Polyphenols from Green tea revert CCl₄-Induced liver oxidative stress and inflammation associated with high fat diet by activation of NRF-2 and TIMP-1 pathways to neutralize ROS

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Research Article

Keywords: Camellia sinensis, Catechins, Carbon tetrachloride, Oxidative stress, Inflammatory cytokines, Liver fibrosis

Posted Date: March 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1414824/v1>

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Abstract

Hepatic disorders are progressed by oxidative stress, chronic inflammation, hepatocyte death and increased transforming growth factor. Herein we investigated the phytochemical screening of green tea (*Camellia sinensis*) and analyzed its anti-oxidant and anti-inflammatory potential on liver biomarkers that elaborate therapeutic efficacy of polyphenols (catechins) in rat model associated with CCl₄ induced liver injury along high fat diet. Pro-inflammatory cytokines are stimulated by either acute or chronic liver injury and critically triggered the production of inflammation, steatosis, fibrosis and liver carcinoma. We designed HPLC work to isolate the green tea phytochemical constituents quantitatively in a remarkable therapeutic range. Anti-inflammatory and antioxidant activities were carried out in vivo using treatment with green tea polyphenol extract (GTPE 600 mg/kg) that significantly reduced ($P < 0,001$) pro-inflammatory cytokines like tumor necrosis factor α (TNF α) and interleukin 6 (IL-6) in CCl₄-induced hepatic injury in Wistar albino rats. Hepatoprotective potential of GTPE was evaluated through biochemical parameters (complete blood count, liver functional tests and lipid profile), oxidative stress biomarkers (MDA, CAT, GPx and SOD) as well as histopathological analysis. Study of NRF-2 and TIMP-1 pathways through qRT-PCR provided strategy to delay the progression of liver injury into liver fibrosis by neutralizing reactive oxygen species and reverting inflammatory cytokines with the help of GTPE in therapeutic dose. Pharmacological confirmation of current study indicated that green tea possessed incredible anti-oxidative and anti-inflammatory properties, suggesting that polyphenols providing active components (catechins) of crude extract.

Introduction

In recent years, phytotherapy acts as a novel methodology with minimized side effects. Nutraceuticals from plant origin have become a potential source for restorative therapy (Daliu et al. 2019). After water the second most used drink all over the world is tea (Wei et al. 2012). It possesses an immense amount of phytoconstituents like catechins, proteins, carbohydrates, vitamins and minerals (Noormandi et al. 2015). The word “tea” alludes to green tea (GT) in China. Yunnan province of China is the indigenous source of various species of **sinensis**. Suitable region for best development of *Camellia sinensis* (family Theaceae, locally called tea plant, tea shrub and tea tree) is tropical and subtropical regions with sufficient precipitation, slightly acidic soil and proper seepage (Anand et al. 2015).

Fatty liver, in which fats accumulate inside the liver cells, that results in cell steatosis and sometimes steatohepatitis, and can further convert into cirrhosis through fibrosis. Cirrhosis is considered to be constitute one-third of worldwide deaths (Higashi et al. 2017). Fibrotic disease related deaths are estimated to be 45% in the developed countries. It has been estimated that world widely 6% of the adults in developed countries have non-alcoholic steatohepatitis with up to 40% of these individuals progressing to advanced fibrosis (Abeysekera et al. 2020). Liver fibrosis is the activation of HSC and its trans-separation into myofibroblast-like cells. HSC activation is started by development factors and fibrogenic cytokines discharged by activated epithelial cells, including cholangiocytes, hepatocytes and inflammatory cells, among them the transforming growth factor beta 1 (TGF β 1) is the key controller

(Ragab et al. 2019). Abnormal fat accumulation due to high fat diet results in a production of various metabolic diseases (Karczewski et al. 2018). Extra macronutrients, inflammatory agents like IL-6 and TNF- α and suppressed production of adiponectin, prompt to oxidative stress and inflammation (Bagherniya et al. 2022).

Toxic dose of carbon tetrachloride (CCl₄) metabolized into trichloromethyl radical (CCl₃•) in the presence of cytochrome P450. This radical is not only react with lipids, proteins and nucleic acid but also disrupt the cellular mechanisms to lead alteration in lipid metabolism, increased production of ROS and decreased efficiency of antioxidant defendants and build oxidative stress that result in chronic liver diseases (Scholten et al. 2015). Moreover, excessive production of free radicals leads to predisposition of inflammatory cytokines with activation of TGF β 1 in such critical injuries (Hassan et al. 2021).

Green tea possesses an amazing range of pharmacological and phytotherapeutical advantages, including anti-inflammatory, antioxidative, hypolipidemic and anticancer activities due to the presence of flavonoids and other polyphenols. Prolong intake of tea catechins, decreases the risk of liver disease and could be beneficial against inflammation, oxidative stress, steatosis, fibrosis and high-fat diet-induced obesity (Azzahra et al. 2012).

Conventional and current anti-inflammatory drug (steroids and NSAIDs) are synthetic drugs. To overcome their reported drug related toxicities and adverse effects, newer drugs from herbal sources with little or no side effects are needed to synthesize against chronic inflammation (Ma et al. 2020; Pahwa et al. 2020). Considering the medicinal properties, current study was aimed to evaluate the phytochemical screening and analyze the anti-inflammatory and antioxidant potential of green tea polyphenol extract on liver biomarkers.

Materials And Methods

Collection of plant

Green tea (*Camellia sinensis*) was collected from the Botanical Garden and identified under the voucher number 1218-21-03 by the head of Botany Department of University of Agriculture, Faisalabad, Pakistan and plant name was checked with <http://www.theplantlist.org>.

Preparation of GTPE

Distilled boiled water was added to crudely crush dried green tea leaves in the ratio 10:1 (with periodically stirring to deactivate enzymes). Whatman filter paper was used to collect the filtrate (repeated three times). At 42°C, concentrate the green tea solution by hot air oven. Equal volume of dichloromethane used to decaffeinate the filtrate. Ethyl acetate and minute amount of ascorbic acid were added to prevent oxidation. Separating funnel was used to separate polyphenols (Bharadwaz and Bhattacharjee 2012).GTPE was freeze dried and stored for further analysis.

Estimation of catechins by UV-Vis spectroscopy

10 g of powdered leaves of green tea was added to of distilled water (100 ml) at 100°C temperature and stirred with magnetic stirrer for 1h and then filtered. Equal volume of chloroform was used to wash the tea infusion in a separator funnel to remove pigments, caffeine and other non-polar impurities. This washing was repeated five times, the absorbance of the aqueous phase obtained was taken at maximum wavelength (230 nm). The *absorption spectra* of pure catechins and catechins in green tea were measured using Multiskan Sky with touchscreen and µDrop Plate with wavelength range 200-1000 nm by using UV-visible spectrometer. 1 ml from the stock solution was added into a 10 ml volumetric flask and made up to the mark with distilled water to produce 10 µg/ml solution (Fig 3.6). This was then scanned in the spectrophotometer through range wavelengths (240 – 400 nm) to obtain the wavelength of maximum absorption (Ibrahim et al. 2017).

Characterization of green tea by HPLC

Green tea extract was prepared with slight modifications according to the method of (He et al. 2015) and stored at 4°C in darkness. C (99% purity), EC (99.5% purity), ECG (97% purity), EGC (95% purity), EGCG (99% purity), GC (95.5% purity), and caffeine (97% purity), these reference compounds were purchased from Sigma Chemical Co. For the detection of bioactive compounds, UV–visible detector was used at the wave-length of 210 nm (Wangkarn et al. 2021). A HPLC system equipped with LC-10AT pump, ultraviolet detector model SPD-10AV (Shimadzu Europa GmbH, Duisburg, Japan) and Shim-Pack CLC-ODS (C-18), 25 cm × 4.6 mm, 5 µm column were used for characterization.

Experimental animals

The recent study was carried out on thirty-two (32) female wistar albino rats, weighing 140–200 g. They were acclimatized for period of two weeks before the initiation of experiment. Normal basal diet and tap water *ad libitum* were given under controlled condition (23 ± 2 °C) and 12:12 h light-dark cycle. The experiment was conducted with preliminary approval by the Directorate of Research and Advance Studies and with consent of Bioethics Committee, University of Agriculture, Faisalabad under issued ethical certificate wide letter no. 1720/ORIC.

Induction of liver fibrosis and experimental layout

The animals were randomly divided into four groups (n=8) after adaptation period. Group 1: Normal Control (Normal diet only); Group 2: Positive Control (High-fat diet+ CCl₄); Group 3: Standard (High-fat diet + CCl₄ + **Silymarin** 200 mg/kg); Group 4: GTPE (High-fat diet + CCl₄ + Green tea polyphenols 600 mg/kg). The CCl₄/olive oil (3 ml per 1000g body weight) induced liver injury via intraperitoneal (i.p.) injection once after 5 days, only 2 doses of CCl₄ were countable to induce toxicity. Treatment was started along 2nd dose of CCl₄ and given on daily basis. At the end of experiment the rats were decapitated by anesthetic overdose and samples of blood and liver were collected. Biopsy samples were preserved in 10% formalin solution for histopathological studies (Huang et al. 2012).

Measurement of biochemical factors

Blood samples were centrifuged for 10 min at 4000 rpm and resultant serum was stored at -80° C for biochemical investigations. Hemoglobin, total count of red blood corpuscles, total count of white blood cells and platelets were analyzed with hematology auto analyzer MICROS-60 France, as per manufacturer's instructions. Serum samples were analyzed for liver function tests and lipid profile (in order to assess any abnormality in functioning of liver) by following method of Parasuraman et al. (2010).

Determination of antioxidant enzymes

Liver tissue was homogenized by using ice-cold PBS, pH 7.1. Fluctuations were monitored in anti-oxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) by following method of (Popov et al. 2003). Malondialdehyde (MDA) was determined through thiobarbituric acid assay by using colorimetric technique, following Bio-diagnostic kits guidelines (Cusabio Biotech Co., Ltd.).

Estimation of liver inflammatory cytokines

The serum levels of TNF- α and IL-6 (cat. no. CSB-E04639m) were determined by using ZELLBIO GmbH ELISA kit (Germany) according to the manufacturer's instructions. The cytokine assays were performed in duplicate.

Histopathological examination

Animals were decapitated on 28th day of research trial. Liver tissue samples were collected and added in 10% buffering solution of formalin and store for overnight at 4°C for histopathological analysis by following the method of Huang et al. 2012.

Quantitative real-time PCR assay

Quantitative real-time PCR analysis was performed by using Maxima SYBR Green and Thermo Fisher kit (Majeed et al. 2021). Expressions of NRF-2 and TIMP-1 were studied. Beta actin was acted as a housekeeping gene and internal control. Therefore, genes were amplified by using oligonucleotides (Primers) sequence that mentioned as following:

NRF-2F TCCCGAATGGAACCGAGACT

NRF-2R TTCATCCACGGGAAAGGGAG

TIMP-1F ACAGCTTTCTGCAACTCG

TIMP-1R CTATAGGTCTTTACGAAGGCC

All reactions were carried in triplicates. Following formula was used to analyze relative gene expression

Statistical analysis

Analysis of results was tabulated as mean ± SEM along statistically applied one-way analysis of variance (ANOVA) by using graph pad prism. Tukey’s multiple comparisons test was followed in case of significant differences among control, positive, standard and treated I groups ($P \leq 0.001$).

Results

Estimation of GTPE by UV-Vis spectroscopy

UV-Vis spectrophotometer used to measure absorbance verses wavelength of each solution of green tea polyphenol extract in distilled water. The UV absorption spectrum of green tea polyphenol extract with different concentrations recorded at room temperature (Fig. 1) revealed maximum absorption at 230 nm when scanned between 200 nm and 400 nm.

Isolation and characterization of green tea phytoconstituents

In order to isolate and characterize the major constituents of green tea, we performed High Pressure Liquid Chromatography (HPLC) of green tea aqueous extract. The observed result has been revealed the retention time of different components of green tea polyphenol at 210 nm presented in Fig. 2. The retention order of the green tea constituents was found to be 1: Gallic acid; 2: GC; 3: EGC; 4: caffeine; 5: EC; 6: EGCG; 7: C; 8: GCG; 9: ECG (Table. 1).

Table 1: HPLC report of phytoconstituents present in the aqueous extract of Green tea

Sr. No.	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.890	516.848	6.222	5.5	2.1	1.47
2	4.270	1140.124	33.929	12.1	11.2	0.56
3	4.580	1761.601	67.890	18.7	22.4	0.32
4	5.660	2152.136	70.286	22.8	23.2	0.39
5	7.340	448.911	11.109	4.8	3.7	0.58
6	8.540	978.070	42.225	10.4	14.0	0.34
7	9.490	1324.221	58.849	14.0	19.5	0.34
8	10.610	175.468	4.278	1.9	1.4	0.88
9	12.080	465.950	4.051	4.9	1.3	1.71

Effect of GTPE on plasma biochemical markers

In CCl₄ induced liver injury along high fat diet model, the effect of GTPE on selected biochemical parameters in current study was represented in Table 2, 3 and 4. Treatment with GTPE (600mg/kg) became responsible for depletion in ALT, AST, total bilirubin, total cholesterol, triglycerides, low density lipoprotein and white blood cells levels and raise the levels of albumin, high density lipoprotein, RBCs, Hb, and platelets as compare to the positive control (PC) group (P < 0.001).

Table 2. The effect of GTPE on CCl₄-treated + high fat diet induced alterations in serum hepatic enzymes in female albino rats

Parameters	Normal Control	Positive Control	Standard	GTPE
ALT (U/L)	34.875 ± 2.467d	90.750 ± 2.963a	38.125 ± 2.409c	47.125 ± 1.540bc
AST (U/L)	288.625 ± 24.156cd	677.750 ± 43.619a	291.500 ± 24.463cd	369.625 ± 17.037bc
Total Proteins (mg/dl)	6.413 ± 0.152a	4.738 ± 0.287c	5.725 ± 0.198b	5.388 ± 0.156b
Total Bilirubin (mg/dl)	0.621 ± 0.023bc	0.896 ± 0.024a	0.636 ± 0.031bc	0.700 ± 0.033b

Data were expressed as means ± SEM. Based on ANOVA and Tukey's test, Positive Control group showed significant difference as compared to Normal, Standard and GTPE groups (P<0.001). ALT = Alanine Transaminase, AST = Aspartate Transaminase

Table 3. The effect of GTPE on CCl₄-treated + high fat diet induced alterations in serum lipid profile in female albino rats

Parameters	Normal Control	Positive Control	Standard	GTPE
Total Cholesterol (mg/dl)	47.625 ± 4.049b	49.625 ± 4.170a	47.750 ± 3.342b	48.750 ± 3.599ab
Triglyceride (mg/dl)	38.425 ± 2.845bc	55.125 ± 2.594a	45.000 ± 2.745b	51.875 ± 2.271ab
LDL (mg/dl)	20.338 ± 3.535bc	39.625 ± 4.480a	25.625 ± 2.725b	28.500 ± 3.391b
HDL (mg/dl)	27.350 ± 2.545a	25.550 ± 1.546b	27.188 ± 2.116a	27.115 ± 0.946a

Data were expressed as means ± SEM. Based on ANOVA and Tukey's test, Positive Control group showed significant difference as compared to Normal, Standard and GTPE groups (P<0.001). HDL = High-Density Lipoproteins, LDL = Low-Density Lipoproteins

Table 4. The effect of GTPE on CCl₄-treated + high fat diet induced alterations in complete blood count in female albino rats

Parameters	Normal Control	Positive Control	Standard	GTPE
RBCs(10 ⁶ /μL)	4.835 ± 0.106a	3.331 ± 0.288c	4.475 ± 0.250ab	4.106 ± 0.193b
WBCs(10 ³ /μL)	8.950 ± 0.761c	15.513 ± 0.553a	10.238 ± 0.753b	12.175 ± 0.542b
Hb (g/dl)	10.350 ± 0.453a	7.688 ± 0.330c	9.550 ± 0.377ab	9.275 ± 0.366ab
Platelets Count (10 ⁵ /μL)	3.943 ± 0.228a	2.786 ± 0.273c	3.800 ± 0.181a	3.513 ± 0.129ab

Data were expressed as means ± SEM. Based on ANOVA and Tukey's test, Positive Control group showed significant difference as compared to Normal, Standard and GTPE groups (P<0.001). RBC's = Red Blood Cells, WBC's = White Blood Cells, Hb = *Hemoglobin*

Effect of GTPE on oxidative stress factors

Toxic dose of CCL₄ and high fat diet promote inhibition of antioxidant NRF-2 signal pathway due to oxidative liver injury. We analyzed levels of CAT, SOD, and GPx in PC group and compared them with NC group having healthy rats showed a significant reduction in their levels due to the elicitation of oxidative stress (P < 0.001). However, treatment with 600 mg/kg GTPE could optimized the action of CAT, SOD, and GPx as compared to PC group (Fig. 3. A, B and C). In case of MDA, GTPE treatment reduced the occurrence of lipid peroxidation (reduction in MDA level). Moreover, silymarin also elevated the antioxidant activity of CAT, SOD, and GPx and reduced the induction of lipid peroxidation in standard group (Fig.3. D).

Effect of GTPE on inflammatory markers

The serum levels of TNF-α and IL-6 were analyzed by comparing positive group with others. 600mg/kg dose of GTPE exhibited a reduction in serum level of inflammatory markers in treated I group after induction of liver oxidative stress and inflammation, which was similar to standard group (silymarin treated) shown in fig. 4 A & B.

Effect of GTPE on expression level of NRF-2 and TIMP-1

Green tea exerts its hepatoprotective effects by modulating NRF-2 and TIMP-1 signal pathways. Liver mRNA expression of NRF-2 was considerably up-regulated in standard and GTPE groups compared to the positive control group (Fig. 5. A). On the contrary, liver mRNA expression of TIMP-1 was down-regulated in standard and GTPE groups as compared to the positive control group (Fig. 5. B).

Histopathological status of liver tissue related to GTPE

Histopathological examination of liver tissue displayed the irregular surface area, indicating CCl₄-induced hepatocellular impairment in PC group such as vacuolization of cells, expansion of dissepimental space, increased percentage of tissue necrosis and fibrosis as compared to NC group. However, GTPE treated group with dose 600 mg/kg to minimize the process of necrosis and suppress the action of oxidative stress. GTPE group can be compared with standard group in case of effectiveness (Fig. 6).

Discussion

On the basis of diet and genetics, several animal models indicating features of non-alcoholic steatohepatitis due to administration of CCl₄ and high fat diet (HFD), which resulted in liver fibrosis due to chronic inflammation and oxidative stress (Kubota et al. 2013). Current research aimed to introduce a liver fibrosis along fatty liver in four weeks showing timely alterations by following two strategies, first fed on HFD to cause fatty liver and secondly administered CCl₄ in toxic dose (3ml/kg = 1.5ml CCl₄ plus 1.5ml olive oil) only twice with five days gap after first week of trial to induce chronic oxidative stress and inflammation in the liver. However, administration of toxic dose of CCl₄ to obese rat with fatty liver induced not only steatosis, but also recruit inflammatory cells, ballooning of hepatocytes, pericellular fibrosis, perisinusoidal fibrosis, hypertriglyceridemia and increased in serum AST and ALT levels (Van Herck et al. 2017). Inflammation initiates and controls fibrosis resulting in the release of products for remodeling or degrading the natural basement membrane matrix within the liver (Mehal et al. 2007).

The leaves of the *Camellia sinensis*, an individual from the Theaceae family are infused to make green tea that has anti-inflammatory, antioxidant, anti-carcinogenic, protein-denaturing, anti-mutagenic and antibacterial characteristics (Melok et al. 2018). Polyphenols and caffeine are the most significant chemical present in green tea, which are of extensive pharmacological hugeness (Bharadwaz and Bhattacharjee 2012).

Absorption spectrum of green tea recorded at room temperature using UV-VIS spectrophotometer as shown in Fig. 1, the absorption peaks characterizing green tea extraction in known dilutions were different. Green tea extract was characterized by main absorption peak of all dilutions located at 230 nm (Araya-Farias et al. 2014). It was also clearly seen that the intensity of light within measured wavelength range depends on the tea concentrations. The absorbance increases slightly with increasing green tea concentration in all wavelengths (Atomssa and Gholap 2015).

High performance liquid chromatography was done for quantitative analysis of polyphenols (catechins) in Fig. 2, the major antioxidants in extracts including gallic acid, GC, EGC, caffeine, EC, EGCG, C, GCG, ECG (Li et al. 2021). These findings suggested appreciable antioxidant and anti-inflammatory activity which serves as a hepatoprotective and hypocholesterolemic ingredients (Das et al. 2021). As natural antioxidants, catechins can be ameliorated liver diseases by overcome the load of inflammation (Ojeaburu et al. 2021).

Rats injected with CCl₄, the activities of ALT and AST were significantly ($p < 0.001$) raised. Virtually, CCl₄-induced damage to liver tissue could elevate serum ALT and serum AST levels (Fahim and Sathi, 2018). Total proteins and total bilirubin were significantly decreased when compared to those values in control rats. The ranges of serum ALT and AST were also decreased while levels of total proteins and total bilirubin elevated towards normal range (Ciardullo et al. 2021). In our study there was a decrease in RBC's, Hb, WBC's, neutrophils and platelets count in albino rats after high fats diet administration along with CCl₄, raises the level of oxidative stress in liver and induces hepatotoxicity (Wang et al. 2019).

GTPE was responsible for a significant depletion in total cholesterol (TC), triglycerides (TG) and low density lipoprotein values as compare to PC group (Table 3). The reduction in values suggested a protective effect of GTPE on liver against CCl₄-induced toxicity (Mahmoud et al. 2012). Antioxidant defense system of enzymes such as malondialdehyde, catalase, superoxide dismutase and glutathione peroxidase is disturbed due to CCl₄-induced liver damage (Mahmoodzadeh et al. 2017). GTPE administered group returns to the normal due to the antioxidant ability of catechins. The antioxidant potential due to the excessive amount of catechins in GTPE has great attribution towards antioxidant defense system of the liver.

Green tea polyphenol extract demonstrated anti-inflammatory and antioxidant dose- response effect. Both TNF- α and IL-6 are classic indicators of pro-inflammatory processes, which are secreted by macrophages and responsible for the activation of T cells. Excessive accumulation of fats in the liver not only induced oxidative stress but also activated the inflammation response (Xia et al. 2019). For prevention and treatment of inflammatory disorders, we must halt the production of both cytokines in an effective way by using therapeutic dose (600mg/kg GTPE) herbal drug with few or no side effects (Wu et al. 2022).

Histopathological results were confirmed the hepatoprotective effect of GTPE on CCl₄-induced liver disease along HFD. Liver samples after decapitation indicated fatty liver, vacuolization of cells, increased percentage of necrosis and fibrosis (Mahmoodzadeh et al. 2017). However, in current study GTPE group showed less degeneration and healing process after necrosis in the liver. It proved that GTPE can protect damaged fatty liver and restore the parenchyma cells of the liver (Shareef et al. 2021). On the basis of effectiveness, replenishment of liver tissues in GTPE treated group can be compared with silymarin treated group.

In the presence of GTPE, the antioxidant pathway of NRF-2 was activated to restore the liver functioning, which even protect liver stellate cells from hepatosteatosis and liver fibrosis (Xu et al. 2019). The up-regulation of TIMP-1 was indicated the presence of hepatic fibrogenesis due to CCl₄ toxicity and considered to initiate fibrosis due to extracellular matrix degradation (Lachowski et al. 2019). Our study suggested that disruption of mRNA expression level in NRF-2 and TIMP-1 genes resulted in the proliferation of hepatic stellate cells in an in-vivo model.

Conclusions

Our research concluded that high fats diet and CCl₄ can lead to liver damage and severe toxicity, but it can be reduced by herbal treatments like use of green tea polyphenols as nutraceutical supplement. GTPE neutralized the ROS by the activation of NRF-2 and TIMP-1 pathways and improved anti-inflammatory and antioxidant defense mechanism. Thus, this herb have significant power to reduce the chronic inflammation and oxidative stress and effective in preventing the liver illness.

Abbreviations

ANOVA = Analysis of variance

ALT = Alanine Transaminase

AST = Aspartate Transaminase

C = Catechin

CAT = Catalase

CBC = Complete Blood Count

CCl₄ = Carbon tetrachloride

EC = Epicatechin

ECG = Epicatechingallate

ECM = Extracellular Matrix

EGC = Epigallocatechin

EGCG =Epigallocatechin gallate

GA = Gallic Acid

GC = Gallocatechin

GCG = Gallocatechingallate

GPx = Glutathione Peroxidase

GT= Green Tea

GTPE = Green Tea Polyphenol Extract

Hb = *Hemoglobin*

HDL = High-Density Lipoproteins

HSC = Hepatic Stellate Cell

LDL = Low-Density Lipoproteins

MDA = Malondialdehyde

NASH = Non-alcoholic Steatohepatitis

NC = Normal Control

PC = Positive Control

RBC's = Red Blood Cells

ROS = Reactive Oxidative Species

SG = Standard Group

SOD = Superoxide Dismutase

TC = Total Cholesterol

TG = Triglyceride

TGFβ 1 = Transforming Growth Factor beta 1

WBC's = White Blood Cells

Declarations

Data Availability

Not applicable.

Aknowlwdgement

Authors acknowledged the respected lab staff of Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan for collaboration in the lab and research facilities.

Funding

There was no funding by any funding agency.

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Contributions

Wafa Majeed made experimental design and participated in data collection. Hafiza Arooj Kanwal analyzed phytochemical constituents of plant extract through UV-Vis spectroscopy and HPLC and performed the histopathological examination as well as analyzed and interpreted measurements of biochemical parameters, antioxidant enzymes and inflammatory biomarkers. Ambreen Mehmood studied gene expression via qrt-PCR. All authors read and approved the final manuscript.

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Conflict of interest

Authors declare no conflict of interest.

Ethics approval

The experiment was conducted with preliminary approval by the Directorate of Research and Advance Studies and with consent of Bioethics Committee, University of Agriculture, Faisalabad, Pakistan under issued ethical certificate wide letter no. 1720/ORIC.

References

1. Abeysekera KW, Fernandes GS, Hammerton G, Portal AJ, Gordon FH, Heron J, Hickman M (2020) Prevalence of steatosis and fibrosis in young adults in the UK: a population-based study. *Lancet Gastroenterol Hepatol* 5:295–305. [https://doi.org/10.1016/S2468-1253\(19\)30419-4](https://doi.org/10.1016/S2468-1253(19)30419-4)
2. Alhakmani F, Kumar S, Khan SA (2013) Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pac J Trop Biomed* 3:623–627. [https://doi.org/10.1016/S2221-1691\(13\)60126-4](https://doi.org/10.1016/S2221-1691(13)60126-4)
3. Anand J, Upadhyaya B, Rawat P, Rai N (2015) Biochemical characterization and pharmacognostic evaluation of purified catechins in green tea (*Camellia sinensis*) cultivars of India. *Biotech* 5:285–294. <https://doi.org/10.1007/s13205-014-0230-0>
4. Araya-Farias MA, Gaudreau E, Rozoy, Bazinet L (2014) Rapid HPLC-MS method for the simultaneous determination of tea catechins and folates. *J Agric Food Chem* 62(19):4241–4250
5. Atomssa T, Gholap AV (2015) Characterization and determination of catechins in green tea leaves using UV-visible spectrometer. *J Eng Technol Res* 7(1):22–31
6. Azzahra LF, Fouzia H, Mohammed L, Noureddine B (2012) Antioxidant response of *Camellia sinensis* and *Rosmarinus officinalis* aqueous extracts toward H₂O₂ stressed mice. *J Appl Pharm* 2:7–12. DOI: 10.7324/JAPS.2012.2708
7. Bagherniya M, Mahdavi A, Abbasi E, Iranshahy M, Sathyapalan T, Sahebkar A (2022) The effects of phytochemicals and herbal bio-active compounds on tumour necrosis factor- α in overweight and obese individuals: a clinical review. *Inflammopharmacology* 1–20. <https://doi.org/10.1007/s10787-021-00902-y>
8. Bharadwaz A, Bhattacharjee C (2012) Extraction of Polyphenols from Dried Tea Leaves. *Int J Sci Eng Res* 3:134–138
9. Ciardullo S, Monti T, Perseghin G (2021) Prevalence of Liver Steatosis and Fibrosis Detected by Transient Elastography in Adolescents in the 2017–2018 National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* 19:384–390. <https://doi.org/10.1016/j.cgh.2020.06.048>
10. Daliu P, Santini A, Novellino E (2019) From pharmaceuticals to nutraceuticals: Bridging disease prevention and management. *Expert Rev Clin Pharmacol* 12:1–7. <https://doi.org/10.1080/17512433.2019.1552135>
11. Das D, Tamuly S, Das PM, Dutta B, Barman C, Kalita DJ, Boro R, Agarwal S (2021) Green tea leaves extract with low concentration of EGCG can provide health benefits without causing renal damage. *Acta Aliment* 50:369–382
12. Fahim NF, Sathi ZS (2018) Assesment of hepatoprotective activity of roots and barks of *Achyranthes aspera* in carbon tetrachloride-induced hepatotoxicity in rats. *Malays J Halal Res* 1:23–26. <http://doi.org/10.26480/mjhr.02.2018.23.26>
13. Hassan AA, Moustafa EM, El-Khashab IH, Mansour SZ (2021) Mangosteen hinders gamma radiation-mediated oxidative stress and liver injury by down-regulating TNF- α /NF- κ B and pro-fibrotic factor TGF- β 1 inducing inflammatory signaling. *Dose-Response* 19(2):15593258211025190

14. He X, Li J, Zhao W, Liu R, Zhang L, Kong X (2015) Chemical fingerprint analysis for quality control and identification of Ziyang green tea by HPLC. *Food Chem* 171:405–411. <https://doi.org/10.1016/j.foodchem.2014.09.026>
15. Higashi T, Friedman SL, Hoshida Y (2017) Hepatic stellate cells as key target in liver fibrosis. *Adv Drug Deliv Rev* 121:27–42. <https://doi.org/10.1016/j.addr.2017.05.007>
16. Huang HL, Wang YJ, Zhang QY, Liu B, Wang FY, Li JJ, Zhu RZ (2012) Hepatoprotective effects of baicalein against CCl₄-induced acute liver injury in mice. *World J Gastroenterol* 18:6605–6613. doi: 10.3748/wjg.v18.i45.6605
17. Ibrahim Y, Musa A, Yakasai IA (2017) Spectrophotometric method for determination of catechins in green tea and herbal formulations. *Niger J Pharm Sci* 16(1):25–30
18. Karczewski J, Śledzińska E, Baturo A, Jończyk I, Maleszko A, Samborski P, Dobrowolska A (2018) Obesity and inflammation. *Eur Cytokine Netw* 29(3):83–94
19. Kubota N, Kado S, Kano M, Masuoka N, Nagata Y, Kobayashi T, Miyazaki K, Ishikawa F (2013) A high-fat diet and multiple administration of carbon tetrachloride induces liver injury and pathological features associated with non-alcoholic steatohepatitis in mice. *Clin Exp Pharmacol Physiol* 40:422–430
20. Lachowski D, Cortes E, Rice A, Pinato D, Rombouts K, Rio HA (2019) Matrix stiffness modulates the activity of MMP-9 and TIMP-1 in hepatic stellate cells to perpetuate fibrosis. *Sci Rep* 9(1):1–9. <https://doi.org/10.1038/s41598-019-43759-6>
21. Li J, Chen Q, Zhai X, Wang D, Hou Y, Tang M (2021) Green tea aqueous extract (GTAE) prevents high-fat diet-induced obesity by activating fat browning. *Food Sci Nutr* 9(12):6548–6558
22. Ma B, Xu H, Zhuang W, Wang Y, Li G, Wang Y (2020) Reactive oxygen species responsive theranostic nanoplatfrom for two-photon aggregation-induced emission imaging and therapy of acute and chronic inflammation. *ACS Nano* 14(5):5862–5873. <https://doi.org/10.1021/acsnano.0c01012>
23. Mahmoodzadeh Y, Mazani M, Rezagholizadeh L (2017) Hepatoprotective effect of methanolic Tanacetum parthenium extract on CCl₄-induced liver damage in rats. *Toxicol Rep* 4:455–462. <https://doi.org/10.1016/j.toxrep.2017.08.003>
24. Mahmoud MF, Fahmy A, Auf MA (2012) Evaluation of the hepatoprotective effect of green tea extract and selenium on CCL₄-induced fibrosis. *e-SPEN J* 7:23–29. <https://doi.org/10.1016/j.eclnm.2011.12.004>
25. Majeed W, Aslam B, Iftikhar A, Awan AM, Javed F, Daud M, Shahab N, Syed M, Iqbal H (2021) Acacia nilotica polyphenol extract restores glucose homeostasis by upregulating the insulin secretion and lowering the oxidative stress through down regulation of c-Jun N-terminal kinase (JNK) signaling cascade. *J King Saud Univ Sci* 33:101–474. <https://doi.org/10.1016/j.jksus.2021.101474>
26. Mehal WZ, Friedman SL (2007) The role of inflammation and immunity in the pathogenesis of liver fibrosis. In: Gershwin ME, Veiriling JM, Manns MP, eds. *Liver Immunol* 7:111–121
27. Melok AL, Lee LH, Mohamed SA, Yussof, Chu T (2018) Green Tea Polyphenol Epigallocatechin-3-Gallate-Stearate Inhibits the Growth of Streptococcus mutans: A Promising New Approach in Caries

28. Nibir YM, Sumit AF, Akhand AA, Ahsan N, Hossain MS (2017) Comparative assessment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh. *Asian Pac J Trop Biomed* 7:352–357. <https://doi.org/10.1016/j.apjtb.2017.01.005>
29. Noormandi A, Dabaghzadeh F (2015) Effects of green tea on *Escherichia coli* as a uropathogen J Tradit. Complement Med 5:15–20. <https://doi.org/10.1016/j.jtcme.2014.10.005>
30. Ojeaburu SI, Oriakhi K (2021) Hepatoprotective, antioxidant and, anti-inflammatory potentials of gallic acid in carbon tetrachloride-induced hepatic damage in Wistar rats. *Toxicol Rep* 8:177–185. <https://doi.org/10.1016/j.toxrep.2021.01.001>
31. Pahwa R, Goyal A, Bansal P, Jialal I (2020) chronic inflammation. In: StatPearls. StatPearls Publishing, Treasure Island (FL). PMID: 29630225
32. Parasuraman S, Raveendran R, Kesavan R, Parasuraman S, Raveendran R, Kesavan R (2010) (2010). Blood sample collection in small laboratory animals. *Journal of pharmacology & pharmacotherapeutics*, 1(2), 87–93. <https://doi.org/10.4103/0976-500X.72350>
33. Popov B, Gadjeva V, Valkanov P, Popova S, Tolekova A (2003) Lipid peroxidation, superoxide dismutase and catalase activities in brain tumor tissues. *Arch Physiol Biochem* 111(5):455. <https://doi.org/10.3109/13813450312331342328>
34. Ragab A, Nasr NE, Kahilo K (2019) Protective effect of glycyrrhizic acid against carbon tetrachloride-induced liver fibrosis in rats: role of integrin subunit β like 1 (itg β 1). *Slov Vet Res* 56:673–679
35. Scholten D, Trebicka J, Liedtke C, Weiskirchen R (2015) The carbon tetrachloride model in mice. *Lab Anim* 49:4–11. <https://doi.org/10.1177/0023677215571192>
36. Shareef SH, Ibrahim IAA, Alzahrani AR, Al-Medhtiy MH, Abdulla MA (2021) Hepatoprotective Effects of Methanolic Extract of Green Tea against Thioacetamide-Induced Liver Injury in Sprague Dawley Rats. *Saudi J Biol Sci* 28:1–10. <https://doi.org/10.1016/j.sjbs.2021.09.023>
37. Van HMA, Vonghia L, Francque SM (2017) Animal models of nonalcoholic fatty liver disease—a starter’s guide. *Nutrients* 9:1072
38. Wang D, Wang Y, Madhu S, Liang H, Bray CL (2019) Total hemoglobin count has significant impact on A1C—Data from National Health and Nutrition Examination Survey 1999–2014. *Prim Care* 13:316–323. <https://doi.org/10.1016/j.pcd.2019.01.002>
39. Wangkarn S, Grudpan K, Khanongnuch C, Pattananandecha T, Apichai S, Saenjurn C (2021) Development of HPLC Method for Catechins and Related Compounds Determination and Standardization in Miang (Traditional Lanna Fermented Tea Leaf in Northern Thailand). *Molecules* 26(19):6052–6063
40. Wei K, Wang LY, Zhou J, He W, Zeng JM, Jiang YW, Cheng H (2012) Comparison of catechins and purine alkaloids in albino and normal green tea cultivars (*Camellia sinensis* L.) by HPLC. *Food Chem* 130:720–724. <https://doi.org/10.1016/j.foodchem.2011.07.092>
41. Wu Z, Sun L, Chen R, Wen S, Li Q, Lai X, Zhang Z, Cao F, Sun S (2022) Chinese Tea Alleviates CCl₄-Induced Liver Injury through the NF- κ B/Nrf2 Signaling Pathway in C57BL-6J Mice. *Nutr* 14(5):972

42. Xia HM, Wang J, Xie XJ, Xu LJ, Tang SQ (2019) Green tea polyphenols attenuate hepatic steatosis, and reduce insulin resistance and inflammation in high-fat diet-induced rats. *Int J Mol Med* 44(4):1523–1530
43. Xu D, Xu M, Jeong S, Qian Y, Wu H, Xia Q, Kong X (2019) The role of Nrf2 in liver disease: novel molecular mechanisms and therapeutic approaches. *Front Pharmacol* 9:1428. <https://doi.org/10.3389/fphar.2018.01428>

Figures

Figure 1

UV spectrum of green tea polyphenols in distilled water at different concentrations

Figure 2

HPLC chromatogram of Green tea aqueous extract

Figure 3

The effect of GTPE on the level of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) enzymes in CCl₄-damaged rats (n =8). Data were expressed as means ± SEM. Based on ANOVA and Tukey's test, Positive Control group showed significant difference as compared to Normal, Standard and GTPE groups (P<0.001).

Figure 4

Inflammatory profile evaluated by quantification of pro-inflammatory cytokines **A** TNF- α and **B** IL-6 with the help of ELISA kit in CCl₄ and HFD-induced liver toxicity group (PC) and estimated the effect of GTPE and silymarin on these inflammatory markers (n =8). Data were expressed as means $\pm$ SEM. Based on ANOVA and Tukey's test, Positive Control group showed significant difference as compared to Normal, Standard and GTPE groups (P<0.001).

Figure 5

The effect of GTPE on the level of NRF2 and TIMP1 genes in CCl4-damaged rats (n =8). Based on ANOVA and Tukey's test, Positive Control group showed significant difference as compared to Normal, Standard and GTPE groups ($P<0.001$).

Figure 6

H&E $\times$ 40 stained microscopic images of liver tissue in the groups under study. A: normal group, B: positive group, C: standard group with 200 mg/kg silymarin and D: GTPE group with 600mg/kg GTPE.