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Assessment of the Effects of *Camellia sinensis* (Green Tea) Extract on Renal, Cardiac, and Pulmonary Tissues in Wistar Rats

Victor Tamunotonye Ibubeleye[1], Faith Onyedikachi Ogar[1], Nwibana Barisuka Kofii[2], Preye David Ogbe[3], Precious Ojo Uahomo[4]* and Owunari Abraham Georgewill[1]

[1]*Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria*

[2]*School of Medical Laboratory Science, Rivers State College of Health Science and Management Technology, Nigeria*

[3]*Department of Pharmacology, Faculty of Basic Clinical Sciences, Niger Delta University, Wilberforce Island, Nigeria*

[4]*Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Port Harcourt, Nigeria*

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***Corresponding author:** Precious Ojo Uahomo, Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

Abstract

Background: Green tea is widely consumed and is rich in phytochemicals reported to have antioxidant, anti-inflammatory, and anticancer properties. However, there is a need to evaluate the effects of green tea on the heart, lung, and kidney. This study investigated the effects of *Camellia sinensis* on the heart, kidney, and lung in Wistar rats.

Methods: Forty-eight adult male Wistar rats were divided into four equal groups and orally administered 250mg/kg, 500mg/kg and 1000mg/kg of *Camellia sinensis* extract or 1ml of distilled water for up to 28 days. Serum electrolytes such as sodium (Na^+), potassium (K^+), chloride (Cl-), kidney function parameters such as urea and creatinine, and lipid profile parameters such as total cholesterol (TC), triglyceride (TAG), high-density lipoprotein-cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were measured at various time points. Effects on heart, kidney, and lung histology were also assessed using standard methods.

Results: The administration of *Camellia sinensis* extract in varying doses led to changes in some biochemical parameters with no significant effects. While heart histology remained unaffected, kidney tissues displayed acute tubular necrosis, especially at higher doses, with signs of recovery by day 28. Lung tissues exhibited some abnormalities, particularly at higher doses, with indications of recovery over time.

Conclusion: These results emphasize the need for consumption of moderate doses of green tea extract and the importance of further research to understand the mechanisms involved in these observed effects on organ histology.

Keywords: Green tea; *Camellia sinensis*; Toxicity; Kidney; Lung; Heart; Histology

Introduction

Green tea, derived from the plant, Camellia sinensis, has been widely consumed for its potential health benefits for centuries [1]. Previous studies have highlighted its potential therapeutic effects and its role in preventing various chronic diseases, including cancer, diabetes, and cardiovascular diseases [2-4]. This beneficial effects of *Camellia sinensis* have been associated with its rich antioxidant, anti-inflammatory, and anticancer properties [5]. However, it is important to consider that "natural" is not always "safe" as concerns have been raised regarding the potential toxicological effects of green tea, particularly on vital organs such as the heart, lungs, and kidneys [5-8].

Cardiovascular diseases remain one of the leading causes of morbidity and mortality worldwide [9]. The heart, being the central pump of the circulatory system, is highly susceptible to injuries caused by oxidative stress and inflammation, which may lead to structural alterations [10]. Previous studies have indicated that the consumption of green tea can modulate several factors related to cardiovascular health, including blood pressure, reduction of

LDL levels, and inhibition of platelet aggregation [11]. However, some studies have reported that high doses of green tea extract can have detrimental effects on the cardiovascular system. A study conducted on rats showed that high doses of green tea extract caused a significant increase in blood pressure and heart rate [5]. Another study conducted in mice showed that green tea extract increased the risk of thrombosis [12]. Therefore, it is important to consider the dose and duration of green tea consumption to avoid any adverse effects on the cardiovascular system.

The kidney is an essential organ in the body as it plays a crucial role in excreting waste products and fluid from the body, which helps to maintain a healthy balance of water, salts, and minerals in the blood [13,14]. It also helps to regulate blood pressure and produce hormones that stimulate the production of red blood cells and maintain bone and muscle health [13,14]. Hence, renal function assessment is of paramount importance since the kidneys help in filtering and eliminating toxins from the body [15].

Green tea and its bioactive compounds have been reported to possess diuretic, antioxidant, and nephroprotective properties [16,17]. However, conflicting findings exist on the potential renal toxicity of green tea, with some studies suggesting a protective effect and others highlighting adverse effects on renal function. A study conducted by Bedrood et al. [5] reported that high doses of green tea extract caused a significant increase in serum creatinine and urea levels, indicating impaired renal function.

Histo-morphological assessments are crucial for elucidating the structural changes induced by green tea consumption in various organs. Histopathological evaluation provides valuable information about cellular changes, tissue damage, and potential toxic effects caused by substances [18]. A study has reported that high doses of green tea extract can have detrimental effects on the histomorphology of the liver and lungs. This study reported that high doses of green tea extract may have caused histopathological changes such as congestion, haemorrhage, and oedema in the lungs [19].

While green tea is widely recognized for its health benefits, the evaluation of its toxicological effects on various organ systems is crucial for ensuring its safety. Hence, this study aimed to evaluate the potential toxicological effects of green tea extract on cardio-pulmonary, and renal functions, as well as histo-morphological changes in these organs in Wistar rats. The findings of this study will provide valuable insights into the safety profile of green tea and contribute to a better understanding of its potential toxicological effects, aiding in the development of evidence-based guidelines for its consumption.

Materials and Methods

Experimental Animals

Forty-eight (48) adults 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 and maintained under a 12-12hours light-dark cycle at a temperature of 23±3 °C throughout the experimental period. Drinking water and feed were provided *ad libitum* to the animals.

Green Tea Aqueous Extraction

Twenty-five (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°C to get concentrated.

Oral Toxicity Testing (LD_{50} determination

In this study, the LD_{50} of the green tea crude extract was determined using the Bruce [20] method as described by Uahomo and Isirima [21]. Based on the results of the acute toxicity study, three different doses of the green tea sample were selected for the sub-acute toxicity study: a high dose of 1000mg/kg, a moderate dose of 500mg/kg, and a low dose of 250mg/kg. 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 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. In addition, ethical approval was obtained from the University of Port Harcourt Research Ethics Committee (UPH/CEREMAD/REC/MM87/040).

Sample collection

Three animals per group were sacrificed after the 7th, 14th, 21st, and 28th 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) [22]. The thorax was opened, and using the cardiac puncture procedure, blood samples were obtained from the heart using a needle. Also, rat blood (5 ml) was drawn from the inferior vena cava under anaesthesia for biochemical analyses.

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Biochemical analysis

The serum electrolytes [sodium (Na^+), potassium (K^+), chloride (Cl^-)] levels were determined using colorimetric methods as previously described by Maruna [23], Trinder [24], Terri and Sesin [25], Skeggs and Hochstrasser [26], respectively. Furthermore, the serum bicarbonate was determined by the back titrimetric method as described by Van Slyke et al. [27], while serum urea and creatinine levels were quantified using the Urease-Berthelot [28] and Jaffe's reaction [29], respectively. Lipid profile including total cholesterol (TC), triglyceride (TAG), high-density lipoprotein-cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were determined using Randox assay kits.

Histopathology Examination

The animals were anaesthetized with diethyl ether and dissected aseptically to remove the heart, kidney, and lungs which were then transferred into 10% chloroform and later trimmed to a size of 2mm to 4mm thickness, to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker [30] and Isirima and Uahomo [31], including, fixation, dehydration, clearing, impregnation, embedding, sectioning, and staining with hematoxylin and eosin (H&E) and finally mounting.

Method of Statistical Analysis

The data obtained were analysed using Statistical Package for Social Science (IBM SPSS, Version 26). The data were expressed as mean ± standard error of mean. Statistical analysis was performed using Analysis of Variance (ANOVA) followed by the Dunnett method to determine significant differences among the groups. Statistical significance was considered at $p<0.05$.

Results

Effect of *Camellia sinensis* Extract on Serum Electrolytes in Wistar rats

Potassium (K) levels showed an increase on day 28 in the 250mg/kg group. No significant changes were observed in the other groups (Table 1). Sodium (Na) levels showed a decrease on day 7 in the 250mg/kg and 1000mg/kg groups. On day 21, there was a significant increase in the 250mg/kg group, and on day 28, there was a significant increase in the 1000mg/kg group (Table 2). Chloride (Cl-) levels showed a decrease on day 14 in the 250mg/kg group and on day 21 in the 500mg/kg group. No significant changes were observed in the other groups (Table 3). Urea levels showed an increase on day 21 in the 500mg/kg and 1000mg/kg groups. No significant changes were observed in the other groups (Table 4). Creatinine levels showed a decrease on day 7 in the 500mg/kg group. On day 21, there was a significant decrease in the 250mg/kg group and a significant increase in the 500mg/kg and 1000mg/kg groups. On day 28, there was a significant increase in the 500mg/kg and 1000mg/kg groups (Table 5). Bicarbonate ion levels showed a significant increase on day 14 in the 500mg/kg and 1000mg/kg groups. On day 21, there was a significant increase in the 500mg/kg and 1000mg/kg groups, and on day 28, there was a significant increase in the 500mg/kg and 1000mg/kg groups (Table 6). Overall, the administration of *Camellia sinensis* at different doses resulted in changes in potassium, sodium, chloride, urea, creatinine, and bicarbonate ion levels in Wistar rats. The significance of these changes was dose dependent.

Table 1: Effect of *Camellia sinensis* on Serum Potassium (K) (mmol/l) in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	4.37±0.52	4.37±0.52	4.37±0.52	4.37±0.52
250mg/kg	3.03±0.03	3.63±0.44	3.67±0.09	4.90±0.46
500mg/kg	3.97±0.62	3.43±0.22	4.03±0.38	4.03±0.38
1000mg/kg	3.37±0.19	3.43±0.23	3.53±0.15	3.63±0.26

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at $p \leq 0.05$ when compared with the control.

Table 2: Effect of *Camellia sinensis* on Sodium (Na) (mmol/l) result in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	152.33±7.97	152.33±7.97	152.33±7.97	152.33±7.97
250mg/kg	119.33±1.33	129.00±5.86	143.33±2.03	159.33±7.22
500mg/kg	138.33±11.86	129.33±5.78	141.00±5.69	141.00±7.23
1000mg/kg	127.00±6.11	129.67±6.74	133.33±5.24	134.33±6.69

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at $p \leq 0.05$ when compared with the control.

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Table 3: Effect of *Camellia sinensis* on Chloride (Cl-) (mmol/l) result in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	60.00±1.53	60.00±1.53	60.00±1.53	60.00±1.53
250mg/kg	62.00±2.00	54.67±1.45	61.33±2.60	55.33±3.28
500mg/kg	65.67±1.86	52.00±2.08	58.67±2.03	56.67±2.33
1000mg/kg	54.67±0.88	56.33±1.45	52.67±1.86	53.00±3.61

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Table 4: Effect of *Camellia sinensis* on Urea result in Wistar rats (mmol/l).

Group	Day 7	Day 14	Day 21	Day 28
Control	4.83±0.26	4.83±0.26	4.83±0.26	4.83±0.26
250mg/kg	4.27±0.48	4.37±0.49	4.73±0.37	7.27±0.19
500mg/kg	3.43±0.35	4.33±0.35	5.27±0.50	5.23±0.55
1000mg/kg	4.97±0.97	5.73±0.67	4.67±0.41	5.60±1.10

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Table 5: Effect of *Camellia sinensis* on Creatinine (µmol/L) result in Wistar rats .

Group	Day 7	Day 14	Day 21	Day 28
Control	98.00±4.93	98.00±4.93	98.00±4.93	98.00±4.93
250mg/kg	86.00±8.02	86.67±10.14	95.67±7.88	149.00±3.79
500mg/kg	70.67±5.81	90.00±6.03	106.33±10.27	119.33±11.98
1000mg/kg	99.00±18.15	116.00±14.00	95.67±7.31	115.67±23.21

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Table 6: Effect of *Camellia sinensis* on Bicarbonate ion (HCO3-) (mmol/l) result in Wistar rats .

Group	Day 7	Day 14	Day 21	Day 28
Control	23.67±1.45	23.67±1.45	23.67±1.45	23.67±1.45
250mg/kg	25.67±1.45	27.00±1.15	26.00±1.15	26.00±1.15
500mg/kg	24.00±1.73	27.00±0.58	29.33±0.67*	29.00±0.58*
1000mg/kg	26.00±1.15	27.33±1.76*	28.00±1.15*	27.33±1.76*

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Effect of *Camellia sinensis* on lipid profile parameter

Table 7 presents the effect of *Camellia sinensis* on total cholesterol levels in Wistar rats. In the group receiving a dose of 250mg/kg, there is a marginal rise in total cholesterol levels on days 14, 21, and 28 when compared to the control group. Nevertheless, it is important to note that this increase lacks statistical significance. Conversely, in the group administered with 500mg/kg, total cholesterol levels exhibit an elevation on days 14, 21, and 28 in comparison to the control group. The increase observed on day 21 is statistically significant. Meanwhile, in the 1000mg/kg group, there is a notable and statistically significant increase in total cholesterol levels on days 14, 21, and 28 when contrasted with the control group.

Table 8 presents the effect of *Camellia sinensis* on triglyceride levels in Wistar rats. Within the 250mg/kg group, there is a slight reduction in triglyceride levels on day 7 in comparison to the control group. However, on day 14, an increase is observed, and on days 21 and 28, there are subsequent decreases, but none of these fluctuations hold statistical significance. Moving to the 500mg/kg group, there is an upswing in triglyceride levels on day 21 in contrast to the control group. Nevertheless, it is crucial to emphasize that this increase does not attain statistical significance. Conversely, in the 1000mg/kg group, there is a drop in triglyceride levels on day 7 when compared to the control group. On days 14 and 21, an increase is witnessed, and on day 28, there is a subsequent decline. Importantly, none of these changes reach statistical significance.

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Shifting our focus to the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels as presented in Tables 9 and 10, within the 250mg/kg group, a slight increase is noted on day 7 in comparison to the control group. On day 14, a statistically significant rise is observed, and this upward trend continues on days 21 and 28 when contrasted with the control group. Meanwhile, in the 500mg/kg group, there is an increase in HDL levels on days 7, 14, and 21 when compared to the control group. However, it is essential to note that these increases do not achieve statistical significance. In contrast, in the 1000mg/kg group, there is no statistically significant alteration in HDL levels compared to the control group, while for LDL, within the 250mg/kg group, a minor increase in low-density lipoprotein (LDL) levels is seen on day 14 compared to the control group. On day 21, there is a decrease, followed by another increase on day 28 in contrast to the control group. None of these variations, however, reach statistical significance. Transitioning to the 500mg/kg group, there is a decline in LDL levels on day 7 when compared to the control group. On day 14, there is an increase, which continues days 21 and 28 in comparison to the control group. Similar to the 250mg/kg group, none of these changes attain statistical significance. In the 1000mg/kg group, there is no significant alteration in LDL levels compared to the control group. In summary, it is noteworthy that higher doses of *Camellia sinensis* (green tea) supplementation tend to result in increased total cholesterol and HDL levels in Wistar rats. However, there is no consistent or statistically significant impact on triglyceride levels or LDL levels.

Table 7: Effect of *Camellia sinensis* on Total Cholesterol (mmol/l) result in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	2.80±0.06	2.80±0.06	2.80±0.06	2.80±0.06
250mg/kg	3.03±0.20	3.37±0.32	3.23±0.09	3.27±0.19
500mg/kg	2.70±0.17	3.10±0.36	3.33±0.41	3.63±0.03*
1000mg/kg	2.97±0.12	3.77±0.43*	3.73±0.2*3	3.93±0.12*

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Table 8: Effect of *Camellia sinensis* on Triglycerides (mmol/l) result in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	0.91±0.13	0.91±0.13	0.91±0.13	0.91±0.13
250mg/kg	0.78±0.06	1.23±0.17	1.28±0.13	1.07±0.06
500mg/kg	1.19±0.04	1.16±0.16	1.63±0.12	1.22±0.08
1000mg/kg	0.79±0.15	1.05±0.11	1.35±0.17	0.98±0.04

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Table 9: Effect of *Camellia sinensis* on High Density Lipoprotein (HDL) (mmol/l) result in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	1.04±0.09	1.04±0.09	1.04±0.09	1.04±0.09
250mg/kg	1.17±0.04	1.44±0.15	1.93±0.29	1.60±0.28
500mg/kg	1.54±0.14	1.41±0.22	1.70±0.25	1.69±0.19
1000mg/kg	1.06±0.04	1.87±0.05	1.75±0.19	1.33±0.08

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

Table 10: Effect of *Camellia sinensis* on Low Density Lipoprotein (LDL)(mg/dl) result in Wistar rats.

Group	Day 7	Day 14	Day 21	Day 28
Control	2.16±0.07	2.16±0.07	2.16±0.07	2.16±0.07
250mg/kg	2.21±0.26	2.48±0.21	1.92±0.26	2.15±0.8
500mg/kg	1.70±0.30	2.22±0.42	2.37±0.54	2.48±0.19
1000mg/kg	2.26±0.18	2.37±0.43	1.99±0.28	2.05±0.17

Values are expressed as mean ± Standard Error of the Mean (n=3); *significant at p ≤ 0.05 when compared with the control.

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Effect of *Camellia sinensis* on histo-morphology of the Heart

The histological analysis of the heart tissue samples indicates that the administration of green tea extract at the specified doses (250mg/kg, 500mg/kg, and 1000mg/kg) does not result in observable histological changes, damage, or abnormalities in the heart tissue of the experimental animals over the course of the study. These findings suggest that the green tea extract does not appear to have adverse effects on the heart histology in the tested Wistar rats.

Effect of *Camellia sinensis* on histo-morphology of the Kidney

The histological analysis of the kidney tissue samples indicates that the administration of green tea extract, especially at higher doses (500mg/kg and 1000mg/kg), leads to mild to moderate acute tubular necrosis in the tested Wistar rats, as observed on days 14 and 21. However, by day 28, the kidney tissue appears to recover, showing no significant histological abnormalities.

Effect of *Camellia sinensis* on histo-morphology of the Lungs

The histological analysis of the lung tissue samples indicates that the administration of green tea extract, especially at higher doses (500mg/kg and 1000mg/kg), leads to lung abnormalities characterized by interstitial oedema, lymphocytic infiltrate, and alveolar changes. These effects are more pronounced on days 14 and 21 but tend to lessen by day 28, suggesting some degree of recovery in the lung tissue.

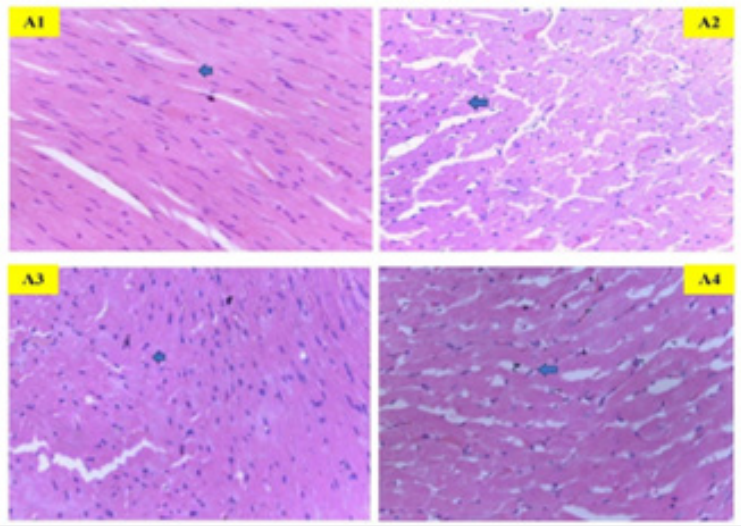

Figure 1: Photomicrograph of the Heart on day 7. (A1) Histological effect of Green tea on the heart on day 7 control. Section of cardiac muscle shows normal myofibres (blue). (A2) Histological effect of Green tea on the heart on day 7 treated with 250mg/kg. Section of cardiac muscle shows normal myofibres (blue). (A3) Histological effect of green tea on the heart on day 7 treated with 500mg/kg. Section of cardiac muscle shows normal myofibres (blue). (A4) Histological effect of green tea on the heart on day 7 treated with 1000mg/kg. Section of cardiac muscle shows normal myofibres (blue). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

Discussion

Green tea, derived from the leaves of *Camellia sinensis*, has been a subject of extensive research owing to its potential health benefits [7,32,33]. Rich in antioxidants, particularly polyphenols like catechins, green tea has been associated with various health advantages, including its cardioprotective and antioxidant properties [34-36]. Antioxidants help protect the body from oxidative stress and reduce the risk of chronic diseases such as cardiovascular disease [34-36]. However, as with any compound, the effects of green tea extract can vary depending on the dose and duration of exposure [33,34]. Hence this study evaluated the toxicological effects of *Camellia sinensis* (Green Tea) on cardiopulmonary, renal function and histo-morphological changes in Wistar Rats.

The study found that the administration of *Camellia sinensis* at varying doses resulted in changes in potassium, sodium, chloride, urea, creatinine, and bicarbonate ion levels in Wistar rats. These changes were observed to be dose-dependent, indicating that the dosage of green tea extract plays a crucial role in its effects on blood biochemistry. Ben Saad et al. [37] reported a significant increase in serum electrolyte parameters such as urea, creatinine and uric acid in animals treated with nicotine, which is indicative of kidney damage and a possible malfunction or failure of the kidneys and was ameliorated by treatment with *Camellia sinensis* extract. The result of this present study reported no significant effect of green tea on serum electrolyte parameters which is indicative of a normal kidney function which corroborates the nephroprotective capacity reported by Ben Saad et al. [37].

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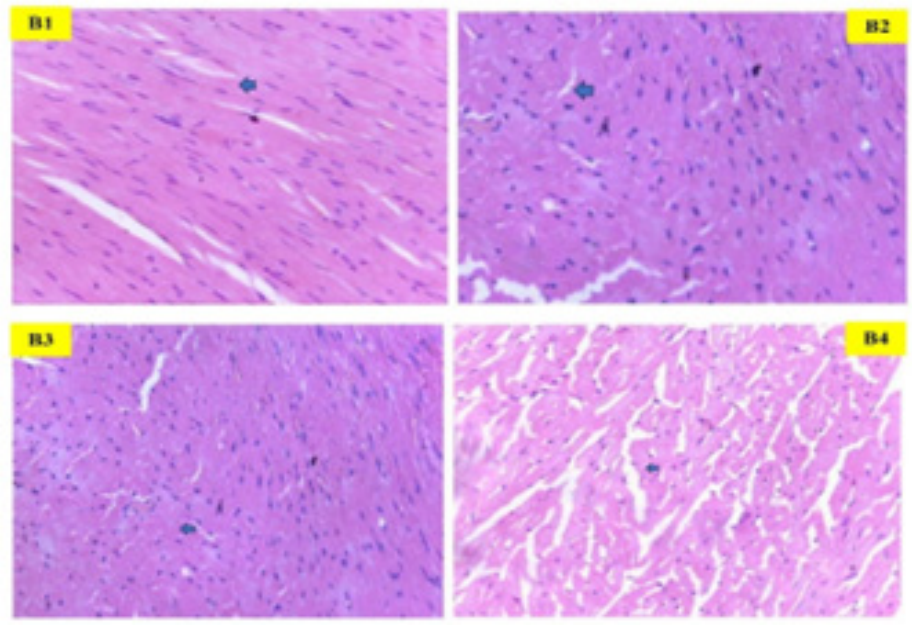

Figure 2: Photomicrograph of the Heart on day 14. (B2) Histological effect of Green tea on the heart on day 14 control. (B2) Histological effect of green tea on the heart on day 14 treated with 250mg/kg. Section of cardiac muscle shows normal myofibres (blue). (B3) Histological effect of green tea on the heart on day 14 treated with 500mg/kg. Section of cardiac muscle shows normal myofibres (blue). (B4) Histological effect of green tea on the heart on day 14 treated with 1000mg/kg. Section of cardiac muscle shows normal myofibres (blue). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

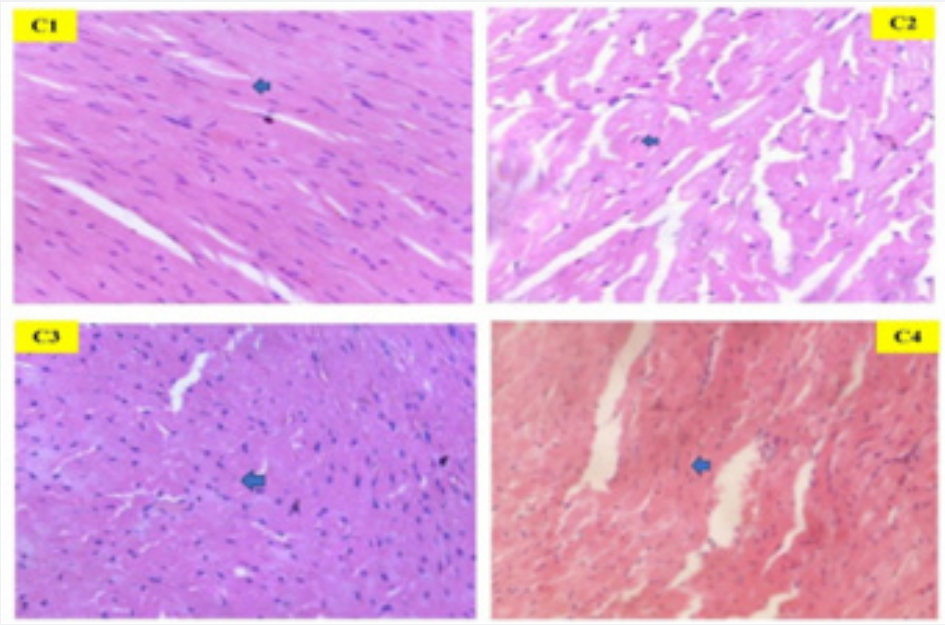

Figure 3: Photomicrograph of the Heart on day 21. (C1) Histological effect of Green tea on the heart on day 21 control. (C2) Histological effect of green tea on the heart on day 21 treated with 250mg/kg. Section of cardiac muscle shows normal myofibres (blue). (C3) Histological effect of green tea on the heart on day21 treated with 500mg/kg. Section of cardiac muscle shows normal myofibres (blue). (C4) Histological effect of green tea on the heart on day 21 treated with 1000mg/kg. Section of cardiac muscle shows normal myofibres (blue). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

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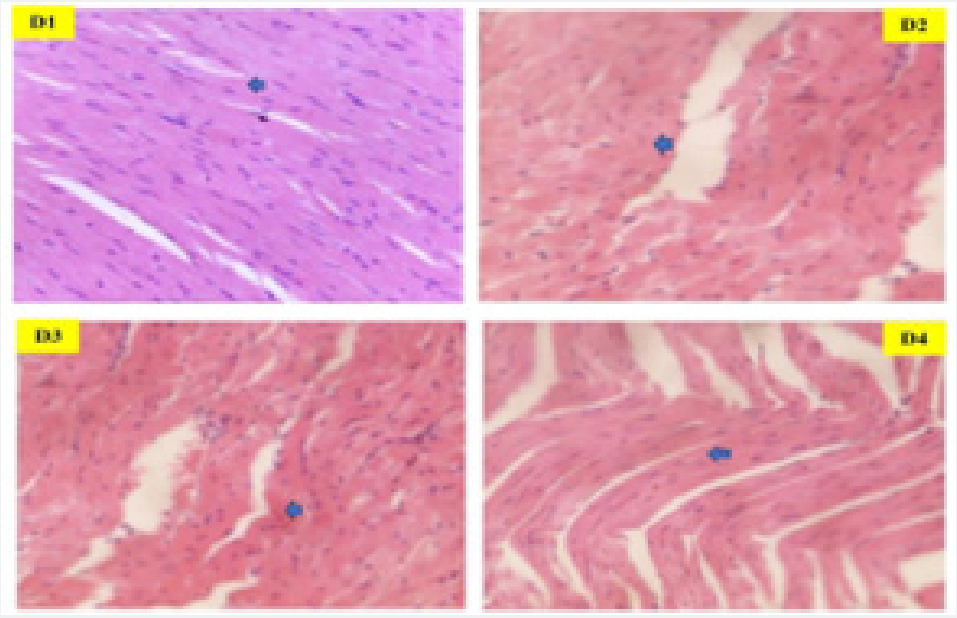

Figure 4: Photomicrograph of the Heart on day 28. (D1) Histological effect of Green tea on the heart on day 28 control. (D2) Histological effect of green tea on the heart on day 28 treated with 250mg/kg. Section of cardiac muscle shows normal myofibres (blue). (D3) Histological effect of green tea on the heart on day 28 treated with 500mg/kg. Section of cardiac muscle shows normal myofibres (blue). (D4) Histological effect of green tea on the heart on day 28 treated with 1000mg/kg. Section of cardiac muscle shows normal myofibres (blue). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

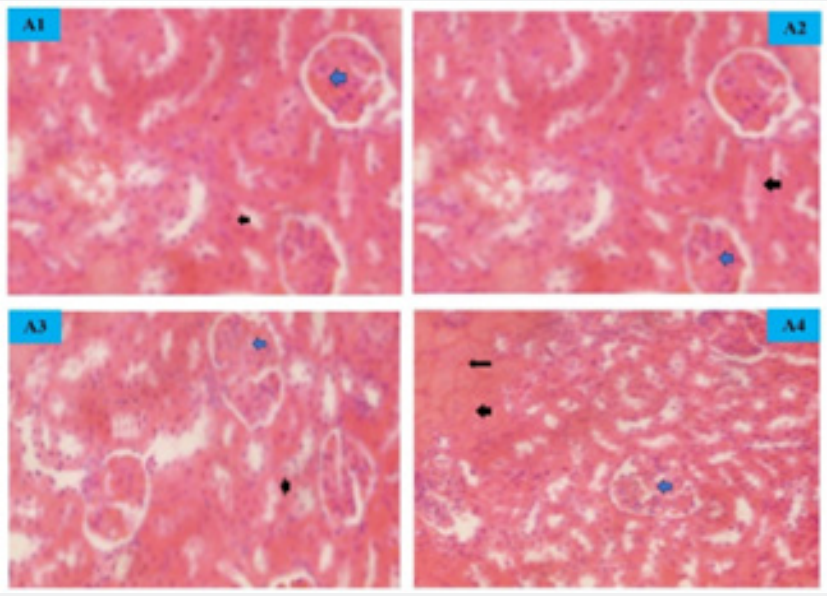

Figure 5: Photomicrograph of the Kidney on day 7. (A1) Histological effect of green tea on the kidney on day 7 control. Section of kidney showing normal glomeruli (blue) and normal tubules (black). (A2) Histological effect of green tea on the kidney on day 7 treated with 250mg/kg. Section of kidney showing normal glomeruli (blue) and normal tubules (black). (A3) Histological effect of green tea on the kidney on day 7 treated with 500mg/kg. Section of kidney showing normal glomeruli (blue) and normal tubules (black). (A4) Histological effect of green tea on the kidney on day 7 treated with 1000mg/kg. Section of kidney showing normal glomeruli (blue) with some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of mild acute tubular necrosis (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

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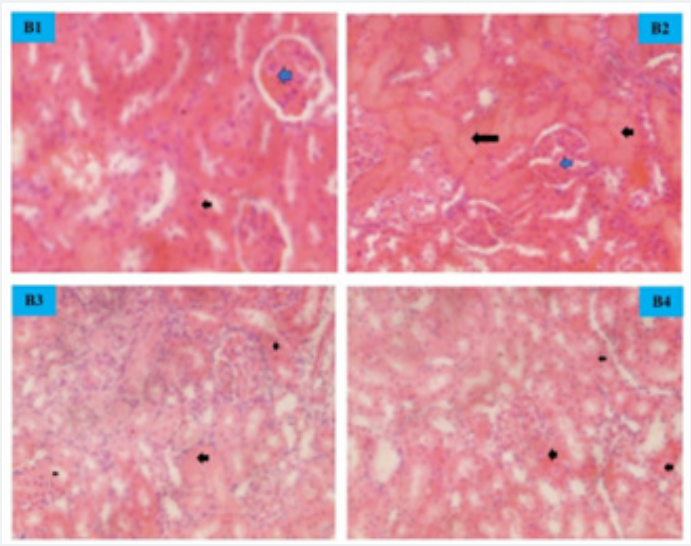

Figure 6: Photomicrograph of the Kidney on day 14. (B1) Histological effect of green tea on the kidney on day 14 control. Section of kidney showing normal glomeruli (blue) and normal tubules (black). (B2) Histological effect of green tea on the kidney on day 14 treated with 250mg/kg. Section of kidney showing normal glomeruli (blue) with some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of mild acute tubular necrosis (black). (B3) Histological effect of green tea on the kidney on day 14 treated with 500mg/kg. Section of kidney showing some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of moderate acute tubular necrosis (black). (B4) Histological effect of green tea on the kidney on day 14 treated with 1000mg/kg. Section of kidney showing some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of moderate acute tubular necrosis (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

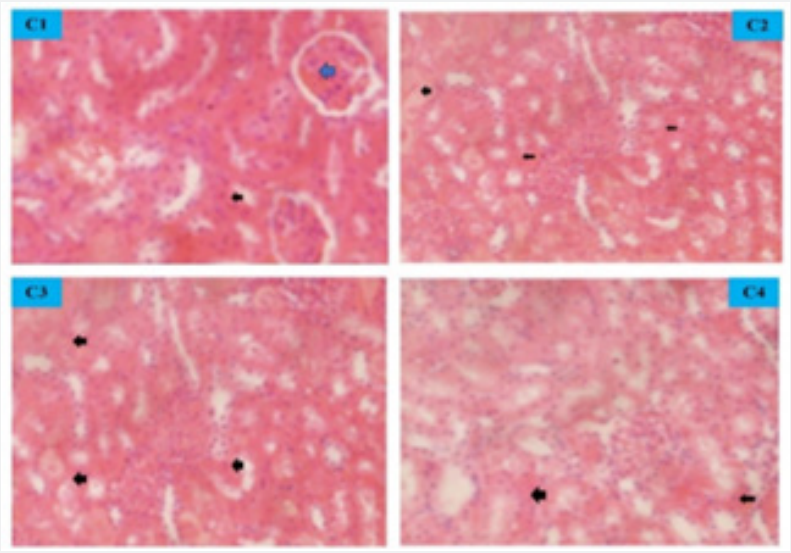

Figure 7: Photomicrograph of the Kidney on day 21. (C1) Histological effect of green tea on the kidney on day 21 control. Section of kidney showing normal glomeruli (blue) and normal tubules (black). (C2) Histological effect of green tea on the kidney on day 21 treated with 250mg/kg. Section of kidney showing some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of moderate acute tubular necrosis (black). (C3) Histological effect of green tea on the kidney on day 21 treated with 500mg/kg. Section of kidney showing some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of moderate acute tubular necrosis (black). (C4) Histological effect of green tea on the kidney on day 21 treated with 1000mg/kg. Section of kidney showing some of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of moderate acute tubular necrosis (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

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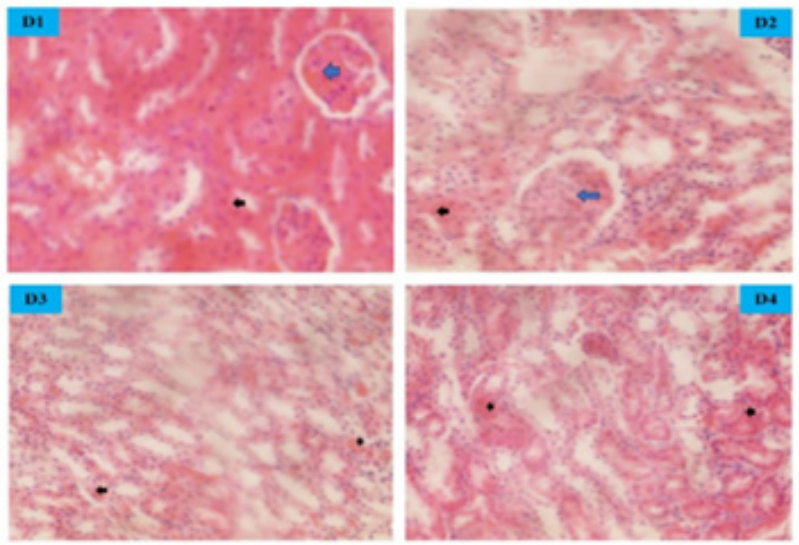

Figure 8: Photomicrograph of the Kidney on day 28. (D1) Histological effect of green tea on the kidney on day 28 control. Section of kidney showing normal glomeruli (blue) and normal tubules (black). (D2) Histological effect of green tea on the kidney on day 28 treated with 250mg/kg. Section of kidney showing few of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of mild acute tubular necrosis (black). The glomerulus seen appears normal (blue). (D3) Histological effect of green tea on the kidney on day 28 treated with 500mg/kg. Section of kidney showing with few of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of mild acute tubular necrosis (black). (D4) Histological effect of green tea on the kidney on day 28 treated with 1000mg/kg. Section of kidney showing with few of the tubules lined by cells that are anucleated with increased cytoplasmic eosinophilia suggestive of mild acute tubular necrosis (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

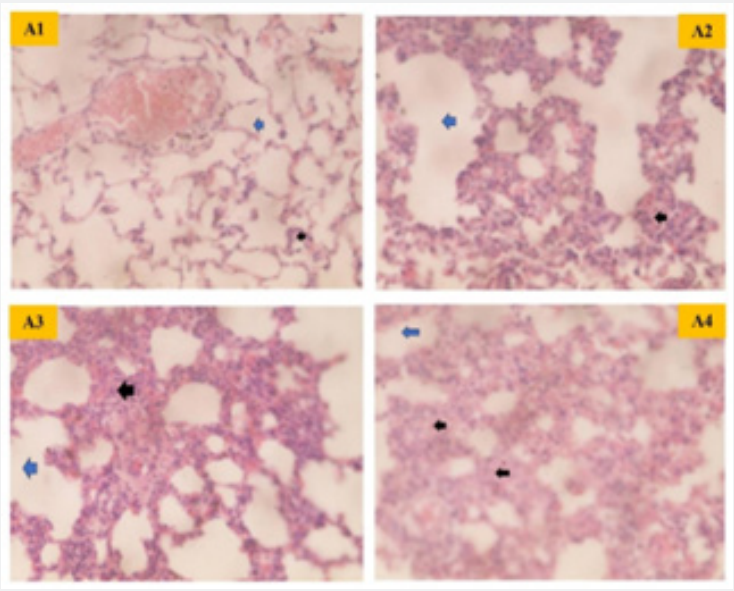

Figure 9: Photomicrograph of the Lung on day 7. (A1) Histological effect of green tea on the lungs on day 7 of control. Section of the lungs shows normal alveoli spaces (blue) and interstitium (black). (A2) Histological effect of green tea on the lungs on day 7 treated with 250mg/kg. Section of the lungs shows normal alveoli spaces (blue) and oedematous and widened interstitium (black). (A3) Histological effect of green tea on the lungs on day 7 treated with 500mg/kg. Section of the lungs shows normal alveoli spaces (blue) and oedematous and widened interstitium with mild lymphocytic infiltrate (black). (A4) Histological effect of green tea on the lungs on day 7 treated with 1000mg/kg. Section of the lungs shows normal alveoli spaces (blue) and oedematous and widened interstitium with mild lymphocytic infiltrate (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

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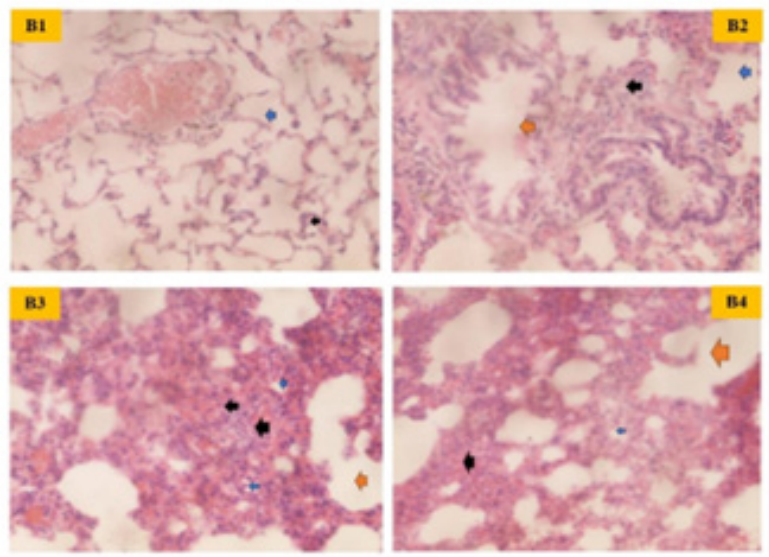

Figure 10: Photomicrograph of the Lung on day 14. (B1) Histological effect of green tea on the lungs on day 14 of control. Section of the lungs shows normal alveoli spaces (blue) and interstitium (black). (B2) Histological effect of green tea on the lungs on day 14 treated with 250mg/kg. Section of the lungs shows normal alveoli spaces (blue), normal bronchioles (orange) and oedematous and widened interstitium with mild lymphocytic infiltrate (black). (B3) Histological effect of green tea on the lungs on day 14 treated with 500mg/kg. Section of the lungs shows collapse of the alveoli spaces in areas (blue) with compensatory dilation of the alveoli (orange). The interstitium is oedematous and widened with moderate lymphocytic infiltrate (black). (B4) Histological effect of green tea on the lungs on day 14 treated with 1000mg/kg. Section of the lungs shows collapse of the alveoli spaces in areas (blue) with compensatory dilation of the alveoli (orange). The interstitium is widened and oedematous with moderate lymphocytic infiltrate (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

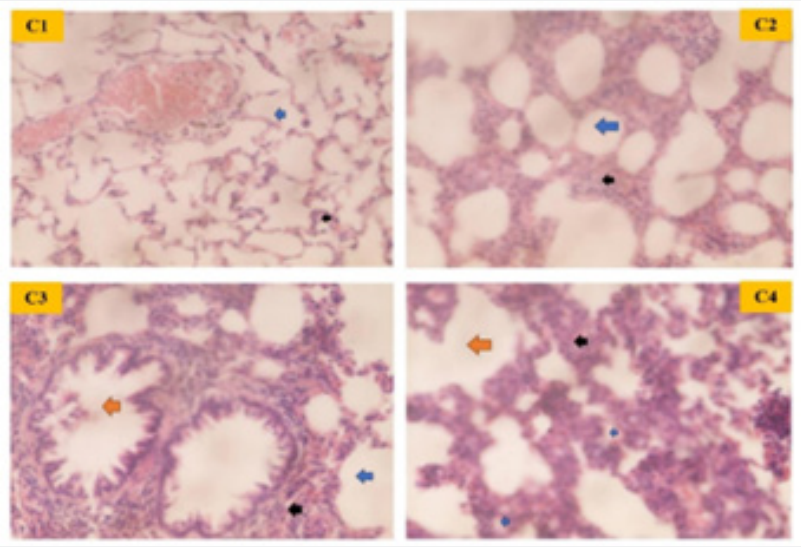

Figure 11: Photomicrograph of the Lung on day 21. (C1) Histological effect of green tea on the lungs on day 21 of control. Section of the lungs shows normal alveoli spaces (blue) and interstitium (black). (C2) Histological effect of green tea on the lungs on day 12 treated with 250mg/kg. Section of the lungs shows normal alveoli spaces (blue)). The interstitium is widened and oedematous with moderate lymphocytic infiltrate (black). (C3) Histological effect of green tea on the lungs on day 12 treated with 500mg/kg. Section of the lungs shows normal alveoli spaces (blue), normal bronchioles (orange) and oedematous and widened interstitium with mild lymphocytic infiltrate (black). (C4) Histological effect of green tea on the lungs on day 12 treated with 1000mg/kg. Section of the lungs shows collapse of the alveoli spaces in areas (blue) with compensatory dilation of the alveoli (orange). The interstitium is widened and oedematous with moderate lymphocytic infiltrate (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

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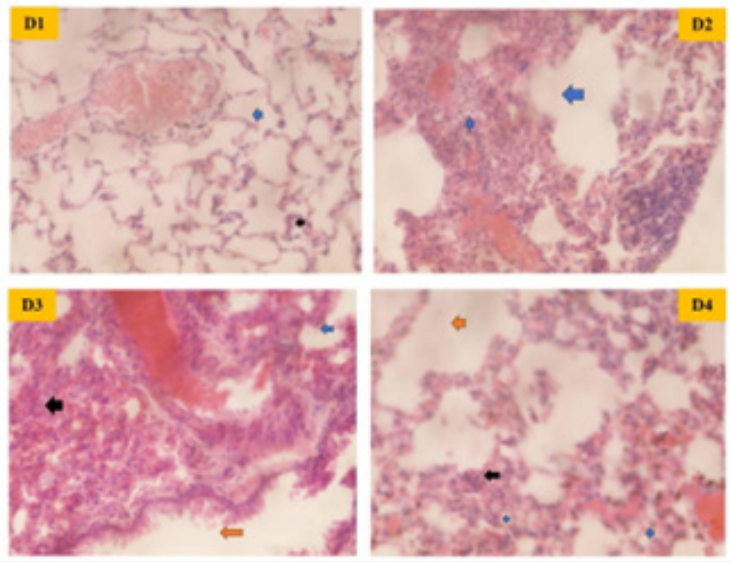

Figure 12: Photomicrograph of the Lung on day 28. (D1) Histological effect of green tea on the lungs on day 28 of control. Section of the lungs shows normal alveoli spaces (blue) and interstitium (black). (D2) Histological effect of green tea on the lungs on day 28 treated with 250mg/kg. Section of the lungs shows normal alveoli spaces(blue). The interstitium is widened and oedematous with moderate lymphocytic infiltrate (black). (D3) Histological effect of green tea on the lungs on day 28 treated with 500mg/kg. Section of the lungs shows normal alveoli spaces (blue) and normal bronchioles (orange). The interstitium is widened and oedematous with moderate lymphocytic infiltrate and haemorrhage (black). (D4) Histological effect of green tea on the lungs on day 28 treated with 1000mg/kg. Section of the lungs shows collapse of the alveoli spaces in areas (blue) with compensatory dilation of the alveoli (orange). The interstitium is widened and oedematous with mild lymphocytic infiltrate (black). Stain/Magnification: Hematoxylin and Eosin stain at x400 magnification.

Discussion

The findings revealed that the 250mg/kg group showed marginal increases in total cholesterol levels on days 14, 21, and 28, although these increases were not statistically significant. In contrast, the 500mg/kg group exhibited a significant elevation in total cholesterol levels on day 21, while the 1000mg/kg group showed statistically significant increases on days 14, 21, and 28 compared to the control group. This suggests that higher doses of green tea extract may lead to elevated total cholesterol levels. Regarding triglyceride levels, the study showed inconsistent and non-significant changes across different doses of green tea extract. The 250mg/kg group exhibited minor fluctuations in triglyceride levels, while the 500mg/kg and 1000mg/kg groups displayed no significant alterations when compared to the control group. These findings indicate that green tea extract supplementation has limited impact on triglyceride levels in Wistar rats. In terms of HDL levels, the 250mg/kg group demonstrated a statistically significant increase on day 14, which continued days 21 and 28 compared to the control group. However, the 500mg/kg group exhibited increases in HDL levels that did not reach statistical significance. The highest dose (1000mg/kg) did not significantly affect HDL levels.

Short-term exposure to green tea extract at a dosage of 250mg/kg displayed some favourable outcomes, such as increasing HDL levels. This suggests a potential positive impact on cardiovascular health. However, the marginal rise in total cholesterol levels may raise concerns when considering prolonged exposure to this dosage. It is essential to note that the increase lacks statistical significance, which suggests that it may not be a cause for significant concern. However, prolonged exposure to higher doses (500mg/kg and 1000mg/kg) of green tea extract resulted in notable and statistically significant increases in total cholesterol levels. This raises caution about prolonged consumption of higher doses of green tea, as elevated total cholesterol levels are associated with an increased risk of cardiovascular disease. Hence, is crucial to carefully consider the appropriate dosage and duration of green tea extract supplementation to mitigate potential adverse effects.

It has been reported that catechins such as epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG) are abundant in *Camellia sinensis* extract and contribute to the regulation of cholesterol metabolism [38]. According to a report by Anaeigoudari et al. [39], dietary supplements of *Camellia sinensis* extract (5%) significantly reduced water intake and food consumption and lowered the serum total LDL-c and TG levels. The tea extracts also significantly reduced lipid synthesis and blood glucose level, but increased glucose tolerance in mice. This corroborates the finding in the present study where *Camellia sinensis* did not result in a significant increase in lipid profile parameters. Also, Luo et al. [38] reported that *Camellia sinensis* extract stimulated LDL-

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cholesterol (LDL-c) uptake by activating the LDL receptor (LDLR). Furthermore, it significantly downregulated TG synthesizing enzyme genes and reduced intracellular TG accumulation. Hence, it is possible that there was no significant increase in lipid profile parameters in the present study due to this effect.

The histological analysis of heart tissue samples revealed no observable changes, damage, or abnormalities when green tea extract was administered at different doses (250mg/kg, 500mg/kg, and 1000mg/kg). This suggests that green tea extract may not have any adverse effects on heart histology in Wistar rats. Antioxidants present in green tea, such as catechins and flavonoids, have been previously reported to protect against oxidative stress and inflammation, which can contribute to heart disease [8,40]. These antioxidants may have played a role in maintaining the normal histology of heart tissue.

In contrast to the heart tissue, the histological analysis of kidney tissue samples indicated acute tubular necrosis in Wistar rats, particularly at higher doses of green tea extract (500mg/kg and 1000mg/kg). However, by day 28, the kidney tissue appeared to recover with no significant histological abnormalities. While short-term exposure to green tea extract may induce mild to moderate kidney damage, prolonged exposure seems to allow for tissue recovery. It is worth noting that the antioxidants in green tea may play a role in reducing oxidative stress-induced kidney damage.

The administration of high doses of green tea extract (500mg/kg and 1000mg/kg) resulted in lung abnormalities characterized by interstitial edema, lymphocytic infiltrate, and alveolar changes. These effects were more pronounced on days 14 and 21 but tended to lessen by day 28, suggesting some degree of recovery in the lung tissue. The damaging effects observed on lung histology could be attributed to the higher concentration of green tea extract, indicating a potential dose-dependent response. Further investigation is required to understand the exact mechanisms behind these lung abnormalities.

Short-term exposure to green tea extract may lead to mild kidney damage and lung abnormalities, as observed in this study. However, it is important to note that prolonged exposure appeared to promote recovery in both kidney and lung tissues. Green tea contains antioxidants such as catechins and flavonoids, which have been reported to protect against oxidative stress and inflammation [41,42]. These antioxidants may have played a role in reducing the oxidative stress-induced kidney damage and promoting tissue recovery. This suggests that the beneficial effects of green tea on the kidney may be mediated through its antioxidant properties [40]. While green tea extract did not induce adverse effects on heart histology, it caused mild to moderate kidney damage and lung abnormalities at higher doses. However, tissue recovery was observed with prolonged exposure to the extract. These findings emphasize the need for caution when consuming green tea extract at high doses and underscore the importance of further research to fully elucidate the underlying mechanisms involved in these observed effects.

Conclusion

The investigation unveiled several crucial insights into the effects of green tea extract at various doses on the physiology of Wistar rats. Notably, it was observed that the administration of *Camellia sinensis* at different doses led to notable changes in blood electrolytes and lipid profile parameters. These changes were demonstrated to be dose-dependent, underlining the significance of the dosage of green tea extract in influencing these parameters. Also, short-term exposure to green tea extract, even at higher doses, demonstrated mild to moderate kidney damage and lung abnormalities in Wistar rats. Nonetheless, it is encouraging that prolonged exposure appeared to facilitate tissue recovery in both the kidney and lung tissues. This suggests that the antioxidant-rich composition of green tea, including catechins and flavonoids, likely contributed to mitigating oxidative stress-induced damage and promoting tissue repair. While green tea extract did not adversely affect heart histology, the study underscores the need for caution when consuming higher doses and emphasizes the importance of additional research to fully comprehend the underlying mechanisms involved in these observed effects.

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