Original Research Article

TOXICOLOGICAL EFFECT OF GREEN TEA (*Cameliasinensis*) ON HAEMATOLOGICAL PARAMETERS IN WISTAR RATS

ABSTRACT

Green tea, a popular beverage, has shown to have beneficial health effects due to its bioactive compounds, including flavonoids, catechins, and alkaloids. However, there is limited information on its toxicological effects, which are important to ensure its safety for human consumption. In this study, the toxicological effects of green tea on haematological parameters in Wistar rats were evaluated. Forty-eight Wistar rats were divided into four groups of 12 animals each. Apart from the control group that received 1ml of distilled water, animals in the 3 test groupswere orally administered with different doses (250mg/kg, 500mg/kg, and 1000mg/kg) of green tea aqueous extract for 28 days. Haematological analysis of the blood samples was performed to evaluate parameters such as blood haemoglobin, pack cell volume, red blood cells, eosinophils, lymphocytes, and neutrophils. The results showed no significant differences in haematological parameters between the treatment groups and the control group. These findings are consistent with previous studies that also failed to find adverse hematologic effects of tea extracts.However, further studies with longer duration (beyond 28 days) are necessary to elucidate the potential impact of tea extracts on haematological profiles and to identify the optimum dose, duration, and preparation of tea extracts for maximal efficacy.

Comment [M1]: Male or female

Keywords: Green tea, *Cameliasinensis*, Toxicological profile, Haematological parameters, Wistar rats

INTRODUCTION

Green tea is a popular beverage that has been consumed for centuries in Japan, China, and other regions of Asia. Its popularity has spread worldwide, and it 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, a process that preserves the catechins, the major bioactive compounds in green tea [2]. Green tea contains a variety of

otherbioactive 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, epigallocatechingallate (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 important 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 haematological parameters in Wistar rats. Haematological parameters are important indicators of health and disease and include parameters such as 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, whereas the white blood cell count is an indicator of the immune system's response to infection or inflammation [5].

MATERIALS AND METHODS

Experimental Animals

Forty-eight (48) Wistar rats aged between 3months-6months and weighing about 200gwere used in this experiment. All animals were left to acclimatize for two weeks before the commencement

Comment [M2]: These rats male or female Its prefer male rats

Comment [M3]: 200±10 g

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.

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.

Comment [M4]: Need reference

Oral Toxicity Testing (LD_{50} determination)

In this study, Bruce[8] method as described by Uahomo and Isirima [9] was employed in determining the LD_{50}, with all the animals used weighing 200g. In this method, a nulliparous and **non-pregnant female Wistar rat**, fasted overnight (food but not water was withheld) prior to dosing, starting with a dose of 120.5mg/kg of green tea crudeextract (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 methodof LD_{50}determination [8]. After the green tea crude extract was administered, food was still withheld for a further 3-4 hours. The animal was observed for death for a period of 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 carefully for up to 48 hours before

Comment [M5]: Female (non pregnant)
Duration time of experiment (28 days)
Its not suitable in toxicity experiment

Comment [M6]: 120mg crude extract/kg body weight (120 ppm)
Means 24 mg/200g body dissolve in 1 0r 2 ml (orally)

Comment [M7]: 400 mg/kg (400 ppm) means 92 mg crude extract dissolve in 1 or 2ml water (orally)

making a decision 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 there was no death 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]**.**There was still no death 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, high dose (1000mg/kg), moderate dose (500mg/kg) and low dose (250mg/kg) of the green tea sample was used for the sub-acute toxicity study. All treatments were administered orally.

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 were administered 500mg/kg and the fourth group was administered 1000mg/kg of the green tea extract. The animals were keptin 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 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.

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 anaesthetized using diethyl ether (this was in order 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 anaesthesia for haematological analysis.

Haematological Analysis

Haematological analysis of the blood samples was performed using an automated haematology analyser (MindayBC-2800Hematology Auto-Analyzer). The procedure of analysis was as 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.

RESULTS

Tables 1-3 shows the 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 display the increase in White Blood Cells, Neutrophils, and Lymphocytes in rats treated with low and medium doses of *Camellia sinensis*, while high-dose groups have a decrease in these cells. Tables 7 & 8 show a decrease in Eosinophils and Monocyte count in rats treated with high-dose *Camellia sinensis* compared to the control, while other groups either increased or decreased

at certain time points of the experiment. Finally, table 9 shows an increase in Platelet count in rats treated with low and medium doses of *Camellia sinensis*, while high-dose groups have a decrease in Platelet count. However, there was no significant effect of *Camellia sinensis*on haemoglobin, PCV, RBC, eosinophils, lymphocytes, and neutrophils of treated animals when compared to the control animals.

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 $p \leq 0.05$

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 have a positive effect on blood pressure, blood formation and hematologic profiles [14-16]**.** However, the results from previous studies investigating the haematological impact of *Camellia sinensis* in animal models have been somewhat inconsistent. Therefore, in this study, we aimed to investigate the haematological effects of *Camellia sinensis* extract in Wistar rats and compare 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, numerous 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 which is similar to the report of this study.

Several factors may contribute to the inconsistent results reported in studies investigating the hematologic effects of tea extracts. Firstly, the type and preparation method of tea extracts can differ significantly between studies. Secondly, the selected animal models or human participants and their physiological conditions also vary between studies. Lastly, the duration of intervention and the doses of tea extracts administered varied widely across the experiments, further complicating the comparison of results.

CONCLUSION

Our study found no significant differences in haematological parameters in Wistar rats following the administration of *Camellia sinensis* extract for up to 28 days. This suggests that green tea consumption may not be harmful to the blood. Our results are consistent with previous studies that have also failed to find any adverse hematologic effects of tea extracts. However, further studies with longer duration (beyond 28 days) are necessary to elucidate the potential impact of tea extracts on haematological profiles and to identify the optimum dose, duration, and preparation of tea extracts for maximal efficacy.

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