



Research Article

The Therapeutic Potential of *Allium sativum* on Sleep Deprivation – Induced Haematological Toxicity in Adult Female Wistar Rats

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ABSTRACT

Sleep deprivation (SD) is a potent inducer of oxidative stress that disrupts haematological homeostasis through elevated reactive oxygen species (ROS) production and systemic inflammation. This study evaluated the haematological consequences of SD and the potential ameliorative effects of *Allium sativum* (AS) aqueous extract in a rodent model. Twenty-five female Wistar rats (150–200 g) were randomly assigned to five groups (n = 5 per group) following a two-week acclimatization period with *ad libitum* access to food and water. Group I served as the normal control, while Group II was subjected to sleep deprivation without treatment. Groups III, IV, and V received AS extract orally at doses of 100, 200, and 400 mg/kg, respectively. Following the experimental period, animals were anesthetized and sacrificed for blood collection and haematological analysis. Sleep-deprived rats exhibited significant reductions in red blood cell (RBC) count (5.80 ± 0.12), packed cell volume (PCV) (38.30 ± 0.65), and haemoglobin (Hb) concentration (12.10 ± 0.10), alongside elevations in red cell distribution width (RDW) (22.80 ± 0.16) and mean corpuscular haemoglobin (MCH) (21.28 ± 0.47), indicative of impaired erythropoiesis and increased erythrocyte fragility. These haematological disturbances were associated with inflammatory responses, iron dysregulation, suppressed erythropoietin synthesis, and oxidative damage to erythrocyte membranes. Treatment with AS extract resulted in marked improvements ($p < 0.05$) in haematological parameters, including normalization of RDW (19.70 ± 0.19 , 15.20 ± 0.15) and MCH levels (20.90 ± 0.47 , 20.34 ± 0.20) and stabilization of RBC membranes (6.16 ± 0.33 , 6.20 ± 0.10). The observed effects are linked to the antioxidant, anti-inflammatory, and erythropoietic properties of *Allium sativum*, likely mediated by bioactive compounds such as allicin and polyphenols. These findings highlight the therapeutic potential of *Allium sativum* in alleviating SD-induced haematological toxicity.

Keywords: *Allium sativum*; Garlic; Haematological parameters; Red cell distribution width; Sleep deprivation

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INTRODUCTION

Haematological toxicity constitutes a critical biomarker of systemic dysfunction, arising when physiological stressors or xenobiotic insults perturb hematopoietic processes and compromise blood cell

homeostasis. Such toxicity is characterized by quantifiable deviations in erythrocytic, leukocytic, and thrombocytic indices, which collectively reflect impaired hematopoiesis and the activation of oxidative-inflammatory pathways. Owing to the

intrinsic sensitivity of the hematopoietic system, haematological parameters are widely employed as sentinel indicators for evaluating systemic toxicity and therapeutic efficacy (Bissinger *et al.*, 2019; Rybalkina *et al.*, 2021).

Analogously, sleep represents a fundamental regulatory process conserved across phylogeny, indispensable for maintaining physiological equilibrium and organismal health (Kocevska *et al.*, 2021). Despite its ubiquity, the mechanistic underpinnings of sleep remain incompletely resolved, even though systematic investigation dates back to the 19th century (Andersen, 2020). Prevailing explanatory frameworks including the inactivity hypothesis, energy conservation hypothesis, restoration hypothesis, and brain plasticity hypothesis capture distinct facets of sleep function, yet none provides a comprehensive account of its complexity. A more integrative perspective emerges from synthesizing these models (Brinkman, 2022). Recent conceptual advances propose that sleep may be understood within the paradigm of metaregulation, wherein it functions as an adaptive state of neural quiescence or a default operational mode of central nervous systems, thereby reframing its restorative and regulatory roles (Vyazovskiy, 2015).

Sleep deprivation (SD), encompassing both diminished duration and disrupted architecture of sleep, has emerged as a widespread concern in modern society, predominantly driven by technological proliferation and shifting lifestyle norms (Neculicioiu *et al.*, 2022). Across age groups, substantial variability in sleep deficits has been attributed to environmental exposures, behavioral patterns, and intrinsic physiological mechanisms (Kocevska *et al.*, 2021). Although a definitive classification of SD as an epidemic remains contested (Colten, 2006), the deleterious health outcomes associated with inadequate sleep are unequivocally documented. SD is implicated in a range of pathological conditions, including excessive daytime somnolence, obesity, type 2 diabetes, psychiatric morbidity, cardiovascular disease, and neurodegenerative disorders (Garbarino *et al.*, 2021; Andersen, 2020). Beyond its systemic effects, sleep plays a pivotal role in cellular regeneration, skeletal integrity (Mapfumo, *et al.*, 2022), and immunological regulation (Besedovsky *et al.*, 2019). Recent evidence

also underscores sleep's regulatory influence on hematopoiesis the dynamic process through which hematopoietic stem cells (HSCs) and progenitor cells (HSPCs) proliferate and differentiate into mature blood cell lineages, including leukocytes, erythrocytes, and thrombocytes (Cool *et al.* 2019).

In many regions of Africa, particularly within tropical zones, socioeconomic challenges such as poverty and limited access to formal education significantly hinder the availability and utilization of conventional Western medical services (Yeh *et al.*, 2024). Consequently, there has been a notable reliance on traditional herbal remedies as accessible alternatives. These preparations are often favored due to their affordability, perceived efficacy, minimal side effects, and low toxicity profiles, making them especially appealing in resource-limited settings (Craig, 1999). The growing global interest in medicinal plants has prompted increased scientific investigation into their pharmacological properties and safety. This research provides clinicians with evidence-based data to guide patients in making informed decisions regarding herbal medicine use (O'Hara *et al.*, 1998). Among the numerous botanicals under study, species within the *Allium* genus such as garlic (*Allium sativum*) and onion (*Allium cepa*) have garnered considerable attention. These plants are widely utilized not only as culinary ingredients and flavoring agents but also in traditional medicine across various cultures (Banerjee & Maulik, 2002).

Garlic, in particular, holds a prominent place in African ethnomedicine and is known by various local names, including *Ayo* (Igbo), *Ayuu* (Yoruba), and *Tafarnuwa* (Hausa) (Pittler & Ernst, 2007). Its widespread use and cultural significance have made it a subject of interest in modern biomedical research. The therapeutic effects of garlic are attributed to its ability to modulate key biological processes, including reduction of cardiovascular and cancer risk factors, enhancement of immune responses, detoxification of xenobiotics, hepatoprotection, antimicrobial activity, and antioxidant defense mechanisms (Banerjee & Maulik, 2002; Augusti, 1996; Olaniyan *et al.*, 2013). Despite the extensive documentation of the therapeutic properties of *Allium sativum* (garlic), there remains a paucity of data regarding its haematological effects, particularly in the context of physiological stressors such as sleep deprivation. This gap in knowledge underscores the need for targeted

investigations using an appropriate experimental model. Given garlic's affordability and its potential as a complementary approach for managing a wide range of health conditions, optimizing its application remains a significant challenge for researchers worldwide (Ozougwu n.d). In light of this, the present study was undertaken to assess the haematological impact of aqueous garlic extract in sleep-deprived female Wistar rats.

MATERIALS AND METHODS

Materials

The following materials were utilized in the study: white transparent plastic cages (40cm x 35 cm), ethylenediaminetetraacetic acid (EDTA), plain sample bottles, syringes, oral cannulas, gloves, and a weighing machine was obtained from Department of Human Physiology, Ahmadu Bello University, Zaria.

Plant Collection

Fresh garlic bulbs were collected from a Samaru market in Zaria, Kaduna State, Nigeria. The bulbs were identified using standard keys 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 bulbs were cut into small pieces, ground and sieved. It was weighed using a weighing scale.

Preparation of Extract

The *Allium sativum* bulbs powder (100 g) was dissolved in 250 ml distilled water and covered. After 48 hours, the mixture was filtered using a nylon sieve into a small container, and the residue was spread in a container and allowed to dry. Re-extraction with fresh 250 ml of distilled water was conducted for 24 hours. The pooled and dried extract was used for this study. These aqueous extracts were administered to respective animals daily by oral gavage for four weeks (El-Demerdash *et al.*, 2005).

Experimental Animals

The experiments were carried out in the laboratory of Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria, at standard laboratory conditions of temperature and humidity. A total of Twenty-five (N=25) apparently healthy female Wistar albino rats (*Rattus norvegicus*) weighing between 120–150 g was used for the study. The animals were obtained from the Animal Unit of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University,

Zaria. They were housed under standard laboratory conditions at room temperature. They were fed with standard commercial rat chow and water *ad libitum*. Pelletized growers feed was purchased from Vital Feed a subsidiary of UAC Nigeria Limited in Zaria and were used throughout the research work. Pelletized growers feed 25kg contains the following ingredients; cereals/grains, vegetable protein, premix (vitamins/minerals) essential amino acids, salt, antioxidant, anti-toxins, prebiotic and enzymes. Constituent percentage includes; Crude Protein (13%), Fat (8%) Crude Fibre (15%), Calcium (0.9%), Phosphorus (0.35%) and Metabolized energy (2600Kcal/kg).

Experimental Design and Ethical Approval

The animals were weighed (Model: XY100C, Serial Number: 1404273, Changzhou Xingyun) and randomly divided into five groups, each consisting of five animals (n=5). Group I was designated as the normal control and received distilled water (1ml/kg), while Group II served as the negative control (SD-untreated) and was exposed to Sleep deprivation for 18 hours. Groups III, IV, and V was exposed to Sleep deprivation for 18 hours and 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 (Njinga *et al.*, 2020). All experimental protocols were in accordance with the Research policies of Ahmadu Bello University Animal Use and Care (ABUAUC) governing the use of experimental animal research purposes. All administrations were given through oral route for 28 days.

Experimental Induction of Paradoxical Sleep Deprivation

Rats were acclimated to the glass tank for one hour each day over three consecutive days before the addition of water. The water level was maintained at 3 cm below the platforms (Rizki *et al.*, 2020). 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 hours a day over 7 days (Spano *et al.*, 2019).

Animal Sacrifice

After 28 days of *Allium sativum* administration, the animals were sacrificed using sodium phenobarbital (60 mg/kg body weight, intraperitoneally) (Tobar-Leitão *et al.*, 2021). Blood samples were collected via

cardiac puncture and placed in a plain serum bottle. The serum was prepared by spinning blood samples for 20 minutes at a speed of 3500 rpm using a bench centrifuge. A clear supernatant was used.

Haematological Indices

Blood parameters were analyzed using an automatic haematological assay analyzer, the Advia 60 Hematology System (Bayer Diagnostics Europe Ltd., Ireland). The assessment was carried out according to the method described by Faulkner & King, (1970).

Data Analysis

Data obtained from the study were analyzed and expressed as mean \pm SEM. Statistical analysis was carried out using version 23 of the IBM Statistical Package for Social Sciences (SPSS). A one-way analysis of variance (ANOVA) was carried out, followed by *Tukey's post hoc* test, to determine the differences among the groups. Values with a $p < 0.05$ were considered statistically significant. GraphPad Prism 8 software (version 8.0.2) was used for charts.

RESULTS

Red blood cell (RBC) and Packed Cell Volume (PCV)

In Figure 1a, the RBC count was significantly decreased in the SD-untreated group compared to the NC. Treatment with AS extract at 200 and 400

mg/kg significantly increased RBC count ($p = 0.05$) compared to the SD-untreated group. In the group given AS extract at 400 mg/kg, the RBC count was significantly higher ($p = 0.05$) compared to the SD+AS (200 mg/kg)-treated group. In Figure 1b the PCV in the SD-untreated group was significantly decreased ($p = 0.05$) compared to the NC. In all the groups treated with AS extract, PCV was significantly ($p = 0.05$) increased compared to the SD-untreated group.

Some Haematological Parameters in Adult Female Wistar Rats Exposed to Paradoxical

Sleep Deprivation

Hb in Figure 2a significantly reduced ($p = 0.05$) in the SD-untreated group compared to the NC. At 200 and 400 mg/kg, Hb significantly increased compared to SD-untreated and SD+AS (100 mg/kg) groups. In figure 2b, mean corpuscular haemoglobin (MCH) was significantly reduced in the SD-untreated group compared to NC and significantly improved in the AS 100 mg/kg treated group. In Figure 2c Mean corpuscular haemoglobin concentration (MCHC) was significantly reduced in the SD-untreated and SD+AS 100 mg/kg groups compared to the NC. Mean corpuscular haemoglobin concentration (MCHC) was significantly higher in the group that was given AS at 400 mg/kg compared to the SD-untreated.

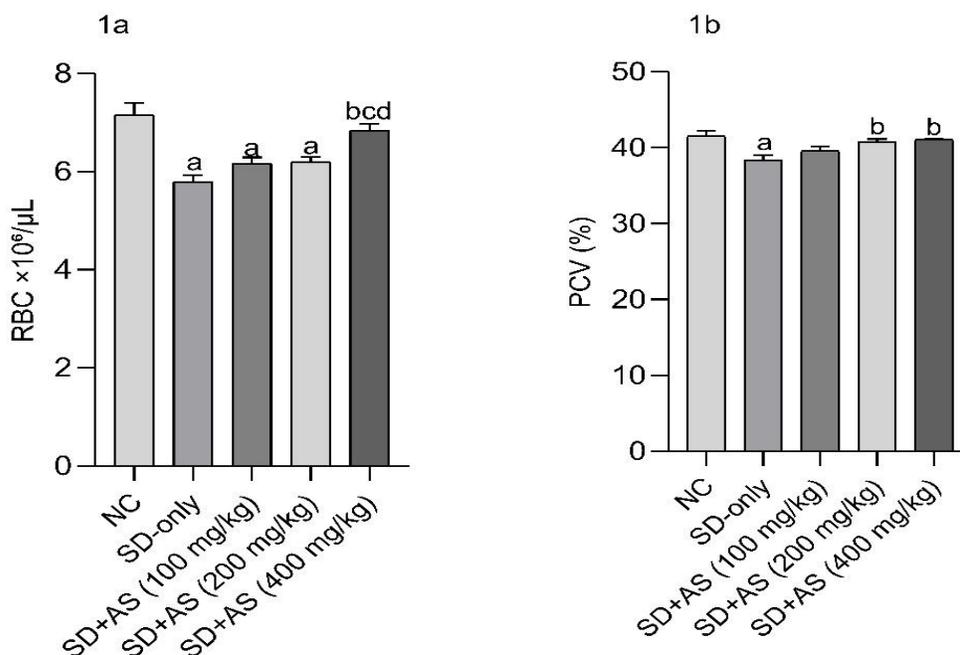


Figure 1: Results of AS extract on RBC [1a] and PCV [1b] in adult female Wistar rats exposed to paradoxical sleep deprivation. NC = Normal control, SD = sleep deprivation, AS = *Allium sativum*. Superscripts; a= $p < 0.05$ vs NC, b= $p < 0.05$ vs SD-untreated, c= $p < 0.05$ vs SD+AS (100 mg/kg)

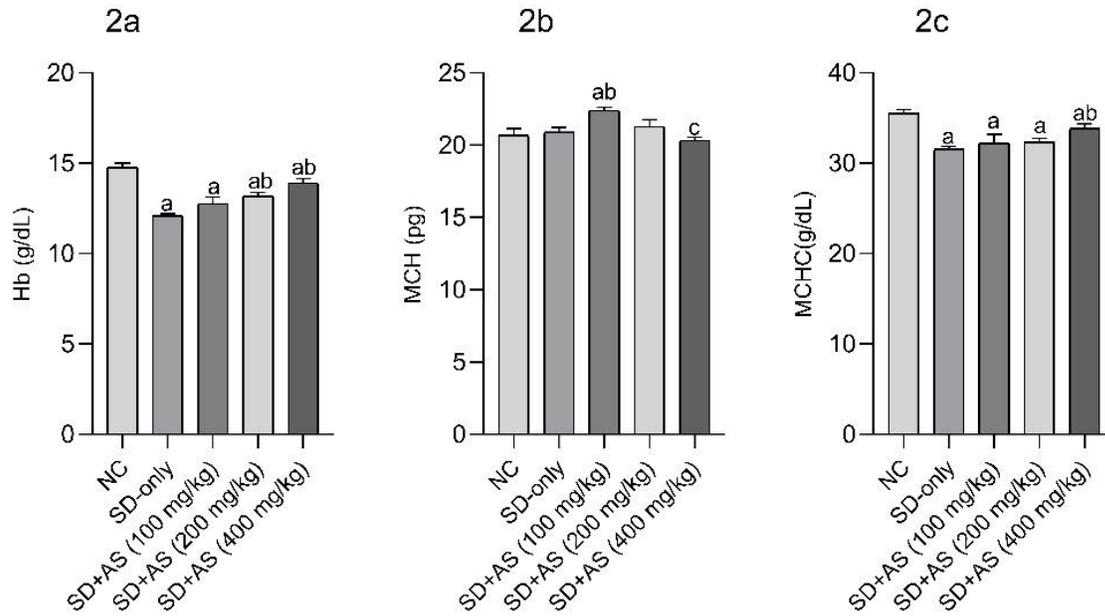


Figure 2: Results of AS extract on Hb [2a], MCH [2b], and MCHC [2c] in adult female Wistar rats exposed to paradoxical sleep deprivation. NC = Normal control, SD = sleep deprivation, AS = *Allium sativum*. Superscripts; a= $p < 0.05$ vs NC, b= $p < 0.05$ vs SD-untreated, c= $p < 0.05$ vs SD+AS (100 mg/kg).

Mean Corpuscular Haemoglobin (MCH) and Red Cell Distribution Width (RWD-cv)

In Figure 3b, mean corpuscular haemoglobin (MCH) was significantly increased in the SD-untreated group compared to NC and significantly improved in the AS 200 mg/kg treated groups. mean corpuscular haemoglobin (MCH) was significantly higher in the

group that was given AS at 100 mg/kg compared to the SD-untreated. In Figure 3b, Red cell distribution width was significantly higher in the SD-untreated group compared to the NC ($p = 0.05$). Treatment with AS extract at 100, 200 and 400 mg/kg significantly reduced the red cell distribution width compared to the SD-untreated group.

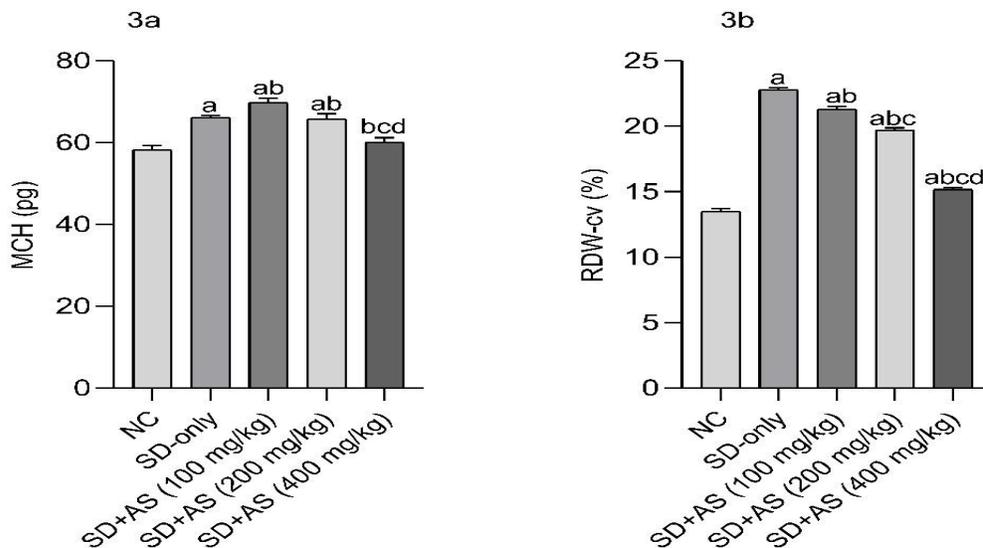


Figure 3: Results of AS extract on MCH [3a] and Red Cell Distribution Width [3a] in adult female Wistar rats exposed to paradoxical sleep deprivation. NC = Normal control, SD = sleep deprivation, AS = *Allium sativum*. Superscripts; a= $p < 0.05$ vs NC, b= $p < 0.05$ vs SD-untreated, c= $p < 0.05$ vs SD+HS (100 mg/kg)

DISCUSSION

Sleep deprivation has emerged as a significant contributor to oxidative stress, largely attributed to the heightened generation of reactive oxygen species (ROS), which disrupt cellular equilibrium and trigger widespread inflammatory responses (Pandey *et al.*, 2018). Among the various indicators used to assess hematopoietic activity, haematological parameters offer critical insights into blood composition and function (Aprioku & Obianime, 2008). Packed cell volume (PCV), in particular, denotes the fraction of blood volume occupied by red blood cells in samples drawn from capillary, venous, or arterial sources. It serves as a key diagnostic marker for detecting haematological disorders such as anemia and polycythemia, and is also useful in evaluating changes related to hemodilution or hemoconcentration (Ezeigwe *et al.*, 2022). In our study, rats subjected to sleep deprivation demonstrated a marked decline in both red blood cell (RBC) count and PCV. These changes suggest a disruption in erythropoietic processes and red cell stability, likely driven by inflammatory cascades associated with sleep loss. Inflammatory cytokines can interfere with iron regulation, inhibit erythropoietin production, and accelerate the removal of erythrocytes through enhanced phagocytic activity in the liver and spleen. Additionally, the oxidative burden induced by sleep deprivation promotes eryptosis an apoptotic-like mechanism in erythrocytes further exacerbating the decline in red cell parameters and contributing to anemia-like manifestations (Bissinger *et al.*, 2019; Katsumi *et al.*, 2021).

Interestingly, administration of *Allium sativum* (AS) extract appeared to mitigate these deleterious effects, as evidenced by improved haematological indices including normalization of RDW and MCH levels, and stabilization of RBC membranes in treated groups. The observed modulation of pro- and anti-inflammatory biomarkers suggests that AS may exert protective effects through its bioactive compounds, particularly allicin and polyphenols. Allicin has demonstrated erythropoietic potential by stimulating erythropoietin activity (Rybalkina *et al.*, 2021), while polyphenols are known to stabilize iron homeostasis and enhance red blood cell production (Xu *et al.*, 2021). These mechanisms likely underlie the restoration of RBC, haemoglobin (Hb), and PCV levels in AS-treated animals.

Furthermore, red cell distribution width (RDW), a marker of anisocytosis and erythrocyte size variability, was notably elevated in the sleep-deprived untreated group, consistent with increased hemolysis (Ananthaseshan *et al.*, 2022). Earlier research confirmed this finding, showing significant red blood cell breakdown in the SD group as indicated by the osmotic fragility test. Treatment with AS extract significantly reduced RDW, suggesting membrane stabilization and reduced lipid peroxidation effects attributed to the antioxidant properties of AS, particularly its ability to protect the polyunsaturated fatty acid-rich RBC membrane from oxidative damage (Spengler *et al.*, 2014; Al Balushi *et al.*, 2019). Additionally, allicin may inhibit haemoglobin polymerization, further preserving red cell integrity (Oluwole, 2001).

Sleep deprivation has a notable impact on red blood cell parameters, including mean corpuscular haemoglobin (MCH), and may play a role in the development of anemia and reduced oxygen-carrying capacity (Liu *et al.*, 2009). The observed rise in MCH following sleep deprivation is consistent with earlier studies, such as (Agena *et al.*, 2024), which reported elevated MCH levels in sleep-deprived subjects. However, AS treatment significantly reversed this trend, lowering both MCH and mean corpuscular haemoglobin concentration (MCHC). These results support earlier studies indicating that allicin can modulate MCH levels and reduce haematological toxicity (Wilkinson & Yamazaki, 2018; Orororo & Asagba, 2022). Collectively, these findings highlight the therapeutic potential of *Allium sativum* in counteracting sleep deprivation-induced haematological disturbances through its anti-inflammatory, antioxidant, and erythropoietic properties.

CONCLUSION

Result suggests that the administration of *Allium sativum* modulates some haematological parameters in paradoxical sleep-deprived adult female Wistar rats probably due to its constituent antioxidant properties. Thus, its regular consumption may be helpful for health.

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