Research Article

Zoheir Amrouche*, Lazhari Tichati, Latifa Khattabi, Mohammed Messaoudi, Ayomide Victor Atoki*, Marie-Laure Fauconnier, Hassiba Laribi-Habchi

Toxicological effects of green tea catechin extract on rat liver: Delineating safe and harmful doses

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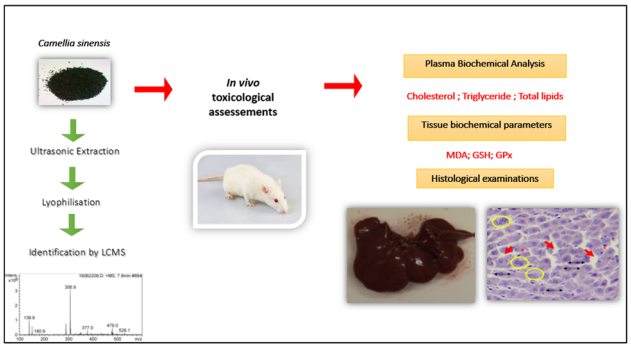
Abstract

Purpose – Catechins, the bioactive compounds found in green tea, are known for their beneficial health effects, but overconsumption may result in adverse effects. Thus, this study aimed to examine the influence of green tea (*Camellia sinensis* L.) catechin extract (GTCE) on liver metabolism and structure in rats.

Methods – GTCE was phytochemically characterized by LC-MS spectrometry. An *in vivo* study was conducted on female rats separated into four groups of five each, i.e., a control group and three catechin-treated groups D1, D2, and D3 received, respectively, 0.4, 0.6, and 0.8 g/kg b.w./day of GTCE by gavage for 28 days. The effects of GTCE were monitored through the analysis of plasma lipid, oxidative stress markers, reduced glutathione (GSH) content, glutathione peroxidase (GPx) activity, and malondialdehyde (MDA) level in the liver. Furthermore, histopathological examinations of liver tissue were conducted.



Graphical Abstract

Results – LC-MS analyses of GTCE revealed the presence of five phenolic compounds with a predominance of epigallocatechin-3-gallate (EGCG) at 60.1%. Exposure to catechins with an elevated dose (0.8 g/kg) caused oxidative damage in the liver, as indicated by a considerable increase ($p < 0.05$) in MDA levels, a decrease in GSH content, and GPx activity ($p < 0.05$), along with a decrease ($p < 0.05$) in plasma total cholesterol, triglycerides, and total lipids in comparison to the control group. These changes were confirmed by histological examination.

Conclusion – Although catechins offer known health benefits, high doses may induce oxidative stress and liver damage.

Keywords: *Camellia sinensis* L., catechins, LC-MS, liver, oxidative stress, rats, toxicity

*** Corresponding author: Zoheir Amrouche,** Biological Department, Faculty of Nature and Life Science, University of Khemis Miliana, Aïn Defla 44000, Algeria; Functional Analysis of Chemical Process Laboratory, Chemistry Department, University of Blida1, Blaida 9000, Algeria, e-mail: Zoheir.amrouche@yahoo.fr
*** Corresponding author: Ayomide Victor Atoki,** Department of Biochemistry, Kampala International University, Ishaka, Uganda, e-mail: atokiav@kiu.ac.ug
Lazhari Tichati: Environment and Biodiversity Research Division, Environmental Research Center, Alzon Castle, Boughazi Said Street, PB 2024, Annaba, 2300, Algeria
Latifa Khattabi: Biotechnology Research Center, (CRBt), Constantine, 25000, Algeria; Laboratory in Immunology and Biological Activities of Natural Substances, Nature and Life Sciences University of Constantine 1, Constantine, 25000, Algeria
Mohammed Messaoudi: Nuclear Research Centre of Birine, AinOussera, Djelfa, Algeria
Marie-Laure Fauconnier: Laboratory of Chemistry of Natural Molecules, Gembloux AgrobioTech, University of Liège, 4000 Liège, Belgium
Hassiba Laribi-Habchi: Functional Analysis of Chemical Process Laboratory, Chemistry Department, University of Blida1, Blaida, Algeria

Abbreviations

EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GT	Green tea
GTCE	Green tea catechin extract
MDA	Malondialdehyde

1 Introduction

Green tea (GT) is obtained from *Camellia sinensis* L., a member of the Theaceae family. It is known to be the most commonly consumed drink in the world after water and for its health benefits, including the chemoprevention effect [1,2]. Owing to its good biological activity, including pharmacological and antioxidant properties, it is attracting increasing interest [3,4]. The antioxidant properties of GT are attributed to important phytochemicals including phenolic compounds such as catechins, phenolic acids, and caffeine [5,6]. Catechins (flavan-3-oils), the primary phenolic compounds in GT, inhibit cell proliferation and exert potent anti-radical activity by acting as natural antioxidants [7,8]. It consists of catechin, epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and epigallocatechin gallate (EGCG) [9,10]. EGCG is the predominant catechin in GT and a potent antioxidant inhibiting oxidative damage disorders such as cancer, cardiovascular and neurological diseases, [11,12] obesity, and diabetes [13,14].

The liver is an essential organ that performs major functions, including protein synthesis, energy metabolism, glycogen storage, and drug detoxification [15]. Hepatic disorders often result from various substances such as pharmaceuticals, chemicals, and natural products like plant extracts. These substances mainly damage the liver by forming free radicals, which increase lipid peroxidation (LP), leading to liver damage [16]. Some studies suggest that EGCG directly influences lipid metabolism by inhibiting intestinal lipid absorption, increasing LDL receptor expression, and activating AMPK, which promotes fatty acid oxidation [17,18]. However, at high doses, EGCG has been associated with liver failure [19,20], which may indirectly affect the lipid levels. Liver damage can alter lipid metabolism due to disrupted hepatic function, leading to reduced cholesterol and triglyceride levels. In this context, several previous studies have shown that EGCG from GT at high doses has been shown to induce liver injury and oxidative damage [16,21,22]. EGCG induced hepatotoxicity by depleting antioxidant defense systems like antioxidant enzyme activities and altering liver biochemical markers [23,24].

Our study was designed to identify the chemical profile of green tea (*Camellia sinensis* L.) catechin extract (GTCE) using LC-MS. Furthermore, an *in vivo* study was performed to evaluate the biochemical variations, antioxidant status, and histological aberrations in the liver of female rats after treatment with GTCE at 0.4, 0.6, and 0.8 g/kg body weight (b.w).

2 Materials and methods

2.1 Plant material and extraction

The commercial GT leaves were suspended in ten volumes of distilled water (w/v; 1:10) in a 50 mL tube, followed by ultrasonic treatment at 95°C for 15 min [25,26]. The resulting infusions were centrifuged (5,000 *g* for 15 min at 4°C) and left to stand for 10 min, after which they were filtered through 0.45 µm filter membranes (Sartorius) and the top solution was collected and lyophilized. The lyophilized powder was kept in the dark at 4°C until assay.

2.2 LC-MS analysis

LC-MS analysis was performed using an Agilent 1100 Series HPLC system equipped with a diode array detector and coupled to an HCT linear ion trap mass spectrometer. Chromatographic separation was achieved on an EC/3 Nucleodur 100-3 C18 ec column (Macherey-Nagel, Germany). The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). A linear gradient of acetonitrile in water (5–95% B) was applied over 40 min. The sample injection volume was 20 µL. Mass spectra were recorded over a mass-to-charge (*m*/*z*) range of 100–600 under the following MS conditions: capillary voltage of 4,500 V, nebulizer pressure of 50 psig, drying gas flow rate of 10 L/min, and drying temperature of 365°C. Analyses were conducted in the positive ionization mode. Compounds in the GT extracts were identified based on their *m*/*z* values, UV/Vis absorption spectra, and retention times, and were compared with reference data reported in the literature [27]. Peak identification was further confirmed by mass spectrometry.

2.3 *In vivo* study

2.3.1 Animals and treatment

Female rats from the Wistar strain (Pasteur Institute, Algeria), weighing 125–130 g and aged 1 month, were placed in an animal house at a temperature of 22°C, a photoperiod of 12 h day/12 h night, and relative humidity of 50–60%. The rats were given a standard diet (supplied by ONAB, Bejaia, Algeria). Throughout the study, the rats had access to drinking water *ad libitum*. All experimental procedures were carried out in accordance with the International Guidelines for the Care and Use of Laboratory Animals (Council of European Communities, 1986).

2.4 Experimental procedure

After a 2-week adaptation period, animals were grouped into four groups of five rats each. The first group was designated as control and was given 1 mL of distilled water per day by oral gavage for 28 days. The groups (D1), (D2), and (D3) received oral treatment with GTCE at dose levels of 0.4, 0.6, and 0.8 g/kg/kg b.w., respectively, for 28 days. The test doses and treatment period were chosen based on a previous study [18].

2.5 Blood and tissue processing

After 28 days of treatment, the rats in each group were subjected to overnight fasting and then anesthetized with light ether, and then they were sacrificed by cervical decapitation. Blood samples were collected using heparinized tubes and subjected to centrifugation (3,000 *g* for 15 min), and the resulting plasma was maintained at −20°C in aliquots for biochemical assays, including total lipids, triglycerides, and cholesterol. The liver was recovered, washed with saline solution (NaCl 0.9%), and weighed. One portion of the liver was fixed in formalin solution for histological analysis, while the other portion was stored at −20°C for assessing oxidative stress markers [28].

2.5.1 Tissue preparation

Liver tissue was suspended in three volumes of phosphate buffered saline (1/3 w/v, pH 7.4), homogenized, and centrifuged at 10,000 *g* for 15 min at 4°C. The supernatant fraction was employed to estimate oxidative stress markers [29].

2.5.2 Biochemical assays

The total lipid, cholesterol, and triglyceride levels of the plasma were measured using Spinreact (Spain) commercial kits.

2.6 Oxidative stress marker evaluation

2.6.1 LP

LP was assessed by measuring the amount of malondialdehyde (MDA), the end product of LP, according to the technique of Buege and Aust [30] with slight modifications [31], based on TBA (thiobarbituric acid) reactivity. The absorbance at 532 nm was then determined. The amount of MDA formed was expressed as nmol MDA/mg protein.

2.6.2 Reduced glutathione (GSH) levels

Liver GSH levels were measured by the colorimetric method reported by Khattabi et al. [32] by recording the absorbance values at 412 nm resulting from the formation of 2-nitro-5-mercapturic acid from the reduction of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) by the SH groups present in GSH. The amount of GSH was expressed in nmol GSH/mg protein.

2.6.3 Glutathione peroxidase (GPx) activity

GPx activity in the liver homogenate was determined using the Flohé and Günzler [33] method, and the absorbance at 412 nm was recorded. GPx activity was expressed as nmol glutathione degraded/min/mg protein.

2.7 Histological examinations

Liver sections, previously fixed in formalin, were processed through graded alcohol baths followed by paraffin embedding. Liver sections were then cut at 4–5 µm thickness, stained with H&E (hematoxylin-eosin stain) following the technique of Houlot [34] and then examined under an optical microscope.

2.8 Statistical analysis

Data were analyzed using GraphPad Prism 7.0. The results are presented as mean ± SEM (standard error of mean) and were evaluated using ANOVA followed by Tukey's *post-hoc* test. Differences were considered significant at $p < 0.05$.

3 Results

3.1 Phenolic composition of GTCE

3.1.1 GT extract and phenolic composition

The results of LC-MS analyses of the GT extract are shown in Figures 1 and 2. The main phenolic compounds in GT are

catechins (Figure 2) with predominance of (−) epigallocatechin-3-gallate (EGCG) 60.1 ± 0.17% followed by (−) epigallocatechin (EGC) 12 ± 0.25%, (−) epicatechin gallate (ECG) 13 ± 0.45%, and (−) epicatechin (EC) 5 ± 0.2%.

3.2 Effects of GTCE on rat's health

The death of two rats was recorded in the group treated at a dose of 800 mg/kg on the 20th day of the experiment

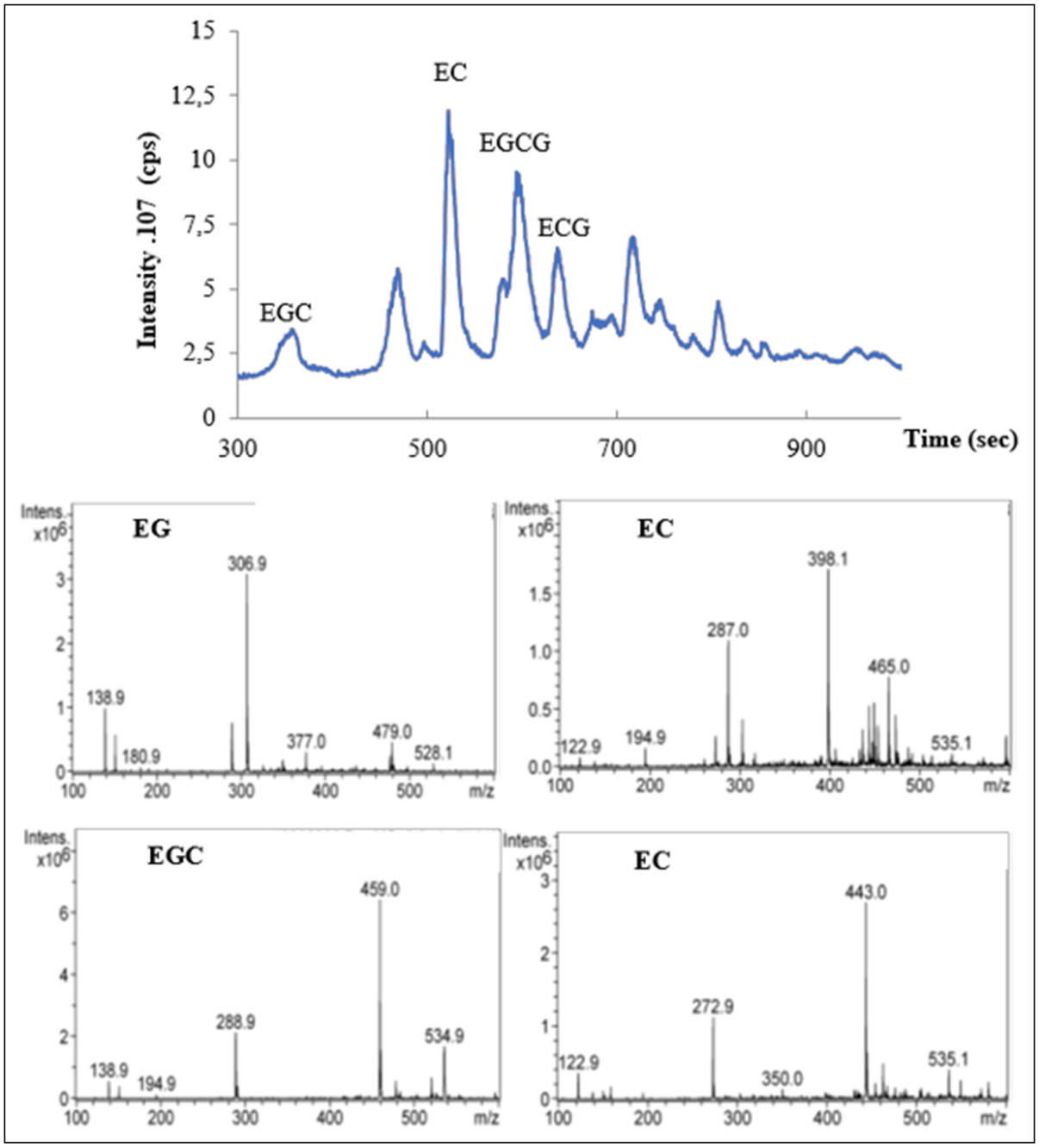
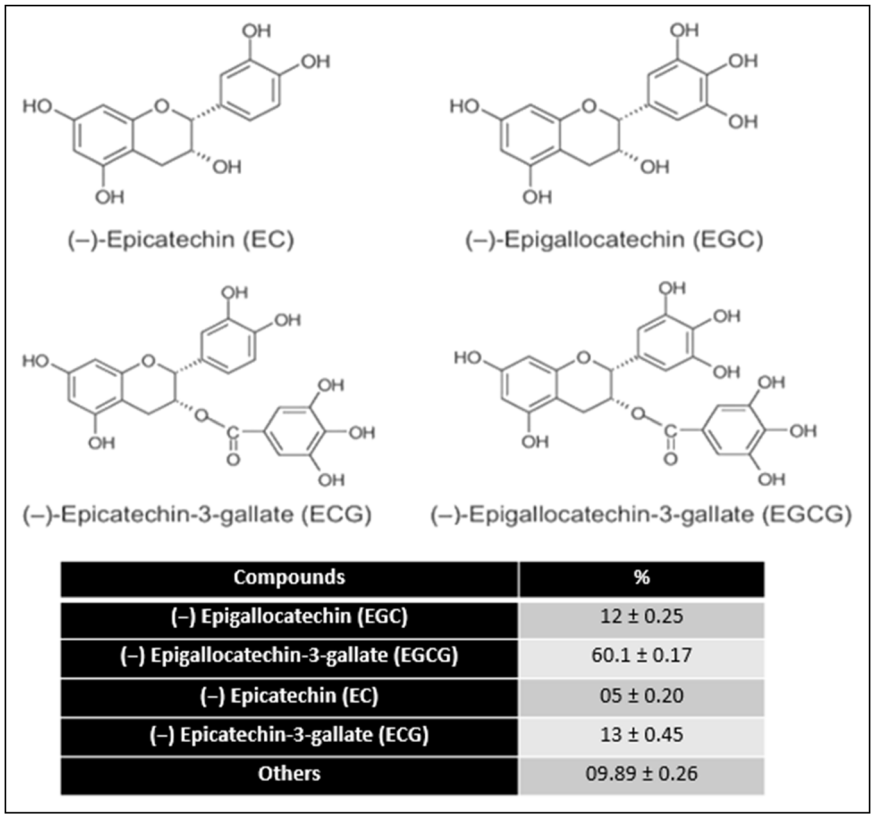


Figure 1: Chromatograms of LC-MS phenolic profile of GTCE. EGC: epigallocatechin; EC: epicatechin; EGCG: epigallocatechin-3-gallate; and ECG: epicatechin-3-gallate.



Compounds	%
(–) Epigallocatechin (EGC)	12 ± 0.25
(–) Epigallocatechin-3-gallate (EGCG)	60.1 ± 0.17
(–) Epicatechin (EC)	05 ± 0.20
(–) Epicatechin-3-gallate (ECG)	13 ± 0.45
Others	09.89 ± 0.26

Figure 2: Proportion and structure of major catechin polyphenols GT. The results are expressed as averages of three independent measurements ± SEM.

and a decrease in the body weight from the starting day of the test, regardless of the administered dose of GTCE. This reduction was more accentuated in a group treated with 800 mg/kg during 28 days of experimentation.

3.3 Effects of GTCE on the body and absolute and relative liver weights

Changes in the body weight of the studied groups are shown in Table 1. The data showed a significant decrease

Table 1: Body weight, weight gain, and absolute and relative liver weights of control and experimental rats

Parameters	Experimental groups			
	Control	D1	D2	D3
Initial weight (g)	132.1 ± 3.6	131.7 ± 5.45	130.9 ± 5.40	130.1 ± 5.5
Final weight (g)	185.8 ± 6.7^{a}	165.2 ± 5.6^{a}	156.4 ± 4.5^{b}	115.3 ± 7.2^{c}
Weight gain (%)	53.7 ± 2^{a}	33.5 ± 1.5^{a}	26.5 ± 1.3^{b}	−14.8 ± 1.4^{c}
Absolute liver weight (g)	3.56± 0.12^{a}	3.26 ± 0.15^{a}	3.05 ± 0.11^{a}	6.05 ± 0.26^{c}
Relative liver weight (g/100 g body weight)	1.92 ± 0.02^{a}	2.00 ± 0.05^{a}	2.03 ± 0.04^{a}	2.51 ± 0.17^{c}

Values are expressed as mean ± SEM; each group consisted of five rats. D1: rats treated with 400 mg/kg b.w. of GTCE; D2: rats treated with 600 mg/kg b.w. of GTCE; D3: rats treated with 800 mg/kg b.w. of GTCE; values in the same row with different letters are significantly different at $p < 0.05$.

($p < 0.05$) in the body weight of rats treated with 800 mg/kg GTCE compared to control rats. No significant difference in the body weight was noted between the group treated at both doses (400 and 600 mg/kg) compared to the controls.

As indicated in Table 1, the absolute and relative liver weights were significantly increased ($p < 0.05$) in GTCE at a dose of 800 mg/kg compared to controls.

3.4 Effect of GTCE on the lipid biochemical parameters

3.4.1 Cholesterol

The cholesterol concentrations in control and treated rats at the three tested doses of GTCE are shown in Table 2. Our results show that treatment with GTCE induces a significant decrease ($p < 0.05$) in the concentration of cholesterol in rats treated with GTCE at a 800 mg/kg dose compared to the control group. This value is not significant ($p > 0.05$) in treated rats at doses of 400 and 600 mg/kg not statistically significant ($p > 0.05$).

3.4.2 Triglycerides

The effects of GTCE on the concentration of triglycerides in the control and treated rats are presented in Table 2. The results showed a significant decrease ($p < 0.05$) in triglyceride concentration at both doses 600 and 800 mg/kg of GTCE. In contrast, a non-significant increase ($p > 0.05$) in triglyceride concentration was recorded in rats that received 400 mg/kg of GTCE compared to the control group.

3.4.3 Total lipids

Plasma concentration of the total lipids was significantly decreased ($p < 0.05$) after treatment with both doses (600 and 800 mg/kg) (Table 2). However, a significant increase ($p < 0.05$) in total lipids in rats treated with 400 mg was observed compared to the control.

3.5 Oxidative stress parameters

3.5.1 MDA level

The liver MDA levels in the control and treated groups are shown in Figure 3a. In fact, GTCE at a dose of 800 mg/kg/day significantly increased ($p < 0.05$) the level of MDA. However, no significant difference in the MDA hepatic levels was noticed between the groups treated at doses of 400 and 600 mg/kg and the control group.

3.5.2 GSH level

Figure 3b shows the hepatic GSH levels in the control and treated groups. The administration of GTCE at a dose of 800 mg/kg induced a significant decrease ($p < 0.05$) in the concentration of GSH. In contrast, a significant increase ($p < 0.05$) in GSH levels was recorded in rats treated with both doses (400 and 600 mg/kg) compared to control rats.

3.5.3 GPx activity

Liver GPx activity in the studied groups is shown in Figure 4. Based on the obtained results, the enzymatic activity of GPx was significantly decreased ($p < 0.05$) at a dose of 800 mg/kg. Furthermore, our results showed a non-significant increase in GPx activity ($p > 0.05$) at doses 400 and 600 mg/kg.

3.6 Gross macroscopic observations

The examination of the gross appearance of the liver of experimental rats (Figure 5) showed that the liver color of

Table 2: Plasma biochemical parameters in control and experimental rats

Parameters	Experimental groups			
	Control	D1	D2	D3
Cholesterol (mg/L)	0.72 ± 0.15	0.6 ± 0.081[a]	0.4 ± 0.091[a]	0.2 ± 0.081[b]
Triglycerides (mg/L)	1.62 ± 0.23[a]	1.52 ± 0.14[a]	1.25 ± 0.07[a]	0.45 ± 0.07[b]
Total lipids (mg/L)	3.13 ± 0.27[a]	3.7 ± 0.14[a]	2.65 ± 0.07[c]	0.65 ± 0.13[b]

Values are expressed as mean ± SEM; each group consisted of five rats. D1: rats treated with 400 mg/kg b.w. of GTCE; D2: rats treated with 600 mg/kg b.w. of GTCE; D3: rats treated with 800 mg/kg b.w. of GTCE; values in the same row with different letters are significantly different at $p < 0.05$.

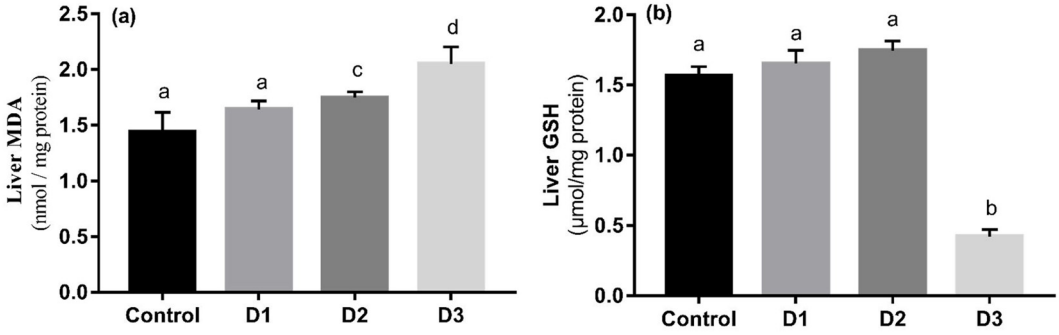


Figure 3: Hepatic GSH levels in the control and treated groups. Values are expressed as mean ± SEM; each group consisted of five rats. D1: rats treated with 400 mg/kg b.w. of GTCE; D2: rats treated with 600 mg/kg b.w. of GTCE; D3: rats treated with 800 mg/kg b.w. of GTCE; MDA: malondialdehyde; GSH: reduced glutathione. Values in the same row with different letters are significantly different at $p < 0.05$.

rats treated with GTCE at 800 mg/kg differed from other treatments at 400 and 600 mg/L as well for controls (Figure 5). The pro-oxidant effect may exist at the dose of 800 mg/L due to the macroscopic change in the liver.

3.7 Light microscopy of liver

The observation under an optical microscope Leica of the liver sections of the control rats showed normal cellular architecture and hepatocytes polyhedral in a form of trabeculae with granular cytoplasm organized around the centrilobular vein (Figure 6a). Figure 6b–d shows microscopic changes in sections of livers of animals treated with increasing doses of GTCE (400, 600, and 800 mg/kg). The histological examination in rats treated with the three tested doses showed varying degrees of hepatic histological lesions, especially in rats treated with a dose of 800 mg/kg GTCE compared to rats treated with 600 mg/kg (Figure 6b) and 400 mg/kg (Figure 6c). Binucleated hepatocytes, vacuolar degeneration, parenchymal cells hypertrophy, and sinusoid dilation and congestion were also observed.

4 Discussion

Despite the wide consumption of GT as a beverage, and its beneficial effects proven by several studies, scientific data on its toxic effects at high doses are available [10,35]. In this context, the present study aimed to evaluate the impact of

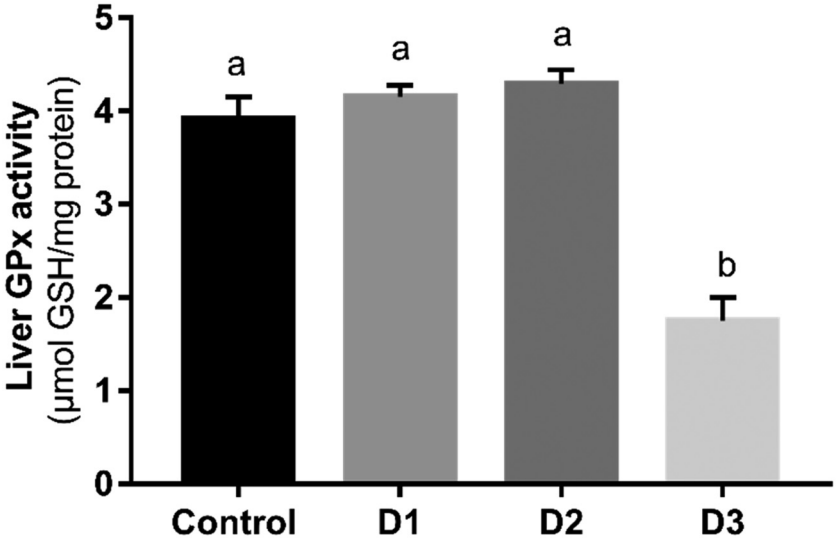


Figure 4: Liver GPx activity in the studied groups. Values are given as mean ± SEM; each group consisted of five rats. D1: rats treated with 400 mg/kg BW of catechins; D2 rats treated with 600 mg/kg b.w of GTCE; D3 rats treated with 800 mg/kg b.w of GTCE; MDA: malondialdehyde; GSH: reduced glutathione. Values on the same row with different letters are significantly different at $p < 0.05$.

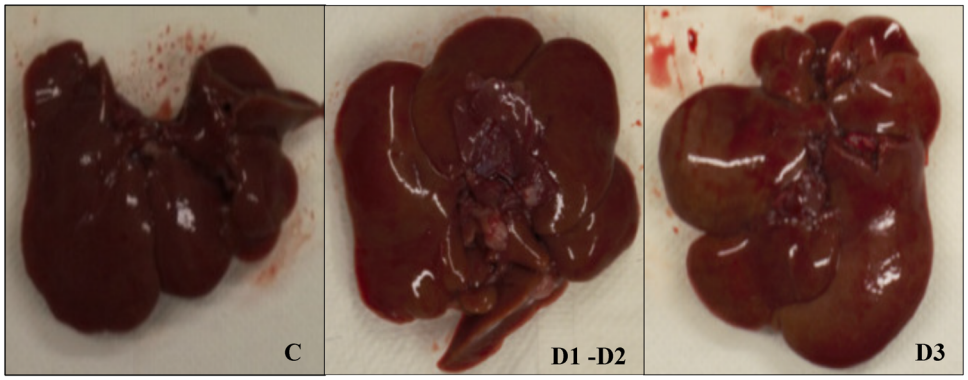


Figure 5: Rat liver after 28 days of GTCE treatment. C: control; D1: rats treated with 400 mg/kg b.w of GTCE; D2 rats treated with 600 mg/kg b.w of GTCE; D3 rats treated with 800 mg/kg b.w of GTCE.

GTCE at increasing doses on the liver over a 28-day period. For this reason, a phytochemical study was performed in order to characterize its chemical composition. The results showed that the phenolic composition in GT is catechins, EGCG being the highest, accounting for 60–65% of the entire catechin content [36]. EGCG is the major catechin in GT and accounts for 50–80% [37,38]. Furthermore, chemical modification of the EGCG pharmacophore may modify relative therapeutic activities so that combinatorial supplementation may synergistically enhance the beneficial health effects [3,39].

In animal experiments, the changes in the behavior and body weight are important revealing indices of the health status [40], and this change is correlated with the animal's physiological state [41]. In this study, treating rats with GTCE at a dose of 800 mg/kg significantly decreased the body weight. The observed reduction can be attributed to the anti-obesity effect, which inhibits the weight gain and the formation of adipose tissue [42], as reflected by reduced plasma triglyceride, cholesterol, and lipid levels in our study.

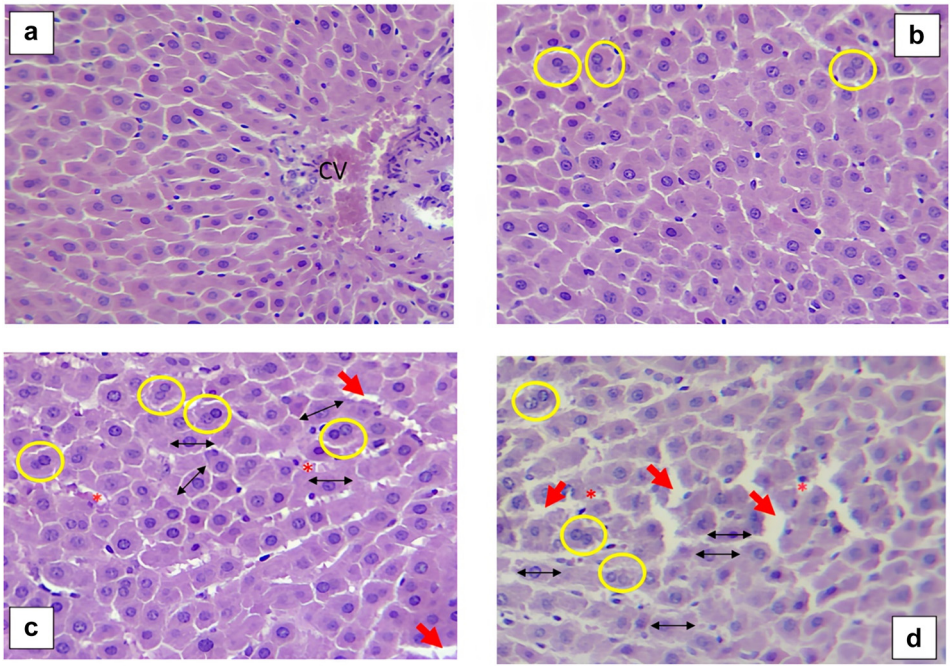


Figure 6: Effect of GTCE on histopathological changes in the liver. Controls (a), treated with 400 mg/kg GTCE (b); treated with 600 mg/kg (c), and treated with 800 mg/kg GTCE (d) after 28 days of treatment: sections were stained using the hematoxylin–eosin method; magnification: ×100. CV: centrilobular vein. Red arrow: sinusoid dilation and congestion, yellow circle: binucleated hepatocytes, black double arrow: parenchymal cells hypertrophy, red asterisk: vacuolar degeneration.

Relative organ weights are an essential part of the toxicological risk assessment of drugs, chemicals, and biologics [43]. The results of the present study reveal that GTCE at doses of 800 mg has a negative impact on the liver, resulting in an increase in the absolute and relative liver weights. The observed increase in organ weights can be attributed to an increase in hepatic metabolism following high doses administered. Here, it has been reported that GTCE at doses > 800 mg or at repeated doses causes liver damage [35].

Our results show that treatment with GTCE induces a significant decrease ($p < 0.05$) in the concentration of cholesterol in rats treated with a dose of 800 mg/kg. According to Sánchez-Muniz et al. [44], the decrease in cholesterol concentration is attributed to a reduction in the activity of lecithin cholesterol acyl transferase (LCAT), which is itself due to the reduction in the biosynthesis of HLVs at the liver level. On the other hand, Holloway and Rivers [45] explained that the decrease in cholesterol is due to the activation of 7-α-hydrolase, an enzyme responsible for the transformation of cholesterol into bile acids. The same phenomenon was observed after treatment with 400 mg/kg nigra leaf extract in female Wistar rats, resulting in decreased cholesterol and high-density lipoprotein (HDL) [46]. These results proved that polyphenols have a hypolipidemic effect and induce a decrease in chronic damage in rats. In contrast, rats treated with 400 mg/L resulted in an increase in the plasma content of insignificant triglyceride ($p > 0.05$). The increase in triglyceride content was reported by Yuan and Kitts [47]. They speculated that the supplementation of 130 g of phenolic extracts in non-fasting rat food promotes increased triglyceride concentration because phenolic extracts tend to increase the concentration of triglycerides in comparison with controls. This effect was not predicted, and they assumed that phenolic (catechins) extracts slowed the kinetics of lipid absorption, or exposed to a lipotropic action on the liver.

The assessment of the cytotoxic effect of GT catechins and the hypothesis of a pro-oxidizing effect on body growth, liver, and serum lipids in growing female rats were investigated. Additionally, growth retardation was observed in all rats treated with polyphenols relative to controls regardless of catechins at doses 400, 600, and 800 mg/kg. Previous studies conducted on rodents showed a decrease in lipid liver accumulation, particularly triglycerides and cholesterol following tea consumption [48]. In addition, in a double-blind, placebo-controlled clinical trial, it was reported that high doses of EGCG (856.8 mg/day) reduced plasma cholesterol and LDL levels [49].

However, the mechanisms involved in this decline in liver lipids are still far from clear. Indeed, it has been shown that tea decreases the genes involved in lipogenesis and increases those involved in oxidation, but the precise mechanisms involved in this regulation are not clearly described. At the cellular level, several studies have examined the lipotropic effect of GT catechins, particularly EGCG, but few studies have investigated the lipotropic effects of a global tea extract, let alone a mixture of several teas [50,51]. Some studies suggest that EGCG directly influences lipid metabolism by inhibiting intestinal lipid absorption, increasing LDL receptor expression, and activating AMPK, which promotes fatty acid oxidation [17,18]. However, at high doses, EGCG has been associated with liver failure [19,20], which may indirectly affect the lipid levels. Liver damage can alter lipid metabolism due to disrupted hepatic function, leading to reduced cholesterol and triglyceride levels.

Oxidative stress can advance oxidative damage involving cellular proteins, lipids, DNA, and other molecules in a way that could lead to abnormal cell functions. The results obtained in our study for the oxidative stress portion showed a well-defined increase in the liver tissue MDA concentration in rats treated with the catechins at a dose of 800 mg/L compared to rats treated with 400 and 600 mg/L and normal control. MDA is the most widely used parameter for assessing oxidative lipid damage, although it is known that oxidative damage to amino acids, proteins, and DNA also results in MDA release [52]. The observed increase in the liver MDA confirmed the hepatotoxic effect of GTCE at higher doses. This study shows that GTCE causes a significant decrease in GSH levels in the rat liver. The significant depletion of GSH levels is associated with a decrease in GPx activity ($P < 0.05$). In this context, several studies reported the damaging effect of EGCG at higher doses on hepatic tissues *in vivo* and *in vitro* [24,53,16]. Regarding tissue aberrations, the hepatic tissue sections demonstrated the presence of vacuolization, binucleated cells, and prominent congestion and sinusoidal dilatation, which notably for groups D3 and D2, was previously regarded as a side effect of cytotoxic factor activity. Its accumulation is now recognized as a critical initiating event that triggers metabolic disruptions or stress responses, ultimately resulting in cell death [32]. The binucleate cells are frequently observed in several organs, such as the liver, salivary glands, and endometrium, although their functional significance remains unclear. The heightened presence of binucleate hepatocytes during the necro-inflammatory phase of progressive chronic hepatitis and in cirrhosis, coupled with their absence in hepatocellular carcinoma, suggests that they might serve as an indicator of hepatic disease severity rather than arising from cell cycle errors [54]. Sinusoidal dilatation is primarily characterized by the enlargement of hepatic sinusoids, typically identified as a prominent histopathological feature. Initially considered

an artifact or nonspecific change, growing evidence suggests that sinusoidal dilatation is linked to inflammatory diseases, portal hypertension and vascular disorders [55]. Hypertrophy refers to the enlargement of cells due to increased synthesis of structural components, rather than the accumulation of water, glycogen, or lipids. When a significant number of cells grow in size, this can lead to a noticeable increase in tissue or organ mass. Exposure to high levels of toxic metabolites induces various tissue changes. In the liver, these include hepatocyte hypertrophy, hepatitis, hepatocellular necrosis, and hepatocellular death [56]. Effectively, previous findings suggest that induced oxidative stress in liver hepatocytes has increased bile duct hyperplasia, as hepatocytes surrounding the bile ducts display positive signals to a significant extent [57].

5 Conclusion

The chemical composition analysis of GTCE using LC-MS identified EGCG as the most abundant component. Catechins demonstrated cytotoxic effects on lipid parameters, leading to a reduction in lipid accumulation, particularly triglycerides and cholesterol. However, the mechanisms underlying this lipid reduction in the liver remain poorly understood. The findings of our study indicate that high doses of GTCE induce hepatotoxicity, characterized by elevated oxidative stress, histological alterations, and, in severe cases, the death of some rats.

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References

[1] Li YH, Wu Y, Wei HC, Xu YY, Jia LL, Chen J, et al. Protective effects of green tea extracts on photoaging and photommunosuppression. Skin Res Technol. 2009;15(3):338–45.
[2] Siddiqui IA, Asim M, Hafeez BB, Adhami VM, Tarapore RS, Mukhtar H. Green tea polyphenol EGCG blunts androgen receptor function in prostate cancer. FASEB J. 2011;25(4):1198–207.
[3] Bose A. Interaction of tea polyphenols with serum albumins: A fluorescence spectroscopic analysis. J Lumin. 2016;169:220–6.
[4] Rubab S, Rizwani GH, Bahadur S, Shah M, Alsamadany H, Alzahrani Y, et al. Determination of the GC–MS analysis of seed oil and assessment of pharmacokinetics of leaf extract of Camellia sinensis L. J King Saud Univ-Sci. 2020;32(7):3138–44.
[5] Sang S, Tian S, Wang H, Stark RE, Rosen RT, Yang CS, et al. Chemical studies of the antioxidant mechanism of tea catechins: Radical reaction products of epicatechin with peroxyl radicals. Bioorg Med Chem. 2003;11(16):3371–8.
[6] Kaya-Dagistanli F, Tanriverdi G, Altin A, Ozyazgan S, Ozturk M. The effects of alpha lipoic acid on liver cells damages and apoptosis induced by polyunsaturated fatty acids. Food Chem Toxicol. 2013;53:84–93.
[7] Thawonsuwan J, Kiron V, Satoh S, Panigrahi A, Verlhac V. Epigallocatechin-3-gallate (EGCG) affects the antioxidant and immune defense of the rainbow trout, Oncorhynchus mykiss. Fish Physiol Biochem. 2010;36(3):687–97.
[8] Gan RY, Li HBin, Sui ZQ, Corke H. Absorption, metabolism, anticancer effect and molecular targets of epigallocatechin gallate (EGCG): An updated review. Crit Rev Food Sci Nutr. 2018 Apr;58(6):924–41.
[9] Sano M, Tabata M, Suzuki M, Degawa M, Miyase T, Maeda-Yamamoto M. Simultaneous determination of twelve tea catechins by high-performance liquid chromatography with electrochemical detection. Analyst. 2001;126(6):816–20.
[10] Hu J, Webster D, Cao J, Shao A. The safety of green tea and green tea extract consumption in adults–results of a systematic review. Regul Toxicol Pharmacol. 2018;95:412–33.
[11] Tomás-Barberán FA, Andrés-Lacueva C. Polyphenols and health: Current state and progress. J Agric Food Chem. 2012;60(36):8773–5.
[12] Cao SY, Zhao CN, Gan RY, Xu XY, Wei XL, Corke H, et al. Effects and mechanisms of tea and its bioactive compounds for the prevention

and treatment of cardiovascular diseases: An updated review. Antioxidants. 2019;8(6):166.
[13] Wu CH, Lu FH, Chang CS, Chang TC, Wang RH, Chang CJ. Relationship among habitual tea consumption, percent body fat, and body fat distribution. Obes Res. 2003;11:1088–95. North American Assoc for the Study of Obesity.
[14] Iso H, Date C, Wakai K, Fukui M, Tamakoshi A, Mori M, et al. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med. 2006 Apr;144(8):554–62.
[15] El-Bakry HA, El-Sherif G, Rostom RM. Therapeutic dose of green tea extract provokes liver damage and exacerbates paracetamol-induced hepatotoxicity in rats through oxidative stress and caspase 3-dependent apoptosis. Biomed Pharmacother. 2017;96:798–811.
[16] Ouyang J, Zhu K, Liu Z, Huang J. Prooxidant effects of epigallocatechin-3-gallate in health benefits and potential adverse effect. Oxid Med Cell Longev. 2020;2020(1):9723686.
[17] Raederstorff DG, Schlachter MF, Elste V, Weber P. Effect of EGCG on lipid absorption and plasma lipid levels in rats. J Nutr Biochem. 2003 Jun;14(6):326–32. [cited 2025 Mar 14] https://pubmed.ncbi.nlm.nih.gov/12873714/.
[18] Li K, Zhou X, Yang X, Shi X, Song X, Ye C, et al. Subacute oral toxicity of cocoa tea (Camellia ptilophylla) water extract in SD rats. Int J Food Sci Technol. 2015 Nov;50(11):2391–401.
[19] Grajecki D, Ogica A, Boenisch O, Hübener P, Kluge S. Green tea extract-associated acute liver injury: Case report and review. Clin Liver Dis. 2022 Dec;20(6):181–7. [cited 2025 Mar 14]. https://pubmed.ncbi.nlm.nih.gov/36523867/.
[20] Wang X, Yang L, Wang J, Zhang Y, Dong R, Wu X, et al. A mouse model of subacute liver failure with ascites induced by step-wise increased doses of (-)-epigallocatechin-3-gallate. Sci Rep . 2019 Dec;9(1):1–9. [cited 2025 Mar 14] https://www.nature.com/articles/s41598-019-54691-0.
[21] Bedrood Z, Rameshrad M, Hosseinzadeh H. Toxicological effects of Camellia sinensis (green tea): A review. Phyther Res. 2018 Jul;32(7):1163–80.
[22] Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, et al. Scientific opinion on the safety of green tea catechins. EFSA J. 2018;16(4):e05239. doi: 10.2903/j.efsa.2018.5239.
[23] Molinari M, Watt KDS, Kruszyna T, Nelson R, Walsh M, Huang WY, et al. Acute liver failure induced by green tea extracts: Case report and review of the literature. Liver Transplant. 2006 Dec;12(12):1892–5. journals.lww.com.
[24] Wang D, Wang Y, Wan X, Yang CS, Zhang J. Green tea polyphenol (-)-epigallocatechin-3-gallate triggered hepatotoxicity in mice: Responses of major antioxidant enzymes and the Nrf2 rescue pathway. Toxicol Appl Pharmacol. 2015;283(1):65–74.
[25] Amrouche Z, El-Hadi D, Fauconnier ML, Benrima-Guendouz A, Bitam A. Oxidative stress of bioactive molecules antioxidant (EGCG) polyphenols extract of green tea (Camellia sinensis L.). AgroBiologia. 2018;8(1):727–34, http://agrobiologia.net/editions/AGROBIOLOGIAVOL-8-N-01_final.pdf.
[26] Amrouche Z, Blecker C, Laribi-Habchi H, Fauconnier ML, El-Hadi D. Antioxydant activity, oxidative stability properties of Colza oil, comparison of mechanical agitated and ultrasonic extraction on green tea catechins of Camellia sinensis L. Algerian J Environ Sci Technol. December edition. 2021;7:2167–76, www.aljest.org.
[27] Lin LZ, Chen P, Harnly JM. New phenolic components and chromatographic profiles of green and fermented teas. J Agric Food Chem. 2008 Sep;56(17):8130–40.
[28] Tichati L, Trea F, Ouali K. The antioxidant study proprieties of Thymus munbyanus aqueous extract and its beneficial effect on 2, 4-Dichlorophenoxyacetic acid -induced hepatic oxidative stress in albino Wistar rats. Toxicol Mech Methods. 2021;31(3):212–23. [cited 2025 Mar 14] https://pubmed.ncbi.nlm.nih.gov/33371761/.
[29] Lekouaghet A, Boutefnouchet A, Bensuici C, Gali L, Ghenaiet K, Tichati L. In vitro evaluation of antioxidant and anti-inflammatory activities of the hydroalcoholic extract and its fractions from Leuzea conifera L. roots. South Afr J Botany. 2020;132:103–7.
[30] Buege JA, Aust SD, Biomembranes -, Part C. Biological oxidations. Methods Enzymol. 1978;52:302–10. http://www.sciencedirect.com/science/article/pii/S0076687978520326.
[31] Khattabi L, Khaldi T, Bahri L, Mokhtari MB, Bouhenna MM, Temime A, et al. Insights about the deleterious impact of a carbamate pesticide on some metabolic immune and antioxidant functions and a focus on the protective ability of a Saharan shrub and its anti-edematous property. Open Chem. 2024;22(1):20240022.
[32] Khattabi L, Chettoum A, Hemida H, Boussebaa W, Atanassova M, Messaoudi M. Pirimicarb induction of behavioral disorders and of neurological and reproductive toxicities in male rats: Euphoric and Preventive effects of Ephedra alata Monjauzeana. Pharmaceuticals. 2023;16(3):402.
[33] Flohé L, Günzler WA. Assays of glutathione peroxidase. In: Methods in Enzymology. Vol. 105, Elsevier; 1984. p. 114–20. doi: 10.1016/S0076-6879(84)05015-1.
[34] Houlot R. Techniques d'histologie et de cytologie. Edition Ma. 1984;186:1–85.
[35] Oketch-Rabah HA, Roe AL, Rider CV, Bonkovsky HL, Giancaspro GI, Navarro V, et al. United States Pharmacopeia (USP) comprehensive review of the hepatotoxicity of green tea extracts. Toxicol Rep. 2020;7:386–402.
[36] Yang CS, Hong J. Prevention of chronic diseases by tea: Possible mechanisms and human relevance. Annu Rev Nutr. 2013 Jul;33:161–81.
[37] Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate. Cancer Res. 2006;66(5):2500–5. doi: 10.1158/0008-5472.CAN-05-3636.
[38] Tang GY, Zhao CN, Xu XY, Gan RY, Cao SY, Liu Q, et al. Phytochemical composition and antioxidant capacity of 30 chinese teas. Antioxidants. 2019;8(6):180.
[39] Giunta B, Hou H, Zhu Y, Salemi J, Ruscin A, Shytle RD, et al. Fish oil enhances anti-amyloidogenic properties of green tea EGCG in Tg2576 mice. Neurosci Lett. 2010;471(3):134–8.
[40] Ezeja MI, Anaga AO, Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala in rats. J Ethnopharmacol. 2014;151(3):1155–64.
[41] El Kabbaoui M, Chda A, El-Akhal J, Azdad O, Mejrhit N, Aarab L, et al. Acute and sub-chronic toxicity studies of the aqueous extract from leaves of Cistus ladaniferus L. in mice and rats. J Ethnopharmacol. 2017;209:147–56.
[42] Huang YW, Liu Y, Dushenkov S, Ho CT, Huang MT. Anti-obesity effects of epigallocatechin-3-gallate, orange peel extract, black tea extract, caffeine and their combinations in a mouse model. J Funct Foods. 2009;1(3):304–10.
[43] Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, et al. Society of Toxicologic Pathology position paper: Organ weight recommendations for toxicology studies. Toxicol Pathol. 2007;35:751–5.

[44] López-Varela S, Sánchez-Muniz FJ, Cuesta C. Decreased food efficiency ratio, growth retardation and changes in liver fatty acid composition in rats consuming thermally oxidized and polymerized sunflower oil used for frying. Food Chem Toxicol. 1995;33(3):181–9.
[45] Holloway D, Rivers JM. Influence of chronic ascorbic acid deficiency and excessive ascorbic acid intake on bile acid metabolism and bile composition in the guinea pig. J Nutr. 1981;111(3):412–24.
[46] Volpato GT, Calderon IMP, Sinzato S, Campos KE, Rudge MVC, Damasceno DC. Effect of Morus nigra aqueous extract treatment on the maternal–fetal outcome, oxidative stress status and lipid profile of streptozotocin-induced diabetic rats. J Ethnopharmacol. 2011 Dec;138(3):691–6.
[47] Yuan YV, Kitts DD. Dietary (n-3) fat and cholesterol alter tissue antioxidant enzymes and susceptibility to oxidation in SHR and WKY rats. J Nutr. 2003;133(3):679–88.
[48] Axling U, Olsson C, Xu J, Fernandez C, Larsson S, Ström K, et al. Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. Nutrition Metab. 2012;9:1–18.
[49] Chen IJ, Liu CY, Chiu JP, Hsu CH. Therapeutic effect of high-dose green tea extract on weight reduction: A randomized, double-blind, placebo-controlled clinical trial. Clin Nutr. 2016 Jun;35(3):592–9. [cited 2025 Mar 14] https://pubmed.ncbi.nlm.nih.gov/26093535/.
[50] Kim JJ, Tan Y, Xiao L, Sun YL, Qu X. Green tea polyphenol epigallocatechin-3-gallate enhance glycogen synthesis and inhibit lipogenesis in hepatocytes. BioMed Res Int. 2013;2013(1):920128.
[51] Goto T, Saito Y, Morikawa K, Kanamaru Y, Nagaoka S. Epigallocatechin gallate changes mRNA expression level of genes involved in cholesterol metabolism in hepatocytes. Br J Nutr. 2012;107(6):769–73.
[52] Sundaram R, Naresh R, Shanthi P, Sachdanandam P. Modulatory effect of green tea extract on hepatic key enzymes of glucose metabolism in streptozotocin and high fat diet induced diabetic rats. Phytomedicine. 2013;20(7):577–84.
[53] Kucera O, Mezera V, Moravcova A, Endlicher R, Lotkova H, Drahota Z, et al. In vitro toxicity of epigallocatechin gallate in rat liver mitochondria and hepatocytes. Oxid Med Cell Longev. 2015;2015:476180.
[54] Grizzi F, Chiriva-Internati M. Human binucleate hepatocytes: Are they a defence during chronic liver diseases? Med Hypotheses. 2007;69:258–61.
[55] Shen H, Dong J, Xia L, Xu J, Xu L. Rat liver sinusoidal dilatation induced by perfusion in vitro through portal vein alone, hepatic artery alone, and portal vein together with hepatic artery. Int J Clin Exp Med. 2016;9(6):11284–91.
[56] Ozcelik D, Ozaras R, Gurel Z, Uzun H, Aydin S. Copper-mediated oxidative stress in rat liver. Biol Trace Elem Res. 2003;96:209–15.
[57] Lee YH, Lim CH, Chung YH. Oxidative stress and sex difference in liver of rats exposed to cyclohexanone. Int J Morphol. 2018;36(3):881–5.