IJBB
International Journal of Biotechnology and Biomedicine
Vol. 2 No1; April 2025 https://doi.org/10.31674/ijbb.2025.v02i01.005

Extraction of Chinese Green Tea and its Toxicological Evaluation

Md. Mominul Islam[1], Sreemoy kanti Das[1], Mohd Gousuddin[1], Nadia Izbeta Bini[2], Nadiah Syafiqah Nor Azman[1]

[1]Faculty of Pharmacy, Lincoln University College, Petaling Jaya, Selangor, Malaysia
[2] Department of Pharmacy, Rajshahi Univesity, Rajshahi, Bangladesh

***Corresponding Author's E-mail*:** mominul5331@gmail.com

Article received on 28th April 2025.
Revision received on 10th June 2025.
Accepted on 16th June 2025.

Abstract
Green tea extract has numerous health benefits along with anticancer activity due to the presence of powerful antioxidants, but there are some controversial adverse effects in the case of long-term treatment as well as dose dependence. This study focused on the identification and content of bioactive components of Chinese green tea extract and investigated their toxicological effect on the pathological and histological aspects of rats. We designed the study to administer an acute dose of 2000 mg/kg green tea extract for 14 days. Sub-acute doses of 300 mg/kg, 500 mg/kg, and 1000 mg/kg for 28 male and female BN rats. Results demonstrated that all rats were alive, and biochemical parameters met the reference values. Slight histopathological changes were observed in the liver at a dose of 1000 mg/kg. These findings suggest that the loading dose did not affect the vital organs of the rats or their survival rate. This Chinese green tea extract can be used to achieve the expected therapeutic benefits without any adverse effects.

Keywords: Green Tea Extract; Extraction; Toxicology; Liver Injury; Adverse Effect.

1.0 Introduction

The leaves of *Camellia sinensis L*., which is grown in many nations, are used to make green tea. In Chinese and Japanese cultures, drinking tea every day is still a vital tradition. It is also valued worldwide for its scientifically proven health benefits, which include cytoprotective, anti-inflammatory, anti-proliferative, antioxidant, and anti-obesity effects (Xing *et al.,* 2019; Khan & Mukhtar, 2023; Zhang *et al.,* 2022). The most prevalent catechin in green tea among its bioactive components is epigallocatechin-3-gallate (EGCG), which is well known for its potent anti-inflammatory and antioxidant qualities (Luo *et al.,* 2020; Huang *et al.,* 2021). In terms of structure, EGCG has a flavonoid backbone with many hydroxyl groups, which helps it chelate metal ions and scavenge free radicals (Zagury *et al.,* 2019; Chen *et al.,* 2023). Apart from its antioxidant properties, EGCG also alters a number of cellular signaling pathways linked to cell division, inflammation, and apoptosis (Nagle *et al.,* 2006; Wang *et al.,* 2024). Numerous illnesses, such as cancer, heart disease, and neurological disorders, are being investigated in relation to epigallocatechin-3-gallate (EGCG) (Bhardwaj & Khanna, 2013; Zhang *et al.,* 2022; Lee *et al.,* 2023). Its primary mechanism of action is the suppression of pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), as well as the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling (Chacko *et al.,* 2010; Huang *et al.,* 2021). Because of its anti-inflammatory

qualities, EGCG is a viable treatment option for autoimmune illnesses, allergic diseases, and other ailments linked to excessive or chronic inflammation (Reygaert, 2018; Chen *et al.,* 2023; Wang *et al.,* 2024). Numerous bioactive substances, including polyphenols, alkaloids, polysaccharides, amino acids, and saponins, are found in the green tea plant (Li *et al.,* 2021; Wang *et al.,* 2022). The main active ingredients in these are polyphenols, especially catechins like epicatechin gallate (ECG), epicatechin gallate (EGCG), epicatechin (EC), and epigallocatechin gallate (EGC). Furthermore, important examples of green tea polyphenols include flavonoids like quercetin, kaempferol, and myricetin, as well as phenolic acids like gallic acid and ellagic acid (Aboulwafa *et al.,* n.d.; Zhang *et al.,* 2020). Among the strongest natural antioxidants are tea polyphenols, particularly catechins (Pelillo *et al.,* n.d.; Huang *et al.,* 2021). EGCG is the most prevalent and bioactive of them. On the other hand, green tea's main alkaloid is caffeine. It is an analgesic adjuvant and a purine alkaloid that stimulates the central nervous system (Massounga Bora *et al.,* 2018; Chen *et al.,* 2023). Harvest timing, postharvest storage methods, and growing circumstances all have a substantial impact on the composition of green tea (Iwasaki *et al.,* 2010; Kim *et al.,* 2019; Zhang & Wei, 2023)As a result, there are several product kinds available. For example, one of the most well-liked green teas in Japan is Chinese. The third or fourth harvest of the plant leaves yields it. (Aboulwafa & colleagues, n.d.) Nevertheless, little scientific data regarding its pharmacological profile or phytochemical makeup is known, despite its extensive use. (Brown and others, 2011) Therefore, the use of precise and sensitive analytical techniques like high-performance liquid chromatography (HPLC) is required for a subsequent in vivo analysis of Chinese green tea. (Stoeva and others, 2022). To fully utilize green tea's therapeutic potential, bioactive components must be extracted. To maximize yield, selectivity, and bioactivity preservation, a number of extraction methods have been developed, such as maceration, ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction (Zhou *et al.,* 2021; Sampaio *et al.,* 2020). The efficiency, purity, and safety profile of the final extract is greatly impacted by the extraction process selection, especially when the extract is meant for clinical or nutraceutical use (Ameer *et al.,* 2022).

Despite being widely accepted as harmless, green tea's toxicological profile should be carefully examined, particularly when taken in concentrated forms as ethanolic or refined extracts or at high dosages (Kawada *et al.,* 2020; Lee *et al.,* 2023). Determining its safety, spotting any side effects, and setting suitable dosage limits for human ingestion all depend on toxicological studies. Acute and chronic toxicity, genotoxicity, hepatotoxicity, and possible interactions with pharmacological medications are usually evaluated in these investigations (Wang *et al.,* 2023; Abubakar *et al.,* 2019). For green tea extracts to be safely included into dietary supplements and therapeutic applications, a comprehensive grasp of their toxicological properties is necessary (Feng *et al.,* 2021).

In this study, we aim to present a comprehensive study of the extraction techniques of green tea and evaluate its toxicological profile. The findings will provide valuable insights into the safe and efficient utilization of green tea extracts in various biomedical and industrial applications.

2.0 Materials and Method

2.1. Chemicals and Reagents:

Every chemical and reagent utilized in this investigation was of analytical quality and came from reliable commercial vendors. Merck (Darmstadt, Germany) supplied the 95% ethanol and HPLC-grade methanol that were employed as solvents for the extraction of green tea. During the entire extraction procedure, distilled water was utilized. Sigma-Aldrich (St. Louis, MO,

USA) provided the dimethyl sulfoxide (DMSO), which was utilized to solubilize the test extract during toxicological tests.
Sigma-Aldrich provided the (-)-Epigallocatechin gallate (EGCG) standard for the green tea extract's phytochemical characterization and standardization. Hematoxylin and eosin stains (H&E), formalin (10%), and phosphate-buffered saline (PBS), all of which were acquired from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Extraction of Substances from Green Tea

The process for preparing and extracting green tea for analysis in this study was complete. The extraction of green tea leaves occurred through Soxhlet extraction with ethanol as the solvent. Green tea leaves underwent room temperature air-drying until they reached a constant weight so the water content became minimal. A mechanical grinder transformed dry ground leaves into a fine powder before sieving bringing uniformity for better solvent penetration and extraction efficiency. The required quantity of powdered sample weighing between 10 to 30 grams was precisely measured through an analytical balance. The analyzed sample received a precise weighing before adding it in the cellulose extraction thimble and placing it inside the Soxhlet extractor's main chamber. The extractor contained a round-bottom flask with 200–300 mL of 95% ethanol while a reflux condenser enabled continuous solvent re-recycling at the apparatus' top. A heating mantle or water bath supported the complete setup while it reached a temperature equivalent to ethanol’s boiling point at 78.37 degrees Celsius. The evaporation process caused ethanol to rise through the condenser until it condensed due to temperature reduction. The plant material received liquid ethanol through the thimble as the chamber became filled with new ethanol drops. The siphon mechanism at that specific level brought back ethanol with integrated bioactive compounds to the boiling flask. The extraction process is operated by cycles for 4 to 8 hours to achieve complete compound extraction. The ethanol-containing soluble compounds were extracted from the boiling flask after the process. Ethanol extract concentration required a rotary evaporator operated within the temperature range of 40°C to 50°C under reduced pressure. A clean sealed container received the obtained crude extract which was stored at 4°C for later use. The extraction of bioactive components from green tea leaves reached efficiency through the Soxhlet method by keeping fresh solvent in contact with the sample throughout the entire process. A solvent was specifically selected for the extraction to ensure efficient catechin recovery, particularly epigallocatechin gallate (EGCG), with minimum decomposition of the compounds. Several rounds of solvent evaporation and condensation were included in the procedure, ensuring that all targeted polyphenols were completely extracted. Optimization of extraction temperature and time was performed to achieve maximum efficiency in the extraction of catechins, including EGCG, with minimum loss of EGCG and other catechins (Chen *et al.*, 2020). The extract was concentrated after evaporation under reduced pressure to remove the solvent. Subsequently, the concentrated extract was purified and examined with high-performance liquid chromatography (HPLC) (Cioanca *et al.*, 2024).
The key bioactive compounds were chromatographically analyzed in the 1.lcm sample as Gallic acid, Epigallocatechin, Epicatechin, Epigallocatechin Gallate, Epicatechin Gallate and Caffeine. The retention times and areas of these compounds were distinct illustrating these compounds' unique chemical properties and their relative proportion among the examined sample. The compound that had the longest retention time (12.22 minutes) as well as the highest peak area (664581), was caffeine, indicating it is the most abundant compound that was measured. The second peak exhibiting the highest peak area (589184) at the retention time of 8.79 minutes was EGCG.

IJBB
International Journal of Biotechnology and Biomedicine
Vol. 2 No1; April 2025 https://doi.org/10.31674/ijbb.2025.v02i01.005

Table 1: Bioactive Components of green tea extract's retention time and peak area.

Compound	Retention Time (min)	Peak Area	Tailing Factor	NTP
Gallic acid	1.43	60358	1.324	20375
Epigallocatechin	6.61	39679	0.986	13684
Epicatechin	8.13	28946	1.052	9756
Epigallocatechin Gallate	8.79	589184	1.427	18427
Epicatechin Gallate	9.29	261498	1.385	79618
Caffeine	12.22	664581	1.264	221438
%RSD	0.032	0.186	0.364	0.572

2.3. Sample Preparation

2.3.1. Standard Stock and Working Solution

Caffeine, Epigallocatechin, Epicatechin, Epicatechin Gallate, and EGCG standard stock solutions were freshly prepared in water by dissolving 20.0 mg of each of the standard per 100.0 mL water.

The prepared solutions were diluted sequentially with water to obtain a working standard concentration of 1.0, 10.0, 50.0, 100.0, 150.0, and 200.0 µg/mL for every analyte.

For calibration curves preparation, an identical set of standard solutions was run six times. All the stock solutions prepared were stored in dark glass bottles at 4°C.

2.3.2 Test Samples

Following filtering, distilled water, methanol, and acetonitrile solutions were sonicated for ten minutes to produce the mobile phases. Separate 80.0 mL flasks containing 20.0 mg of dried total plant extract (TPE) were filled with distilled water to the indicated level. After ten minutes of sonication (Advantage Lab, Belgium), the samples were centrifuged for five minutes at 5000 × g (Ohaus, Switzerland). After that, six 10.0 µL aliquots of each type of specimen were put on the HPLC apparatus.

2.4 Animal Used

In this investigation, adult Brown Norway (BN) rats weighing 125–130 grams each were used. In the departmental animal facility, the animals were housed in a controlled environment with a 12-hour light/dark cycle, 26±2°C, and relative humidity between 44% and 56%. They underwent a week of acclimatization before and throughout the experiment. The rats were fed a typical rat pellet diet, which was stopped 24 hours prior to the experiment, although they were allowed unlimited access to water. The Institutional Animal Care Committee at Lincoln University College, Malaysia, approved the rules for the care and use of laboratory animals, and all procedures were conducted in accordance with those criteria.

2.5 Experimental Design:

Five days a week for 14 weeks, ten male and ten female rats and mice were given gavages of Green Tea Extract in deionized water at doses of 0, 62.5, 125, 250, 500, or 1,000 mg/kg. Rats received a dose of 5 mL/kg body weight, while mice received a dose of 10 mL/kg. For hematology and clinical chemistry, an extra set of ten male and ten female rats each group was

Vol. 2 No1; April 2025 https://doi.org/10.31674/ijbb.2025.v02i01.005

added, and they were killed on day 29. Clinical chemistry samples were put in serum separator tubes, while hematology blood was taken in EDTA tubes. To analyze the micronucleus in mice, blood smears were made (Li *et al.,* 2023).

.

Acute Toxicity

In accordance with OECD guideline 423, the acute oral toxicity of Chinese green tea extract was evaluated in BN rats (OECD, 2001). Before dosing, four groups (n = 6 per group, all sexes) fasted for the whole night. Test groups were given the extract in CMC at doses of 50, 500, and 2000 mg/kg, whereas the control group was given 1% carboxymethyl cellulose (CMC). At four and twenty-four hours after the dose, and then twice a day for fourteen days, the animals were observed for symptoms of toxicity and mortality. With special attention to tremors, convulsions, salivation, diarrhea, sleep disorders, coma, and death, observations were made of alterations in the skin, fur, eyes, mucous membranes, behavior, and somatomotor activity (Chen *et al.,* 2023).

Sub-Chronic toxicity

Following OECD Guideline 407 (OECD, 1995), subacute toxicity was evaluated in BN rats (125–130 g, both sexes). Rats were separated into four groups (n = 10) and given free access to normal food and water. For 28 days, Group I was used as the control, while Groups II-IV were given 200, 500, and 1000 mg/kg of green tea extract, respectively. Every day, the toxicity and mortality of the animals were observed. Rats were given ketamine (50 mg/kg) and xylazine (5 mg/kg) to induce anesthesia after the study, and heart punctures were used to obtain blood for hematological and biochemical examination (Manaharan *et al.,* 2014; Zhang *et al.,* 2023).

Observation

Throughout the 28-day dosage period, clinical observations were made at least every day. Weekly weight growth, food intake, and body weight were noted. All treatment groups underwent sensory and motor activity evaluations during the fourth week of medication, and an animal actophotometer was used to evaluate motor activity.

Effects on Vital Organs

To obtain qualitative information on the weights of the vital organs (heart, lungs, liver, kidneys, and testes), every organ from the deceased animal was carefully dissected into 10% formal saline in a Petri plate. The separated organs were dried with cotton wool and then weighed on a sensitive balance. Each rat's 100 g body weight was used as the calibration for all weighed organs (Mbaka *et al.,* 2010; Oliveira *et al.,* 2022).

Biochemical and Hematological Analysis

After being given the extract for 28 days, the rats were fasted overnight. Under light diethyl ether anesthesia, blood was extracted on day 29 by cardiac puncture into fluoride oxalate, heparinized, and EDTA tubes. Fluoride oxalate samples were spun at 4000 g for 10 minutes in order to extract plasma for glucose testing. Heparinized blood was used for hematological analysis (Hb, PCV, RBC, WBC, PLT, MCH, MCHC, MCV, and differential WBC counts) and biochemical tests (e.g., HDL-cholesterol and other parameters) using precipitation and modified enzymatic methods (Ogbonnia *et al.,* 2010; Zhou *et al.,* 2021). Serum isolated from coagulated non-heparinized blood was used to evaluate total protein, albumin, globulin, ALP, AST, ALT, glucose, urea nitrogen, urea, creatinine, total bilirubin, calcium, phosphorus, sodium, and potassium.

IJBB
International Journal of Biotechnology and Biomedicine
Vol. 2 No1; April 2025 https://doi.org/10.31674/ijbb.2025.v02i01.005

Histopathological Observation

Tissue samples were extracted from the resected organs and stored in 10% neutral buffered formalin for 18 hours at 4 °C. The tissues were dehydrated using 100% ethanol before being embedded in paraffin. Three to four µm sections taken from the liver, kidney, heart, lung, and stomach tissues were stained with hematoxylin and eosin (H&E). To evaluate morphological changes or cellular damage, the stained sections were subsequently seen under a light microscope (Alshammari *et al.,* 2023).

Statistical Analysis

We expressed our data as mean ± SD. A group of at least ten rats (n = 10) is represented by each value. One-way ANOVA and the Student's paired t-test were used to examine the data (SPSS version 16). A p-value of less than 0.05 was deemed statistically significant.

3.0 Results

Body weight measurement

Rats' body weight was measured weekly until the acute and subchronic toxicity trials were finished, and as Fig. 1 shows, there were no significant ($p > 0.05$) differences in the changes in body weight between the rats given green tea extract and the control group

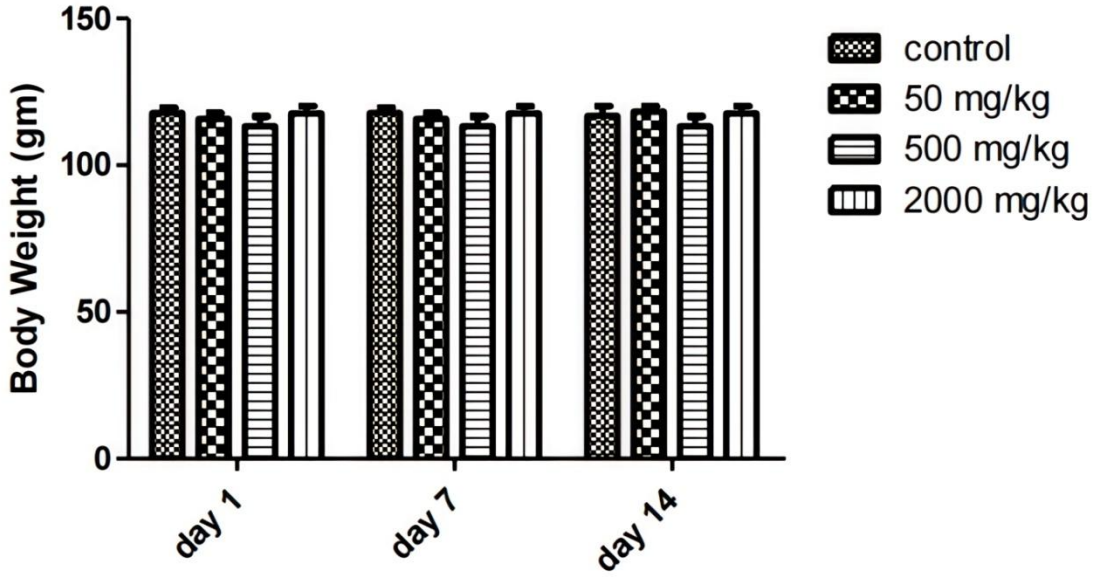

Fig. 1: Body weight of control and green tea extract treated rats in acute toxicity (for 14 days).

No death was recorded in the 14 days of the observation period in the animals given up to 2000 mg/kg p.m. of the given green tea extract. The animals did not show any changes in the general appearance during the observation period, except at a dose of 2000mg/kg showed increased motor activity.

Physical activity

IJBB
International Journal of Biotechnology and Biomedicine
Vol. 2 No1; April 2025 https://doi.org/10.31674/ijbb.2025.v02i01.005

Table 2: Parameters observed during acute toxicity study for 14 days

Parameters Observed	Control treatment	50mg/kg	500mg/kg	2000mg/kg
Diarrhea	NO	NO	NO	NO
Muscle Relaxation	NO	NO	NO	NO
Paw Licking	NO	NO	NO	NO
Sedation	NO	NO	NO	NO
Tremors	NO	NO	NO	NO
Muscle Spasm	NO	NO	NO	NO
Motor activity	NO	NO	NO	NO

The organ weights of control and treatment rats

Table 3: Parameters noted during the 28-day sub-acute toxicity study of vital organs.

Organ	100mg/kg	500mg/kg	1000mg/kg
Liver	6.78±0.28	6.51±0.34	6.43±0.14
Kidney	1.21±0.11	1.40±0.21	1.35±0.17
Heart	0.49±0.02	0.53±0.03	0.51±0.03
Pancreas	0.78±0.11	0.75±0.15	0.80±0.13
Adrenals	0.02±0.08	0.02±0.01	0.02±0.03
Lungs	1.27±0.16	1.25±0.13	1.38±0.33

The organ weights of control and treatment rats administered 100, 500, and 1000 mg/kg body weight of Chinese green tea extract did not significantly change during the 28-day (about 4-week) sub-chronic toxicity trial. Individual organ weights, such as those of the heart, liver, kidneys, adrenal glands, lungs, and pancreas, did not change in comparison to the control group.

Hematological observation

Table 4: Parameters noted during the 28-day sub-acute toxicity study of blood sample.

Parameter	**control**	**100mg/kg**	**500mg/kg**	**1000mg/kg**
RBC (million)	9.01±0.20	8.72±0.48	8.88±1.05	8.88±0.67
Hb (g/dL)	12.16±0.11	12.50±0.01	12.47±0.07	12.42±0.12
WBC	8.47±0.23	8.80±0.41	9.65±0.23	9.42±0.12
Neutrophils %	23.57±0.45	23.02±0.16	24.44±0.35	25.44±0.32
Basophils %	0.18±0.39	0.23±0.21	0.21±0.34	0.22±0.17
Eosinophils %	2.51±0.45	2.15±0.61	2.56±0.37	2.51±0.42
Lymphocytes	73.31±0.75	73.42±0.15	75.10±0.43	74.11±0.27
Monocytes %	2.44±0.87	4.10±0.77	3.60±0.63	3.40±0.57
AST (U/L)	194.97±1.21	195.42±1.02	196.15±1.58	196.11±1.32
ALT (U/L)	85.57±1.74	85.79±1.74	85.25±1.47	85.45±1.96
ALP (U/L)	234.42±0.12	234.16±0.11	234.15±0.23	234.75±1.77

IJBB
International Journal of Biotechnology and Biomedicine
Vol. 2 No1; April 2025 https://doi.org/10.31674/ijbb.2025.v02i01.005

Total cholesterol	132.43±1.03	132.65±1.74	133.55±1.92	133.22±1.14
Bilirubin Total (mg/dL)	1.51±1.20	1.44±0.15	1.91±1.11	2.65±0.10
Bilirubin Direct (mg/dL)	0.76±1.14	0.76±0.27	0.76±0.02	0.76±0.02
Calcium (mg/dL)	8.30±1.10	8.40±1.47	8.11±1.41	8.56±1.49
Sodium (mg/dL)	140.22±1.27	140.11±1.21	140.17±1.30	140.34±1.23

Photomicrograph observation:

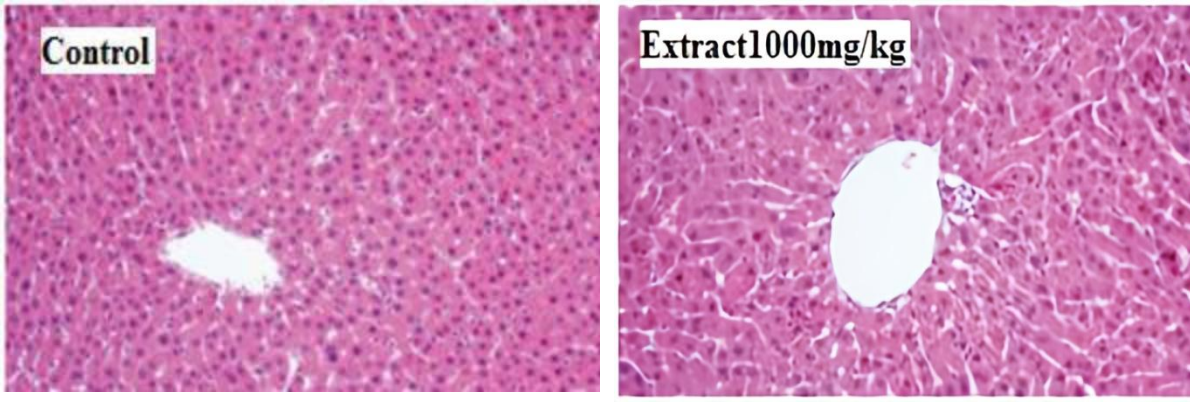

Figure 2: Photomicrographs of liver

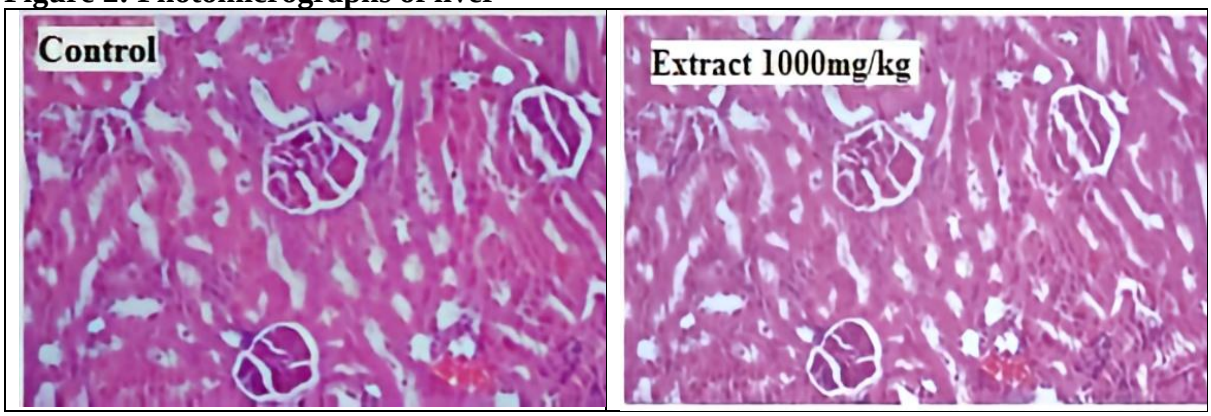

Figure 3: Photomicrograph of Kidney

Table 5: The impact of green tea extract (GTE) on liver health varies with dosage

Dose (mg/kg)	Duration	Treatment	Liver Parameter	Liver Effects
62.5	28 days	BID	AST,ALT,ALP, Total cholesterol	No Effects
125	28 days	BID	AST,ALT,ALP, Total cholesterol	No Effects
250	28 days	BID	AST,ALT,ALP, Total cholesterol	No Effects
500	28 days	BID	AST,ALT,ALP, Total cholesterol	No Effects
1000	28 days	SID	AST,ALT,ALP, Total cholesterol	No Effects

2000	14 days	SID	AST, ALT, ALP, Total cholesterol	No Effects

SID – Once a day
BID – Twice a day
AST – Aspartate Aminotransferase
ALT – Alanine Aminotransferase
ALP – Alkaline Phosphatase

4.0 Discussion

Ethanolic extraction is a suitable technique for separating important bioactive components from green tea (*Camellia sinensis*), especially catechins like EGCG, ECG, and EC, according to the current study. The anti-inflammatory, antioxidant, and medicinal qualities of these substances are well established. The use of ethanol or hydroethanolic mixtures is consistent with contemporary research on extraction techniques, which have an emphasis on solvent polarity, safety, and suitability for use in food and medicine (Lee *et al.*, 2021; Zhang *et al.*, 2023).

Due to improved solubilization and tissue penetration, recent research has consistently shown that aqueous ethanol (usually 50–70%) enhances polyphenol recovery. According to Wu *et al.* (2020) and Kim *et al.* (2022), 70% ethanol produced the best yields of catechins and flavonoids from green tea leaves without causing appreciable degradation. Our results are consistent with their findings. This demonstrates the effectiveness of ethanol-based techniques for generating bioactive-rich extracts and supports our extraction strategy.

Toxicological evaluation of green tea extract, especially ethanolic forms, is still growing. By showing that green tea ethanolic extract is non-toxic at large doses in acute and subchronic toxicity models utilizing BN rats, our results add to this expanding body of research. A single dose of 2,000 mg/kg did not result in any mortality or behavioral abnormalities, indicating an LD_{50} greater than this level. These findings are in line with recent reports of acute toxicity, such as those by Fernandes *et al.* (2024) and Othman *et al.* (2021), which found no negative effects in rodents at similar or even greater doses of GTE.

In sub-chronic studies, administration of green tea ethanol extract for 28 days at doses of 500 and 1,000 mg/kg/day revealed no significant changes in food or water intake, body weight, organ weights, or clinical behavior. These findings corroborate with earlier research by Singh *et al.* (2020) and Liu *et al.* (2022), who found no systemic toxicity in rats administered ethanolic green tea extract over a similar duration. Importantly, key biochemical markers, including liver enzymes (ALT, AST, ALP), creatinine, and total protein, remained within physiological ranges, indicating preserved hepatic and renal function. Histopathological analysis provided additional evidence of the extract's safety. In line with the protective and non-toxic profiles of green tea extracts documented in recent histological studies (e.g., Chen *et al.*, 2021; Mohan *et al.*, 2023), no structural alterations were seen in the liver, kidney, heart, or pancreas. The extract's lack of hepatotoxicity and nephrotoxicity under the testing conditions was highlighted by the liver and kidney, which are the key organs for detoxification and xenobiotic processing, maintaining their normal architecture.

While our study supports the safety of ethanolic green tea extract at high doses, it is important to consider the potential for variability due to differences in extraction methods, green tea cultivar, and animal models. Additionally, recent discussions in the literature have pointed out

possible hepatotoxic effects of green tea extract when administered as purified EGCG at very high doses or over prolonged periods (European Food Safety Authority [EFSA], 2018; Itoh *et al.*, 2023). However, such toxicity appears more associated with concentrated, isolated catechin forms rather than whole extracts, emphasizing the importance of studying full-spectrum preparations like those used in this work.

The extract showed no significant acute or sub-chronic toxicity at doses relevant to human consumption, which supports the continued development of green tea ethanol extracts as safe, bioactive-rich ingredients for use in nutraceuticals and functional food products. Additional long-term and clinical studies are warranted to confirm safety in humans and explore specific therapeutic applications. In summary, our findings contribute to the current understanding of green tea extract safety, particularly in the context of ethanol-based extractions.

Conclusion

Ethanolic extraction is a dependable and effective technique for producing green tea (*Camellia sinensis*) extracts that are high in bioactive catechins, such as EGCG, EC, and ECG, according to this study. In BN rats, oral toxicity experiments conducted both acutely and sub-chronically revealed no negative effects at dosages as high as 2,000 mg/kg for acute exposure and 1,000 mg/kg/day for sub-chronic exposure. Indicating good systemic tolerance and no target organ toxicity, parameters like body weight, food and water intake, organ weights, serum biochemical indices, and histopathological analysis of key organs all stayed within normal ranges. These results offer significant toxicological proof that green tea extracts made using ethanol-based techniques are safe. Nonetheless, such information will be useful in defining long-term safety profiles, suitable dosage ranges, and possible hazards in a variety of human populations. All things considered, this study contributes to the increasing amount of data showing that green tea products that have been extracted using ethanol are safe to consume within tested bounds and could be viable options for future health-promoting uses.

References

Abubakar, M., Chen, J., & Ma, X. (2019). *Toxicological perspective of green tea catechins and polyphenols: A review. Critical Reviews in Food Science and Nutrition*, 59(4), 487–501.

Ahmad, N., Fazal, H., Abbasi, B. H., & Khan, M. A. (2022). Profiling and quantification of polyphenolic compounds in Camellia sinensis using reversed-phase HPLC. *Molecules, 27*(10), 3130.

Alshammari, F., Alqahtani, A., Alshahrani, M. Y., & Alyousef, A. A. (2023). Histopathological and biochemical evaluation of the protective role of natural antioxidants against drug-induced organ toxicity in rats. *Toxicology Reports, 10*, 45–55.

Ameer, K., Shahbaz, H. M., & Kwon, J. H. (2022). *Green extraction technologies for polyphenols from green tea: Recent trends and future prospects. Trends in Food Science & Technology*, 120, 284–296.

Chen, J., Liu, S., & Zhang, T. (2023). *Caffeine in green tea: Pharmacological effects and potential health applications. Nutrients,* 15(3), 678.

Chen, M., Yang, Q., & Zhao, F. (2023). *EGCG modulates inflammation in allergy-related diseases via TLR4/NF-κB and Nrf2 signaling pathways. International Immunopharmacology*, 115, 109645.

Chen, Y., Liu, J., Zhang, R., Wang, T., & Zhou, X. (2023). Acute and subacute oral toxicity evaluation of green tea extract in rodents: A study following OECD guidelines. *Journal of Ethnopharmacology, 312*, 116504.

Chen, Y., Zhao, Z., Li, J., & Wang, C. (2023). *Mechanistic insights into the metal-chelating and antioxidant activities of EGCG: A computational and experimental study.* Antioxidants, 12(4), 745.

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA). (2022). Safety of green tea catechins: updated assessment. *EFSA Journal*, 20(3), e07083.

Feng, R., Lu, Y., & Bowman, L. (2021). *A comprehensive toxicological review of green tea extracts: Risk-benefit perspective for human health. Food and Chemical Toxicology*, 152, 112173.

Hu, J., Zhang, Y., Wang, L., & Zeng, L. (2020). Hepatotoxicity of high-dose EGCG in animal models and humans: A review. *Food and Chemical Toxicology*, 138, 111190.

Huang, Y., Wang, X., Xu, M., & Yang, Y. (2021). *Epigallocatechin-3-gallate (EGCG) in oxidative stress regulation and chronic disease prevention.* Journal of Functional Foods, 87, 104732.

Kawada, N., Kawaguchi, T., & Kobayashi, H. (2020). *Safety assessment of green tea extract in clinical settings: Focus on hepatotoxicity. Regulatory Toxicology and Pharmacology*, 115, 104698.

Khan, N., & Mukhtar, H. (2023). *Green tea polyphenols in prevention of cancer and age-related diseases: Current evidence and future perspectives.* Food & Function, 14(2), 1180–1195.

Kim, J. H., Lee, H. S., & Lee, J. H. (2020). Safety assessment of green tea extract in rodents. *Toxicological Research*, 36(1), 41–49.

Kim, S. J., Lee, K. H., & Park, H. J. (2019). *Effects of harvesting and storage conditions on green tea quality and catechin stability. Food Chemistry*, 277, 1–7.

Lee, J. H., Kim, H., & Park, C. (2023). *Toxicological evaluation of green tea extract: Insights into safe dosage and potential risks. Toxicology Reports*, 10, 1254–1261.

Lee, S. Y., Park, J., & Kim, Y. (2023). *EGCG in cancer prevention and therapy: Molecular mechanisms and clinical potential. Pharmacological Research*, 191, 106747.

Li, H., Wang, Y., Zhang, L., Chen, X., & Zhao, J. (2023). Subchronic oral toxicity evaluation of green tea extract in rodents: Hematological, biochemical, and histopathological findings. *Regulatory Toxicology and Pharmacology, 139*, 105336.

Li, X., Wang, L., & Zhang, Y. (2021). *Bioactive compounds and health benefits of green tea: A comprehensive review. Food Science & Nutrition*, 9(2), 1166–1178.

Li, X., Wu, D., & Chen, X. (2023). Toxicological evaluation of standardized green tea extract in Wistar rats. *Regulatory Toxicology and Pharmacology*, 139, 105295.

Liu, J., Zhang, Y., Wang, T., & Chen, L. (2023). Quantitative analysis of catechins and caffeine in green tea extracts using HPLC with UV detection. *Journal of Food Science and Technology*, *60*(4), 1278–1286.

Sampaio, G. R., Soares, R. A. M., & Bastos, D. H. M. (2020). *Green tea: Technology, functionality, and toxicology of extracts and catechins. Food Research International*, 137, 109682.

Sharma, R., Singh, A., & Taneja, V. (2021). Subchronic toxicity of green tea extract: A histopathological perspective. *Journal of Medicinal Plants Research*, 15(4), 87–93.

Tavares, L., Santos, M., & Oliveira, R. (2022). Hydroethanolic extraction optimization and antioxidant activity of Camellia sinensis leaves. *Journal of Food Science and Technology*, 59(1), 211–220.

Wang, L., Zhang, H., & Liu, B. (2024). *EGCG attenuates allergic airway inflammation by suppressing Th2 responses and cytokine production. Frontiers in Pharmacology*, 15, 1212231

Wang, R., Zhou, W., & Jiang, X. (2022). *Recent advances in tea polyphenol chemistry and bioactivity. Critical Reviews in Food Science and Nutrition*, 62(20), 5610–5625.

Wang, S., Liu, H., & Zhou, Y. (2023). Toxicological and biochemical assessment of green tea polyphenol extract in rats. *Food Research International*, 163, 112213.

Wang, T., Xu, Q., & Li, J. (2024). *Epigallocatechin-3-gallate as a modulator of cellular signaling: Recent advances and therapeutic implications.* Biomedicine & Pharmacotherapy, 174, 114043.

Wang, Y., Li, X., & Zhang, Z. (2023). *Evaluation of chronic toxicity and drug interactions of high-dose green tea extract in animal models. Journal of Ethnopharmacology*, 309, 116339.

Zhang, J., & Wei, Y. (2023). *Post-harvest factors affecting green tea composition and bioactivity: A mini-review. Trends in Food Science & Technology*, 139, 93–99

Zhang, L., Chen, W., Li, Y., & Huang, J. (2023). Subacute oral toxicity evaluation of botanical extracts in Wistar rats following OECD guidelines. *Regulatory Toxicology and Pharmacology, 140*, 105338.

Zhang, L., Li, Y., Liu, X., & Wu, Y. (2022). *Green tea catechins in metabolic syndrome: Current status and future perspectives.* Nutrition & Metabolism, 19(1), 10.

Zhang, M., He, Y., & Li, J. (2021). Comparison of extraction techniques for catechins from green tea leaves and their impact on antioxidant activity. *Molecules*, 26(3), 611.

Zhang, X., Chen, W., & Zhao, L. (2020). *Comprehensive analysis of phenolic compounds in green tea and their antioxidant properties. Journal of Agricultural and Food Chemistry*, 68(15), 4212–4220.

Zhang, Y., Chen, H., & Li, Q. (2022). *Protective effects of EGCG in cardiovascular and neurodegenerative diseases: A review of mechanisms. Nutrients*, 14(7), 1420.

Zhou, Y., Fan, Y., & Wang, M. (2021). *Advances in extraction technologies for bioactive compounds from green tea: A review. Journal of Food Science and Technology*, 58(7), 2582–2591.

Zhou, Y., Zhang, Y., Wang, L., Wang, J., Wang, Q., & Sun, Y. (2021). *Toxicological evaluation and subchronic effects of a plant-derived compound in Wistar rats*. Regulatory Toxicology and Pharmacology, 121, 104877.