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Original Article

Comparative effects of different supplemented dietary doses of chlorophyll on blood parameters of experimental male rats

Efeitos comparativos de diferentes doses dietéticas suplementadas de clorofila nos parâmetros sanguíneos de ratos machos experimentais

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Abstract

Chlorophylls are organic pigments that are a part of our daily diet, particularly in light of the increased popularity of more eco-friendly and healthy practices. Since altering oxidative equilibrium seems to be connected to the emergence of numerous illnesses, the antioxidant capacities of both groups of lipophilic compounds have been studied. The objective was to evaluate adding dietary chlorophyll at two concentrations—30 and 60 mg/ml—would improve blood characteristics in rats. Supplemented dietary chlorophyll showed significantly increased WBCs, RBCs, granulocytes, lymphocytes, HGB, HCT MCHC, and Platelets. it nonsignificant effect on RDW, MPV, and Eosinophil. These findings support a significant rise in critical hematological parameters at two separate time intervals, 14 and 28 days following dietary chlorophyll supplementation, at dosages of 30 and 60 mg/ml. After 30 and 60 mg/ml, platelet count, PCT, lymphocytes, and monocytes substantially (p0.001) rose. In light of these findings, critical hematological indicators markedly rise in response to exogenous dietary chlorophyll. To strengthen blood parameters and enhance blood features and prevent anemia, dietary chlorophyll is advised.

Keywords: chlorophyll, blood parameters, liquid chlorophyll, rats, dietary.

Resumo

As clorofilas são pigmentos orgânicos que fazem parte da nossa dieta diária, especialmente à luz da crescente popularidade de práticas mais ecológicas e saudáveis. Como a alteração do equilíbrio oxidativo parece estar ligada ao surgimento de inúmeras doenças, nesse sentido, as capacidades antioxidantes de ambos os grupos de compostos lipofílicos têm sido estudadas. O objetivo deste trabalho foi avaliar se a adição de clorofila na dieta em duas concentrações – 30 e 60 mg/ml – melhoraria as características do sangue em ratos. A clorofila na dieta suplementada mostrou aumento significativo de leucócitos, hemácias, granulócitos, linfócitos, HGB, HCT MCHC e plaquetas. E demonstraram efeito não significativo sobre RDW, MPV e eosinófilos. Essas descobertas apoiam um aumento significativo nos parâmetros hematológicos críticos em dois intervalos de tempo distintos, 14 e 28 dias após a suplementação dietética de clorofila, em dosagens de 30 e 60 mg/ml. Após 30 e 60 mg/ml, a contagem de plaquetas, PCT, linfócitos e monócitos aumentou substancialmente (p0,001). À luz destas descobertas, os indicadores hematológicos críticos apresentam aumento acentuado em resposta à clorofila exógena na dieta. Para melhorar os parâmetros sanguíneos e as características do sangue e prevenir a anemia, recomenda-se, a partir deste estudo, a utilização de clorofila na dieta.

Palavras-chave: clorofila, parâmetros sanguíneos, clorofila líquida, ratos, dietético.

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1. Introduction

Chlorophylls are natural pigments found in our food, particularly in light of consumers' rising propensity for more eco-friendly and healthier lifestyle choices (Pérez-Gálvez et al., 2020). In a study conducted by Tagauov et al. (2023), the blood parameters of rats exposed to liquid chlorophyll were evaluated. The study demonstrated that different doses of liquid chlorophyll can improve the blood parameters of rats. This suggests that chlorophyll may have beneficial effects on blood health, which warrants further research into this area. As this study further increases our understanding of the potential health benefits of chlorophyll, it also highlights the importance of examining factors such as dose, diet, and age to further understand the potential implications for humans. Study was done to evaluate liquid chlorophyll safety as a potentially medicinal product. These findings suggest that liquid chlorophyll may have a significant effect on the blood parameters of rats and that additional investigations is necessary to investigate the safety of liquid chlorophyll as a potential medicinal product (Tagauov et al., 2023). Significant studies were performed concerning chlorophyll and its importance in various disciplines (Rocha et al., 2009; Pinheiro et al., 2022).

Fresh fruits and vegetables include chlorophyll a and b, but thermally handled foods contain metal-free pheophytins and pyropheophytins (Ferruzzi and Blakeslee, 2007). As a healthy nutritional supplement, chlorophyll from underused greens in fresh vegetables, supplements, liquid solutions, extracts, or pills may be applied (Vivek et al., 2013).

The majority of green pigments present in plants are made up of chlorophyll (Chl), of which chlorophyll-a (Chl-a) makes up roughly seventy-five percent (Lanfer-Marquez, 2003). Chl-a has a hydrophobic character and is an entirely unsaturated, asymmetric macrocyclic molecule, which adds to its low solubility in hydrophilic fluids (Lanfer-Marquez, 2003). Consequently, chlorophyll might be administered as a nutrient in the form of supplements, liquid solutions, extracts, or tablets; adding into biocompatible micellar copolymers, such as P123, ensures the monomerization of the hydrophobic PS and the preservation of its photophysical features for in vivo and in vitro analyses (van Nostrum, 2004).

The liver and stomach accumulate Chl-a and its metabolites (Szczygieł et al., 2008; Xodo et al., 2012) indicates these substances may influence these organs. Antioxidant action in chlorophylls reduces ROS and chelates metal ions, preventing oxidative damage of DNA and lipid peroxidation (Shelton and Vincent, 1963; Lanfer-Marquez, 2003; İnanç, 2011; Pangestuti and Kim, 2011). Porphyrin enables chlorophylls to act as hydrogen donors, interrupting the chain reaction (Endo et al., 1985). Chlorophyll, especially from marine seaweeds, were studied for its biological and physiological benefits (Pangestuti and Kim, 2011). Natural pigments, particularly chlorophylls, are healthy. Anti-inflammatory, anti-obesity, anti-angiogenic, and neuroprotective activities have been reported (Pangestuti and Kim, 2011).

The current research intends to compare the impact of two amounts of dietary chlorophyll supplements on the rat males' hematological parameters.

2. Experimental Procedures

2.1. Liquid chlorophyll

Our research was intend to compare the consequences of using two levels of dietary chlorophyll supplements on the rat males' hematological parameters (Natures Sunshine, 2022). The water-soluble extract known as "liquid chlorophyll ES" is made from chlorophyll that is extracted from alfalfa (sodium-copper salt of chlorophyll). Beta-carotene, vitamins C, E, and K, as well as other nutrients of natural origin are all concentrated in liquid-chlorophyll, which is also a source of both chlorophyll-a and chlorophyll-b. Furthermore, it contains a lot of minerals and trace elements. We'll use 30 and 60 mg/ml as our two concentrations. Research Ethics Committee No. (REC96/2022), with the date of April 9, 2022, granted their permission.

2.2. Sample size calculations

Twenty-one rats were calculated to be sufficient to detect the effect size of 0.386, at a power 0.80 at 0.05 significance level. Sample size was calculated by G*Power ver. 3.1.9.6 (Cohen, 1988; Faul et al., 2007, 2009).

2.3. Animals and experiment design

Twenty-one non-purebred white male rats its weight from 200 and 230 g/individual were used in the studies, which were done in accordance with sample size estimations. Three groups were created from the experimental rats; A 10 (L dose of isotonic saline solution were given to the group-I control group's food. 10 mL of liquid chlorophyll with a 30 mg/ml concentration were given orally to experimental rats in group-II as a food supplement. Experimental rats in groups III (A2) and II (A1) will take a food supplement of 10 (L of liquid chlorophyll containing 60 mg/ml. Following routine care, animals were fed on a regular basis and monitored for variations in body-weight and other characteristics. 14 and 28 days after therapy, a sample of peripheral blood were taken.

2.4. Hematological indices

Using a Coulter Automated Cell Counter, RBC hematological indices such as RBC count, mean corpuscular volume (MCV), mean corpuscular Hgb (MCH), mean corpuscular Hgb concentration (MCHC), Hgb, and Hct were determined. Various hematological indices will also be estimated after 14 and 28 days of dietary intervention (Coulter AcT, Beckman Coulter, New York, NY, USA) (Tchir et al., 2013).

3. Statistical Analyses

Different treatment groups were compared at various investigational times in the statistical analysis.

Using Microsoft Excel 2016, the information was gathered, examined, corrected, and arranged in tables and figures. To determine if the data are parametric or nonparametric, outlier identification and statistical normality tests were used to the data. Data analysis will include both graphical and numerical explanations for descriptive statistics. Repeated measures-ANOVA or equivalent nonparametric analyses were used for inferential statistics to evaluate and compare three distinct treatments and investigational times at significance levels of 0.05. ANOVA were used to compare treatment groups, and Tukey's HSD or an equivalent post hoc analysis were used for nonparametric data. Data analysis performed using IBM-SPSS ver. 28.0 for Mac OS (Knapp, 2017).

4. Results

Evaluation of various blood parameters was presented as mean ±standard deviation (±SD). WBC, monocytes, monocytes (%), lymphocytes, lymphocytes (%), and granulocytes was presented in Figure 1. The WBC x 10^9/L in control, 30 and 60 mg/ml of dietary chlorophyll after 14 days presented a mean(±SD) of 8.10±0.22, 8.52±0.16, and 8.77±0.08; respectively. Nevertheless, after 28 days recorded a mean (±SD) of 8.12±0.03, 9.02±0.17, and 9.54±0.14. The Monocytes x 10^9/L in control, 30 and 60 mg/ml of chlorophyll after 14 days showed a mean (±SD) of 0.20±0.00, 0.20±0.00, and 0.30±0.00; respectively. Though, after 28 days recorded a mean (±SD) of 0.20±0.00, 0.40±0.01, and 0.30±0.00; respectively. The Monocytes (%) in control, 30 and 60 mg/ml of feeding with chlorophyll after 14 days recorded a mean (±SD) of 1.00±0.02, 1.00±0.02, and 3.00±0.04; respectively. After 28 days recorded a mean (±SD) of 1.00±0.02, 1.00±0.02, and 3.00±0.04; respectively (Figure 1C).

The Lymphocytes x 109/L in control, 30 and 60 mg/ml of feeding with chlorophyll after 14 days recorded a mean (±SD) of 11.30±0.17, 12.70±0.19, and 13.30±0.20; respectively (Figure 1D). After 28 days recorded a mean (±SD) of 11.39±0.09, 13.20±0.20, and 13.30±0.20; respectively. Lymphocytes (%) in control, 30 and 60 mg/ml of chlorophyll after 14 days showed a mean (±SD) of 66.00±0.99, 71.00±1.07, and 73.00±1.10; respectively. Though, after 28 days recorded a mean (±SD) of 65.00±1.00, 73.00±1.10, and 73.00±1.10; respectively (Figure 1E).

The Granulocytes x 109/L in control, 30 and 60 mg/ml of dietary chlorophyll after 14 days showed a mean (±SD) of 2.50±0.04, 2.80±0.04, and 2.90±0.04; respectively. after 28 days recorded a mean (±SD) of 2.54±0.05, 2.90±0.04, and 2.90±0.04; respectively (Figure 1F).

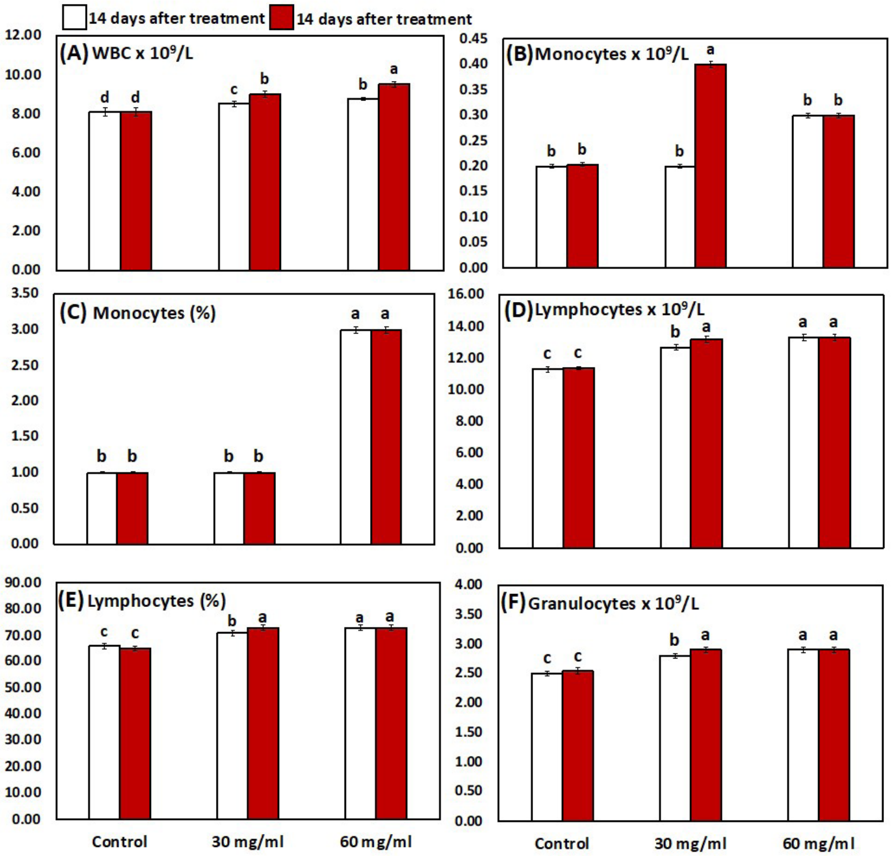

Figure 1. WBC, Monocyte (10^9/L), Monocytes (%), Lymphocytes (10^9/L), and Lymphocytes (%), and Granulocyte (10^9/L), granulocyte (%), presented as mean and standard error. Bars followed by different letters are significantly different according to Tukey's honest significant difference (HSD).

The RBCs x 1012/L in control, 30 and 60 mg/ml of dietary chlorophyll after 14 days showed a mean (±SD) of 7.03±0.03, 7.54±0.03, and 7.66±0.05. after 28 days recorded a mean (±SD) of 7.03±0.06, 7.68±0.07, and 7.66±0.05; respectively (Figure 2A). The HGB g/L in control, 30 and 60 mg/ml of chlorophyll after 14 days showed a mean (±SD) of 131.00±1.97, 137.00±2.06, and 140.00±2.10; respectively. after 28 days recorded a mean (±SD) of 131.67±0.58, 142.00±2.13, and 140.00±2.10; respectively (Figure 2B).

The HCT % in control, 30 and 60 mg/ml chlorophyll after 14 days showed a mean (±SD) of 41.27±0.39, 42.90±0.64, and 43.50±0.65; respectively. after 28 days recorded a mean (±SD) of 41.17±0.29, 43.70±0.66, and 43.50±0.65; respectively (Figure 2C).

The MCV fL after 14 days recorded a mean (±SD) of 58.45±0.15, 59.93±0.12, and 60.00±0.10; respectively. after 28 days recorded a mean (±SD) of 58.20±0.20, 60.80±0.35, and 61.33±0.06; respectively (Figure 2D). the MCH pg recorded a mean (±SD) of 19.90±0.17, 20.20±0.30, and 20.83±0.32; respectively. after 28 days recorded a mean (±SD) of 20.00±0.10, 21.40±0.32, and 21.24±0.23; respectively (Figure 2E).

The MCHC g/L after 14 days recorded a mean (±SD) of 330.33±1.53, 339.00±0.90, and 340.17±0.31; respectively. After 28 days recorded a mean (±SD) of 330.33±2.52, 339.27±0.74, and 340.43±0.42; respectively (Figure 2F).

The PLT x 10^9/L after 14 days recorded a mean (±SD) of 487.17±0.76, 497.33±1.53, and 503.33±2.31; respectively. after 28 days recorded a mean (±SD) of 488.63±0.55, 510.00±1.00, and 509.01±0.87; respectively (Figure 3A).

The RDW after 14 days recorded a mean (±SD) of 8.40±0.01, 8.10±0.10, and 8.23±0.06; respectively. after 28 days recorded a mean (±SD) of 8.46±0.05, 8.40±0.10, and 8.37±0.06; respectively. The RDW % after 14 days recorded a mean (±SD) of 15.64±0.08, 15.48±0.13, and 15.28±0.13; respectively. after 28 days recorded a mean (±SD) of 15.60±0.10, 15.34±0.05, and 15.31±0.11; respectively (Figure 3B).

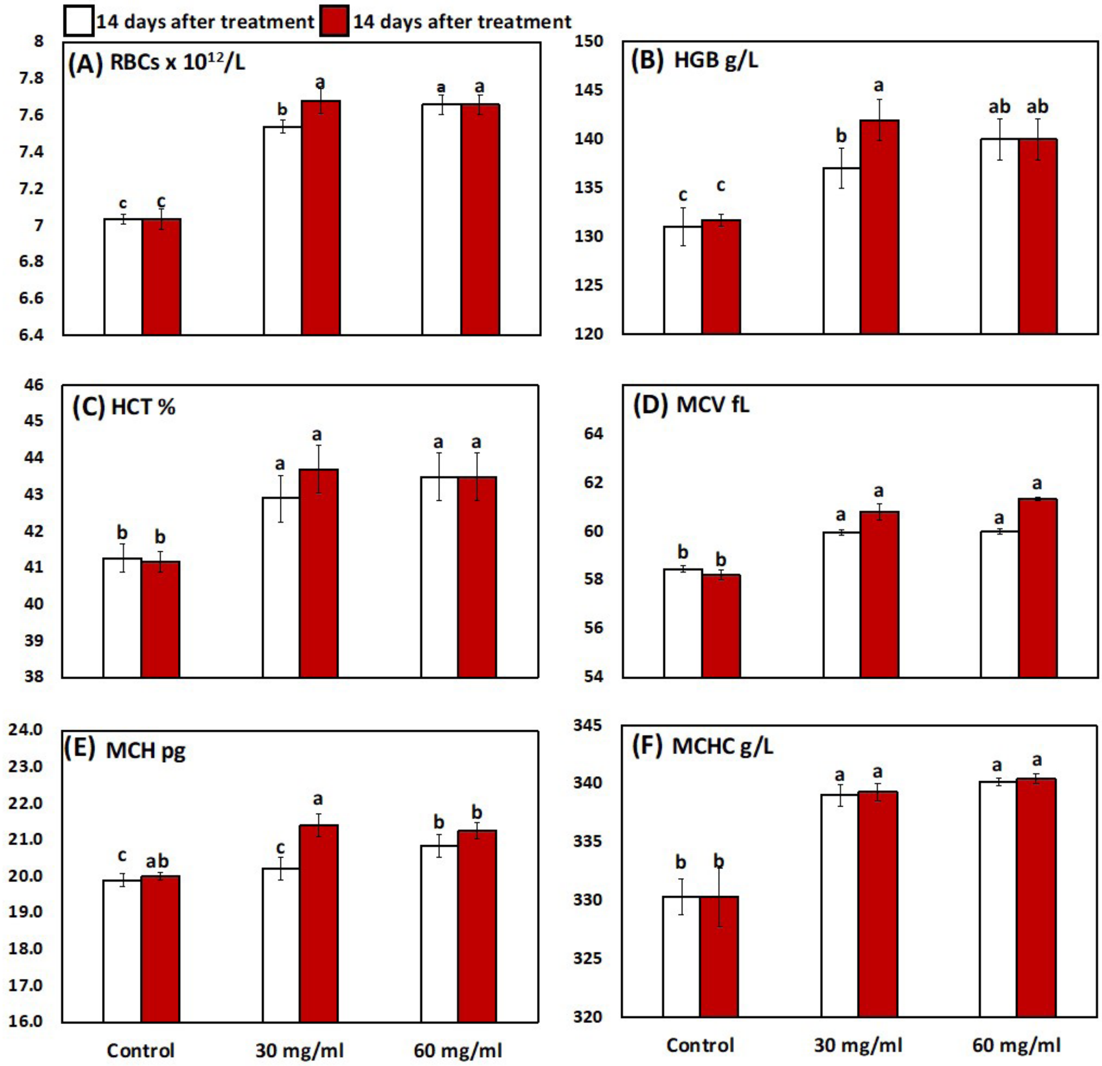

Figure 2. Various blood parameters in experimental rats after 30 and 60 mg/ml. Bars followed by different letters are significantly different according to Tukey's HSD.

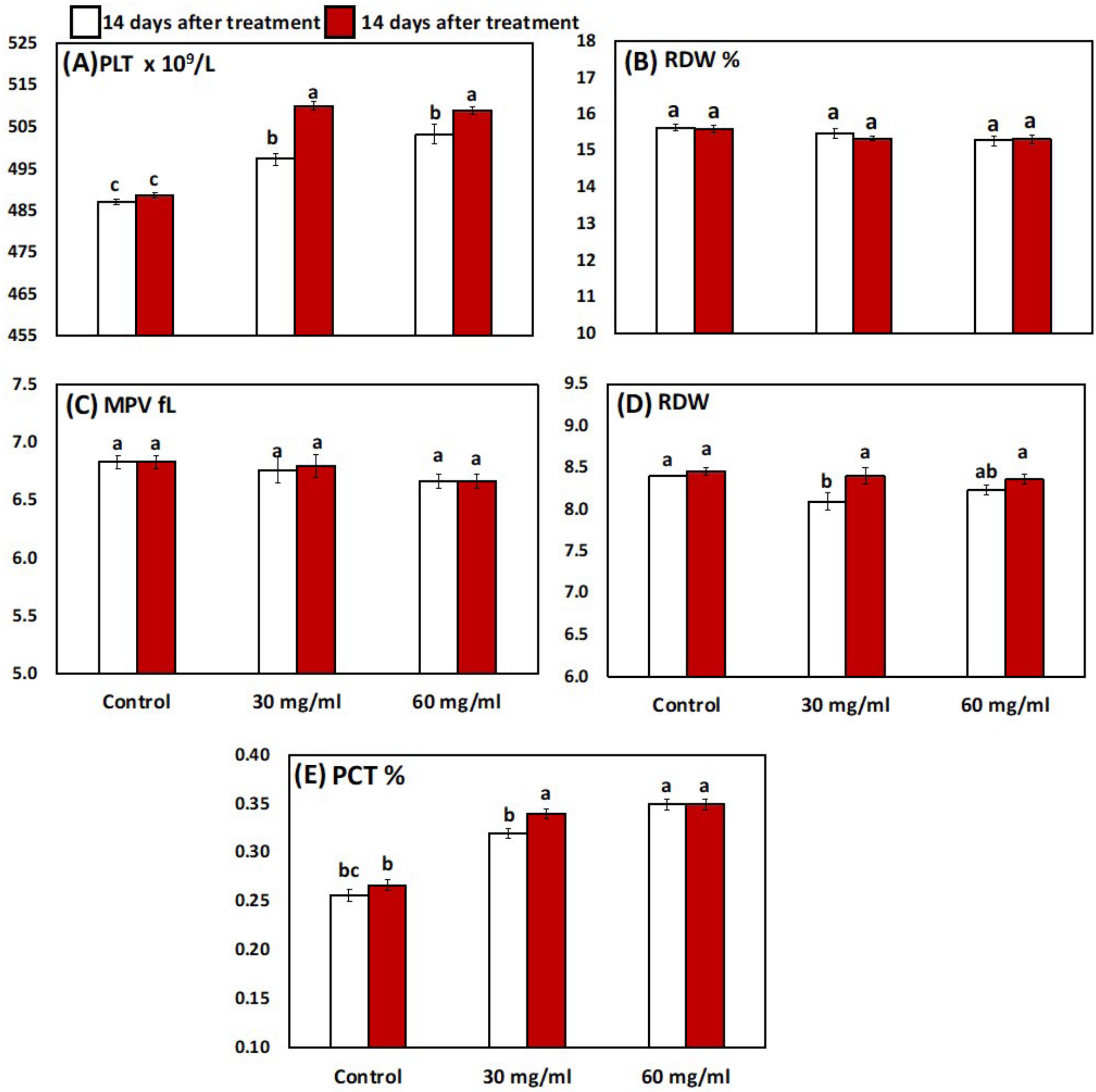

Figure 3. Various blood parameters in experimental rats after 30 and 60 mg/ml. Bars followed by different letters are significantly different according to Tukey's HSD.

The MPV fL after 14 days recorded a mean (±SD) of 6.83±0.06, 6.77±0.12, and 6.67±0.06; respectively. after 28 days recorded a mean (±SD) of 6.83±0.06, 6.80±0.10, and 6.67±0.06; respectively (Figure 3C).

The PCT % in after 14 days recorded a mean (±SD) of 0.26±0.01, 0.32±0.00, and 0.35±0.01; respectively. after 28 days recorded a mean (±SD) of 0.27±0.01, 0.34±0.01, and 0.35±0.01; respectively (Figure 3E).

The levels of WBC, Lymphocytes, Lymphocytes (%), Monocyte, Monocyte (%), Granulocyte, Granulocyte %, RBC, HGB, HCT%, MCV fL, MCH, MCHC, RDW %, PLT x 109/L, MPV fL, PCT %, Segmento-nuclear neutrophils, Basophils, Lymphocytes, Monocytes, was presented in Table 1. Their level was significant with dietary chlorophyll as revealed by multivariate analysis of variance (MANOVA) (Table 2).

RDW, Rod nuclear neutrophils, and eosinophils showed non a non-significant response to dietary chlorophyll, as assessed by multivariate analysis of variance (MANOVA) (Table 2).

Table 1 and Figure 4 show the correlation coefficient (r) and two-tailed significance tests for the connection between chlorophyll level and time against several blood parameters (p-value). Dietary chlorophyll significantly and positively increased WBC, Lymphocytes, Monocytes, Lymphocytes (%), Monocytes (%), Granulocytes (%), HGB, MCH, MCHC, PLT, and Lymphocytes. RDW, MPV and eosinophil showed no correlation with dietary chlorophyll supplements. Figure 4 shows a heatmap of the correlation coefficients with the colors denoting positive, negative, and significant correlations. Blue denoted a positive correlation, red a negative correlation, and boxed colors a significant connection.

Table 1. The relationship between exogenous dietary chlorophyll concentration and time on various blood parameters is presented as a correlation coefficient (r) and two tailed significance test (p-value).

parameter	Chlorophyll		Time		Chlorophyll x Time	
	r	*p*	r	*p*	r	*p*
White Blood Cells count (WBCs)	0.821	*<.001****	0.422	0.081	0.908	*<.001****
Lymphocytes	0.910	*<.001****	0.114	0.652	0.903	*<.001****
Lymphocytes (%)	0.875	*<.001****	0.048	0.851	0.851	*<.001****
Monocyte	0.548	*0.019**	0.447	0.063	0.655	*0.003***
Monocyte (%)	0.866	*<.001****	0.000	1.000	0.828	*<.001****
Granulocyte	0.887	*<.001****	0.133	0.598	0.887	*<.001****
Granulocyte %	0.870	*<.001****	0.264	0.290	0.909	*<.001****
Red Blood Cell (RBC) count	0.882	*<.001****	0.080	0.751	0.866	*<.001****
Hemoglobin level (HGB)	0.784	*<.001****	0.209	0.404	0.811	*<.001****
Hematocrit (HCT, %)	0.497	*0.036**	0.492	0.038*	0.619	*0.006***
Mean Corpuscular Volume (MCV, fL)	0.831	*<.001****	0.281	0.259	0.877	*<.001****
Mean corpuscular hemoglobin (MCH)	0.707	*0.001****	0.453	0.059	0.808	*<.001****
Mean corpuscular hemoglobin concentration (MCHC)	0.892	*<.001****	0.014	0.955	0.857	*<.001****
Red blood cell distribution width (RDW)	-0.398	0.102ns	0.610	0.007**	-0.202	0.421
Red blood cell distribution width (RDW, %)	-0.833	*<.001****	-0.136	0.590	-0.837	*<.001****
Platelet (PLT x 10^9/L)	0.821	*<.001****	0.365	0.137	0.891	*<.001****
Mean platelet volume (MPV, fL)	-0.716	*<.001****	0.058	0.818	-0.667	*0.002***
The procalcitonin test (PCT, %)	0.612	*0.007***	0.000	1.000	0.586	*0.011**
Rod nucleus. neutrophils	0.408	0.093ns	-0.333	0.176	0.293	0.238
Segmentonuclear neutrophils	0.524	*0.025**	-0.360	0.143	0.396	0.104
Eosinophils	0.000	>0.999ns	-0.447	0.063	-0.131	0.605
Basophils	0.866	*<.001****	0.000	1.000	0.828	*<.001****
Lymphocytes	0.612	*0.007***	-0.400	0.100	0.469	*0.050**
Monocytes	0.913	*<.001****	-0.149	0.555	0.829	*<.001****

*Significant at p<0.05. **Significant at p<0.01. ***Significant at p<0.001. ns: non-significant at p>0.05. italic numbers mean significant at p<0.05.

Table 2. Multivariate analysis of variance summarizing the effect of dietary chlorophyll and time of treatment on various blood parameters of male rats.

	Chlorophyll		Time		Chlorophyll x Time	
	F	*p*	F	*p*	F	*p*
WBC	0.82	***<.001*****	0.42	**0.081 ns**	0.91	***<.001*****
Lymphocytes	0.91	***<.001*****	0.11	**0.652ns**	0.90	***<.001*****
Lymphocytes (%)	0.88	***<.001*****	0.05	**0.851ns**	0.85	***<.001*****
Monocyte	0.55	***0.019***	0.45	**0.063ns**	0.66	***0.003*****
Monocyte (%)	0.87	***<.001*****	0.00	**>0.999ns**	0.83	***<.001*****
Granulocyte	0.89	***<.001*****	0.13	**0.598ns**	0.89	***<.001*****
Granulocyte %	0.87	***<.001*****	0.26	**0.290ns**	0.91	***<.001*****
RBC	0.88	***<.001*****	0.08	**0.751ns**	0.87	***<.001*****
HGB	0.78	***<.001*****	0.21	**0.404ns**	0.81	***<.001*****
HCT%	0.50	***0.036****	0.49	**0.038***	0.62	***0.006*****
MCV fL	0.83	***<.001*****	0.28	**0.259ns**	0.88	***<.001*****
MCH	0.71	***<.001*****	0.45	**0.059ns**	0.81	***<.001*****
MCHC	0.89	***<.001*****	0.01	**0.955ns**	0.86	***<.001*****
RDW	-0.40	**0.102ns**	0.61	***0.007*****	-0.20	**0.421ns**
RDW %	-0.83	***<.001*****	-0.14	**0.590 ns**	-0.84	***<.001*****
PLT x 109/L	0.82	***<.001*****	0.37	**0.137ns**	0.89	***<.001*****
MPV fL	-0.72	***<.001*****	0.06	**0.818ns**	-0.67	***0.002*****
PCT %	0.61	***0.007*****	0.00	**>0.999ns**	0.59	***0.011****
Rod nucleus. neutrophils	0.41	**0.093ns**	-0.33	**0.176ns**	0.29	**0.238ns**
Segment nuclear neutrophils	0.52	***0.025****	-0.36	**0.143ns**	0.40	**0.104ns**
Eosinophils	0.00	**>0.999ns**	-0.45	**0.063ns**	-0.13	**0.605ns**
Basophils	0.87	***<.001*****	0.00	**>0.999ns**	0.83	***<.001*****
Lymphocytes	0.61	***0.007*****	-0.40	**0.100ns**	0.47	***0.050****
Monocytes	0.91	***<.001*****	-0.15	**0.555ns**	0.83	***<.001*****

Note: WBC, white blood cells count; RBC, red blood cells count; HGB, hemoglobin; HCT, hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red blood cell distribution width; PLT, Platelet; MPV, Mean platelet volume; PCT, The procalcitonin test. *Significant at p<0.05. **Significant at p<0.01. ***Significant at p<0.001. ns: non-significant at p>0.05. italic number represent significant at *p*<0.05

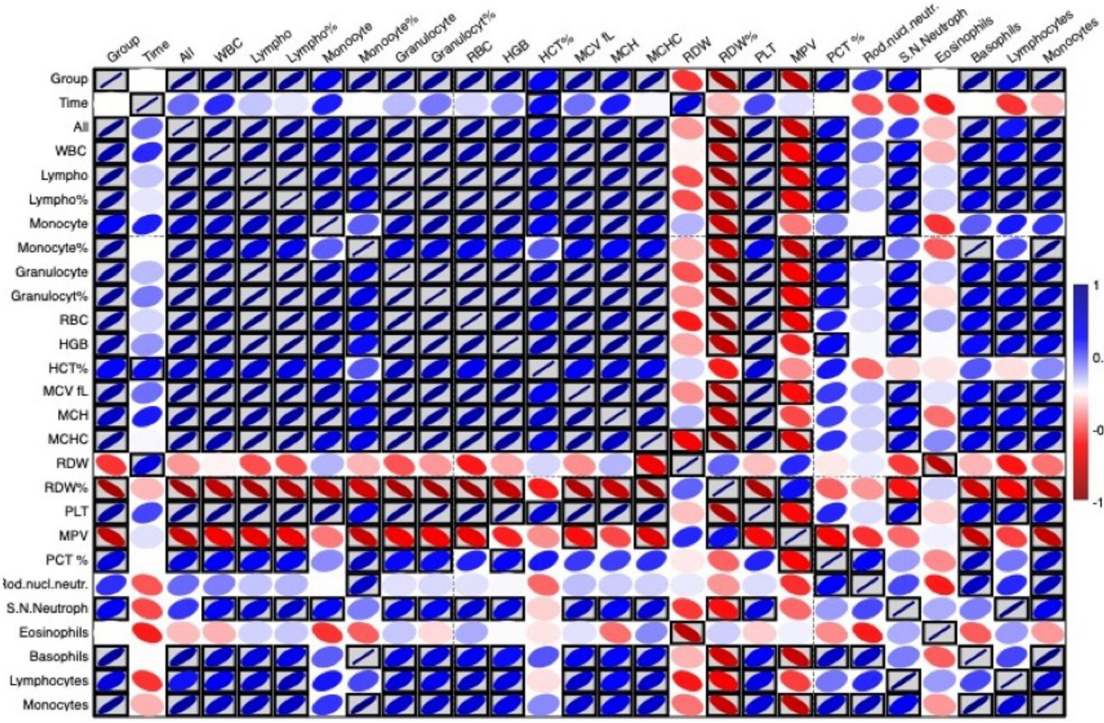

Figure 4. Heat map showing the interrelationships between variables.

5. Discussion

The supplemented dietary chlorophyll in the current study in two doses 30 and 60 mg/L presented a significant direct correlation with white blood cells, WBC, Lymphocytes, Monocytes, Lymphocytes (%), Monocytes (%), Granulocytes (%), HGB, MCH, MCHC, PLT, and Lymphocytes. The obtained significant effects of supplemented chlorophyll were in consistence with a recent previous research by Tagauov et al. (2023). Furthermore, these noteworthy chlorophyll findings suggest that utilizing chlorophyll from biological sources has a considerable positive impact on health. These findings support Pangestuti and Kim (2011) who detailed a number of key impacts of natural pigments, including chlorophyll. Though, it opposes study by Cugliari et al. (2018), researchers looked into the purported anti-anemic capabilities of chlorophyll in order to confirm its impact on blood count and iron in endurance athletes. They said that there was no discernible variation in the hemoglobin and other blood parameters, but that the blood platelets had increased (Cugliari et al., 2018).

By lowering pain and tiredness, the rise in platelet-related parameters may have a favorable impact on endurance performance. more research into the purported ergogenic benefits and anti-anemic qualities is advised (Cugliari et al., 2018). Platelet-rich plasma has anti-inflammatory and anabolic properties (Cugat et al., 2015) and several studies demonstrate its efficiency in promoting muscle damage repair (Cunha et al., 2014), tendon injury (Hamid et al., 2014) and in osteoarthritis treatment (Raeissadat et al., 2015). According to a recent research, the amount of time spent running a half marathon and MPV are significantly correlated (Lippi et al., 2014), PLTS, MPV, and PDW seem to have no meaningful association with VO2Max, resistance, or running speed in short-term performance at maximal effort, however (Alis et al., 2016). These findings imply that platelets might influence medium-term performance by encouraging the gradual release of growth factors, which would relieve muscular pain and/or fatigue, or that MPV increase could be attributed to a higher platelet turnover that might reflect other chronic physical adaptation without necessarily having a direct ergogenic effect. only the experimental group in the current research saw a substantial improvement, pointing to the function chlorophyll plays in influencing the aforementioned aspect.

Antioxidant functions are among the ways that dietary chlorophyll affects blood parameters. ROS, which targets macromolecules including membrane lipids, proteins, and DNA and causes a variety of health problems like cancer, diabetes mellitus, aging, and neurological illnesses, may cause harm to the human body. Antioxidants can protect the body from this damage (Ngo et al., 2011).

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