groups randomly, group 1 which served as the control group was given only food and water without sleep deprivation, the group 2 rats were deprived from sleep for a period of 20hrs daily for 7days according to a standard protocol and were not treated with, and the third and fourth groups were sleep deprived and were given lauric acid (at 50mg/k Lauric acidg and 100mg/kg respectively) 4hrs before sleep deprived protocol for a period of 20hrs daily for 7days. The animals were all sacrificed a day after the seventh day of the experiment and the blood samples were collected via cardiac puncture with a 5ml syringe into sample bottles for hematological analysis.

Results: The results of the present study revealed a statistically significant ($p < 0.05$) reduction in the mean value of Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) in the group subjected to sleep deprivation compared to the control group and non-significant decrease was also observed in the mean values of Red Blood Cell (RBC) Count and Mean Corpuscular Haemoglobin Concentration (MCHC) in the sleep-deprived group compared to the control. The groups administered with 50 mg/kg and 100 mg/kg of Lauric acid 4 hrs and subjected to sleep deprivation for 20 hrs daily for 7 days showed increased mean values in RBC, PCV, Mean Corpuscular Volume, Haemoglobin Concentration, Mean Corpuscular Haemoglobin and MCHC and were statistically significant ($p < 0.05$) except for RBC and MCHC compared to control group and the sleep deprived group.

Conclusion: In conclusion, the present study demonstrated significant decrease in PCV, Hb, MCV, MCH and a non-significant reduction in the mean values of RBC count and MCHC in the sleep-deprived male Wistar rats and the effects were significantly ameliorated in the groups treated with 50 mg/kg and 100 mg/kg of Lauric acid 4 hrs before being subjected to 20 hrs sleep deprivation daily for 7 days.

Keywords:
Sleep Deprivation; Lauric Acid; Hematological parameters; Wistar Rats

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[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Kaduna State University, Kaduna, Nigeria; [2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria.

Address for Correspondence:
Avidime O.M.
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Kaduna State University, Kaduna, Nigeria.
nenemakoju@yahoo.com

INTRODUCTION

Sleep plays a vital role in maintaining good health, as its timing, length, and quality affect metabolic control, emotion management, performance, memory consolidation, brain recovery, and learning (Perry *et al.*, 2013). Sleep deprivation is a prevalent issue affecting modern society, with detrimental effects on various physiological processes, including immune function, metabolism, and cardiovascular health (Irwin, 2019; Palagini *et al.*, 2020). Studies have demonstrated that implementing interventions such as brief periods of rest, moderate physical activity and intake of natural supplements can mitigate the noxious effect of sleep deprivation and enhance the quality of sleep, hence resulting in improved mental and physical well-being (Tanaka & Shirakawa 2004).

Multiple studies have consistently demonstrated that a lack of sleep can have notable impacts on blood-related factors and the body's ability to clot. Abd Elhadi *et al.* (2024), observed fluctuations in lymphocyte, eosinophil, and neutrophil counts, as well as changes in coagulation factors. Liu *et al.* (2009), also reported sleep deprivation (SD) can result in increased levels of white blood cells and neutrophils,

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as well as reduced prothrombin and activated partial thromoplastin durations, indicating a state of hypercoagulability. A direct correlation exist between the amount of time spent sleeping and the levels of hematocrit in the blood. Longer sleep was found to increase both hematocrit and haemoglobin levels (Wang *et al*., 2020). However, partial sleep deprivation was found to have no significant impact on red blood cell count, haemoglobin levels, or hematocrit during intermittent activity (Mejri *et al*., 2014).

Lauric acid is a natural biological agent found in various kinds of foods, such as fruits, seeds, and breast milk (Alves *et al*., 2017). Lauric acid possesses pleiotropic biological and antioxidant activities which has been reported in various oxidative stress processes (Ahola *et al*., 2017).

Previous literature on sleep deprivation (SD) and biological responses (e.g., hematological) are unclear, contradictory or inconclusive and several of them reported that partial sleep deprivation and total sleep deprivation increase WBC and their subpopulations, while some reported no rise or reduction in these measurements, and that hematocrit (HCT) and red blood cells (RBC) also seem unaffected by sleep deprivation (Mejri *et al*., 2014). Therefore, this study aimed at assessing the effect of Lauric acid on some hematological parameters and their indices in sleep-deprived male Wistar rats.

MATERIALS AND METHOD

Study Location

The study was carried out in the Laboratory of Experimental Physiology, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Chemicals and Drugs

Lauric acid (50ml/kg and 100ml/kg), distilled water, Ketamine and Diazepam, Methylated Spirit, Dettol, Dilution fluids (Sodium Citrate, Sodium Chloride), Formalin, Mercuric Chloride. All chemicals were commercially obtained and were analytically graded.

Experimental Animals and Design

Sixteen (16) healthy male Wistar rats between the ages of 7-8weeks weighing 100-160g were used for this study. The Wistar rats were purchased from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. The animals were kept in the animal house for acclimatization and fed for 2 weeks. They were housed in white transparent vivarium plastic cages and fed on standard commercial feeds with free access to water (*ad libitum*).

The animals were randomly divided into four groups (n= 4). Group 1 served as normal control administered with distilled water (1mL/Kg body weight). Group 2 was the sleep- deprived group and left untreated with lauric acid. Group 3 and 4 were administered with Lauric acid 50mL/Kg body weight and 100 mL/Kg body weight, respectively for 4hr before being subjected to sleep deprivation for 20 hrs.

Induction of Sleep Deprivation

Before the commencement of sleep deprivation, the rats were given time to adjust to the ambient experimental environment by introducing them to the tank environment for five hours on the first day. Then the tank was filled with water at 3 cm below the surface of the platforms to prevent the rats from lying down without becoming partially submerged (Neville *et al*., 2022).

The method of inducing sleep deprivation involved placing the rats on a narrow platform in water, a modified version of the "flowerpot" technique, to prevent sustained sleep, requiring constant movement to avoid falling into the water. This sleep deprivation protocol was carried out for 20 hours per day for a period of 7 days (Koban *et al*., 2008).

Determination of Hematological Parameters

Automated Analysis of Complete Blood Count (CBC)

Blood samples were analyzed using an automated hematology analyzer (Mindray BC-5000 Auto 5-Diff Hematology Analyzer model) to determine Complete Blood Count (CBC) parameters, including Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) (Johnson *et al*., 2019).

Measurement of Haemoglobin Concentration and Hematocrit

Haemoglobin concentration was measured using a spectrophotometric method after conversion to cyanmethaemoglobin by the haemoglobinometer, while hematocrit was determined by centrifuging blood samples in capillary tubes to measure the percentage of blood volume occupied by red blood cells (Green & Adams, 2017).

Statistical Analysis

Data analysis was done using one-way analysis of variance (ANOVA), which was followed by *Tukey's* post-hoc test to compare the level of significance between the test group and the control. The results were expressed as mean $\pm$ standard error. SPSS version 20 was used for the analysis and values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of lauric acid on red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration in Male Wistar Rats Exposed to 7 days of Sleep Deprivation.

Table 1: shows the result of lauric acid on red blood cell count, packed cell volume, haemoglobin concentration, mean

corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in sleep-deprived male Wistar rats. The results of the present study revealed a statistically significant ($p < 0.05$) reduction in the mean value of Packed Cell Volume (PCV) (22.00±0.82%), Haemoglobin concentration (Hb) (7.33±0.27 g/dL), Mean Corpuscular Volume (MCV) (42.08±1.74 fL) and Mean Corpuscular Haemoglobin (MCH) (14.01±0.58 pg) in the group subjected to sleep deprivation compared to the control group and non-significant decrease was also observed in the mean values of Red Blood Cell (RBC) Count (5.24±0.12 million cell/µL) and Mean Corpuscular Haemoglobin Concentration (MCHC) (33.28±0.06 g/dL) in this sleep-deprived group compared to the control. The groups administered with 50 mg/kg and 100 mg/kg of Lauric acid 4 hrs and subjected to sleep deprivation for 20 hrs daily for 7 days showed increased mean values in RBC, PCV, Mean Corpuscular Volume, Haemoglobin Concentration, Mean Corpuscular Haemoglobin and MCHC and were statistically significant ($p < 0.05$) except for RBC and MCHC compared to control group and the sleep deprived group.

Table 1: Effect of lauric acid on red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration in Male Wistar Rats Exposed to 7 days of Sleep Deprivation

	Parameters (Mean ±SEM)					
GROUPS	RBC	PCV	Hb	MCV	MCH	MCHC
Control	5.38±0.14	49.25±2.29^{a}	16.43±0.77^{a}	91.78±5.46^{a}	30.61±1.84^{a}	33.35±0.03
Sleep Deprived	5.24±0.12	22.00±0.82^{b}	7.33±0.27^{b}	42.08±1.74^{b}	14.01±0.58^{b}	33.28±0.06
LA 50mg/kg	5.38±0.12	39.50±3.28^{c}	13.18±1.09^{ac}	73.55±6.29^{c}	24.53±2.09^{ac}	33.33±0.03
LA 100mg/kg	5.70±0.35	37.50±1.56^{c}	12.5±0.52^{ac}	66.39±4.52^{ac}	22.13±1.49^{c}	33.32±0.04

RBC = Red blood cell count (million cell/µL), PCV = packed cell volume (%), Hb= Haemoglobin concentration (g/dL), MCV = Mean Corpuscular volume (fL), MCH = Mean corpuscular haemoglobin (pg), MCHC = Mean Corpuscular haemoglobin Concentration (g/dL). All data were expressed as Mean ± SEM, n=4. ^{a,b,c}superscript letters show statistically significant different, $P < 0.05$

DISCUSSION

The current study observed no significant differences in the control group and the group subjected to sleep deprivation and also the administration of Lauric Acid (LA) 4 hrs before sleep deprivation in male Wistar rats for seven days showed no difference in the mean value of red blood cell count. This non-significant differences observed across the groups may suggests that LA did not have a short while effect on RBC production, as reported by Lim *et al*. (2017), which they found in their study that administration of LA did not significantly increased RBC synthesis outcome in Wistar rats. Although, the 100mg/kg dose of LA resulted in a higher mean RBC count compared to the 50mg/kg dose, compared to the control group and the sleep deprived group.

The significant decrease in hematocrit observed in the sleep-deprived group in this study compared to the control group reflects an impaired physiological defense state against the RBC membrane by oxidative stress which probably leads to their haemolysis and reduction in number. Reduced hematocrit levels can be an indication of anaemia or other blood related disorders, suggesting that the condition modeled in the sleep-deprived group compromised erythropoiesis or an increased destruction of red blood cells. Upon administration of Lauric acid 4 hr before sleep deprivation in the treatment groups significantly increased the hematocrit levels compared to the sleep-deprived group. This finding is in accordance with the findings of Nagao & Yanagita, (2010), which reported that LA administration improved RBC production and its hematocrit in Wistar rats subjected to oxidative stress condition. These properties likely helped to restore hematocrit level closer to normal in the treated group.

The current study also revealed a significant decrease in haemoglobin level in the sleep-deprived group compared to the control group. Treatment with LA at dose 50 mg/kg and 100 mg/kg in the treatment groups resulted in improved mean value of haemoglobin levels compared to the sleep-deprived group. The significant reduction in haemoglobin levels in the sleep-deprived group suggests some underlying stress in the RBC production process, which resulted in poor yield of haemoglobin contents. The finding in the treatment groups in this study is in line with the study of Wasan *et al*. (2008), which found that LA administration in Wistar rats improves the life span of erythrocytes, thus preserving haemoglobin concentrations. This ameliorative properties of LA could probably be due to its antioxidant properties for prevention of F^{2+} to F^{3+} and restrict Fenton reactions and keep the iron in their ferrous state and their incorporation in the RBC production processes.

This study also observed reduction in MCV in the sleep-deprived group compared to the control group, which suggests impaired erythropoietic process, and a tendency for producing smaller red blood cells, which is an indication of microcytic anaemia . This condition could probably resulted from factors such as iron deficiency due to interruption in iron absorption or by oxidative stress conditions that might affect the synthesis and maturation of red blood cells. Reduced MCV is often associated with inability of the erythropoietic system to produce sufficient mature and functional erythrocytes as reported by Johnson-Wimbley & Graham, 2011. The administration of LA particularly at 50 mg/kg and 100 mg/kg, showed a significant improvement in MCV, suggesting that LA has protective or restorative effects on RBC maturation and decreased microcytosis.

The study revealed significantly decrease in MCH in the sleep-deprived group compared to the control group. The drastic reduction in MCH value in this group suggests a form of hypochromic anaemia , where the haemoglobin content within

the red blood cells is severely reduced. This condition is often associated with conditions such as iron deficiency or diseases that impair erythropoiesis (Johnson-Wimbley & Graham, 2011). The reduction in MCH value may be due to a decrease in haemoglobin synthesis or increased oxidative stress damaging RBCs, leading to less haemoglobin per cell. The increase in MCH in LA treated groups compared to the sleep-deprived group suggested LA might have enhanced erythropoiesis and haemoglobin synthesis in these groups, as RBCs are able to carry more haemoglobin per cell. This improvement could be due to the anti-inflammatory and antioxidant effects of LA, which have been shown to improve RBC function by reducing oxidative stress which is in consonance with the report of Wasan *et al*. (2008). They found that short-chain fatty acids mitigate oxidative stress in animal models and improved systemic functions.

The non-statistically significant value of MCHC observed in the sleep-deprived group compared to the control group suggesting that the state of sleep deprivation in this study did not induce a significant effect on the MCHC. This may indicate that there could be changes in RBC count or size, the haemoglobin concentration per cell remained relatively stable. According to Dacie & Lewis (2001), the stability of MCHC in some pathological states are often maintained unless there is severe haemolytic processes in haemoglobin production. However, the slight increase in MCHC observed in the LA 50 mg/kg and 100 mg/kg groups might be attributed to the antioxidant properties of LA which ultimately enhance RBC membrane stability and improve haemoglobin retention within the cells, similar to the finding of Nago & Yanagita (2010), which reported that medium-chain fatty acids like LA are beneficial in prevention and treatment of the metabolic oxidative stresses.

Conclusion: The present study demonstrated significant decrease in PCV, Hb, MCV, MCH and a non-significant reduction in the mean values of RBC count and MCHC in the sleep-deprived male Wistar rats and the effects were significantly ameliorated in the groups treated with 50 mg/kg and 100 mg/kg of Lauric acid 4 hours before being subjected to sleep deprivation.

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