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Hibiscus sabdariffa calyx extract alters inflammatory cytokines, oxidative stress biomarkers and hematological parameters in paradoxically sleep-deprived adult female Wistar rats

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Sleep deprivation negatively impacts well-being including increased stress levels, reduced cognitive performance and weakened immune systems. Twenty five female Wistar rats weighing 150–200 g were split into five groups of five rats each. Group I served as the normal control, while Group II was the negative control (sleep-deprived untreated). Groups III, IV, and V were administered varying doses of the HS extract via gavage at 100, 200 and 400 mg/kg respectively. The animals were anaesthetized and sacrificed, and blood samples were collected for biochemical assessment. HS notably reduced oxidative stress ($P< 0.05$) by improving the activities of key enzymatic antioxidants, including SOD, GPx, and CAT, while lowering ROS production in comparison to the SD-untreated group. Additionally, HS treatment led to a significant increase ($P< 0.05$) in the levels of interleukin-10 (IL-10) and brain-derived neurotrophic factor (BDNF), alongside a marked decrease in TNF-alpha levels when compared to the SD-untreated groups. The study demonstrated that HS treatment significantly ($P< 0.05$) improved hematological indices. In conclusion, HS extract modulates oxidative stress and inflammation via its effect on key antioxidant enzymes; SOD, GPx and CAT, inflammatory biomarkers; IL-10 and TNF-alpha as well as BDNF. It also improves hematological indices in female sleep-deprived animals.

Keywords: *Hibiscus sabdariffa*, Reactive oxygen species, Interleukin-10, Tumor necrosis factor alpha; Brain-derived neurotrophic factor, Sleep-deprived

Modern civilizations are experiencing a decline in sleep length, affecting overall health and immune system function, as research highlights the negative consequences of sleep deprivation[1]. Sleep boosts immunity, and enhances the body's ability to fight inflammation and infections[2,3]. Insufficient sleep can lead to immune system changes, causing chronic inflammation and increasing the risk of infectious diseases like cancer, autoimmune disorders, neurodegenerative diseases, and cardiometabolic health[4,1]. There are two different kinds of sleep deprivation: acute and chronic. Acute sleep deprivation is a condition in which a person's regular sleep length is significantly reduced[5]. Oxidative stress can disrupt biological processes[6]. The body uses both enzymatic and non-enzymatic antioxidants to address this problem. Enzymatic and non-enzymatic defenses protect against free radicals[7]. Inflammation activates both the innate and adaptive immune systems[8] and is usually brief and strictly controlled[9]. Excessive tumour necrosis factor-alpha (TNF-α) signaling can result in chronic inflammation and autoimmune disorders[10,11]. Research has shown that sleep deprivation can increase both circulating and hypothalamic levels of TNF-α[12-14]. Interleukin-10 (IL-10), is a potent cytokine[15,16], implicated in sleep deprivation-induced modifications[17]. Research has demonstrated an inverse relationship between reactive oxygen species and BDNF[18-20], and is a promising therapeutic target for ischemic injuries due to its neuroprotective effects, which include anti-apoptotic, anti-inflammatory, anti-neurotoxic, and regenerative properties[21,22]. The interaction between stress and sleep can significantly influence BDNF levels, increasing BDNF expression[23].

Sleep is crucial in hematopoiesis and other related processes like bone restoration and cellular

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proliferation[24,25], growth and proliferation of hematopoietic stem cells, progenitor cells, leucocytes, thrombocytes and erythrocytes[26,27]. Roselle, also known as *Hibiscus sabdariffa* L., is a versatile crop suitable for developing countries due to its ease of cultivation, multi-cropping compatibility, and potential food and fiber applications. In Nigeria, using organic fertilizers can enhance Roselle cultivation[28]. Applying 2.5 tons of manure and 60 kg of nitrogen per hectare significantly increased the dry calyx yield of *Hibiscus sabdariffa*[29]. Another research highlighted that the average productivity of Roselle was about 13.20 q/ha (quintals per hectare) during the 2012-2013 period[30]. HS, known for its antipyretic and anti-inflammatory properties, has been traditionally used in medicine to reduce fever and prevent anemia by blocking cytokines involved in the inflammatory response[31,32]. There have been reports on other natural agents with antioxidant potential. Iridoid glycosides derived from *Scrophularia amplexicaulis* exhibit neuroprotective effects through mechanisms such as cholinesterase inhibition and antioxidative actions[33]. The species *Octaviania asterosperma* has also been shown to possess antioxidative, antimicrobial, and neuroprotective properties in vitro[34]. In addition, curcumin has been reviewed for its multifaceted role in addressing oxidative stress, inflammation, nervous system health, and lipid regulation[35]. The broader implications of propolis on neuropsychiatric conditions through dietary means have been documented[36]. Therefore, this study aimed to investigate the effects of HS extract on inflammatory, oxidative stress, and hematological changes induced by sleep deprivation in adult female Wistar rats.

Materials and Methods

Materials

The following materials were utilized in the study: white transparent plastic cages, ethylenediaminetetraacetic acid (EDTA), plain sample bottles, syringes, oral cannulas, gloves, and a weighing machine (Model: XY100C, Serial Number: 1404273, Changzhou Xingyun). The commercial ELISA kits used included Rat MDA (EU2577), Rat SOD1 (ER0332), Rat GPx-1 (ER0274), Rat CAT (ER0264), Rat TNF-alpha (ER1393), Rat BDNF (ER0008), and Rat IL-10 (ER0033), all sourced from Fine Test, Wuhan, China. Additionally, Rat ROS (CK-bio-20410) was obtained from Shanghai Coon Koon Biotech Co., Ltd., Shanghai, China. The hematological assays were conducted using the Advia 60 Hematology System (Bayer Diagnostics Europe Ltd., Ireland).

Plant identification and extraction

Fresh red calyces of *Hibiscus sabdariffa* L. were procured from a local market in Zaria, Kaduna State, Nigeria. The calyces were authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and assigned voucher number 01056. After shade-drying at 80°C, the calyces were ground and sieved. Subsequently, 25 g of the powdered calyces were dissolved in 250 mL of distilled water to prepare a 10% extract using the cold maceration method. The extract was then filtered, and the filtrate was stored in a refrigerator for further experiments. The percentage yield was calculated using the method described by Aliyu *et al*.[37]

Animal handling and care

Twenty-five adult female Wistar rats (150-200 g body weight) were acquired from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. The animals were kept in clear plastic cages and given free rein to commercial feed for two weeks while they acclimated to their new surroundings.

Experimental design and ethical approval

After the animals were weighed, they were split into five groups at random, with five animals in each group. (n=5). Group I was designated as the normal control and received distilled water, while Group II served as the negative control (SD-untreated). Groups III, IV, and V were treated with varying doses of the extract: 100, 200, and 400 mg/kg, respectively. The dosages of the extract used in this study were determined based on prior research[38]. The experimental procedures were conducted per the approved guidelines of the Ahmadu Bello University Ethical Committee on Animal Use and Care.

Induction of paradoxical sleep deprivation

Rats were acclimated to the glass tank for 1 h each day over three consecutive days before the addition of water. The water level was maintained at 3 cm below the platforms[39]. Sleep deprivation was induced using the column-in-water method, in which rats were placed on platforms designed to prevent sleep, necessitating constant movement to avoid falling. This sleep deprivation protocol lasted for 20 h a day over 7 days[40,41].

Blood sample collection

After the study, the animals were anaesthetized with pentobarbital (60 mg/kg body weight, intraperitoneally)[42]. Using a heart puncture, blood samples were taken and placed in EDTA and plain sample vials for biochemical and hematological assessments.

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 GPx-1 (ER0274) and Rat CAT (ER0264). Serum TNF-alpha, BDNF and IL-10 were assayed using the specie specific ELISA kits according to the manufacturer's manual; Rat TNF-alpha (ER1393), Rat BDNF (ER0008) and Rat IL-10 (ER0033).

Hematological Indices and erythrocyte osmotic fragility

Blood parameters were analyzed using an automatic hematological assay analyzer, the Advia 60 Hematology System (Bayer Diagnostics Europe Ltd., Ireland). The assessment of erythrocyte osmotic fragility was conducted using the methodology outlined by Okonofua *et al.*[43] and Faulkner & King[44].

Statistical analysis

The study's data were evaluated and presented as mean ± SEM. The IBM Statistical Package for Social Sciences (SPSS) version 23 was used to conduct the statistical analysis. To ascertain the differences between the groups, a one-way analysis of variance (ANOVA) was performed and then Tukey's *post hoc* test was performed. A *P*-value of less than 0.05 was deemed statistically significant. Charts were created using GraphPad Prism 8 software (version 8.0.2 [263]; GraphPad Software, San Diego, California, USA).

Results

Serum MDA, GPx, SOD and CAT

In Fig. 1A, serum malondialdehyde concentration (MDA) was significantly higher (*P*= 0.001) in the SD-untreated group compared to the NC. Treatment with HS-extract at 200 and 400 mg/kg significantly (*P*= 0.004, 0.001 respectively) lowered serum MDA compared to the SD-untreated group. Although serum MDA was lower in the HS-extract at 100 mg/kg, it was not statistically significant (*P*= 0.159). In the group treated with 400 mg/kg of the extract, serum MDA was significantly lower (*P*= 0.002) than the SD+HS (100 mg/kg) group. Serum GPx in Fig. 1B was significantly decreased (*P*= 0.001) in the SD-untreated group compared to the NC. In the SD+HS (100, 200 and 400 mg/kg) treated groups, serum GPx was significantly increased (*P*= 0.003, 0.001 and 0.001 respectively) compared to the NC. In Fig. 1C, serum SOD was significantly decreased (*P*= 0.001) in the SD-untreated group compared to the NC and increased significantly (*P*= 0.002, 0.001, 0.001) In the SD+HS (100, 200 and 400 mg/kg respectively) treated groups compared to the

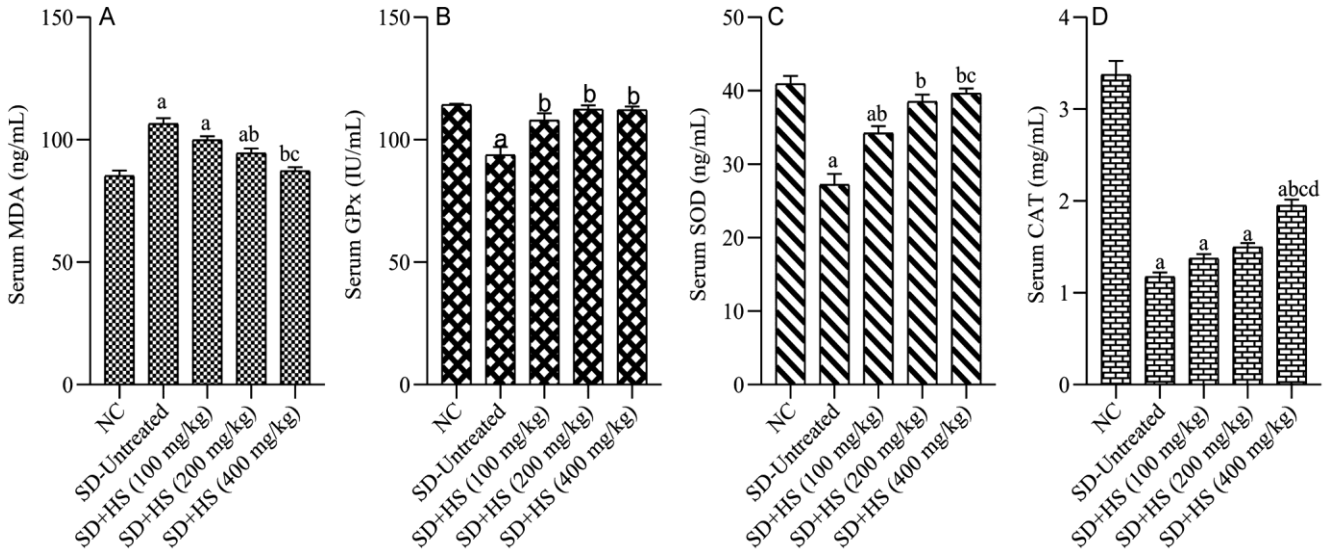

Fig. 1 — Results of HS extract on indicators of blood oxidative stress in adult female Wistar rats subjected to paradoxical sleep deprivation. [SD stands for sleep deprivation, NC for normal control, and HS for *Hibiscus sabdariffa*. Superscripts; a= P<0.05 compared NC, b= *P*<0.05 *vs* SD-untreated, c= *P*<0.05 *vs* SD+HS (100 mg/kg), d= *P*<0.05 *vs* SD+HS (200 mg/kg)]

SD-untreated group. In the group given 400 mg/kg of HS extract, serum SOD was significantly higher (*P*= 0.017) compared to the SD+HS (100 mg/kg)-treated group. In Fig. 1D, serum CAT was significantly decreased (*P*= 0.001) compared to the NC. Comparing the groups administered HS-extract at 400 mg/kg to the SD-untreated group (*P*=0.001), SD+HS (100 mg/kg) (*P*=0.001), and SD+HS (200 mg/kg) (P=0.001), serum CAT in the HS-extract groups increased considerably.

Serum ROS, TNF-alpha, IL-10 and BDNF

In Fig. 2A, serum ROS was significantly higher (*P*= 0.001) in the SD-untreated group compared to the NC. The ROS was decreased significantly (*P*= 0.003, 0.001, 0.001) in all the HS extract-treated groups compared to the SD-untreated group. In the 200 and 400 mg/kg HS extract-treated groups, serum ROS was significantly decreased (*P*= 0.033, 0.001) compared to the SD+HS (100 mg/kg). In Fig. 2B, serum

TNF-alpha was significantly higher in the SD-untreated group compared to the NC (*P*= 0.001). In the groups that were given HS extract at 100, 200 and 400 mg/kg, the level of TNF-alpha was significantly decreased compared to the SD-untreated group (*P*= 0.001, 0.002, 0.001). In the 200 and 400 mg/kg treated groups, TNF-alpha levels were significantly higher than the HS 100 mg/kg treated group. In Fig. 2C, serum IL-10 was significantly reduced (*P*= 0.001) in the SD-untreated group compared to the NC. Treatment with HS extract significantly improved IL-10 in a dose-dependent manner. Serum BDNF in Fig. 2D reduced significantly (*P*= 0.001) in the SD-untreated group compared to the NC. When compared to the SD-untreated group, the HS extract at 200 and 400 mg/kg considerably (*P*=0.014, 0.001) raised serum BDNF.

RBC, Hb, PCV and red cell distribution width

In Fig. 3A, the RBC count was significantly decreased in the SD-untreated group compared to the NC. Treatment with HS extract at 200 and 400 mg/kg significantly increased RBC count (*P*= 0.002, 0.013) compared to the SD-untreated group. In the group given HS extract at 200 mg/kg, the RBC count was significantly higher (*P*= 0.001) compared to the SD+HS (100 mg/kg)-treated group. Hb in Fig. 3B significantly reduced (*P*= 0.001) in the SD-untreated group compared to the NC. Hb significantly increased at 200 and 400 mg/kg compared to SD-untreated and SD+HS (100 mg/kg) groups. In Fig. 3C the PCV in the SD-untreated group significantly decreased (P= 0.001) compared to the NC. In all the groups treated with HS extract, PCV was significantly (*P*= 0.001, 0.002 and 0.001) increased compared to the SD-untreated group. In Fig. 3D, the red cell distribution width was significantly higher in the SD-untreated group compared to the NC (*P*= 0.002). Treatment with HS extract at 100, 200 and 400 mg/kg significantly reduced the red cell distribution width compared to the SD-untreated group.

WBC, lymphocytes, platelets count and platelet cell distribution width

In Fig. 4A, the WBC count was significantly increased in the SD-untreated group compared to the

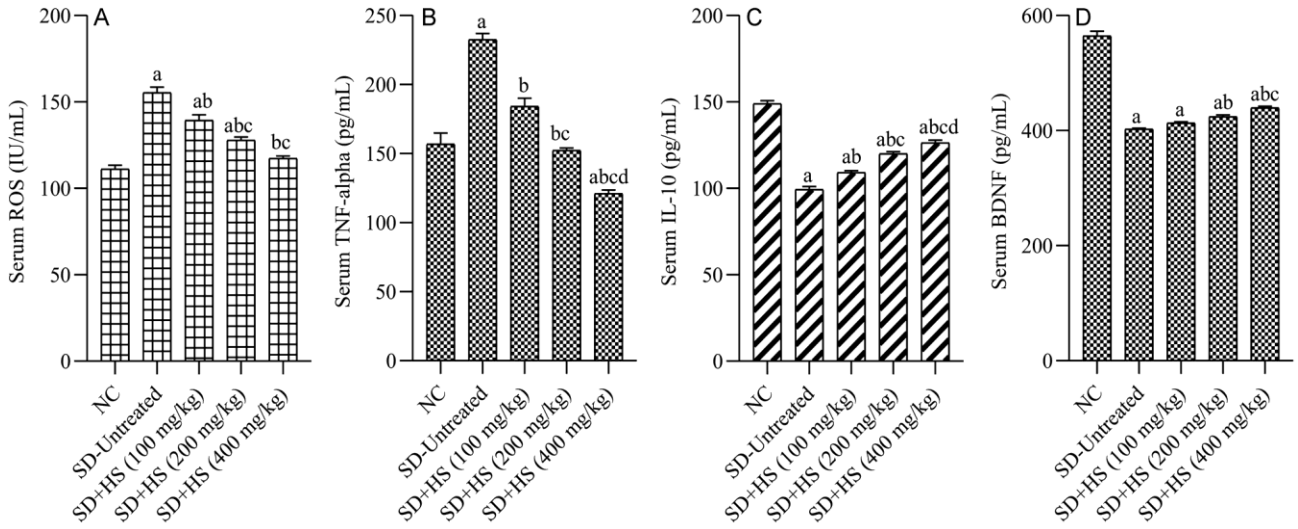

Fig. 2 — Results of HS extract on serum ROS (2A), TNF-alpha (2B), IL-10 (2C), and serum BDNF (2D) in adult female Wistar rats exposed to paradoxical sleep deprivation. [HS = *Hibiscus sabdariffa*; SD = sleep deprivation; and NC = normal control. Superscripts; a= *P*<0.05 *vs* NC, b= *P*<0.05 *vs* SD-untreated, c= *P*<0.05 *vs* SD+HS (100 mg/kg), d= *P*<0.05 *vs* SD+HS (200 mg/kg)]

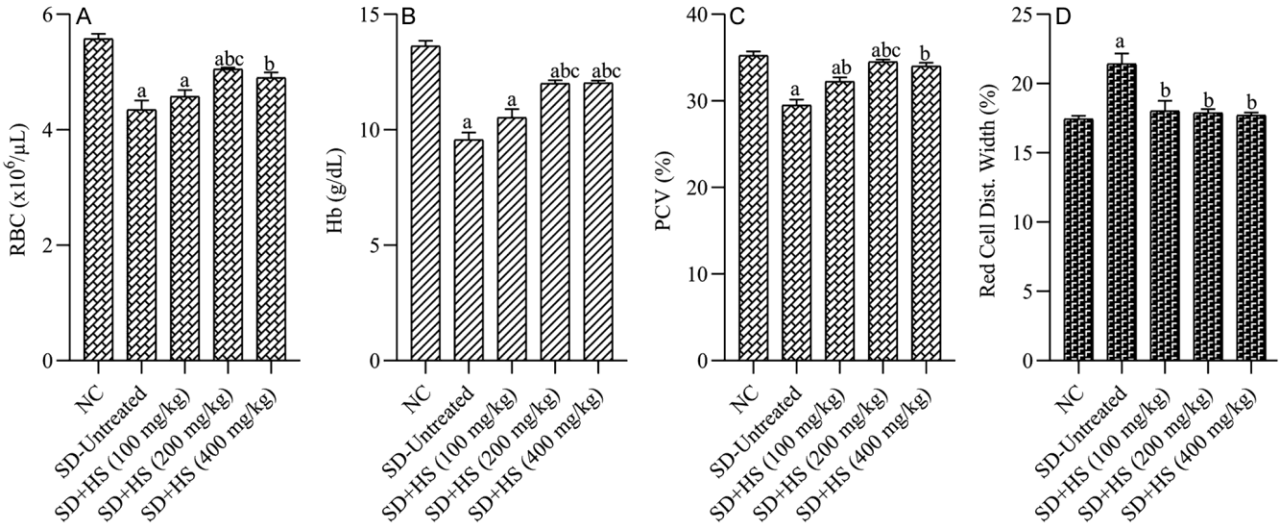

Fig. 3 — Results of HS extract on RBC (3A), Hb (3B), PCV (3C), and Red cell distribution width (3D) in adult female Wistar rats exposed to paradoxical sleep deprivation. [NC = Normal control, SD = sleep deprivation, HS = *Hibiscus sabdariffa*. Superscripts; a= *P*<0.05 *vs* NC, b= *P*<0.05 *vs* SD-untreated, c= *P*<0.05 *vs* SD+HS (100 mg/kg)]

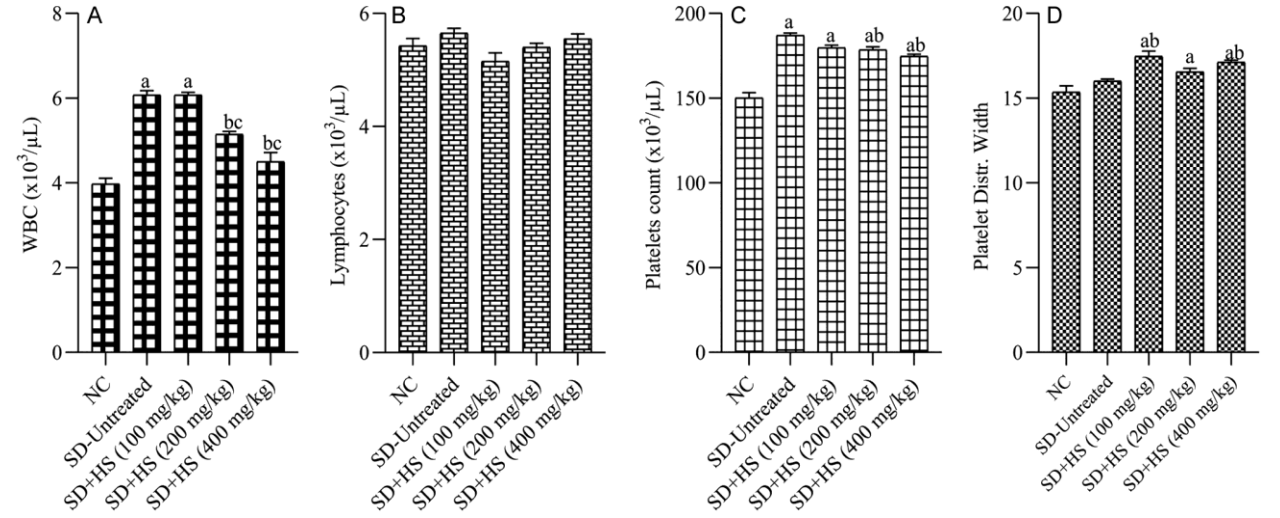

Fig. 4 — Results of HS extract on WBC (4A), lymphocytes (4B), platelets (4C) and platelet distribution width (4D) in adult female Wistar rats exposed to paradoxical sleep deprivation. [NC = Normal control, SD = sleep deprivation, HS = Hibiscus sabdariffa. Superscripts; a= *P*<0.05 *vs* NC, b= *P*<0.05 *vs* SD-untreated, c= *P*<0.05 *vs* SD+HS (100 mg/kg)]

control (*P*= 0.001). WBC in the HS extract at 200 and 400 mg/kg was significantly decreased compared to SD-untreated and SD+HS (100 mg/kg) groups. No significant changes (*P*> 0.05) were observed in the lymphocyte count in the SD-untreated group compared to the NC and in all the treated groups compared to the SD-untreated group in Fig. 4B. The platelet count in Fig. 4C was significantly higher (*P*= 0.001) in the SD-untreated group than in the NC. Treatment with HS extract at 200 and 400 mg/kg significantly reduced platelets compared to the SD-untreated group. Platelet distribution width in Fig. 4D did not change significantly (*P*> 0.05) in the SD-untreated group compared to the NC. In the groups that were given 100 and 400 mg/kg, platelet distribution width was significantly increased compared to the SD-untreated group (*P*= 0.001, 0.001).

Erythrocyte osmotic fragility

In Fig. 5, the percentage of lysed RBCs was significantly higher in the SD-untreated and SD+HS 100 mg/kg groups compared to the NC. The percentage of lysed cells in the HS-treated groups at 200 and 400 mg/kg was significantly lower compared to the SD-untreated group at 0.7 % normal saline. At

0.5 % normal saline, the percentage of lysed cells was significantly higher (*P*= 0.001, 0.023) in the SD-untreated group and SD+HS 100 mg/kg compared to the NC. The percentage of lysed cells in the HS-treated groups at 200 and 400 mg/kg was significantly (*P*= 0.001, 0.001) lower compared to that of SD-untreated and SD+HS 100 mg/kg. At 0.4 % normal saline, the percentage of lysed cells was significantly higher in the SD-untreated compared to NC, and in the HS-treated groups at 200 and 400 mg/kg compared to the SD-untreated. The percentage of lysed cells at 0.3 % normal saline solution was significantly higher in the SD-untreated group. In all the HS-treated groups, the percentage of lysed cells was significantly lower compared to the SD-untreated group.

Hematological parameters in adult female Wistar rats exposed to paradoxical sleep deprivation

Table 1 shows mean corpuscular hemoglobin (MCH) was significantly reduced in the SD-untreated group compared to NC and significantly improved in the HS 200 and 400 mg/kg treated groups. Mean corpuscular volume (MCV) was significantly higher in the SD-untreated group compared to NC. Treatment with HS at 200 and 400 mg/kg significantly reduced it. Mean corpuscular hemoglobin concentration (MCHC) was significantly reduced in the SD-untreated and SD+HS 100 mg/kg groups compared to the NC in Table 1. Granulocytes in the SD+HS 200 mg/kg group were significantly higher compared to the NC. The MPV was significantly higher in the SD-untreated group compared to the NC. In all the HS-treated groups Mean platelet volume (MPV) was significantly decreased compared to the SD-untreated group. Platelet large cell count (PLCC) was significantly higher in the groups that were given HS at 200 and 400 mg/kg compared to the NC. In the HS 400 mg/kg treated group, PLCC was significantly higher in the SD+HS 400 mg/kg group compared to the SD-untreated and SD+HS 100 mg/kg groups. In Table 1, the platelet large cell ratio (PLCR) significantly decreased in the SD-untreated group compared to the NC. Treatment with HS extract significantly increased PLCR in the 200 and 400 mg/kg-treated groups compared to the SD-untreated group

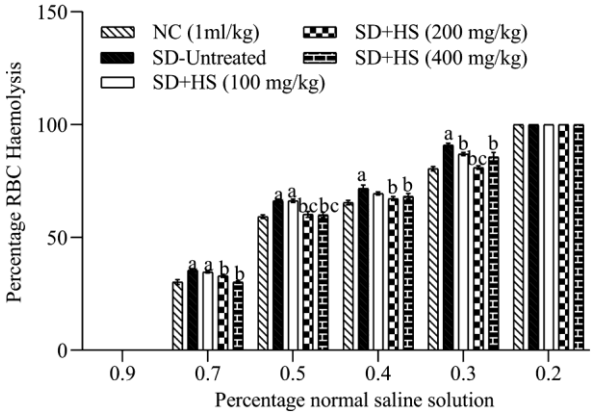

Fig. 5 — Results of HS extract on % hemolysis of RBC in adult female Wistar rats exposed to paradoxical sleep deprivation. [SD = sleep deprivation, NC =normal control, and HS = *Hibiscus sabdariffa*. Superscripts; a= *P*<0.05 *vs* NC, b= *P*<0.05 *vs* SD-untreated, c= *P*<0.05 *vs* SD+HS (100 mg/kg)]

Discussion

Sleep deprivation is a potent oxidative stressor resulting from the increased production of reactive oxygen species[45]. An increase in malondialdehyde levels is caused by increased lipid peroxidation in cell membranes[46]. In this study, we observed a significant increase in levels of MDA (Fig. 1A) and ROS (Fig. 2A) in the SD-untreated group. The elevated levels of MDA and ROS observed in this investigation may be attributed to either a decrease in clearance or a combination of the two mechanisms. Furthermore, lack of sleep might result in specific negative circumstances that support the build-up of

Table 1 — Results of HS extract on hematological parameters in adult female Wistar rats exposed to paradoxical sleep deprivation

Groups	MCH (pg)	MCV (fL)	MCHC (g/dL)	GRAN (10^3µL)	MPV (fL)	PLCC (10^9/L)	PLCR (%)
NC	34.80±0.66	82.80±1.32	38.68±0.85	2.37±0.13	8.50±0.14	35.50±0.65	37.25±0.49
SD-untreated	29.90±0.47^{a}	86.18±0.93^{a}	32.53±1.49^{a}	2.75±0.13	8.95±0.06^{a}	37.50±0.86	33.48±0.21^{a}
SD+HS (100 mg/kg)	32.22±0.41	84.40±0.70	32.65±1.19^{a}	2.45±0.06	8.45±0.05^{b}	39.50±1.19^{a}	33.40±0.81^{a}
SD+ HS (200 mg/kg)	34.23±0.72^{b}	82.37±0.73^{b}	34.80±0.72	2.80±0.04^{a}	8.08±0.13^{ab}	41.00±0.82^{a}	36.65±0.62^{bc}
SD+HS 400 mg/kg	34.88±1.36^{b}	81.72±0.93^{b}	35.37±0.18	2.70±0.04	7.78±0.05^{abc}	43.50±0.87^{bc}	37.35±0.37^{bc}
	P = 0.002	*P* = 3.249	*P* = 0.003	*P* = 0.012	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001
	F = 7.105	F = 7.105	F = 6.372	F = 4.636	F = 21.588	F = 11.938	F = 13.765

[NC = Normal control, SD = sleep deprivation, HS = *Hibiscus sabdariffa*. Superscripts; a= *P*<0.05 *vs* NC, b= *P*<0.05 *vs* SD-untreated, c= *P*<0.05 *vs* SD+HS (100 mg/kg)]

ROS[47]. HS extract treatment, however, lessened the increase in ROS and lipid peroxidation in this investigation. Most of Roselle's pharmacological actions that have been discovered are thought to be mediated by its antioxidant properties[48]. In this study, the activity of HS on oxidative stress could be attributed to increased antioxidant enzyme activity: GPx (Fig. 1B), SOD (Fig. 1C), and CAT (Fig. 1D). HS has been shown to possess antioxidant activity[49]. The main constituents of HS are organic acids, anthocyanins, polysaccharides, and flavonoids[50]. Anthocyanins can directly scavenge ROS[51,52]. Indirectly, they can stimulate the synthesis or activity of antioxidant enzymes like GPx, SOD, and CAT, as observed in the results of this study. Anthocyanins have been shown to increase SOD production[53]. Therefore, the observed mitigated oxidative stress could also be due to the inhibition of ROS-forming enzymes such as nicotinamide[54,55]. In the present study, the increased level of IL-10 in the groups treated with HS extract could also account for the improved oxidative stress balance, which is in favour of the antioxidants. This is because IL-10 has antioxidant activity[56].

TNF-α is a cytokine with diverse effects on various cell types and plays a crucial role in regulating inflammatory responses. It has been implicated in the development of certain inflammatory conditions[57]. In our study, we noted a marked increase in TNF-α levels in animals subjected to sleep deprivation (SD) (Fig. 2B). Research indicates that sleep deprivation can disrupt immune function and elevate levels of inflammatory markers, including interleukin-6 (IL-6) and TNF-α[58,59]. The elevated TNF-α levels in the SD group may be linked to the relationship between melatonin and TNF-α[60]. Sleep deprivation is known to decrease melatonin production, which is a potent antioxidant[61]. Consequently, lower melatonin levels could lead to increased TNF-α production. Our findings also showed that treatment with (HS) reduced circulating TNF-α levels. The polyphenols found in HS are effective in mitigating TNF-α induced inflammation[62] which may explain the outcomes observed in our study. Anthocyanins present in HS and their metabolites have been shown to reduce TNF-α[63,64]. Other studies have reported decreased TNF-α with anthocyanins administration[65,66]. The production of TNF-α is suppressed by increased IL-10 synthesis[67]. Thus, the reduced TNF-α in animals treated with HS in this study could be via the IL-10 suppression pathway. TNF-α is key to the underlying causes of neurodegenerative disorders, and polyphenols have been tagged as inhibitors of TNF-α[68]. Sleep deprivation and disorders are causes of neurodegenerative diseases[69]. Thus, the findings of this present study showcase the potential of HS extract in mitigating sleep deprivation-induced inflammation and toxicities.

IL-10 is a cytokine known for its potent anti-inflammatory properties, essential for protecting the host from injury and maintaining tissue homeostasis[70]. In our study, the group suffering from sleep deprivation (SD) without therapy showed significantly lower serum IL-10 levels. This decrease in the anti-inflammatory cytokine IL-10 in the blood was directly linked to poor sleep quality[71,72]. The findings of our study showed that HS extract administration resulted in a significant rise in circulating IL-10 levels. This may be explained by the polyphenolic chemicals in HS extract, which are known to increase the mRNA expression of IL-10[73]. The benefits of HS could also be attributed to anthocyanins, which are capable of promoting the production of anti-inflammatory cytokines[74]. Quantifying circulating BDNF in whole blood, plasma, or serum[75] is possible. BDNF protein is also detectable outside the nervous system in several non-neuronal tissues, such as endothelial cells[76] and leucocytes[77]. In the present study, serum BDNF was assayed. In the current investigation, we found a substantial drop in the serum level of BDNF in the SD-untreated group. There have been reports of an interplay between stress and sleep and its impacts on the BDNF level[23]. Treatment with HS significantly improved serum BDNF in our study which could be from the inactivation of the HPA axis, shown to alter BDNF production and pathway[78,79]. Anthocyanins, phenolic acids and phenolic compounds positively influence the levels of BDNF[80,81]. Therefore, the presence of polyphenols in HS[82] could have accounted for the observed activities with HS administration in this study. There is a report of a correlation between BDNF and antioxidant activities[83]. The role of BDNF in sleep-deprived animals is however not lucid. BDNF produced in the brain can cross the brain-blood barrier (BBB) into circulation. Thus, the blood BDNF level correlates with the brain level[84]. Therefore, in this study, the increased serum BDNF would infer that HS may

protect against sleep deprivation-induced neuronal toxicities. BDNF protects the neurons against excitotoxicity[85]. Although other works have reported on the effect of HS on BDNF[86], the result of our findings on serum BDNF in sleep-deprived animals treated with HS is novel.

In the current study, we observed decreased RBC (Fig. 3A), Hb (Fig. 3B) and PCV (Fig. 3C) in the sleep-deprived group. These changes could be associated with SD-induced inflammation. Inflammatory molecules negatively impact the hematological system's cells in several ways, including iron deficiency, decreased erythropoietin production, increased erythrocyte phagocytosis by hepatic and splenic macrophages, and increased eryptosis due to oxidative stress in the bloodstream[87-89]. Therefore, the changes we observed in the pro- and anti-inflammatory biomarkers with HS administration could account for the improved hematological indices observed in the HS-treated groups. Sleep deprivation has been shown to impact Hb concentration[90]. Additionally, short sleep duration is associated with low Hb[91] observed in the sleep-deprived group in this study. Anthocyanins have erythropoietic potential via their pro-activity on erythropoietin[92]. Polyphenols can stabilize iron status and improve erythropoiesis. A study conducted in Northern Ghana reported improvement in iron status of childbearing women with HS administration[93]. However, Peter *et al.*[94] reported no significant effect of HS on the iron status of adults in malaria-endemic areas, although their study also reported the potential of HS to improve hematopoietic parameters. Thus, HS in our study possibly improved RBC, Hb and PCV via its action on iron and consequentially erythropoiesis[95]. This agrees with the findings of Umoren *et al*. who reported improvement in hematological parameters with HS administration[96]. Therefore, in sleep-deprived animals, treatment with HS extract can improve the oxygen-carrying capacity of the red blood cells. Our findings from the results of Hb and MCHC in the groups given HS also suggest the potential of HS in mitigating the combined effect of oxidative stress and sleep deprivation-induced hypoxia which could be detrimental[97].

Red cell distribution width (RDW) measures the variability in the size of circulating erythrocytes (anisocytosis). Elevated RDW has been observed in conditions like RBC hemolysis[98,99]. This is in concert with our study's findings, where we observed marked erythrocyte hemolysis in the SD-untreated group, which was mitigated by the administration of HS extract according to the osmotic fragility test (Fig. 5). RDW has been evaluated in other sleep-related disorders and underlying oxidative stress and inflammation[100]. Our finding is the first to report the RDW in sleep-deprived animals treated with HS-extract. The action of HS on RDW in this study could also be explained by the combination of increased antioxidant activity of the extract leading to decreased lipid peroxidation of the RBC membrane which is susceptible due to the polyunsaturated fatty acid[101-103], as well as reduced inflammation in the HS-treated groups. Anthocyanin extracts play a role in both stabilizing the red blood cell membrane and inhibiting the polymerization of Hb[104]. Stress affects RBCs in several ways, resulting in observable changes in their physiology, morphology, and function[105]. HS administration has been shown to mitigate these changes as seen in our study. The result of RDW in this study further supports the anti-inflammatory activity of the HS extract as RDW is one of the markers of inflammation[106]. Additionally, HS extract could protect against anemia, as shown by the RDW result in our study.

Sleep deprivation is associated with a significant rise in WBC[107]. In this present study, we observed a significant increase in the WBC with sleep deprivation which is consistent with the work of Sochal *et al.*[108]. However, this finding contradicts that of Agena *et al.*[109]. The increase in WBC observed in the sleep-deprived animals could have been a response to the system inflammation as shown by the result of TNF-α. Inflammation is characterized by increased white blood cell count[110]. However, the group treated with HS significantly decreased WBC. This can be explained by the reduced inflammation observed with HS treatment, which resulted in a corresponding decrease in WBC. According to Wilkinson *et al.*[111] antioxidants are associated with WBC dysregulation. Mean corpuscular hemoglobin (MCH) was significantly reduced in the SD-untreated group in the present study. This indicates a reduction in the hemoglobin content of an RBC[112]. This agrees with the work of Agamme *et al.*[113], but converse with the findings of Agena *et al.*[114] which reported no significant change. The reduced MCH in the SD-untreated could have been the effect of inflammation on stored iron utilization for Hb formation. Inflammation impedes the body's use of

stored iron for Hb synthesis[115]. Treatment with HS improved the MCH in the present study. This could be attributed to reduced inflammation, oxidative stress, and a possible augmentation of iron in the body[116]. Increased MCV was observed in the SD-untreated group in the present study. The higher MCV in this group could infer a problem with the liver. High mean corpuscular volume (MCV) indicates health complications like anemia, liver disease and thyroid diseases. Sleep deprivation is associated with liver toxicity[117,118]. Liver complications could cause changes in membrane lipids, affecting the membrane surface area, and leading to increased MCV as observed in this study[119]. Administration of HS extract in the present study returned the MCV to normal. The extract could have achieved this by reversal of higher MCV causality factors as observed in the SD-untreated group. The effect of HS extract on MCV could have been due to its proactivity on vitamin B12[120], which is vital to RBC production[121]. These findings are consistent with that of Orororo & Asagba[123]. Sleep deprivation has been shown to have minimal effects on platelet count[122]. This is consistent with the findings of this current study which showed a significant increase in platelet in the SD-untreated group compared to the NC (Fig. 4C). Polyphenolic compounds and anthocyanins have been shown to improve platelet counts as observed in the result of our study[123]. In the current study, PDW was significantly higher in the HS-treated groups (Fig. 4D). This indicates higher platelet activation with HS administration in sleep-deprived animals. Simple platelet markers that rise with platelet activation are the MPV and PDW. PDW does not rise with straightforward platelet swelling, so it is a more precise indicator of platelet activation[124]. Few studies have been done on how HS administration affects platelet activation in sleep-deprived animals. A laboratory test called the platelet-large cell ratio (PLCR) is used to assess a patient's blood quality. It calculates the proportion of big cells and platelets in the sample. This ratio can offer crucial details regarding a person's chance of contracting specific diseases[125]. Treatment with HS restored PLCR to normal, thus reducing the risk of developing further diseases. However, the mechanism of this activity is not known.

Conclusion

In conclusion, the administration of HS in this study significantly mitigated oxidative stress by decreasing serum MDA, and ROS while augmenting key antioxidant enzymes; SOD, GPx and CAT. It also ameliorated sleep deprivation-induced inflammation by decreasing TNF-α, with a corresponding increase in BDNF and IL-10. HS extract also possesses pro-erythropoietic activity, increased RBC, Hb, PCV, MCH and MCHC), and anti-macrocytic potential; decreased MCV. It also improved the RBC fragility. Thus, HS extract mitigates oxidative stress, modulates inflammatory cytokines and possesses pro-hematopoietic potential in paradoxical sleep-deprived adult female Wistar rats.

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Conflict of Interest

The authors declare no competing interests

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