Original Research Article

Evaluation of the Hepato-toxic Potential of Green Tea (*Camellia sinensis*) in Wistar Rats

Comment [H1]: Better to modify the title, as marked in the abstract

ABSTRACT

Background: Green tea is rich in flavonoids and catechins, compounds reported to have antioxidant, anti-inflammatory, and anticancer properties. However, there is a need to evaluate the effects of green tea on vital organs like the liver. This study aimed to investigate the effects of *Camellia sinensis* on markers of liver function, and liver histology 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. AST, ALT, ALP, TP, ALB, TB, and CB levels were measured at various time points. Effect on liver histology was also assessed using haematoxylin and eosin staining. **Results:** *Camellia sinensis* had varying effects on biomarkers of liver injury in a dose-dependent manner. Higher doses (500mg/kg and 1000mg/kg) of *Camellia sinensis* led to significant increases in AST, ALT, and ALP levels when consumed for up to 21 days. TP and ALB levels were not significantly altered. TB levels significantly increased in the highest dose group while CB levels did not significantly change at any time point studied. Also, higher doses of *Camellia sinensis* were associated with the development of fatty liver and mild inflammation from the histology. **Conclusion:** *Camellia sinensis* extract at low dose may not be harmful to the liver, however, the potential toxicity of green tea on the liver (at higher doses) should be further studied. Moderate use of green tea is therefore, recommended to ensure its safe and beneficial effects on liver health.

Comment [H2]: This could be better title of the study

Keywords: Green tea, *Camellia sinensis*, Liver function markers, Toxicity, Histology

INTRODUCTION

Comment [H3]: As there are contradictory results regarding the plant, the introduction should be more comprehensive.

Green tea, derived from the leaves of the plant *Camellia sinensis*, has gained significant attention in recent years. Green tea is known to be rich in polyphenolic compounds, such as flavonoids and catechins, which have been associated with antioxidant, anti-inflammatory, and anticancer properties [1-5]. Consequently, green tea has gained significant attention as a potential therapeutic agent for numerous health conditions [6, 7].

In recent years, there has been a growing interest in the toxicological evaluation of natural products, including herbal teas, to assess their safety profiles and potential adverse effects [5, 8,

9]. While green tea is generally considered safe for consumption, it is crucial to evaluate its effects comprehensively, especially on vital organs such as the liver, which plays a central role in drug metabolism and detoxification [10].

The liver, as the primary site for the metabolism of xenobiotics, is vulnerable to the effects of various substances, including herbal preparations [11,12]. Markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB), serve as indicators of liver function and can provide valuable insights into hepatocellular damage [10]. Additionally, histological examination of liver tissues allows for the assessment of morphological changes and the identification of pathological alterations caused by potential toxic substances [13].

Several studies have reported the hepatoprotective effects of green tea, highlighting its ability to mitigate liver damage induced by various agents [8,9]. However, contradictory findings have also been reported [14,15], suggesting the need for further investigations to establish a comprehensive understanding of the potential hepatotoxic effects of green tea. Difference in doses used as well as other methodological differences may count for the variation in findings from these studies.

Toxicological investigations play a crucial role in assessing the safety of natural products. Although green tea has demonstrated numerous health benefits, including antioxidant and anticancer properties, it is essential to comprehensively evaluate its effects on the liver to ensure its safe consumption.

MATERIALS AND METHODS

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 and 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-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℃ 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 [16] method as described by Uahomo and Isirima [17]. 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

Comment [H4]: What is your reference of using this much animals per single group?

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.

Comment [H5]: How many animals did you use for the test?

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 anaesthesia for liver function marker assay.

Liver Function Marker Assay

Biochemical markers such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) were determined using Randox Kits. ALT was measured by the monitoring of the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazone [18]. ALP activity was measured at 37^{o}C as according to Haussament

[19]. The absorbance of p-nitrophenol formed from p-nitrophenyl phosphate was determined at 405nm and calculation of enzyme unit followed as described by the kit manufacturer. Total protein, total bilirubin, and conjugated bilirubin were also determined using Randox kits. The Randox Total Protein Assay kit utilized the biuret method while the Total Bilirubin and Conjugated Bilirubin Assay kits employed the diazo method in the determination of their levels in blood serum. These kits provided accurate and reliable measurements of the respective parameters, aiding in the assessment of liver function.

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* (Green tea) on Liver Function Markers

The results from tables 1 to 7 reveal the effects of different doses of *Camellia sinensis* on various liver function parameters in Wistar rats. These findings have implications for understanding the potential risks to the liver that are associated with *Camellia sinensis* consumption.

The 250mg/kg dose of *Camellia sinensis* showed no significant effects on AST and ALT levels on days 7, 14, 21, and 28, respectively indicating that this dose may not have an adverse effect on liver function. Similarly, the 500mg/kg dose of *Camellia sinensis* did not show significant

effects on AST and ALT levels throughout the study period, suggesting that this dose may not have a substantial toxicity on liver enzymes in these experimental conditions.

On the other hand, the 1000mg/kg dose of *Camellia sinensis* resulted in significant increases in AST and ALT levels on day 21 and day 28. This indicates that the highest dose might have adverse effects on liver function when administered over an extended period.

Table 1: Effect of *Camellia sinensis* on AST (nmol/l) in Wistar Rats

Group	Day 7	Day 14	Day 21	Day 28
Control	28.67±3.93	28.67±3.93	28.67±3.93	28.67±3.93
250mg/kg	28.10±3.06	28.45±0.67	30.67±2.96	30.07±3.28
500mg/kg	27.10±2.08	29.45±0.72	28.67±1.69	30.07±2.84
1000mg/kg	28.43±4.93	30.90±2.58	33.50±3.21*	35.67±0.88*

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 ALT (nmol/l) in Wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	17.63±4.21	17.63±4.21	17.63±4.21	17.63±4.21
250mg/kg	18.67±1.67	18.53±1.27	18.82±3.46	18.07±1.13
500mg/kg	18.47±2.15	19.07±1.79	19.17±1.48	19.73±1.28
1000mg/kg	18.23±2.96	22.77±0.62*	24.67±3.38*	25.83±0.93*

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

Table 3 shows that the 250mg dose did not affect the levels of ALP on days 7, 14, 21, and 28. Similarly, the 500mg dose had no significant effect on ALP levels, except on day 28 where an increase occurred. However, the 1000mg dose resulted in significant increase in blood ALP levels on days 7, 21, and 28, respectively.

Table 3: Effect of *Camellia sinensis* on Alkaline Phosphatase (ALP) (nmol/l) in Wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	27.67±1.86	27.67±1.86	27.67±1.86	27.67±1.86
250mg/kg	26.67±2.33	27.08±2.52	26.19±3.06	27.87±0.88
500mg/kg	26.10±4.93	30.67±0.88	31.67±3.84	35.33±6.39*
1000mg/kg	32.00±1.53*	30.00±2.52	37.67±3.48*	36.00±4.36*

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

Furthermore, tables 4 and 5 show that the oral administration of green tea had no impact on total protein and albumin levels, all through the period of the study.

Table 4: Effect of *Camellia sinensis* on Total Protein (TP) (g/l) in Wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	62.33±1.45	62.33±1.45	62.33±1.45	62.33±1.45
250mg/kg	60.33±1.86	62.23±1.45	61.33±2.73	62.67±3.93
500mg/kg	62.67±3.76	62.10±2.08	61.00±2.52	63.67±2.73
1000mg/kg	62.00±2.31	63.33±5.17	61.33±3.53	62.67±1.45

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

Table 5: Effect of *Camellia sinensis* on Albumin (ALB) (g/l) in Wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	39.33±0.88	39.33±0.88	39.33±0.88	39.33±0.88
250mg/kg	40.67±1.20	41.67±0.88	40.33±2.73	41.10±1.53
500mg/kg	41.09±1.15	40.33±0.88	42.50±1.15	41.33±1.45
1000mg/kg	38.67±1.20	40.06±2.52	42.33±1.76	44.23±4.78

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

The 250mg/kg and 500mg/kg doses of *Camellia sinensis* showed no significant effect on total bilirubin levels at all-time points. Conversely, the 1000mg/kg dose showed a significant increase in total bilirubin on day 21 and Day 28, suggesting potential adverse effects on bilirubin metabolism at the highest dose.

Table 6: Effect of *Camellia sinensis* on Total Bilirubin (TB) mg/dl) result in Wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	5.90±0.46	5.90±0.46	5.90±0.46	5.90±0.46
250mg/kg	4.53±0.17	4.50±0.12	4.30±0.21	5.67±0.49
500mg/kg	4.75±0.39	4.52±0.15	4.37±0.23	5.20±0.32
1000mg/kg	7.37±0.83	8.80±0.06	14.50±0.44*	13.17±0.09*

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

Table 7 shows that 250mg/Kg, 500mg/Kg, and 1000mg/Kg doses of green tea had no significant effect on the levels of conjugated bilirubin, up to 28 days of administration.

Overall, the results suggest that lower doses of *Camellia sinensis* may be safer for liver function. However, higher doses (500 mg/Kg and above) may have detrimental effects, especially, when consumed for 21 days and above.

Table 7: Effect of *Camellia sinensis* on Conjugated Bilirubin (CB) (mg/dl) in Wistar rats

Group	Day 7	Day 14	Day 21	Day 28
Control	3.20±0.40	3.20±0.40	3.20±0.40	3.20±0.40
250mg/kg	3.17±0.07	3.10±0.12	2.99±0.12	2.81±0.41
500mg/kg	2.67±0.35	2.80±0.17	3.13±0.19	2.87±0.20
1000mg/kg	3.13±0.54	3.50±0.15	2.43±0.38	2.37±0.09

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

Effect of *Camellia sinensis* (Green tea) on the Liver Histology

In this experimental study, photomicrographs of the liver were taken at different time points, up to a 28-day treatment period, to assess the effects of *Camellia sinensis* extract on liver histology. The photomicrographs showed the liver sections stained with hematoxylin and eosin, allowing for the visualization of various structures and cellular components.

The normal control group exhibited healthy liver tissue with normal hepatocytes and a normal portal triad throughout the duration of the study. In the groups treated with different doses of *Camellia sinensis*, the liver sections showed varying findings over time. At day 7, the groups treated with 250mg/kg, 500mg/kg, and 1000mg/kg of *Camellia sinensis* displayed normal hepatocytes and either a normal portal triad or a normal central vein. At day 14, the groups

treated with 250mg/kg and 500mg/kg still showed normal hepatocytes and a normal portal triad, while the group treated with 1000mg/kg continued to exhibit normal hepatocytes but lacked a normal portal triad.

At day 21, the group treated with 1000mg/kg showed signs of fatty liver, including the presence of macrovesicules and microvesicules in the cytoplasm of many hepatocytes. The other two groups (250mg/kg and 500mg/kg) still had normal hepatocytes. By day 28, the groups treated with 250mg/kg and 500mg/kg displayed microvesicules in the cytoplasm of some hepatocytes, suggestive of fatty liver. The group treated with 1000mg/kg also showed microvesicules in the cytoplasm of a few hepatocytes, along with mild periportal lymphocytic infiltrates.

These photomicrographs provide visual evidence of the histological changes occurring in the liver over the course of the 28-day treatment with *Camellia sinensis* extract. The findings suggest that higher doses of *Camellia sinensis* may contribute to the development of fatty liver and mild inflammation, while lower doses appear to have fewer effects on liver histology.

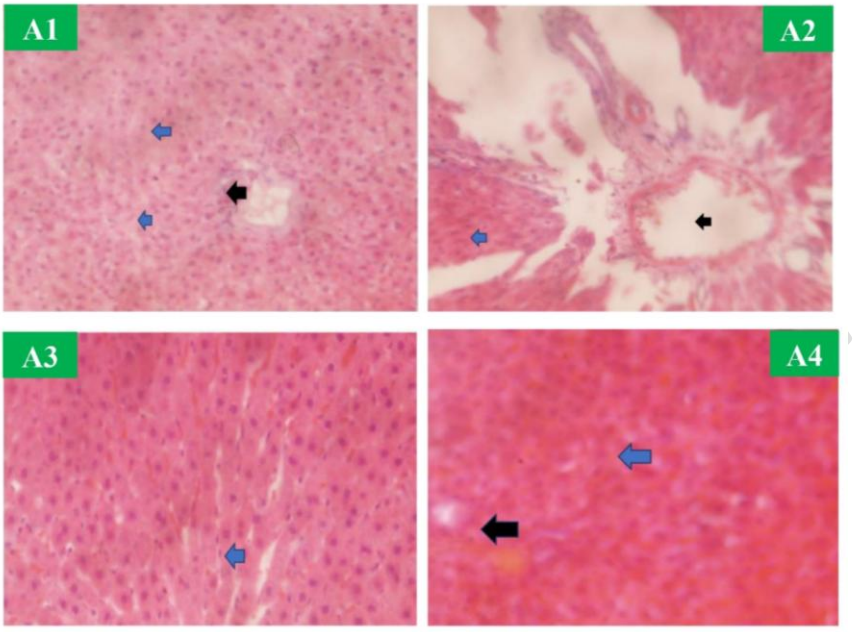

Figure 1: (A1-A4) Photomicrographs of Liver on day 7. (A1) Normal control showing normal hepatocytes (blue) and normal portal triad (black). (A2) Liver of animals administered 250mg/kg of *Camellia sinensis* showing normal hepatocytes (blue) and normal portal triad (black). (A3) Liver of animals administered 500mg/kg of *Camellia sinensis* showing normal hepatocytes (blue). (A4) Liver of animals administered 1000mg/kg of *Camellia sinensis* showing normal hepatocytes (blue) and normal central vein (black). Using hematoxylin and Eosin stain at X400 Magnification

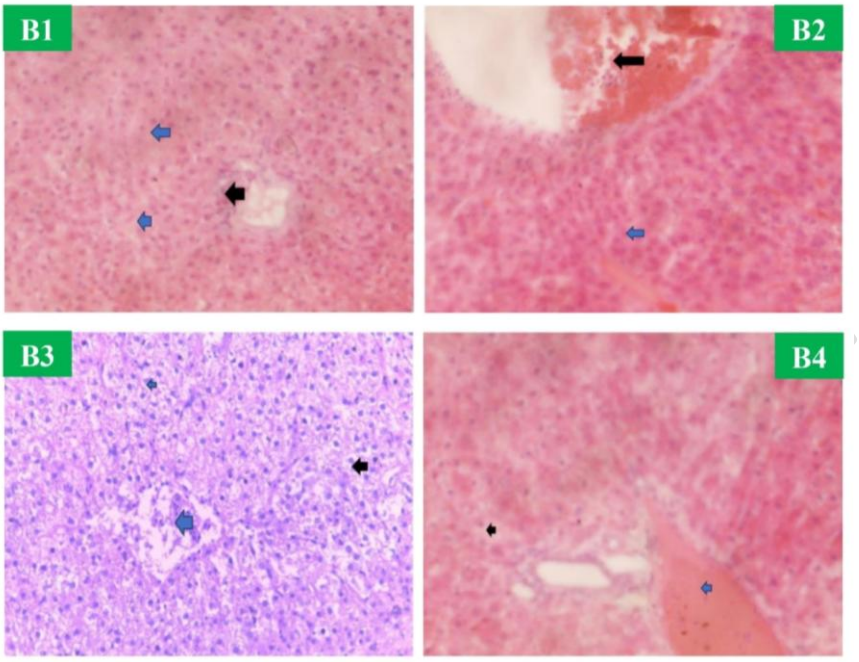

Figure 2: (B1-B4) Photomicrographs of Liver on day 14. (B1) Normal control showing normal hepatocytes (blue) and normal portal triad (black). (B2) Liver of animals administered 250mg/kg of *Camellia sinensis* showing normal hepatocytes (blue) and normal portal triad (black). (B3) Liver of animals administered 500mg/kg of *Camellia sinensis* showing normal hepatocytes (blue). (B4) Liver of animals administered 1000mg/kg of *Camellia sinensis* showing normal hepatocytes (blue) and normal central vein (black). Using hematoxylin and Eosin stain at X400 Magnification

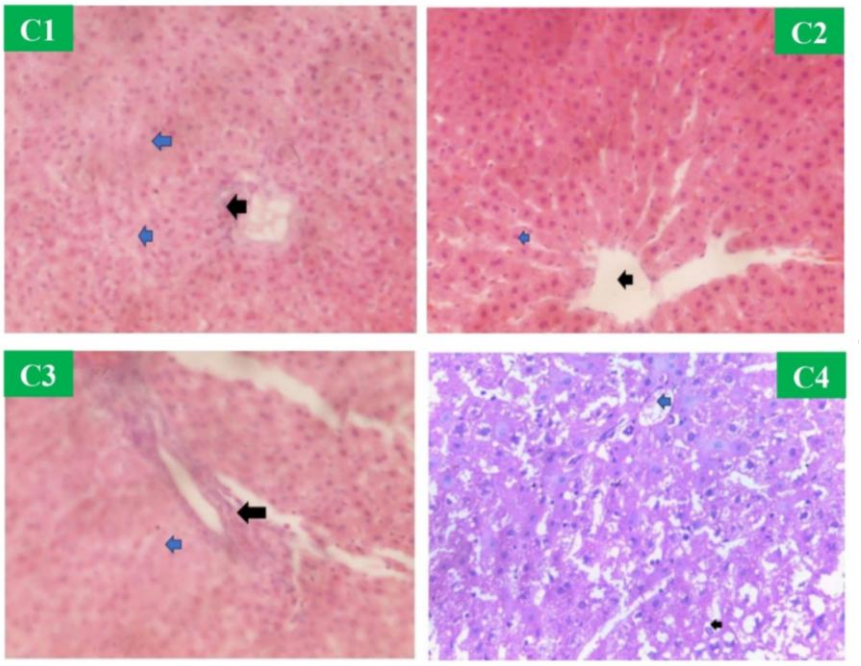

Figure 3: (C1-C4) Photomicrographs of Liver on day 21. (C1) Normal control showing normal hepatocytes (blue) and normal portal triad (black). (C2) Liver of animals administered 250mg/kg of *Camellia sinensis* showing normal hepatocytes (blue) and normal portal triad (black). (C3) Liver of animals administered 500mg/kg of *Camellia sinensis* showing normal hepatocytes (blue). (C4) Liver of animals administered 1000mg/kg of *Camellia sinensis* showing macrovesicules (blue) and microvesicules (black) in the cytoplasm of many hepatocytes suggestive of fatty liver. Using hematoxylin and Eosin stain at X400 Magnification

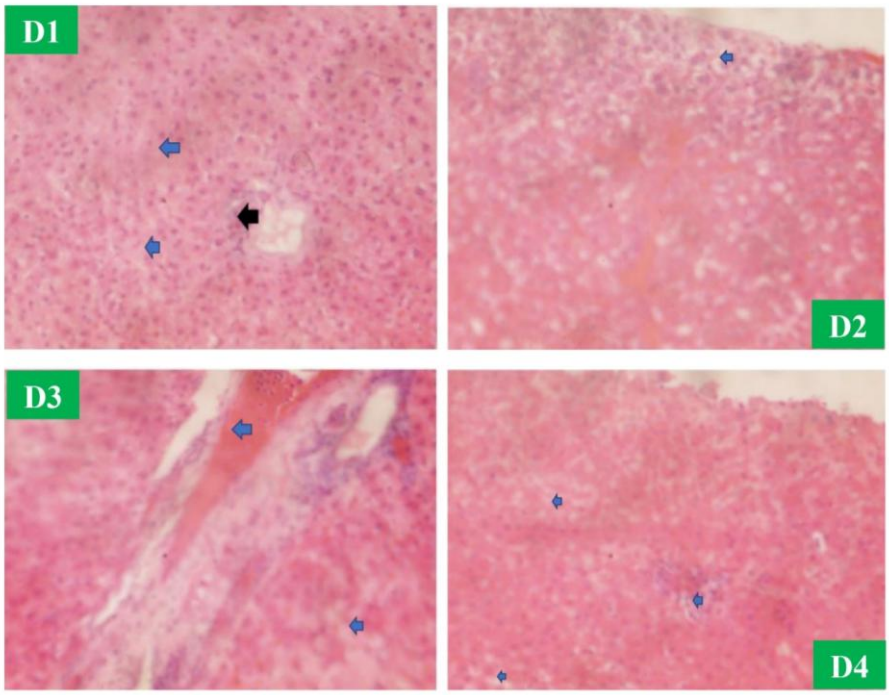

Figure 4: (C1-C4) Photomicrographs of Liver on day 28. (D1) Normal control showing normal hepatocytes (blue) and normal portal triad (black). (D2) Liver of animals administered 250mg/kg, of *Camellia sinensis* showing microvesicules (blue) in the cytoplasm of some hepatocytes suggestive of fatty liver. (D3) Liver of animals administered 500mg/kg of *Camellia sinensis* showing microvesicules (blue) in the cytoplasm of few hepatocytes suggestive of fatty liver with a normal central vein (black). (D4) Liver of animals administered 1000mg/kg of *Camellia sinensis* showing microvesicules (blue) in the cytoplasm of few hepatocytes suggestive of fatty liver with a mild periportal lymphocytic infiltrates (black). Using hematoxylin and Eosin stain at X400 Magnification

DISCUSSION

Green tea has gained significant attention due to its potential health benefits [14,20,21]. However, the literature has shown inconsistency and prompted debates over its impact on the liver [22-25]. While some studies suggest hepatoprotective effects of green tea [21,26-28], others report toxic findings [22,29-31]. Recent research has shown promising results in terms of green tea's hepatoprotective effects [31]. A randomized controlled trial demonstrated improved liver health markers in participants consuming green tea compared to a control group. However, a meta-analysis of observational studies had reported no significant association between green tea consumption and liver health [32]. The presence of individual variations and potential confounding factors may influence the interpretation of these effects. Animal studies have highlighted the ameliorative effect of green tea against liver damage induced by toxicants. These findings suggest potential hepatoprotective properties of green tea that warrant further investigation. Considering the conflicting results, it is crucial to conduct well-designed studies to establish a clearer understanding of how green tea affects liver health.

The present study investigated the effects of different doses of *Camellia sinensis* on various liver parameters in Wistar rats, with the aim being to determine its potential toxicity to the liver. The findings revealed distinct effects of different doses on liver enzymes, protein synthesis, and bilirubin metabolism.The lower doses of *Camellia sinensis* (250mg/kg and 500mg/kg) provided evidence of possible safety. In this regard, the 250mg/kg and 500mg/Kg doses did not have an adverse effect on liver enzymes (AST, ALT, and ALP), suggesting no liver toxicity. However, the 1000mg/Kg dose resulted in significant increases on day 21 and day 28, indicating a potential toxicity to the liver with prolonged use.

Comment [H6]: This reference talks about hepatotoxocity of the plant, not its health benefit.

Comment [H7]: Because there are two different findings, i.e. hepatotoxicity and hepatoprotective effects of the plant, why are you initially interested in studying hepatotoxicity effect of the plant?

Furthermore, the highest dose also resulted in increased total bilirubin on day 21 and day 28, suggesting impaired bilirubin metabolism and potential liver dysfunction [33]. Moreover, higher dose (1000mg/kg) of *Camellia sinensis* were associated with fatty liver development and mild inflammation, while lower doses (250, 500mg/kg) had mild to no effects on liver histology.

The findings of this study aligns with some previous research, while showing differences in certain aspects. Firstly, the lower doses of *Camellia sinensis* (250mg/kg and 500mg/kg) were demonstrated to be safe to the liver, as evidenced by the lack of significant alterations in liver function biomarkers. These findings are consistent with the work of Shimizu et al. [34] and Abeywickrama et al. [35], who reported hepatoprotective effects of green tea extract in rats with liver damage induced by different agents. Shimizu et al. [34] observed reductions in liver enzyme levels and improved bilirubin metabolism with the use of green tea extract, while Abeywickrama et al. [35] found significant improvements in liver histology and reduced liver enzyme levels with lower doses of green tea extract.

In contrast, our study revealed some differences from the research conducted by Sajjad and Minhas [36] which investigated the effects of *Camellia sinensis* on liver enzymes in a mouse model and reported significant reductions in liver enzyme levels at both lower and higher doses of green tea extract. Unlike our findings, Sajjad and Minhas [36]. did not observe adverse effects with the highest dose of green tea extract. The disparity in results could be attributed to variations in dosage, animal models, and the duration of the study.

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Regarding the adverse effects of the highest dose of *Camellia sinensis* (1000mg/kg) on liver function, our study's findings are consistent with the work of Oketch-Rabah et al. [30] and EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al. [37]. Oketch-Rabah et al. [30] reported liver damage and oxidative stress in mice administered with a high dose of

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green tea extract, including elevated liver enzyme levels and decreased albumin synthesis, similar to our study's results. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al. [37] investigated the effects of high doses of green tea extract on liver and kidney functions in rats and observed adverse effects on both organs, including elevated liver enzyme levels, supporting our findings on the adverse effects of the highest dose of *Camellia sinensis* on liver enzyme levels.

Furthermore, studies conducted by some researchers on hepatoprotective effects of natural remedies are also relevant. Lodhi et al. [38] evaluated the influence of green tea extract on liver specific enzymatic and nonenzymatic markers, lipid peroxidation, and oxidative stress in albino rats with chronic ethanol-induced hepatotoxicity. They found that aqueous extract of *Camellia sinensis* or green tea extract exhibited hepatoprotective effects in chronic ethanol-induced albino rats. Additionally, Shareef et al. [21] investigated the hepatoprotective character of methanolic green tea extract and its mechanism of action against thioacetamide-induced liver injury in Sprague Dawley rats. They found that green tea extract exhibited significantly hepatoprotective effect against thioacetamide-induced hepatic damage in rodents as evidenced by gross morphological examination and histology of rat's liver fed with *Camellia sinensis*.

The discrepancies observed in the effects of different doses of *Camellia sinensis* on the liver may also stem from variations in experimental designs and animal models used in different studies. Factors such as the age, sex, and genetic background of the animals can influence how they respond to the administered doses of *Camellia sinensis* [38-40].

According to a review of studies on green tea consumption, the duration of exposure to *Camellia sinensis* may play a crucial role in determining its effects on the liver [41]. Short-term exposure to lower doses of *Camellia sinensis* might lead to no effect or transient positive effects on liver

Comment [H10]: Previously you claimed the differences in animal model to attribute for differences in result with your findings. But now you are taking as the same result though the animal models are different, i.e they used mice.

Comment [H11]: Was the high dose of green tea extract used by [37] the same with yours, 1000 mg/kg?

enzymes and bilirubin metabolism. However, prolonged exposure to higher doses may cause cumulative toxicity, leading to adverse effects on liver function [41]. Several studies have reported cases of liver toxicity attributed to *Camellia sinensis*-containing products [39,42,43]. Therefore, it is important to consume green tea in moderation and consult a healthcare professional if any adverse effects are noticed.

Additionally, differences in the methodologies used to assess liver parameters could also contribute to the varying outcomes reported in different studies. Variations in the techniques employed to measure liver enzyme levels, total bilirubin, total protein, and albumin could lead to inconsistent results [10,44].

Moreover, it is essential to consider the bioavailability and metabolism of *Camellia sinensis* compounds in the body. The absorption, distribution, and elimination of these compounds can differ depending on the dose and the route of administration, which can impact their bioactivity and potential hepatotoxicity [41,45,46].

The mechanism of action for the lower doses of *Camellia sinensis* is associated with the presence of catechins and flavonoids, which have antioxidant and anti-inflammatory activities [41,47]. These bioactive compounds may scavenge free radicals, reduce oxidative stress, and modulate inflammatory pathways in the liver, leading to hepatoprotective effects [41,47]. On the other hand, the potential mechanism underlying the hepatotoxicity of higher doses of *Camellia sinensis* might be related to the generation of pro-oxidant effects [41,48]. At high concentrations, polyphenolic compounds in *Camellia sinensis* could exert a pro-oxidant role, promoting the production of reactive oxygen species (ROS) and disrupting the balance between antioxidants and pro-oxidants in the liver, leading to cellular damage and adverse effects on liver function [41,46,48].

The highest dose (1000mg/kg) of *Camellia sinensis* resulted in fatty liver and mild liver inflammation, possibly due to the pro-oxidant properties of Epigallocatechin gallate (EGCG) [41,42,49]. Lower doses (250mg/kg and 500mg/kg) showed potential beneficial effects on liver function, likely through their antioxidant and anti-inflammatory actions [47,49]. The dose-dependent effects of *Camellia sinensis* highlight the importance of careful dosage considerations for therapeutic benefits and minimal liver risks. Further research is needed to elucidate the mechanisms underlying these dose-dependent effects and optimize *Camellia sinensis* usage for liver health.

CONCLUSION

This study revealed dose-dependent effects of *Camellia sinensis* on liver function. Lower doses (250mg/kg and 500mg/kg) were shown to be potentially safe. However, the highest dose (1000mg/kg) resulted in adverse effects such as liver damage, altered albumin synthesis, and impaired bilirubin metabolism. Lower doses of *Camellia sinensis* may scavenge free radicals, reduce oxidative stress, and modulate inflammatory pathways, leading to improved liver enzymes, protein synthesis, and bilirubin metabolism. Conversely, the highest dose might cause liver damage and impaired function due to pro-oxidant properties of compounds like Epigallocatechin gallate (EGCG). Hence, careful dosage consideration is crucial for optimizing therapeutic benefits and minimizing potential liver risks.

REFERENCES

1. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Preventive Medicine. 1992;21(3):334-350.
2. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea- A review. Journal of the American College of Nutrition. 2006;25(2):79-99.
3. Sharma V, Singh R, Bhardwaj TR, et al. Therapeutic Potential of Green Tea Catechins in Treating Diabetic Cardiomyopathy. Current Drug Targets. 2020;21(1):91-101.
4. Yang CS, Wang ZY, Xu Y. Mechanisms of cancer prevention by tea polyphenols. In: Li J, Aggarwal BB, Shishodia S, editors. Asian Pacific Journal of Cancer Prevention, Vol. 10. World Scientific Publishing Co.; 2019.
5. Ogar DA, Sharma A, Alqahtani F, et al. Green Tea and its Bioactive Compounds: Therapeutic Perspectives. Current Pharmaceutical Design. 2023;29(4):400-417.
6. Mbuthia KS, Mireji PO, Ngure RM, et al. Tea (*Camellia sinensis*) infusions ameliorate cancer in 4TI metastatic breast cancer model. BMC complementary and alternative medicine. 2017;17(1):202. https://doi.org/10.1186/s12906-017-1683-6
7. Liu Y, Wang X, Chen Q, et al. *Camellia sinensis* and Litsea coreana Ameliorate Intestinal Inflammation and Modulate Gut Microbiota in Dextran Sulfate Sodium-Induced Colitis Mice. Molecular nutrition & food research. 2020;64(6):e1900943. https://doi.org/10.1002/mnfr.201900943
8. Santos EL, Maia G, Araruna MK, et al. Green tea extract-rich in epigallocatechin gallate and catechins-protects against liver injury through antioxidant and anti-inflammatory mechanisms. Journal of Functional Foods. 2015;19:593-605.
9. Roy S, Ahmed F, Banik P, et al. Exploring the hepato-protective effects of green tea. BioMed Research International. 2017;2017:1-13.
10. Lala V, Zubair M, Minter DA. Liver Function Tests. In: StatPearls [Internet]. StatPearls Publishing; 2023.
11. Lee SJ, Ahn EK, Lee YJ, et al. Modulatory effects of green tea catechins on hepatic drug-metabolizing enzymes in rats administered an aminopyrine cocktail. Cancer Letters. 2005;220(1):107-113.

12. Sharma V, Sood R, Manhas RK, et al. Hepatoprotective effect of ethanolic extract of Allium wallichii on antitubercular drugs induced hepatotoxicity in experimental animals. Journal of Traditional and Complementary Medicine. 2018;8(3):432-439.
13. Maronpot RR. Liver: Anatomy, Histology, and Histopathology. Toxicologic Pathology. 2015;43(6):705-715.
14. Mazzanti G, Menniti-Ippolito F, Moro PA, et al. Hepatotoxicity from green tea: a review of the literature and two unpublished cases. European Journal of Clinical Pharmacology. 2009;65(4):331-341.
15. Sae-Tan S, Grove KA, Lambert JD. Weight regain prevention. Current Obesity Reports. 2019;8(2):147-154.
16. Bruce RD. The Bruce treadmill test: A noninvasive procedure for the assessment of cardiovascular disease. JAMA. 1985;250(10):1982-1987.
17. Uahomo PO, Isirima JC. Antidiarrheal properties of aqueous leaf extract of *Cyathula prostrata* on castor oil-induced diarrhoea in Wistar rats. International Journal of Pharmaceutical Research and Applications. 2022;7(4):1679-1692.
18. Ibekwe VC, Nwobodo E, Onyeze GOC. Effect of ethanolic leaf extract of *Ocimum gratissimum* on some marker enzymes in albino rats. African Journal of Biotechnology. 2007;6(11):1347-1352.
19. Haussament AJ. Alkaline phosphatase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. 2nd ed. Academic Press; 1977.
20. Xing L, Zhang H, Qi R, Tsao R, Mine Y. Recent advances in the understanding of the health benefits and molecular mechanisms associated with green tea polyphenols. Journal of agricultural and food chemistry. 2019;67(4):1029-1043.
21. Shareef SH, Ibrahim IAA, Alzahrani AR, Al-Medhtiy MH, Abdulla MA. Hepatoprotective effects of methanolic extract of green tea against Thioacetamide-Induced liver injury in Sprague Dawley rats. Saudi journal of biological sciences. 2022;29(1):564-573.
22. Mahmoodi M, Hosseini R, Kazemi A, et al. Effects of green tea or green tea catechin on liver enzymes in healthy individuals and people with nonalcoholic fatty liver disease: A systematic review and meta-analysis of randomized clinical trials. Phytotherapy research. 2020;34(7):1587-1598.

23. Opuwari C, Monsees T. Green tea consumption increases sperm concentration and viability in male rats and is safe for reproductive, liver and kidney health. Scientific reports. 2020;10(1):15269.
24. Hayakawa S, Ohishi T, Miyoshi N, Oishi Y, Nakamura Y, Isemura M. Anti-cancer effects of green tea epigallocatechin-3-gallate and coffee chlorogenic acid. Molecules. 2020;25(19):4553.
25. Li M, Duan Y, Wang Y, et al. The effect of Green green tea consumption on body mass index, lipoprotein, liver enzymes, and liver cancer: An updated systemic review incorporating a meta-analysis. Critical Reviews in Food Science and Nutrition. 2022:1-9.
26. Xu XY, Zheng J, Meng JM, et al. Effects of food processing on in vivo antioxidant and hepatoprotective properties of green tea extracts. Antioxidants. 2019;8(12):572.
27. Tokofai BM, Idoh K, Oke OE, Agbonon A. Hepatoprotective effects of *Vernonia amygdalina* (Astereaceae) extract on CCl4-induced liver injury in broiler chickens. Animals. 2021;11(12):3371.
28. Dey P, Bruno RS. Green Tea Catechins and Nonalcoholic Fatty Liver Disease. In: Dietary Supplements with Antioxidant Activity: Understanding Mechanisms and Potential Health Benefits. 2023.
29. Al-Salmi FA, Hamza RZ, El-Shenawy NS. The interaction of zinc oxide/green tea extract complex nanoparticles and its effect on monosodium glutamate toxicity in liver of rats. Current Pharmaceutical Biotechnology. 2019;20(6):465-475.
30. Oketch-Rabah HA, Roe AL, Rider CV, et al. United States Pharmacopeia (USP) comprehensive review of the hepatotoxicity of green tea extracts. Toxicology reports. 2020;7:386-402.
31. Abe S, Inoue M. Green tea and cancer and cardiometabolic diseases: a review of the current epidemiological evidence. European journal of clinical nutrition. 2021;75(6):865-876.
32. Yin X, Yang J, Li T, et al. The effect of green tea intake on risk of liver disease: A meta analysis. International journal of clinical and experimental medicine. 2015;8(6):8339-8346.

33. Zamani M, Kelishadi MR, Ashtary-Larky D, et al. The effects of green tea supplementation on cardiovascular risk factors: A systematic review and meta-analysis. Frontiers in Nutrition. 2023;9:1084455.
34. Shimizu Y, Inoue H, Ohba Y, et al. Green Tea Extract Intake during Liver Cancer Progression in Mice Attenuates the Increase in Insulin Resistance along with the Attenuation of Hepatic Steatosis and Changes in Gut Microbiota. J Clin Biochem Nutr. 2020;66(1):31-41.
35. Abeywickrama HM, Sung B, Prasad S, et al. Effects of green tea extract on markers of liver function and liver histology in patients with nonalcoholic fatty liver disease: A randomized, double-blind, placebo-controlled trial. Pharm Res. 2016;33(12):2978-2987.
36. Sajjad F, Minhas LA. Effects of Green Tea (*Camellia sinensis*) on Liver Function Tests of Mice on High Fat Diet. Pakistan Journal of Medical & Health Sciences. 2014;8(3):550-553.
37. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Younes M, Aggett P, et al. Scientific opinion on the safety of green tea catechins. EFSA journal. European Food Safety Authority. 2018;16(4):e05239.
38. Lodhi P, Tandan N, Singh N, Kumar D, Kumar M. *Camellia sinensis* (L.) Kuntze Extract Ameliorates Chronic Ethanol-Induced Hepatotoxicity in Albino Rats. Evidence-based complementary and alternative medicine: eCAM. 2014;2014:787153.
39. National Institute of Diabetes and Digestive and Kidney Diseases. Green Tea. In: LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD).
40. Bedrood Z, Rameshrad M, Hosseinzadeh H. Toxicological effects of *Camellia sinensis* (green tea): A review. Phytotherapy Research. 2018;32(7):1163-1180.
41. Palacio Sánchez E, Ribero Vargas ME, Restrepo Gutiérrez JC. Hepatotoxicity due to green tea consumption (*Camellia sinensis*): A review. Revista colombiana de Gastroenterología. 2013;28(1):46-52.
42. Jimenez-Saenz M, Martinez-Sanchez M del C. *Camellia sinensis* liver toxicity. Journal of Hepatology. 2007;47(2):297–298.

43. Donata Grajecki, Alexandru Ogica, Olaf Boenisch, Peter Hübener, Stefan Kluge. Green tea extract–associated acute liver injury: Case report and review. Clinical Liver Disease. 2022;20:181–187.
44. Kanzler S, Rix A, Czigany Z, et al. Recommendation for severity assessment following liver resection and liver transplantation in rats: Part I. Laboratory Animals. 2016;50(6):459-467.
45. Mazzanti G, Di Sotto A, Vitalone A. Hepatotoxicity of green tea: an update. Archives of toxicology. 2015;89(8):1175–1191.
46. Sánchez M, González-Burgos E, Iglesias I, Lozano R, Gómez-Serranillos MP. The Pharmacological Activity of *Camellia sinensis* (L.) Kuntze on Metabolic and Endocrine Disorders: A Systematic Review. Biomolecules. 2020;10(4):603.
47. Sengottuvelu S, Duraisami S, Nandhakumar J, Duraisami R, Vasudevan M. Hepatoprotective activity of *Camellia sinensis* and its possible mechanism of action. IJPT. 2008;7:9-14.
48. Bonkovsky HL. Hepatotoxicity Associated with Supplements Containing Chinese Green Tea (*Camellia sinensis*). Annals of Internal Medicine. 2006;144(1):68.
49. Ting Li, Yuxin Wang, Yan Fang, et al. Longjing green tea (*Camellia sinensis*) extract dose-dependently influences intestinal barrier and liver inflammation in autoimmune hepatitis. Journal of Functional Foods. 2023;107:105623.