**DOSE-DEPENDENT HEMATOLOGICAL EFFECTS OF GREEN TEA (CAMELLIA SINENSIS) EXTRACT IN MALE WISTAR RATS**

**Abstract**

**Background**: Green tea (Camellia sinensis) is widely recognized for its antioxidant, metabolic, and cardiovascular benefits. However, its dose-dependent effects on hematological parameters remain unclear, particularly under non-pathological conditions.

**Aim**: This study aims to evaluate the hematological response to graded doses of green tea extract in healthy male Wistar rats.

**Methods**: In this study, Twenty-eight(28) male Wistar rats (180–220 g) were obtained from the animal facility of the Department of Physiology, Alex Ekwueme Federal University, Nigeria and randomly assigned to four groups (n = 7 each). Group I (control) received distilled water; Groups II, III, and IV received green tea extract orally at 100 mg/kg, 300 mg/kg, and 500 mg/kg body weight, respectively, for 21 days. Hematological parameters—including red and white blood cell indices, hemoglobin concentration, hematocrit, and platelet counts were assessed using an automated hematology analyzer. Data were analyzed using one-way ANOVA and Tukey’s post hoc test (\*p\* < 0.05).

**Results**: No significant differences were observed in RBC count, hemoglobin concentration, hematocrit, WBC count, or platelet indices across groups (p > 0.05). However, mean corpuscular hemoglobin concentration (MCHC) significantly increased in all green tea-treated groups compared to the control (p = 0.003), suggesting enhanced intracellular hemoglobin concentration. Other parameters, including MCV and monocyte percentage, showed non-significant trends approaching statistical relevance.

**Conclusion**: Green tea extract at doses up to 500 mg/kg does not adversely affect hematological parameters in healthy Wistar rats. The observed increase in MCHC may reflect improved erythrocyte function, potentially due to the antioxidant properties of green tea polyphenols. These findings support the hematological safety of green tea and provide a basis for further investigation in disease models or under oxidative stress conditions.

**Keywords**: Camellia sinensis, green tea extract, hematological indices, MCHC, Wistar rats, polyphenols, antioxidant, dose-response

**Introduction**

Hematological indices serve as vital indicators of the physiological and nutritional status of animals. These indices, such as hemoglobin concentration, hematocrit levels, and red blood cell counts, provide insights into the systemic health of an organism and are commonly used to assess the effects of dietary interventions, environmental stressors, and disease states (Hoffbrand et al., 2005). Nutritional status plays a fundamental role in maintaining these physiological functions, and any alteration in diet can significantly impact hematological parameters (Ahmed et al., 2015).

In recent years, there has been growing interest in natural alternatives to synthetic antibiotics and chemical additives, particularly in the field of animal nutrition. Among such natural compounds, medicinal plants with antioxidant properties have gained prominence. One such plant is Camellia sinensis, renowned for its high content of polyphenolic compounds—especially catechins—which can make up to 30% of the dry weight of tea leaves (Namita et al., 2012). Green tea is derived from the Camellia sinensis plant and is made by steaming fresh tea leaves, which preserves catechins, the major bioactive compounds in green tea (Yakubu et al., 2018). These catechins have been associated with various biological activities, including anticancer, hypoglycemic, hypocholesterolemic, and anti-obesity effects (Rahmani et al., 2015; Kanwar et al., 2012). Green tea is one of the oldest and most consumed beverages(Della et al., 2023). It is a popular beverage consumed for centuries in Japan, China, and other regions of Asia. Its popularity has spread worldwide and is now widely consumed in other parts of the world, including the United States, Europe, and Africa (Ogar et al., 2023; Settakorn et al., 2022). Moreover, green tea is widely recognized for its cardiovascular and metabolic health benefits (Winamp & Kristiano, 2016).

Despite the known antioxidant potential of green tea, its effects on hematological indices remain equivocal. While Rahman (2016) reported that green tea possesses antioxidant constituents capable of stabilizing blood cells and mitigating oxidative stress, Lubis et al. (2016) found no significant changes in hemoglobin or hematocrit levels in Wistar rats following green tea administration. In contrast, Bait (2010) observed a reduction in hemoglobin concentration in Sprague Dawley rats exposed to green tea. These conflicting findings may be attributed to differences in animal strain, dosage, extract preparation, or treatment duration. Furthermore, many existing studies have focused on isolated hematological parameters without evaluating the broader profile of blood indices.

The present study aims to assess the effects of green tea extract on a comprehensive range of hematological parameters in male Wistar rats. Specifically, we evaluate whether varying doses (low, moderate, and high) of green tea produce beneficial, neutral, or adverse effects on red and white blood cell indices, haemoglobin levels, and platelet profiles over 21 days.

**MATERIALS AND METHODS**

**Materials**

The materials used in this study included: laboratory animals: 28 male Wistar rats, aluminium cages, water bottles, feeding pans, towels, cotton wool, animal bedding, syringes (5 mL), cannulas, EDTA bottles, face masks, gloves, soaps/sanitisers, animal feed and distilled water, normal saline, and green tea extract.

**Experimental Animals and Handling**

Twenty-eight (28) male Wistar rats weighing approximately 200 g were obtained from the animal facility of the Department of Physiology, Alex Ekwueme Federal University. The rats were housed under standard laboratory conditions with a 12-hour light/dark cycle in a well-ventilated room. Animals were allowed to acclimatize for two weeks prior to experimentation. The bedding was changed every other day, and food and water were provided \*ad libitum\* throughout the experiment.

**Experimental Design**

The rats were randomly divided into four groups (n = 7 per group) as follows:

Group I (Control): Received standard feed and distilled water for 21 days

Group II (Low Dose): Received green tea extract at 100 mg/kg body weight

Group III (Moderate Dose): Received green tea extract at 300 mg/kg body weight

Group IV (High Dose): Received green tea extract at 500 mg/kg body weight

All treatments were administered orally via drinking water for 21 consecutive days.

**Preparation of Green Tea Extract and administration**

Fresh Camellia sinensis leaves were sourced from Taraba State, Nigeria, shade-dried, and pulverized into fine powder. The extract was prepared by boiling 100 g of the powder in 1 litre of distilled water for 15 minutes. The mixture was then cooled, filtered using a muslin cloth, and stored at 4°C. The fresh extract was prepared every three days.

Each rat's body weight was used to calculate the appropriate LD50 dosage (100, 300, or 500 mg/kg). The corresponding volume of extract was administered orally using calibrated syringes. The dosage regimen was selected based on previous studies demonstrating biological activity and safety in rodent models (Lubis et al., 2016; Gad & Zaghloul, 2013; Shoshin et al., 2020).

**Sample Collection**

At the end of the 21-day treatment period, the rats were anaesthetized using ether. Blood samples were collected via cardiac puncture using 5 mL syringes. Samples were transferred into EDTA-coated bottles for hematological analysis.

**Hematological Parameter**

Total Erythrocyte Count (RBC), Hemoglobin HB, Hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Total leukocyte count (WBC) and platelets were estimated using Animal blood counter -ABC vet device (Horiba ABX- France).

**Statistical Analysis**

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by post hoc comparisons using Tukey's test. Statistical significance was set at p < 0.05. All analyses were conducted using SPSS version 14.

**Results and Discussion**

Comparisons of hematological parameters of Wistar rats of the controlgroup and those administered low, intermediate and high doses of *camellia sinensis.*

Table 1 below depicts the mean±SEM of hematological (full blood count) parameters of albino Wistar rats administered low dose (100mg/kg body weight), intermediate dose (300mg/kg body weight) and high dose (500mg/kg body weight) of *camellia sinensis* (green tea). The mean values of the total white blood cell (TWBC) count and platelet count were highest in Wistar rats in group 3 and lowest in group 4 Wistar rats. The mean values of these parameters when compared across the groups were not statistically significant (p>0.05, in each case). The mean values of relative lymphocyte count and the mean cell volume (MCV) were highest in Wistar rats in group 1 and lowest in group 2 Wistar rats, but were not statistically significant when compared across the groups (p>0.05, in each case). The mean value of relative neutrophil count was highest in group 3 rats and lowest in group 1 rats. However, the relative neutrophil count when compared across the groups was not statistically significant (p>0.05, in each case). The mean±SEM of relative monocyte counts and haemoglobin (Hb) concentration were highest in Wistar rats in group 2 and lowest in group 1 Wistar rats. The mean values of the relative monocyte counts and Hb concentration when compared across the groups were not statistically significant (p>0.05, in each case). The mean value of red blood cell count was highest in group 2 rats and lowest in group 3 rats. However, the red blood cell count when compared across the groups was not statistically significant (p>0.05, in each case). The mean value of the packed cell volume (PCV) was highest in Wistar rats in group 1 and lowest in group 4 Wistar rats, but was not statistically significant when compared across the groups (p>0.05, in each case). The mean value of the mean cell haemoglobin concentration (MCHC) was highest in Wistar rats in group 4 and lowest in group 1 Wistar rats. The MCHC was significantly lower in rats in group 1 when compared to rats in Group 2, group 3 and Group 4 (p< 0.05, in each case). However, the MCHC in group 2 rats when compared to those in groups 3 and 4 were not statistically significant (p>0.05, in each case). The value of the mean platelet volume (MPV) was highest in Wistar rats in group 4 and lowest in group 2 Wistar rats, but the MPV was not statistically significant when compared across the groups (p>0.05, in each case).

Table 1: Comparisons of hematological parameters across the groups

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Hematology Parameters | Group 1  (Control) | Group 2  (Low Dose) | Group 3  (Moderate Dose) | Group 4  (High Dose) | F-value | p-value |
| TWBC (X 109/L) | 3.49±0.05 | 2.71±0.58 | 4.18±0.95 | 1.99±0.17 | 2.811 | 0.08 |
| Lymphocyte (%) | 97.80±0.47 | 91.85±1.41 | 93.07±3.52 | 94.27±1.99 | 1.415 | 0.28 |
| Neutrophil (%) | 0.92±0.36 | 4.07±0.53 | 4.73±2.80 | 3.75±1.60 | 1.04 | 0.41 |
| Monocyte (%) | 1.27±0.11 | 4.07±1.01 | 2.20±0.72 | 1.97±0.87 | 3.26 | 0.06 |
| RBC count | 7.93±0.47 | 8.48±0.53 | 7.78±0.39 | 7.96±0.61 | 0.36 | 0.78 |
| Haemoglobin (g/dL) | 16.75±0.88 | 17.45±0.98 | 16.85±0.34 | 16.82±1.25 | 0.12 | 0.95 |
| Packed cell volume (%) | 53.00±2.61 | 52.25±2.78 | 50.75±1.70 | 50.00±1.70 | 0.25 | 0.86 |
| Mean cell volume (fL) | 67.30±0.78 | 61.60±1.25 | 65.40±2.02 | 62.95±1.38 | 3.14 | 0.06 |
| Mean cell haemoglobin (pg) | 21.17±0.14 | 20.57±0.45 | 21.77±0.74 | 21.15±0.45 | 0.98 | 0.43 |
| MCHC (g/dL) | 31.45±0.18 | 33.40±0.18 | 33.30±0.62 | 32.94±0.27 | 8.05 | 0.003 |
| Platelet count (X 109/L) | 508.50±25.25 | 585.00±62.16 | 654.75±58.08 | 435.50±37.09 | 1.35 | 0.31 |
| Mean platelet volume (fL) | 7.93±0.36 | 7.73±0.11 | 7.70±0.11 | 8.00±0.24 | 0.41 | 0.75 |
|  |  | Post Hoc |  |  |  |  |
|  | ½ | 1/3 | 1/4 | 2/3 | 2/4 | 3/4 |
| MCHC | 0.002 | 0.003 | 0.001 | 0.845 | 0.661 | 0.845 |

P-value is significant at p<0.05. Keys: N= 16, Low dose = 100mg/kg body weight of *camellia sinensis*, intermediate dose= 300mg/kg body weight of *camellia sinensis*, high dose =500mg/kg body weight of *camellia sinensis*.

This study investigated the hematological effects of varying doses (100, 300, and 500 mg/kg body weight) of green tea (\*Camellia sinensis\*) extract in male Wistar rats over a 21-day period. The findings demonstrated that most hematological parameters, including red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), white blood cell (WBC) count, differential leukocyte counts, platelet count, and mean platelet volume (MPV), did not differ significantly across treatment groups. However, a statistically significant increase was observed in mean corpuscular hemoglobin concentration (MCHC), particularly in green tea-treated groups compared to the control (\*p\* = 0.003).

The absence of significant changes in RBC count, Hb concentration, and PCV suggests that green tea extract at the doses tested does not adversely affect erythropoiesis or red cell volume. This aligns with findings by Lubis et al. (2016), who reported no substantial hematological alterations in rats administered green tea extract at 100 mg/kg. Similarly, Gad and Zaghloul (2013) demonstrated that green tea extract at moderate doses preserved hematological stability in aged rats. The mild fluctuations observed in RBC and Hb levels across groups were statistically insignificant, indicating a neutral effect under non-pathological conditions.

Interestingly, while mean corpuscular volume (MCV) approached significance (\*p\* = 0.06), it was not enough to confirm a dose-dependent effect. The slight reduction in MCV observed in treated groups may suggest early cellular adaptation to phytochemical exposure, although this finding contrasts with Shoshin et al. (2020), who reported a significant MCV decline at higher doses and longer exposure (30 days). This discrepancy may be attributed to differences in exposure duration, animal strain, or extract preparation.

The significant increase in MCHC across all green tea-treated groups is noteworthy. MCHC represents the average hemoglobin concentration within a single erythrocyte and is often linked to red cell integrity and function. The enhanced MCHC may suggest a beneficial effect of green tea catechins, particularly epigallocatechin gallate (EGCG), which has been shown to stabilize erythrocyte membranes and protect against oxidative damage (Huang et al., 2014; Rahman, 2016). Sae-Tan et al. (2011) also reported that green tea polyphenols can influence erythropoiesis-related gene expression, potentially contributing to improved hemoglobin packing within cells.

White blood cell indices and differential counts remained within normal physiological limits and did not vary significantly between groups. This finding supports prior work by Kim et al. (2014) and Guimarães et al. (2022), who found that green tea extract does not markedly modulate leukocyte profiles in healthy animals. Although monocyte counts showed a trend toward increase in the low-dose group (\*p\* = 0.06), this did not reach statistical significance and requires further investigation.

Platelet indices (platelet count and MPV) were also unaffected by green tea treatment. While the moderate-dose group recorded the highest platelet count and the high-dose group the lowest, these differences were not statistically significant. This is consistent with findings by Rasool et al. (2018) and Sung et al. (2000), who noted that green tea catechins may have mild antithrombotic properties but do not significantly alter platelet production under physiological conditions.

The results suggest that green tea extract, at higher doses (up to 500 mg/kg), is hematologically safe in healthy Wistar rats and may exert a mild functional benefit by enhancing MCHC. The largely neutral impact on other hematological indices supports the plant's adaptogenic and non-toxic profile, at least in the short term and under non-stressed conditions.

**Conclusion**

This study revealed that oral administration of green tea (Camellia sinensis) extract at doses of 100, 300, and 500 mg/kg body weight for 21 days did not produce significant alterations in most hematological parameters of healthy male Wistar rats. The only notable change was a significant increase in MCHC across all treated groups, suggesting a possible influence of green tea constituents on red cell indices. These findings indicate that short-term exposure to green tea extract, even at higher doses, may be hematologically safe in healthy rats. Further studies using longer treatment durations, disease models (e.g., anemia or oxidative stress), and biochemical markers of oxidative injury are needed to determine whether green tea’s hematological effects are more pronounced under pathological conditions.

**Ethical Approval**

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Ethical approval for the study was granted by the Animal Ethics Committee of the Faculty of Basic Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Nigeria.

**Declarations**

Data Availability: There is no data availability statement applicable to this research.

Ethical Approval: The ethical committee approved the protocol of this study according to the rules and guidelines in experimenting at the Department of Physiology, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State with the ethical approval number: 017PY20317

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1.

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