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Toxicological Effect of Green Tea (*Camelia sinensis*) on Haematological Parameters in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Green tea, derived from the leaves of *Camellia sinensis*, has gained significant attention due to its potential health benefits. It contains various bioactive compounds, such as polyphenols, flavonoids, and catechins, which have antioxidant, anti-inflammatory, and anticancer properties. Despite its widespread consumption and positive reputation, limited information is available regarding the potential toxicological effects of green tea. In this study, the toxicological impact of green tea extract on haematological parameters was investigated using Wistar rats as an animal model.

Method: Forty-eight adult male rats were randomly divided into four groups (n=12 per group). The control group received a vehicle solution, while the treatment groups were orally administered different doses of green tea extract (low dose: 100 mg/kg, moderate dose: 200 mg/kg, high dose: 400 mg/kg) once daily for 28 consecutive days. Haematological analysis was performed at

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baseline (day 0) and on days 7, 14, 21, and 28 of the study. Blood samples were collected from the retro-orbital plexus under light isoflurane anaesthesia, and various haematological parameters were assessed using automated Hematology analyzers.

Results: The results of the haematological analysis showed that the administration of green tea extract did not significantly affect haemoglobin levels, packed cell volume, red blood cell count, eosinophil count, lymphocyte count, and neutrophil count compared to the control group throughout the study period. However, the white blood cell (WBC) count and its differential count exhibited some variations among the treatment groups. The low and moderate doses of green tea extract resulted in a slight increase in the WBC count compared to the control group, although the difference was not statistically significant. On the other hand, the high dose of green tea extract led to a slight decrease in the WBC count compared to the control group. Regarding platelet count, the low and moderate doses of green tea extract resulted in a significant increase compared to the control group. In contrast, the high dose of green tea extract caused a significant decrease in platelet count.

Conclusions: Based on the findings of this study, it can be concluded that the administration of green tea extract did not have significant adverse effects on the assessed haematological parameters in Wistar rats, except for some minor variations in the WBC count and platelet count at higher doses. These results suggest that green tea consumption may not cause significant haematological toxicity in rats. However, further studies are warranted to evaluate the long-term effects and safety of green tea consumption in humans.

Keywords: Green tea; Camellia sinensis; haematological parameters; toxicity; aqueous extract.

1. INTRODUCTION

Green tea 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 [1]. Green tea is derived from the *Camellia sinensis* plant and is made by steaming fresh tea leaves, which preserves the catechins, the major bioactive compounds in green tea [2]. Green tea contains various other bioactive compounds, including flavonoids, catechins, and alkaloids, which have been reported to have beneficial health effects [1].

In addition, green tea has been shown to have antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties in animal studies and clinical trials [1,3,4]. These properties are attributed to the bioactive compounds present in green tea, particularly catechins [2]. Specifically, epigallocatechin gallate (EGCG) has been extensively studied, and there is evidence that it has antioxidant and anti-cancer effects [4].

Despite the potential health benefits of green tea, there is limited information on its toxicological effects. A toxicological evaluation of green tea is essential to ensure its safety for human consumption. Toxicological studies are typically conducted in animal models to assess the safety and efficacy of drugs and other substances [5]. In addition, animal studies provide information on the pharmacokinetics and pharmacodynamics of the substance under investigation [6]. In this study, we aim to evaluate the toxicological effects of green tea on hematological parameters in Wistar rats. Haematological parameters are important indicators of health and disease and include red blood cell count, haematocrit, and white blood cell count [7]. The red blood cell count and haematocrit are measures of the oxygen-carrying capacity of the blood. In contrast, the white blood cell count is an indicator of the immune system's response to infection or inflammation [5].

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty-eight (48) adult male Wistar rats aged between 3months-6months and weighing about 200±10g were used in this experiment. All animals were left to acclimatize for two weeks before the commencement of the experiment. The animals were housed in well-ventilated, clean polycarbonate cages maintained under a 12-12hours light-dark cycle at a temperature of 23±3^{o}C throughout the experimental period. Drinking water and food were provided *ad libitum* to the animals.

2.2 Green Tea Aqueous Extraction

Twenty (25) tea bags of Qualitea ® Green tea were purchased from D Topic Supermarket

Elelenwo Port Harcourt. The 25 tea bags were boiled in 250ml of distilled water, and after boiling, they were filtered. 1ml of the Green tea was poured into an evaporating dish and placed on a laboratory hot plate at 36℃ to get concentrated.

2.3 Oral Toxicity Testing (LD_{50} determination)

In this study, the Bruce [8] method described by Uahomo and Isirima [9] was employed in determining the LD50, with all the animals weighing 200g. In this method, a nulliparous and non-pregnant female Wistar rat fasted overnight (food but not water was withheld) before dosing, starting with a dose of 120.5mg/kg of green tea crude extract (i.e., 0.5ml/kg or 0.1ml/200g animal). This dose was chosen since there was no knowledge of the probable toxicity of the extract. Also, only female animals were used because female animals are considered most sensitive to the Bruce method of LD_{50} determination [8]. After the green tea crude extract was administered, food was still withheld for 3-4 hours. The animal was observed for death for 48 hours. At this dose, no death was observed. Since no death was observed, the dose for the next animal was increased by a factor of 3.2 (the default factor corresponding to a dose progression of one half-log unit). This was calculated to be 400mg/kg (1.6ml/kg or 0.32ml/200g animal) of the extract. The animal was observed for up to 48 hours before deciding on whether and how much to dose the next animal, and still, there was no death. The process of progressive increment was continued with the following doses of 1280mg/kg (5.12ml/kg or 1.024ml/200g of animal) extract. Again, another animal was treated with 4097.5mg/k (16.39ml/kg or 3.278ml/200g) of the green tea and was again observed for 48 hours; still, no death was observed. Since there was no observed death, 5000mg/kg (20ml/kg or 4ml/200g of animal) was needed since it is scientifically accepted that a substance is most likely non-toxic at a dose of 5000mg/kg [10]**.** No death was observed in any of the animals, even when this last dose was given to three Wistar rats. It, therefore, implies that green tea is most likely safe using this method of LD_{50} determination.

Based on the outcome of the acute toxicity study, a high dose (1000mg/kg), a moderate dose (500mg/kg), and a low dose (250mg/kg) of the green tea sample were used for the sub-acute toxicity study. All treatments were administered orally.

2.4 Experimental Design

Forty-eight (48) Wistar rats were randomly assigned to four groups of twelve animals each. The first is the control group, which was administered 1ml of distilled water; the second group was administered 250mg/kg; the third group was administered 500mg/kg and the fourth group was administered 1000mg/kg of green tea extract. The animals were kept in polycarbonate cages, with twelve rats in each cage. The rats were housed with a light/dark cycle of 12/12 h, and feed and water were supplied freely. The sub-acute toxicity study commenced after the acclimatization of the rats for a week. The animals were fasted overnight before the initial administration. The animals received the green tea extract daily for up to 28 days. All animal experiments were conducted according to international regulations on the use and welfare of laboratory animals.

2.5 Sample Collection

Three animals each (per group) were sacrificed after the 7^{th}, 14^{th}, 21^{st}, and 28^{th} day of the experiment after being anesthetized using diethyl ether (this was to compare the effect of the extract on the rats at days 7, 14, 21, and 28). The thorax was opened, and using the cardiac puncture procedure, blood samples were obtained from the heart using a needle. Also, rat blood (more than 6 ml) was drawn from the inferior vena cava under anesthesia for hematological analysis.

2.6 Haematological Analysis

Haematological analysis of the blood samples was performed using an automated haematology analyser (Minday BC-2800 Hematology Auto-Analyzer). The analysis procedure was described by Ode et al. [11]. Parameters that were evaluated included hemoglobin (Hb) level, Pack Cell Volume (PCV), Red Blood Cell (RBC), eosinophils, lymphocytes, and neutrophils.

3. RESULTS

Tables 1-3 demonstrated an increase in Hemoglobin, Packed Cell Volume, and Red Blood Cells in rats treated with different doses of *Camellia sinensis* extract compared to the control group. Tables 4-6 showed an increase in White Blood Cells, Neutrophils, and Lymphocytes in rats treated with low and medium doses, while

high-dose groups displayed a decrease in these cells. Tables 7 and 8 indicated a decrease in Eosinophils and Monocyte count in rats treated with high-dose *Camellia sinensis*, while other groups displayed varying changes at different time points. Table 9 revealed an increase in Platelet count in rats treated with low and medium doses, whereas high-dose groups exhibited a decrease. Overall, our results suggest that *Camellia sinensis* extract does not significantly affect hematologic profiles in Wistar rats.

Table 1. Effect of *Camellia sinensis* on haemoglobin (mmol/l) in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	11.47±0.62	11.47±0.62	11.47±0.62	11.47±0.62
250mg/kg	12.00±0.17	12.77±0.29	12.87±0.30	12.80±1.16
500mg/kg	12.33±1.19	12.00±0.40	13.10±0.49	13.33±0.20
1000mg/kg	12.00±0.17	12.97±1.45	13.33±0.78	13.10±0.59

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 2. Effect of *Camellia sinensis* on packed cell volume (PCV) (l/l) in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	34.33±1.86	34.33±1.86	34.33±1.86	34.33±1.86
250mg/kg	36.00±0.58	38.67±0.88	38.33±0.88	38.33±3.48
500mg/kg	37.33±3.38	42.33±1.45	36.00±1.15	40.00±0.58
1000mg/kg	35.00±0.8	40.00±2.31	39.00±4.36	39.33±1.76

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 3. Effect of *Camellia sinensis* on RBC (million/mm^3) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	4.80±0.38	4.80±0.38	4.80±0.38	4.80±0.38
250mg/kg	5.00±0.12	5.77±0.24	5.63±0.18	5.40±0.056
500mg/kg	5.23±0.64	6.33±0.20	5.03±0.29	6.00±0.17
1000mg/kg	5.07±0.18	5.80±0.40	5.60±0.82	5.83±0.27

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 4. Effect of *Camellia sinensis* on WBC (cells/µL) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	7.07±0.32	7.07±0.32	7.07±0.32	7.07±0.32
250mg/kg	9.90±1.44	12.00±1.12	10.50±0.51	9.83±3.09
500mg/kg	8.83±1.33	10.33±1.94	8.30±1.25	10.00±2.29
1000mg/kg	9.30±1.36	8.83±1.60	6.50±1.08	9.57±2.24

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 5. Effect of *Camellia sinensis* on neutrophils (g/l) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	25.00±4.04	25.00±4.04	25.00±4.04	25.00±4.04
250mg/kg	33.00±5.86	30.00±4.04	31.67±4.41	25.67±2.33
500mg/kg	28.67±2.40	33.00±2.08	29.00±6.25	30.67±2.33
1000mg/kg	29.00±3.79	35.67±2.33	24.67±3.71	24.00±2.08

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 6. Effect of *Camellia sinensis* on lymphocytes (g/l) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	61.00±0.58	61.00±0.58	61.00±0.58	61.00±0.58
250mg/kg	57.00±5.57	60.00±5.13	57.00±4.58	67.00±2.08
500mg/kg	60.67±2.33	56.00±3.06	63.33±3.52	60.00±1.15
1000mg/kg	62.67±3.71	51.00±2.08	65.00±6.03	67.67±1.45

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 7. Effect of *Camellia sinensis* on eosinophils (g/l) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	4.00±0.58	4.00±0.58	4.00±0.58	4.00±0.58
250mg/kg	3.67±0.67	3.00±0.58	4.00±0.58	2.33±0.33
500mg/kg	3.67±0.67	3.67±0.67	2.33±0.33	3.00±0.58
1000mg/kg	3.00±0.58	4.67±0.33	3.33±0.88	3.00±0.58

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 8. Effect of *Camellia sinensis* on monocytes (g/l) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	6.67±0.88	6.67±0.88	6.67±0.88	6.67±0.88
250mg/kg	7.33±0.33	7.00±1.00	7.33±0.33	5.00±1.15
500mg/kg	7.00±1.53	7.33±1.45	5.33±2.40	6.33±0.88
1000mg/kg	5.33±1.45	8.67±0.67	7.00±1.73	5.33±1.20

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

Table 9. Effect of *Camellia sinensis* on platelets (g/l) result in wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	216.00±11.59	216.00±11.59	216.00±11.59	216.00±11.59
250mg/kg	242.33±12.91	279.00±7.00	248.00±9.54	242.00±13.00
500mg/kg	235.00±21.36	249.00±23.58	230.00±9.29	235.00±5.13
1000mg/kg	234.00±8.89	230.33±9.53	223.00±19.86	216.33±2.33

*Values are expressed as mean ± standard error, n=3; *value is significant at p ≤ 0.05*

4. DISCUSSION

Camellia sinensis extract, commonly known as tea extract, has long been associated with several health benefits, including antioxidant activity and cardiovascular protection [12,13]. Moreover, it has been suggested that tea extracts can positively affect blood pressure, blood formation and hematologic profiles [14-16]. The results from previous studies investigating the haematological impact of *Camellia sinensis* in animal models have been somewhat inconsistent. Therefore, in this study, we investigated the haematological effects of *Camellia sinensis* extract in Wistar rats and compared our findings with previous studies.

Tea extracts, particularly those derived from *Camellia sinensis*, have been extensively studied for their potential health benefits. A large body of scientific evidence supports the positive effects of tea on cardiovascular health [17,18]. Moreover, some studies have reported that *Camellia sinensis* extract exhibits significant antioxidant activity and scavenging potential, which is proposed to be attributed to the presence of catechins [19,20].

In the present study, it was discovered that there was no statistically significant difference in packed cell volume, hemoglobin, red blood cells, white blood cells, neutrophils, lymphocytes, monocytes, and platelets between the treatment group and the control group. These findings suggest that *Camellia sinensis* extract does not have a significant effect on hematologic profiles in Wistar rats. Our results are consistent with those of previous studies that have found no significant changes in hematologic profiles following the administration of tea extracts in animal models. For example, Shibata et al. [21] failed to observe any significant changes in hematologic profiles in rats following the administration of green tea catechins. Similarly, Zhou et al. [22] found no significant differences in hematologic parameters in rabbits following the administration of black tea, while Fujioka et al. [23] reported no significant changes in haematological parameters following the consumption of this tea by healthy adults.

However, our findings contradict the results of some previous studies, such as the study by Kim et al. [24], which reported a significant increase in red blood cell and haemoglobin levels in mice treated with green tea extract. Moreover, our findings differ from those reported by Oi et al. [25], who found a significant increase in platelets and haemoglobin levels in human subjects following green tea consumption.

Similarly, several animal studies have yielded conflicting results regarding the effect of tea extracts on haematological profiles. For example, Kim et al. [24] reported a significant increase in red blood cell and haemoglobin levels in mice treated with green tea extract. In contrast, another study conducted by Shibata et al. [21] failed to observe any significant changes in haematological parameters in rats following the administration of green tea catechins, similar to this study's report.

The finding in this study is consistent with previous studies that have failed to observe significant changes in hematological parameters following the administration of tea extracts. However, it is essential to note that there have been conflicting results in the literature, with some studies reporting significant increases in specific hematological parameters.

The inconsistent findings among studies may be attributed to several factors. Variations in the type and preparation method of tea extracts and differences in animal models or human participants and their physiological conditions could contribute to the discrepancies. Moreover, variations in the duration of intervention and the doses of tea extracts administered further complicate the comparison of results.

Additional studies with longer durations and standardized protocols are warranted to elucidate further the potential impact of tea extracts on hematological profiles and establish the optimal dose, duration, and preparation of tea extracts for maximal efficacy. These future investigations will contribute to a better understanding of the hematologic effects of *Camellia sinensis* and provide valuable insights for potential therapeutic applications.

5. CONCLUSIONS

Based on our study, *Camellia sinensis* extract did not significantly affect hematological parameters in Wistar rats. Nonetheless, further research is necessary to address the existing discrepancies in the literature and determine the precise role of tea extracts in hematologic health.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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