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Supplementation of *Chlorella vulgaris* Ameliorates the Stress-induced Hematological Alterations in Wistar Rats

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ABSTRACT

Stress has been associated with various diseases and physiological disruptions. *Chlorella vulgaris* is known for its antioxidant properties. This study examined the effects of *C. vulgaris* on the hematological profile of stress-induced Wistar rats. Stress significantly impacted various hematological parameters, including erythrocyte count, hemoglobin concentration, hematocrit levels, white blood cell count, and platelet count. The supplementation of cultivated *C. vulgaris* effectively restored erythrocyte count and suppressed elevated lymphocyte levels, while commercially available *C. vulgaris* and amitriptyline drugs had no significant effects on these parameters. Stress caused smaller-sized erythrocytes but did not significantly affect mean corpuscular volume. Both amitriptyline and *C. vulgaris* restored platelet levels, while mean platelet volume remained unaffected. Overall, *C. vulgaris* showed promise as a therapeutic intervention for countering stress-induced inhibition of erythropoiesis and restoring erythrocyte count, but more research is needed to understand the underlying mechanisms and develop effective strategies for managing stress-related changes in hematological parameters.

Keywords: *Chlorella vulgaris*, hematological profile, rats, stress

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INTRODUCTION

In recent years, there has been a growing global awareness of the importance of overall health, encompassing physical and mental well-being (Saraceno, 2020). Mental health, in particular, has gained significant attention due to its impact on individuals' quality of life. As a pervasive aspect of modern life, stress has been recognized

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as a significant risk factor for various illnesses and serious health conditions (Kessler & Bromet, 2013; Moussavi et al., 2007). The implications of stress extend beyond mental health, as it has been linked to disruptions in the immune system, elevated cholesterol levels, and increased susceptibility to cancer, cardiovascular diseases, and diabetes (Abate et al., 2020; Balkan et al., 2004; Dar et al., 2019; Harris et al., 2017; Wattoo et al., 2008). Chronic stress also impacts neurotransmitter levels in the brain, specifically serotonin and noradrenaline, which play crucial roles in mood regulation. A decline in these neurotransmitter levels can contribute to the development of depression (Goddard et al., 2010; Hammen, 2005; Natarajan et al., 2015). Moreover, stress-induced reductions in antioxidant levels occur within the body, further exacerbating the detrimental effects of stress (Srivastava & Kumar, 2015). These findings underscore the far-reaching impact of stress on the physiological functioning of the human body.

According to data from the Institute of Health Metrics and Evaluation (IHME), an alarming 970 million people worldwide suffered from mental disorder-related issues in 2019 (IHME, 2022). The symptoms associated with stress are wide-ranging and can manifest as anhedonia (loss of pleasure), sleep difficulties, reduced appetite, lack of energy, and other physical and emotional disturbances (Schneiderman et al., 2005; Yaribeygi et al., 2017). Despite the prevalence and severity of stress-related disorders, there persists a misconception among certain segments of the population that stress is not a disease (Willenberg et al., 2020). This misguided belief hampers efforts to raise awareness, promote early intervention, and provide appropriate support for individuals affected by stress and depression.

A range of treatment options are available for managing stress, yet in Indonesia, the utilization of stress treatment remains relatively uncommon. In 2018, it was estimated that approximately 19 million Indonesians, accounting for approximately 9.8% of the overall population, were affected by emotional disorders (Ministry of Health Indonesia, 2022). However, due to various limitations, only a mere 9% of individuals suffering from mental illnesses received professional treatment or had access to mental health facilities (Ministry of Health Indonesia, 2022).

Among the commonly prescribed interventions for individuals with depression or stress are antidepressant medications, which unfortunately come with unpleasant side effects on the body (Almohammed et al., 2022; Carvalho et al., 2016; Faquih et al., 2019). Antidepressant drugs, being non-selective, can lead to significant side effects by affecting neuronal receptors in various parts of the body. These side effects may manifest as heart palpitations, constipation, insomnia, restlessness, migraines, excessive sweating, vomiting, and long-lasting health risks (Ferguson, 2001). The long-term effects include withdrawal symptoms, sexual impairments, and weight increase (Cartwright et al., 2016). Understanding the limitations in stress treatment accessibility and the potential side effects of conventional antidepressant medications highlights the need for alternative approaches that can effectively alleviate stress and its associated symptoms.

Chlorella vulgaris is a species of unicellular green alga characterized by its non-motile nature, eukaryotic cellular structure, and circular shape. It possesses a high chlorophyll concentration, rendering it an exceptional photosynthetic organism. Extensive research has revealed that *C. vulgaris* exhibits notable antioxidant properties (Kumar & Singh, 2019) and is capable of synthesizing various carotenoids, which have been shown to enhance immune function and protect against degenerative diseases (Azlan et al., 2020; Bito et al., 2020; Kwak et al., 2012). Due to its established safety profile and beneficial qualities, *C. vulgaris* has long been utilized as a safe and health-promoting dietary supplement for individuals (Bito et al., 2020). Consequently, considering these attributes, *C. vulgaris* holds potential as an alternative treatment for depression. In light of these characteristics, this study aimed to explore the effects and potential of commercially available and cultivated *C. vulgaris* in modulating the hematological profile of Wistar rats subjected to stress. By examining the effects of *C. vulgaris* on the hematological profile of stressed rats, this study contributes to the understanding of *C. vulgaris*'s potential as an alternative approach to alleviate the physiological effects of stress.

METHODS

The study design received approval from the Animal Ethics Commission Team from the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia, under Ethical Clearance Certificate No: 00042/04/LPPT/VI/2018. Wistar Rats (*Rattus novergicus*), sourced from Laboratorium Penelitian dan Pengujian Terpadu (LPPT) Universitas Gadjah Mada, Indonesia, were divided into 5 groups according to the previously described protocol (Karima & Mulyati, 2019; Soetantyo & Sarto, 2019). The groups for treatment included a no-stress control group, a stress-induced placebo group, and stress-induced groups treated with the antidepressant drug amitriptyline (Indofarma, Indonesia; 2.25 mg/kg of body weight), cultivated *C. vulgaris* (Blue Green Microalga Technology, Indonesia; 153 mg/kg of body weight), or commercially available *C. vulgaris* (CNI Sun Chlorella, Indonesia; 153 mg/kg of body weight). Each group consisted of 5 individuals.

The stress-induced treatments were performed as previously described (Hu et al., 2017; Karima & Mulyati, 2019; Soetantyo & Sarto, 2019; Zhang et al., 2014). Throughout the 42-day experimental period, rats were subjected to seven types of stress treatments (Table 1).

All experimental groups, except the control group, were exposed to the stress treatments. In total, each stress type was applied for six days. The sequential administration of stress types was predetermined, with each type being reapplied after six days from the previous

Table 1
Description of stress treatments

No.	Stress type	Treatment
1	Cold water	Rats were placed in a water tank (25 cm in depth), 5°C (cold) or 45°C (warm) for 3 min, followed by drying up in a lamp-illuminated cage with fresh bedding
2	Warm water	
3	Wet cages	Exposure to 5 cm wet beddings for 24 hr
4	Reversal of dark-light cycle	Rats were subjected to light from 18:00 to 06:00 and covered in dark from 06:00-18:00
5	Ultrasonic exposure	Rats were treated with ultrasonic exposure for 12 hr
6	Tilted cages	The rat cages were tilted at 45° for 24 hr
7	Fasting	Food and water removal for 24-hr

application of the same type to maintain uniformity. Following the completion of the stress treatment phase, from day 43 to day 56, rats were provided with drug supplementation and *C. vulgaris*. The main parameters observed in this study were hematological profiles. These parameters were measured at day 56, at the end of the drug or *C. vulgaris* supplementation. Blood samples were obtained by collecting venous blood from the orbital vein using a micro hematocrit capillary. The hematological parameters of the blood samples were analyzed using a hematology analyzer (Sysmex KX-21, China). The data were analyzed using Microsoft Excel and SPSS 16.0 software through one-way analysis of variance (ANOVA) followed by the post hoc test.

RESULTS AND DISCUSSION

As previously reported (Karima & Mulyati, 2019; Soetantyo & Sarto, 2019), the stress-induced rats exhibited reduced body weight, a significant decrease in sucrose preference, and an increased cholesterol level. The primary focus of this study was to examine the impact of stress and *Chlorella* supplementation on the hematological profile of rats. Figure 1 demonstrates a notable decrease in the number of erythrocytes in the stress-treated group compared to the control group. The data indicates that the stress condition may impair the process of erythrocyte formation in rats. It suggests that stress acts as a disruptive factor, negatively influencing the generation of new erythrocytes. A noteworthy finding of this study is that supplementation with cultivated

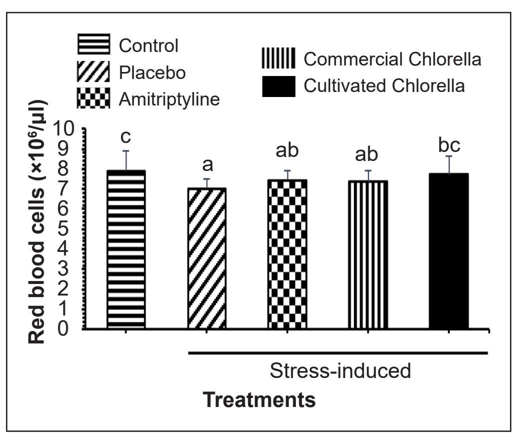

Figure 1. Supplementation of cultivated *Chlorella vulgaris* counteracts the suppressive impact of stress on erythropoiesis
Note. Groups with different letters represent statistically significant differences

C. vulgaris effectively restored the number of erythrocytes in rats experiencing stress.

Intriguingly, this restorative effect was even more pronounced than that observed in the positive control group, which received treatment with amitriptyline. These results strongly suggest that the supplementation of cultivated *C. vulgaris* effectively mitigates the detrimental impact of stress on erythrocyte production. However, it is important to highlight that commercially available *C. vulgaris* did not demonstrate the same ability to rescue the decreased number of erythrocytes induced by stress. This discrepancy could be attributed to several factors. Firstly, variations in the cultivation and processing methods of commercially available *C. vulgaris* products may result in differences in their bioactive composition or nutrient content. The specific combination or concentration of bioactive compounds responsible for stimulating erythrocyte production in cultivated *C. vulgaris* may be absent or present at lower levels in commercially available variants.

Moreover, the quality and purity of commercially available *C. vulgaris* products can vary, and contaminants or impurities introduced during the production or packaging process may interfere with their biological activity. These contaminants could potentially hinder the erythropoietic effects of *C. vulgaris* or introduce other substances that counteract its beneficial properties. Additionally, the timing of harvesting and processing the commercially available *C. vulgaris* may influence its efficacy. The optimal stage of growth or maturation at which *C. vulgaris* exhibits maximum bioactivity for erythropoiesis stimulation may not have been captured during the commercial production process. Further investigations into the specific factors influencing the efficacy of commercially available *C. vulgaris* are warranted to fully understand and address these limitations. Taken together, the results of this study strongly suggest that *C. vulgaris* possesses notable potential as a therapeutic intervention to mitigate the inhibitory influence of stress on erythropoiesis. The ability of *C. vulgaris* to restore the erythrocyte count in stress-induced rats highlights its efficacy in promoting the formation of new erythrocytes, thus aiding in maintaining a healthy erythrocyte population.

Hemoglobin, a respiratory pigment found in red blood cells, plays a crucial role in oxygen transport from the lungs to various tissues in the body (Ahmed et al., 2020). Its iron (Fe)-containing structure facilitates the binding and release of oxygen. Normal hemoglobin levels are typically associated with the overall erythrocyte count and hematocrit values, ensuring efficient oxygen delivery (Ahmed et al., 2020; Gell, 2018). Interestingly, *C. vulgaris* possesses a high concentration of Fe at 749 mg/kg, as Widiyanto et al. (2018) reported. Consequently, *C. vulgaris* shows promise in increasing hemoglobin levels, particularly in individuals experiencing stress.

Figure 2 displays the impact of stress on hemoglobin concentration, which is evident through the significant difference between the control and stress-induced groups. The data illustrates a decrease in hemoglobin concentration under stressful conditions. However, the administration of amitriptyline and the supplementation of *C. vulgaris* showed minimal effect on the recovery of hemoglobin concentration in the stress-induced groups. The decrease in hemoglobin concentration during stress could be attributed to several scientific factors. Stress triggers the release of stress hormones such as cortisol, which can lead to changes in blood volume and hematocrit levels. Additionally, stress-related alterations in erythropoiesis, the red blood cell production process, could contribute to a decrease in hemoglobin concentration. These combined mechanisms may explain the observed decrease in hemoglobin levels in the stress-induced groups despite supplementing amitriptyline or *C. vulgaris*. Further research is necessary to fully elucidate the underlying mechanisms and potential strategies to mitigate stress-induced changes in hemoglobin levels.

Hematocrit, the percentage of red blood cells in the total blood volume, indicates the number of erythrocytes present (Kishimoto et al., 2020). Figure 3 presents the average hematocrit values obtained in our study. Interestingly, our findings reveal a significant decrease in hematocrit levels within the stress-induced group. The decrease in hematocrit observed in the stress-induced group can be closely linked to the decrease in erythrocyte count, as demonstrated in Figure 1. Erythrocytes are the primary component contributing to hematocrit values, and any changes in their quantity can consequently influence hematocrit levels. Thus, the significant decrease in hematocrit levels observed in the stress-induced group may be attributed to the diminished number of erythrocytes. No significant changes in hematocrit levels were observed in the

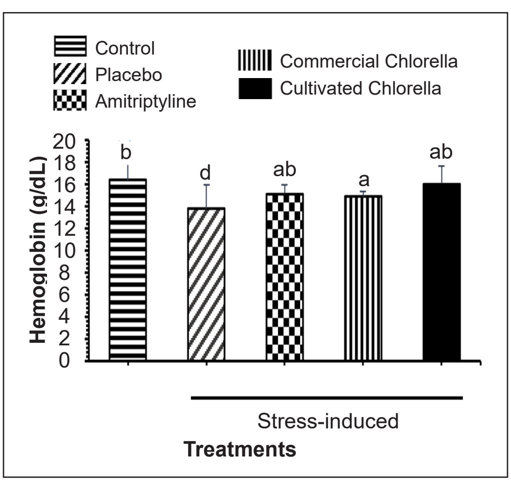

Figure 2. Negative impact of stress treatments on hemoglobin concentration and the minimal effect of *Chlorella vulgaris* supplementation
Note. Groups with different letters represent statistically significant differences

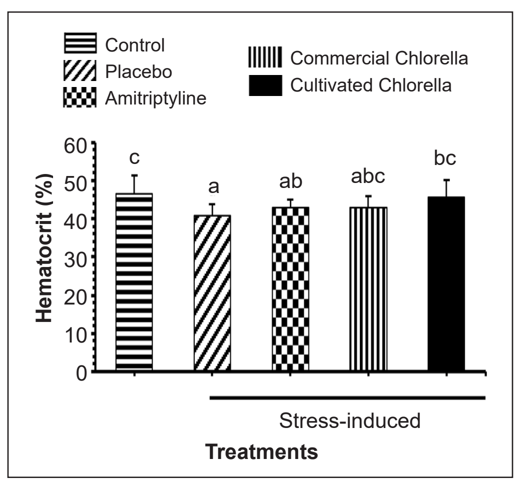

Figure 3. Supplementation of cultivated *Chlorella vulgaris* effectively restores the diminished hematocrit values in stress-induced rats
Note. Groups with different letters represent statistically significant differences

stress group following supplementation with amitriptyline or commercial *C. vulgaris*. However, in contrast, the supplementation of cultivated *C. vulgaris* demonstrated a notable restorative effect on hematocrit values in stressed rats. Similar trends were also observed in Figure 1, showing the positive effect of cultivated *C. vulgaris* on the decreased erythrocyte numbers upon stress exposure. Therefore, these findings provide strong evidence supporting the effectiveness of cultivated *C. vulgaris* supplementation in mitigating the adverse effects of stress on erythrocyte production.

Mean corpuscular volume (MCV) is a widely utilized parameter to assess the average size of erythrocytes (Yavorkovsky, 2021). In our study, depicted in Figure 4, we observed a significant decrease in MCV values of the stress-induced group compared to the control. Surprisingly,

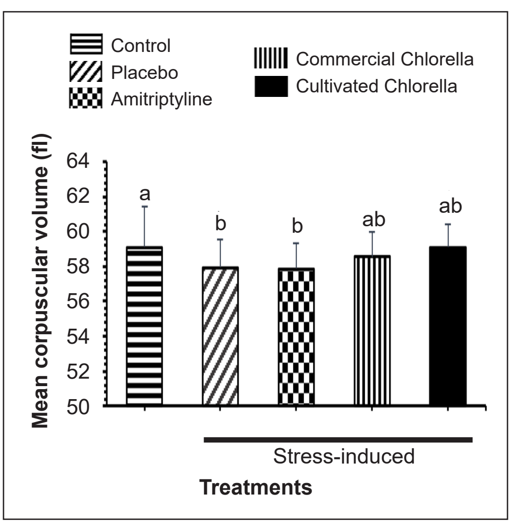

Figure 4. Stress induces a reduction in mean corpuscular volume values in rats
Note. Groups with different letters represent statistically significant differences

supplementation with amitriptyline or *C. vulgaris* exhibited no significant effect on the MCV values of the stress-induced groups. The decrease in MCV observed in the stress-induced groups may be attributed to several plausible reasons. Stress is known to stimulate the release of stress hormones, such as cortisol, which can lead to alterations in erythropoiesis (Lodish et al., 2010). Disturbances in erythropoiesis can result in the generation of smaller-sized erythrocytes, leading to a decrease in MCV. Furthermore, stress-induced oxidative stress and inflammation can influence erythrocyte characteristics, including their size (Maiese et al., 2008; Paulson et al., 2020). Oxidative stress can lead to cellular damage, affecting erythrocyte morphology and potentially leading to smaller-sized erythrocytes, thereby contributing to the decrease in MCV (Ghaffari, 2008; Maiese et al., 2008). Further investigations are warranted to comprehensively understand the underlying mechanisms and explore potential interventions to counteract the stress-induced decrease in MCV.

The mean corpuscular hemoglobin (MCH), which represents the average amount of hemoglobin per red blood cell, was evaluated in our study. Interestingly, despite observing a decrease in the number of erythrocytes and a decrease in hemoglobin concentration in the stress-induced groups, the MCH values did not differ significantly compared to the control group. The lack of significant changes in MCH values between the control and stress-induced groups suggests that stress may not substantially impact the hemoglobin content within individual red blood cells. This observation also demonstrates that supplementation with amitriptyline or *C. vulgaris* did not show any notable effect on MCH values in the stress-induced groups.

The mean corpuscular hemoglobin concentration (MCHC) represents the average hemoglobin concentration within individual red blood cells and is an important parameter in assessing the hemoglobin content (Ahmed et al., 2020; Gell, 2018). The MCHC values for each group are presented in Figure 6. Notably, the supplementation of amitriptyline and *C. vulgaris* in the stress-induced groups exhibited minimal effect on MCHC values. Furthermore, our findings from Figure 5, which illustrates the MCH values, align with the results observed for MCHC, indicating that stress does not induce significant changes in MCHC.

Individuals who experience stress often face a higher susceptibility to pathogen infection due to disruptions in their immune system (Morey et al., 2015; Segerstrom & Miller, 2004). Monitoring the total blood leukocyte count serves as a simple and accessible indicator of immune system activity. In our study, the stress-induced group exhibited elevated white blood cell (WBC) levels compared to the control group (Figure 7). The observed increase in WBC count in the stress group can be attributed to the body's response to stress-related immune dysregulation. Stress promotes the release of stress hormones such as cortisol (Lodish et al., 2010). These hormones play a role in mobilizing immune cells, including white blood cells, to combat potential threats. Therefore, the elevated WBC count in the stress group can be seen as an adaptive immune system response to potential infections.

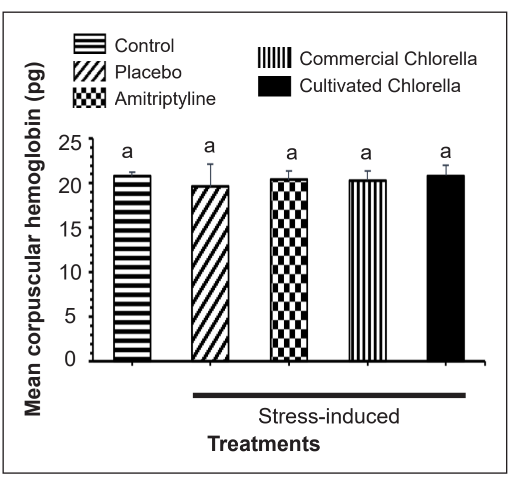

Figure 5. Stress and supplementation of *Chlorella vulgaris* yield no significant impact on mean corpuscular hemoglobin in rats
Note. Groups with different letters represent statistically significant differences

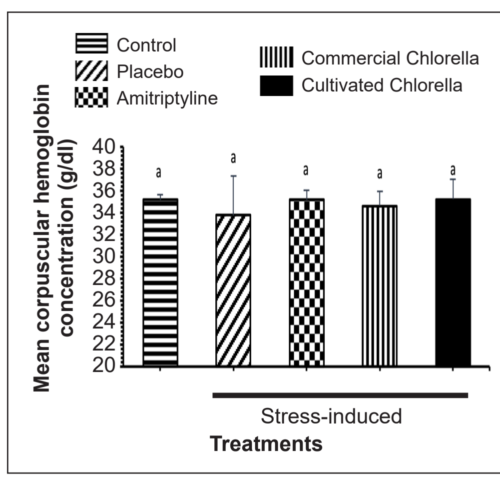

Figure 6. Stress exposure and *Chlorella vulgaris* supplementation exhibit no discernable impact on mean corpuscular hemoglobin concentration in rats
Note. Groups with different letters represent statistically significant differences

However, despite the potential benefits of amitriptyline and *C. vulgaris* supplementation in stress management, our study revealed that neither intervention could effectively reduce the increased WBC levels observed in the stress group. One possible explanation for this lack of effect could be the complex nature of stress-induced immune dysregulation. Stress can influence various immune mechanisms, including cytokine production, lymphocyte function, and cellular communication, which may not be easily modulated solely by amitriptyline or *C. vulgaris* supplementation. Additionally, it is important to consider that the duration and severity of stress exposure, as well as individual variations in stress response, can impact the effectiveness of interventions in modulating immune responses. Further research is needed to explore alternative strategies or combination therapies that may have a more pronounced impact on reducing WBC levels in individuals experiencing stress.

Lymphocytes, a subpopulation of white blood cells, play a crucial role in mounting immune responses against invading antigens (Gasteiger & Rudensky, 2014). As a result, monitoring the number of lymphocytes can provide insights into the ongoing immune responses within the body. Our study demonstrated that the stress-inducing treatments resulted in an elevation of lymphocyte count (Figure 8).

The observed increase in lymphocyte count in response to stress can be attributed to activating the body's immune system. The increase in lymphocyte count indicates the immune system's heightened responsiveness to potential antigens or pathogens in the body. Significantly, our study revealed that the supplementation of *C. vulgaris* was effective in suppressing the elevated levels of lymphocytes, comparable to the effects of the amitriptyline drug. The immunomodulatory properties of *C. vulgaris*, characterized by its bioactive compounds

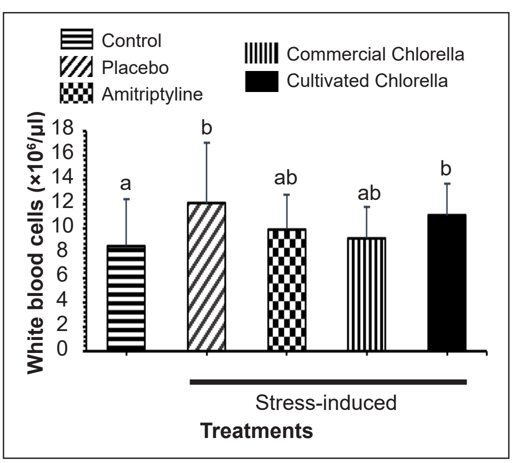

Figure 7. Stress induces an elevation in the white blood cell count in rats
Note. Groups with different letters represent statistically significant differences

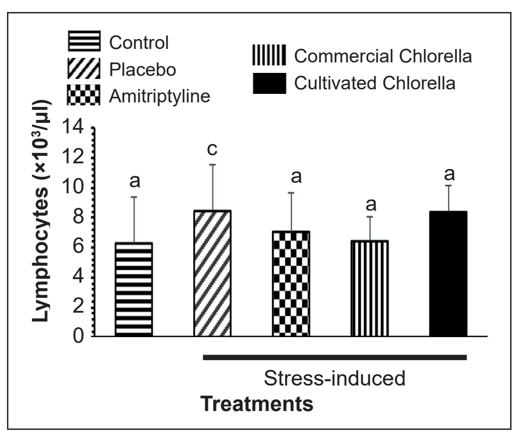

Figure 8. Supplementation of *Chlorella vulgaris* alleviates the elevated lymphocyte numbers in rats subjected to stress
Note. Groups with different letters represent statistically significant differences

and nutritional components, may contribute to its ability to regulate lymphocyte levels. Regueiras et al. (2021) demonstrated that certain components present in *C. vulgaris*, such as polysaccharides and antioxidants, possess anti-inflammatory properties and can modulate immune responses. These properties may help to counterbalance the stress-induced immune activation, resulting in the normalization of lymphocyte levels. However, the specific mechanisms through which *C. vulgaris* exerts its immunomodulatory effects on lymphocytes require further investigation. It is possible that *C. vulgaris* acts by regulating the production and activity of pro-inflammatory cytokines, influencing lymphocyte proliferation and differentiation, or modulating cellular signaling pathways involved in immune responses.

Furthermore, our study observed no significant difference between the effects of cultivated *C. vulgaris* and commercially available *C. vulgaris* in reducing the number of lymphocytes. This finding suggests that both forms of *C. vulgaris*, whether cultivated or commercially sourced, possess comparable immunomodulatory properties. The lack of significant difference between the two forms indicates that the beneficial effects on lymphocyte count reduction are likely attributed to the inherent properties of *C. vulgaris* itself rather than variations in cultivation or processing methods. However, it is important to note that further comparative studies investigating the specific composition and bioactivity profiles of cultivated and commercially available *C. vulgaris* would be beneficial to gain a deeper understanding of any potential differences in their immunomodulatory effects.

Platelets, known as thrombocytes, play a crucial role in blood coagulation following injury to the body, facilitating the formation of blood clots and the cessation of bleeding (Franco et al., 2015). The count of platelets in the bloodstream is a key determinant of the coagulation process and the ability to control bleeding. In the stress group of our study, a significant decrease in platelet count was observed when compared to the control group (Figure 9). The decrease in platelet count observed in the stress group can be attributed to the impact of stress on hematopoiesis, the process of blood cell formation. Stress can disrupt the production and regulation of platelets,

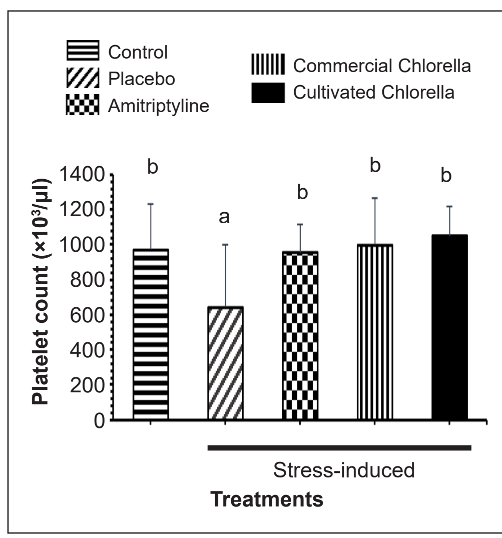

Figure 9. Supplementation with *Chlorella vulgaris* rescues the decreased platelet count in stress-exposed rats

Note. Groups with different letters represent statistically significant differences

potentially affecting their production rate and lifespan. Hormonal changes induced by stress and alterations in the bone marrow microenvironment may contribute to the decrease in platelet count (Cognasse et al., 2019; Koudouovoh-Tripp & Sperner-Unterweger, 2012).

Both *C. vulgaris* supplementation and amitriptyline administration effectively increased platelet levels, restoring them to the normal range. Furthermore, no significant difference was observed between cultivated and commercially available *C. vulgaris* in their capacity to restore platelet levels. It might suggest that the efficacy of *C. vulgaris* in platelet recovery is not influenced by variations in cultivation or processing methods. Both forms of *C. vulgaris* demonstrate comparable effectiveness, indicating that commercially available *C. vulgaris* products can be a reliable option for individuals seeking to restore platelet levels.

Mean platelet volume (MPV), a parameter reflecting the average size of platelets, was included in the study to provide insights into platelet morphology and potential alterations induced by stress (Korniluk et al., 2019). Measurement of MPV allows for assessing platelet size variation, which can indicate platelet activation and function. Interestingly, our findings revealed that stress did not have a significant effect on MPV, as evidenced by the comparison between the stress group and the control group in Figure 10. It suggests that stress-induced changes in platelet count, as observed in Figure 9, were not accompanied by alterations in platelet size. The lack of significant change in MPV may imply that stress predominantly affects platelet production and activation pathways rather than platelet size. Furthermore, despite the increase in platelet count shown in Figure 9, no corresponding changes in platelet volume were observed. This discrepancy may be attributed to the complex regulatory mechanisms involved in platelet production and maturation. It is possible that stress-induced alterations primarily influence platelet formation and release from megakaryocytes rather than affecting their size or volume.

Regarding the supplementation of amitriptyline and *C. vulgaris*, our results indicate that they had no significant effect on MPV. It suggests that these interventions do not directly influence platelet size or

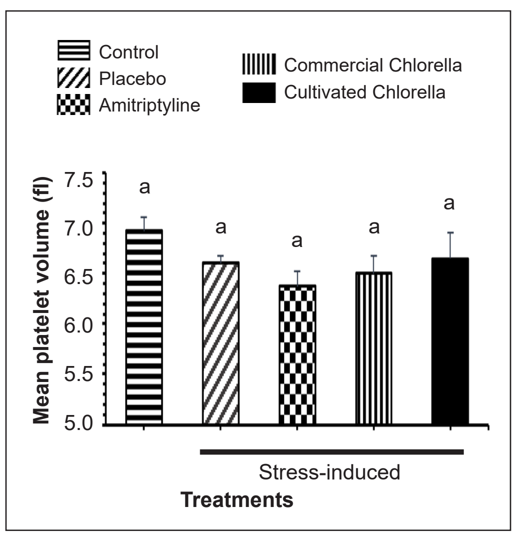

Figure 10. Exposure to stress and supplementation with *Chlorella vulgaris* in rats show no notable impact on mean platelet volume

Note. Groups with different letters represent statistically significant differences

volume. The lack of impact on MPV could be attributed to the specific mechanisms of action of amitriptyline and *C. vulgaris*, which may primarily target platelet function, activation, or other pathways associated with platelet biology rather than altering platelet size. It is important to note that MPV represents an average value, and individual platelets within the population may still exhibit size variations. The lack of significant changes in MPV suggests that stress, as well as the interventions of amitriptyline and *C. vulgaris*, did not induce substantial shifts in platelet size distribution.

CONCLUSION

This study provides compelling evidence of the significant impact of stress on various hematological parameters in rats. Stress leads to decreased erythrocyte count and hematocrit values, which can be effectively restored by supplementation with cultivated *C. vulgaris*, surpassing the positive control group treated with amitriptyline. However, commercially available *C. vulgaris* does not exhibit the same restorative effect. Stress negatively affects hemoglobin concentration, which is likely influenced by stress hormones and altered erythropoiesis. However, neither amitriptyline nor *C. vulgaris* supplementation significantly rescues the negative impact of stress on hemoglobin concentration. In addition, stress induces smaller-sized erythrocytes but does not significantly affect MCH or MCHC.

Notably, WBC count increases under stress, accompanied by elevated lymphocyte levels. *Chlorella vulgaris* effectively suppresses elevated lymphocyte levels, similar to amitriptyline, but does not reduce WBC count. Stress also decreases platelet count, which can be restored by amitriptyline and *C. vulgaris* supplementation. Meanwhile, MPV remains unaffected by stress or interventions, suggesting that stress primarily impacts platelet production and activation. Our findings highlight the potential of *C. vulgaris* as a therapeutic intervention to counteract stress-induced inhibition of erythropoiesis and restore erythrocyte count, although further research is necessary to elucidate underlying mechanisms and develop strategies for managing stress-related hematological changes.

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