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AKADÉMIAI KIADÓ

Green tea leaves extract with low concentration of EGCG can provide health benefits without causing renal damage

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ABSTRACT

Green tea or its concentrated extract is coveted for its health promoting catechin-like polyphenols, especially epigallocatechin-3-gallate (EGCG). However, its amicable efficacy is now being doubted considering the recent occurrence of several cases of hepato- and nephrotoxicity, after the ingestion of EGCG-fortified (≥85–90%) nutritional supplements. Therefore, the current study was carried out to ascertain the effect of green tea leaves extract (GTE), having low EGCG content (73.8%), on liver and kidney functions of male Wistar rats using various *in vivo* experiments and *in vitro* radical scavenging activity. In terms of acute toxicity, GTE was observed to be safe when delivered at a dosage of 2000 mg kg^{-1} body weight (BW). Oral delivery of GTE for 28 days at a concentration of 200 mg kg^{-1} BW/day did not trigger sub-acute toxicity to the liver and kidneys, as per serum biochemical analyses and histopathological examination. In contrast, GTE counteracted the effects of carbon tetrachloride (a potent hepato-degenerative compound) on the liver. Furthermore, increase in high-density lipoprotein—cholesterol with concomitant lowering of serum triglycerides and low-density lipoprotein—cholesterol were noticed in GTE-treated rats. These findings suggest that low EGCG

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containing GTE, with appreciable antioxidant activity (IC_{50} = 53.18–71.28 μg mL^{-1}), can serve as a hepatoprotective, hypolipidemic, and hypocholesterolemic ingredient.

KEYWORDS

green tea extract, epigallocatechin-3-gallate, antioxidant, IC_{50} value, toxicity, blood lipid profile, histopathology

1. INTRODUCTION

With a long-established tradition of consumption in China, India, and East Asia, green tea (*Camellia sinensis*) is one of the most lauded food commodities for its gamut of health benefits. Its array of beneficial properties is attributed to the presence of numerous epistructured galloylated catechin-like phenolic compounds, especially epigallocatechin-3-gallate (EGCG). In tandem with a 'more-is-better' mindset, EGCG of green tea is mostly purified or concentrated for 'over-the-counter' availability in the form of liquid or capsules/tablets as supplementary medications and herbal decoctions. Strikingly, in contrast to the pure or concentrated form of EGCG, crude green tea extracts (GTE) are considered to be far more stable and potent due to the synergistic help imparted by other tea catechins (Schönthal, 2011). However, as the health risks of EGCG emerged lately, the overwhelming benefits of GTE were overlooked, primarily due to its hepatotoxicity and liver failure issues. Hepatotoxicity, inflammatory reactions, hemorrhagic lesions in the stomach, intestines, and even death (Galati et al., 2006; Lambert et al., 2010) have been triggered by GTE with higher-doses of EGCG, as reported by different groups of researchers. Consumption of GTE at moderate doses (expressed as EGCG 270 and 400 mg kg^{-1} body weight (BW) per day) proved fatal (Wang et al., 2015). In contrast, GTE effectively prevented hepatocellular damage in rats caused by 2-nitropropane, galactosamine, carbon tetrachloride (CCl_4), pentachlorophenol, and acetaminophen (Salminen et al., 2012). GTE may also exhibit antioxidant or pro-oxidant activities, depending on the level of the dosage, the interaction with certain other substances, and the biological environment (Hu et al., 2018). On the basis of multiple dosage toxicity studies reported in various peer-reviewed publications from 2005 to 2017 (Park, 2018), it was concluded that the non-observed adverse effect levels (NOAEL) of GTE, EGCG, and catechin fractions were calculated to be approximately 500, 67.8, and 500 mg kg^{-1} BW/day, respectively, indicating that GTE with a low EGCG concentration could help its use, since it would be unwise to completely eliminate a potent bioactive compound like EGCG.

Although the hepatotoxic activity of high EGCG concentrations has been indicated and remains uncontentious, the cardioprotective impact of GTE has been shown to be undeniably positive in multiple animal and human tests (Wolfram, 2007). GTE administration at low doses (1.7 mg catechin kg^{-1} BW) as well as high dosages (250–300 mg kg^{-1} BW) has been shown to be involved in preventing atherosclerosis in mice and ameliorating cardiac dysfunction in rats (Babu et al., 2006). While the alleviating cardiovascular effect of such an elevated dosage was shown, there was no discussion of its concomitant impact on other organs or metabolic processes. There are plenty of alleged health claims for green tea and its EGCG-enriched concentrated derivatives; however, limited research attention is being paid to the effectiveness of GTE with a lower level of EGCG. An effort was made in the present study to verify if GTE is

indeed effective as hepatoprotective, hypolipidemic, and hypocholesterolemic ingredient with low concentration of EGCG. We intended to address this question in *in vivo* system using rat model organism.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All chemicals and reagents used were of analytical grade and procured from HiMedia (India). Standards (catechin and EGCG) were purchased from Sigma-Aldrich (USA), and ALT, AST, ALP, Triglyceride, and total cholesterol kits for serum biochemical analyses were procured from Aspen Laboratories Pvt. Ltd. India. The LDL and HDL kits were obtained from DIATEK Healthcare Pvt. Ltd., Kolkata.

2.2. Extraction of GTE

Fresh tea leaves of second flush were procured from tea gardens of Assam Agricultural University, Jorhat, India (latitude: 26°44′47. 47″N and longitude: 94°12′9. 31″E). The collected leaves were washed, crushed in a blender (Havells, MOMENTA NV 750) using 80% methanol at a ratio of 1:20 (w/v), and then ultrasonicated (50% amplitude, 20 kHz) under chilled water circulation glass cell (4 °C). The macerated mass was centrifuged for 10 min at 1,500 *g* (4 °C) and the supernatant was passed through suction filter, using 0.45 µm pore-sized Millipore membrane. The filtrate was washed thrice with water-chloroform mixture in 1:1 (v/v) ratio, and the separated chloroform phase containing the organic impurities was discarded. The collected aqueous phase was partitioned with water-ethyl acetate mixture in 1:1 (v/v) ratio, wherein the ethyl acetate layer was concentrated under vacuum till dryness using rotary vacuum evaporator (Model No. MVP 10, IKA Germany) and finally re-suspended in ultrapure water. The aqueous suspension was subjected to freeze drying, the recuperated powder (termed as GTE) was stored at –40 °C, and was used as-such for subsequent experiments. For quantification of catechins in GTE, the ethyl acetate residue was dissolved in definite volume of acetonitrile; while for estimation of *in vitro* antioxidant activities, it was dissolved in methanol.

2.3. High performance liquid chromatography (HPLC) analysis of catechins in GTE

HPLC analysis was carried out on a Hitachi Chromaster 5,000 Series instrument (Hitachi, Tokyo, Japan) equipped with an autosampler (Model 5,210). A 10 µl aliquot was injected into a cosmosil $5C_{18}$-MS-II column (4.6 mm ID × 250 mm) through the auto sampler. The mobile phase consisted of 30% of solvent A (2.5% acetic acid in water) and 70% of solvent B (acetonitrile), programmed in an isocratic mode with a flow rate of 1 mL min^{-1}. Fractions were detected using 5,430 Diode Array Detector in a scan mode (200–400 nm). Final concentrations were calculated in comparison with a known standard response.

2.4. Characterisation

FTIR of GTE was obtained from KBr pellets made from 1 mg sample and 300 mg KBr by Cary 630 FTIR spectrometer (Agilent technologies, USA). A total of 32 scans were obtained for each spectrum at a resolution of 4 cm^{-1}.

2.5. *In vitro* antioxidant assays

2.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH scavenging activity was performed in ELISA plate reader (Biotek, Model-Epoch2, USA) as described by Brand-Williams et al. (1995).

2.5.2. Ferric reducing antioxidant power (FRAP) assay. The assay was performed following the protocol of Benzie and Strain (1996).

2.5.3. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. Decolourisation of blue ABTS cation into neutral form by GTE was determined as follows: free radicals were generated by mixing 7 mM ABTS stock in water with 2.45 mM potassium persulphate ($K_2O_8S_2$) (1:1 w/w), and the resulting solution was kept in dark at room temperature for 12–17 h prior to use. $ABTS^+$ solution was diluted with methanol to obtain an absorbance of 0.7 ± 0.005 at 734 nm. To perform the assay, 980 µL of ABTS radical reagent was mixed with 20 µL of the sample or Trolox (0–75 $\mu g\,mL^{-1}$) as standard. The absorbance was measured at 734 nm, after incubating in dark for 30 min. Percentage of $ABTS^+$ inhibition capacity was calculated using the following equation:

$$\%\,ABTS^{+}\text{inhibition} = \left[\left(Abs_{control} - Abs_{sample}\right)\right]/\left(Abs_{control}\right)]\times 100$$

The ABTS scavenging activity of GTE was expressed as the Trolox Equivalent Antioxidant Capacity (TEAC), using the following equation (Shimamura et al., 2014).

$$TEAC = IC_{50}\text{ of Trolox}\left(\mu g\,mL^{-1}\right)/IC_{50}\text{ of sample}\left(\mu g\,mL^{-1}\right)$$

2.6. Animal experiment no. 1: acute toxicity analysis

2.6.1. Experimental animals. In compliance with the OECD Guidelines for Chemical Testing (Acute oral toxicity–acute toxic class method) adopted on 17th December 2001 (Guideline No. 423), the GTE-mediated acute toxicity study was carried out. A total of 6 male Wistar rats weighing 141–173 g BW were caged randomly with saw dust filled floor in a calm and temperature-controlled space (23±4°C). The rats were given free access to proper feed and water (Institutional Animal Ethics Approval No. 770/GO/Re/S/03/CPCSEA/FVSC/AAU/IAEC/19-20/798 dated 23.12.2019).

2.6.2. Experimental designs. The rats were distributed randomly into 2 study groups, comprising of 3 rats per group: Group I, assigned as the control rats, was fed on a standard diet. Group II comprised of rats fed with GTE @ 2000 $mg\,kg^{-1}$ BW prepared using 0.9% NaCl. After dosing GTE @ 2000 $mg\,kg^{-1}$ BW once within the first 30 min, rats were examined individually, periodically over the first 24 h, with extra focus within the first 4 h, and daily follow-up for a total period of 14 days.

2.7. Animal experiment no. 2: sub-acute toxicity analysis

2.7.1. Experimental layout. In adherence with OECD Recommendations for the Testing of Chemicals (Repeated Dose 28-Day Oral Toxicity Study in Rodents) adopted on 3rd October 2008 (Guideline 407), the subacute toxicity analysis was carried out. The rats were split into two

groups ($n = 6$) randomly. GTE @ 200 mg kg^{-1} BW daily for 28 days was dispensed to one group. A 100 µL normal saline solution (0.9% NaCl) was given to the control group. Adopting the typical retro orbital procedure, blood samples were obtained from both the treated and control group at 0, 7, 14, 21, and 28 days, using hematocrit capillary tubes for haematological and blood biochemical analysis. The rats were sedated using mixture of ketamine (40 mg kg^{-1} BW) and xylazine (5 mg kg^{-1} BW) prior to blood collection. The rats were sacrificed by cervical dislocation on the 29th day for obtaining liver, kidney, and spleen for histopathological examination. The organs were stored in 10% buffered formaline. The serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatinine was estimated colorimetrically using the kits provided by Aspen Laboratories Pvt. Ltd. (India). The superoxide dismutase was estimated in haemolysate prepared from whole blood using the method described by Kakkar et al. (1984).

2.8. Hepatoprotective activity analysis against carbon tetrachloride (CCl_4)

A total of 12 rats were randomly categorised into two groups ($n = 6$). GTE at a dose rate of 200 mg kg^{-1} BW was fed to the treated group and 100 µL of 0.9% NaCl was fed to the control group daily. Carbon tetrachloride was fed orally on the 11th day in both classes (control and treated) at a dose rate of 1 mL kg^{-1} BW. Samples of blood and tissue were obtained and handled in the same manner specified above to estimate serum ALT, AST, and ALP and blood SOD.

2.9. Study of hypocholesterolemic and hypolipidemic effect of GTE

Eighteen rats were split randomly into three classes ($n = 6$). One group (coded as HLTE) was retained in the high lipid diet along with GTE, while the second (as NF) and third category (as HL) were maintained in the standard diet and high lipid diet, respectively. The high lipid diet was prepared in compliance with the procedure stated by Muramatsu et al. (1986). Blood samples were obtained from all three groups on 0, 7, and 14 days of the trial using the typical retro-orbital procedure as described above. The serum concentration of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides were estimated colorimetrically. The kits obtained from DIATEK Healthcare Pvt. Ltd. (India) were used for estimation of HDL and LDL concentration and those obtained from Aspen Laboratories Pvt Ltd (India) was used for estimation of total cholesterol and triglycerides.

2.10. Statistical analyses

Data were expressed as mean ± SEM. The differences within a group and between the groups were assessed by ANOVA. The post-hoc analysis was performed by pair-wise *t*-test with Bonferroni correction method at 95% confidence level. The analysis was performed in statistical software R (R Core Team, Austria).

3. RESULTS AND DISCUSSION

3.1. Total catechins, FTIR analysis, and *in vitro* antioxidant activities

Chromatogram of GTE in Fig. 1(A) clearly shows the presence of two major components, namely EGCG and (+)-catechin. GTE contains ~73.77% EGCG and ~26.23% (+)-catechin

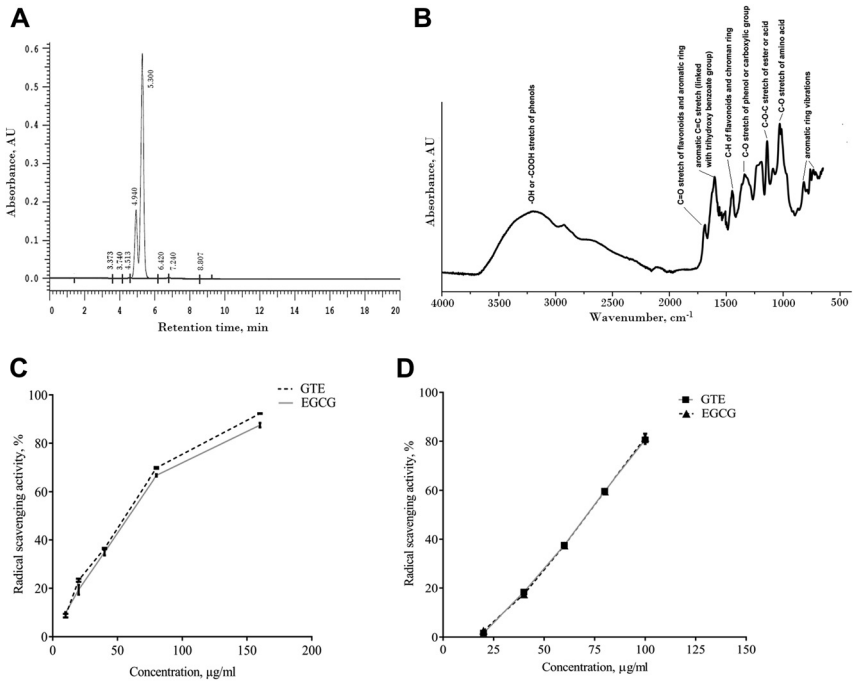

Fig. 1. In vitro antioxidant assays. A: HPLC chromatogram showing presence of EGCG and (+)-catechin in GTE; B: FTIR spectrum of GTE; C: Comparison of DPPH free radical scavenging activity of EGCG and GTE; D: Comparison of ABTS scavenging activity of GTE and EGCG

as the total phenols (w/w). This is much lower than the EGCG content (80–95% by weight) claimed by many commercial extracts and supplements (Oketch-Rabah et al., 2020). FTIR spectrum was acquired to gain insight into the functional facets of GTE. Visual inspection of the spectrum in Fig. 1(B) revealed the occurrence of aromatic ring structures in the sample; a strong –OH absorption band within 3,400–3,600 cm^{-1} conveys the presence of a large number of hydroxyl groups containing polyphenols (Das Purkayastha et al., 2013). Concomitantly, the spectral peaks at 1,690, 1,600, 1,449, 1,338, and 818 cm^{-1} indicates the aromatic entities associated with trihydroxybenzoate or other chroman ring of carbonyl polyphenols (Wang et al., 2014; Senthilkumar et al., 2017; Dahiya et al., 2018). More precisely, the band at 739.9 cm^{-1} is known to be characteristic of EGCG-linked vibration (He et al., 2018). Derived from these facts, it can be assumed that GTE is loaded with catechin-like phenolic compounds.

The IC_{50} values of EGCG and GTE in terms of DPPH scavenging activity were estimated to be 56.95 and 53.18 µg mL^{-1}, respectively ($P < 0.05$). And, IC_{50} values of Trolox, EGCG, and GTE in terms of ABTS inhibition activity were 35.27, 71.42, and 71.28 µg mL^{-1}, respectively. Thus, the efficacy of GTE in scavenging free radicals was found to be at par with that of pure EGCG ($P > 0.05$), but lower than that of Trolox ($P < 0.05$). The IC_{50} value of GTE for DPPH radical quenching was 6.63% higher than that of pure (-)-EGCG ($P < 0.05$); while the IC_{50} score

for ABTS+ scavenging of both GTE and EGCG was comparable ($P > 0.05$). This is clearly evident from Figs 1(C) and 1(D), where GTE showed a dose-dependent increment in the quenching of DPPH and ABTS. Calculated TEAC values of GTE and EGCG were also same (0.49). This can be ascribed to the presence of galloylated EGCG (which has eight phenolic hydroxyl groups) and non-gallated (+)-catechin (with two hydroxyl groups) as the major polyphenols of GTE. Antioxidant potential of catechins is largely affected by the presence of gallate group at the C-3 of C-ring, number and position of –OH groups in the molecule. In case of FRAP assay, GTE presented a reducing power of 9.34 mM Fe^{2+} equivalent/g freeze-dried powder, which was approximately 2-fold lower than that of EGCG (18 mM Fe^{2+} equivalent/g freeze-dried powder), indicating that the constituent catechins have different redox properties due to their chemical configuration (gallate or non-gallate, number of oxidisable –OH moieties, *cis*- or *trans*-epimers, etc.) (Chen and Chan, 1996). EGCG, containing a galloyl group and a B-ring linked with a pyrogallol structure, is expected to exhibit fast reduction of Fe^{3+} to Fe^{2+} (Hotta et al., 2002).

3.2. Acute toxicity analysis

To track the persistent clinical effects on rats after dosing GTE @ 2000 mg kg^{-1} BW, videos were captured and are shown as supplementary files. We did not notice any symptoms of tremors, convulsions, salivation, diarrhea, lethargy, and coma for duration of 14 days. There was no significant difference in the BWs of the treated and untreated (control) groups ($P > 0.05$) measured in the 15-day cycle (Supplementary Table 1).

3.3. Sub-acute toxicity analysis

3.3.1. Effect of GTE on haemato-biochemical parameters. Results of the current investigation indicate that GTE was not a causative agent for any haemato-biochemical liver and kidney toxicity, as apparent from the activity of serum enzymes such as aspartate aminotransferase in Fig. 2(J), serum alanine aminotransferase in Fig. 2(K), serum alkaline phosphatase in Fig. 2(L), serum creatinine level in Fig. 2(M), and haematological parameters (Fig. 2A–2I) (Supplementary Tables 2 and 3). There was no significant difference in the SOD level of GTE-treated and untreated control rats over the treatment period of 28 days ($P > 0.05$), suggesting that GTE does not cause oxidative stress (Fig. 2(N)).

3.3.2. Histopathological examination of liver and kidney. No degenerative alterations were seen among the liver samples obtained from the sacrificed rats belonging to GTE treated group and untreated control group, and the hepatocytes were normally structured in the hepatic cord (Figs 3(A) and 3(B)). There seems to be a slight aberration on renal parenchyma of the treated group; a marginal modification was seen in both the proximal and distal convoluted tubules (Fig. 3(C)) that was not observed in the liver sample of the control (Fig. 3(D)). In the kidneys of GTE administered group, no inflammatory changes could be detected; however, slight congestion was observed in their spleen (Fig. 3(E)), unlike that of the control (Fig. 3(F)). This is not a significant pathological deviation of the spleen, because GTE-mediated spleen atrophy with a depletion of B and T lymphocytes has been reported by Chan et al. (2010) when

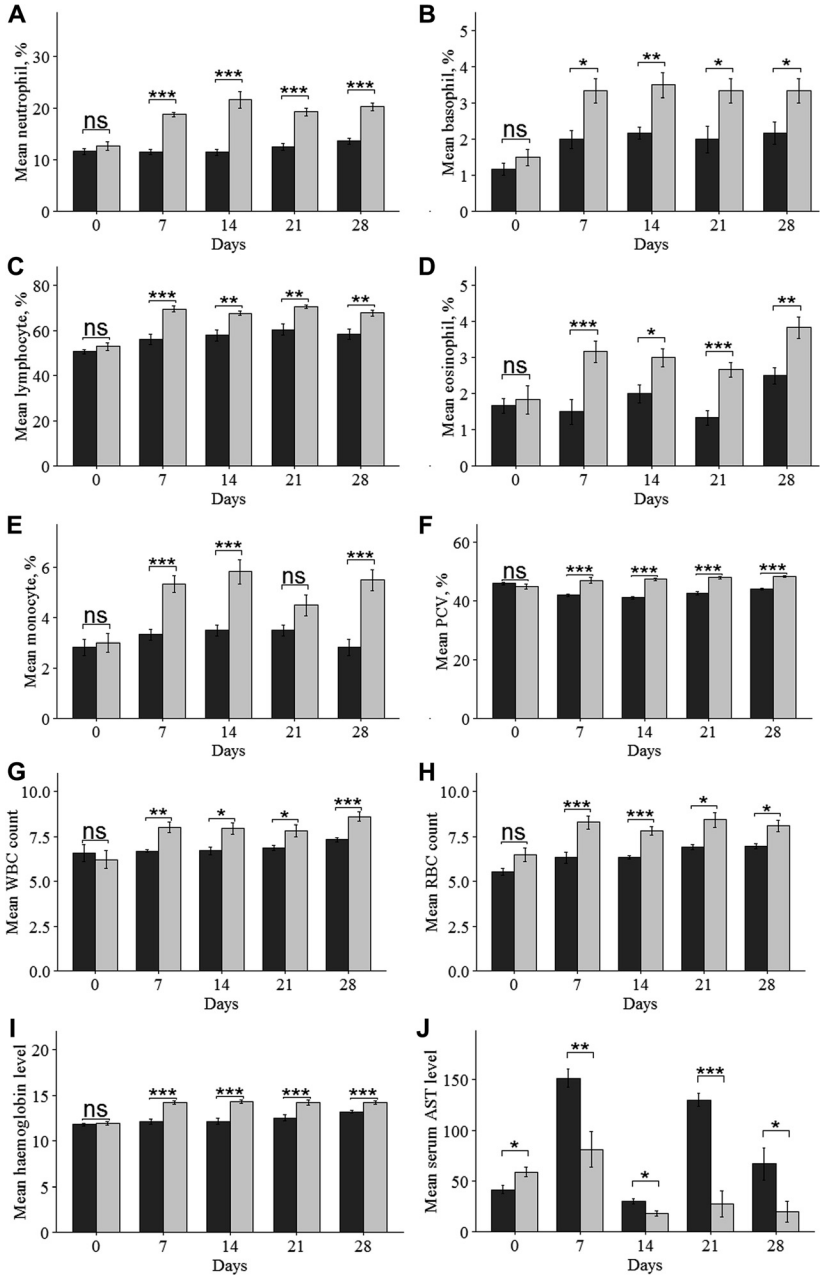

Fig. 2. Effect of 28 day repeated dosing of GTE on blood haematological parameters in control and treated groups of Wistar rat population. A: Neutrophil (%); B: basophil (%); C: lymphocyte (%), D: eosinophil (%); E: monocyte (%); F: packed cell volume (%); G: total WBC count (10^3 μL^{-1}); H: total RBC count (10^6 μL^{-1}); I: haemoglobin level (g dL^{-1}); J: serum AST level (U L^{-1}); K: serum ALT level (U L^{-1}); L: serum ALP level (U L^{-1}); M: serum creatinine level (mg dL^{-1}); N: blood SOD level (U mg^{-1} protein). (***$P < 0.005$, **$P < 0.01$, * $P < 0.05$, ^{ns} $P > 0.05$)

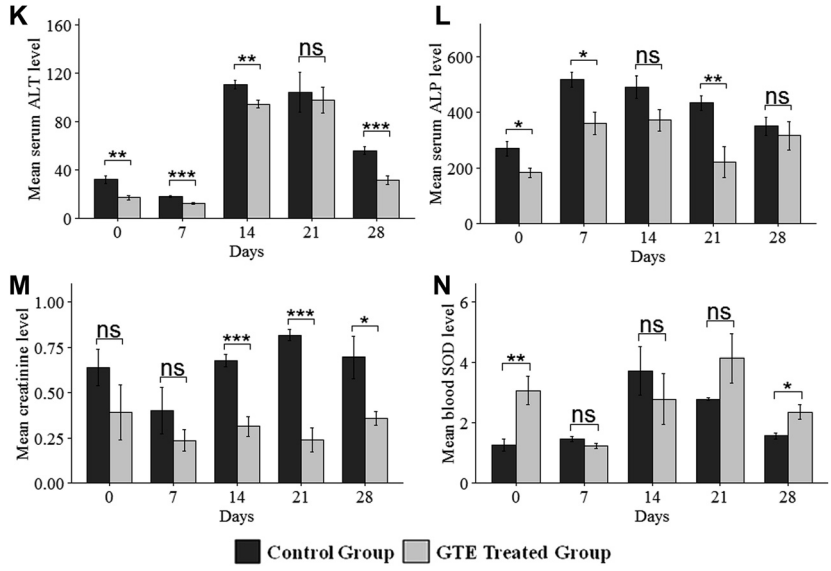

Fig. 2. Continued.

administered at a dosage of 250 mg kg^{-1} BW, which was attributed to inexplicable secondary effect(s) of GTE.

3.4. Experiment no. 3: GTE supplementation attenuate the harmful toxic effect of CCl_4

In line with the findings of Essex et al. (2019), GTE supplementation seemed to ameliorate the hepatotoxic impact of CCl_4 in the treated group. In the test group, CCl_4 caused substantially higher serum ALT, ALP, AST, and SOD levels ($P < 0.05$), as opposed to the GTE treated group (Fig. 4 and Supplementary Table 4). In the control untreated group, CCl_4 brought severe hepatic necrosis, whereas only sinusoidal congestion was present in the GTE treated group (Fig. 5).

3.5. Experiment no. 4: hypocholesterolemic and hypolipidemic effect of GTE

Improvement in the blood lipid profile of the treated group was evident after the ingestion of GTE. The treated population fed with GTE plus high lipid diet (HLTE) had a marked reduction of blood cholesterols, LDL and triglycerides, and an improvement in HDL level against the high-lipid diet (HL) fed control group (Fig. 6). As anticipated, rise in serum LDL, total serum cholesterol, and serum triglycerides were observed in HL category, with concomitant lowering of serum HDL (Supplementary Table 5). On the other hand, no major difference ($P > 0.05$) was found between the HLTE group and the normal feeding (NF) maintenance group, in terms of serum cholesterol (HDL and LDL) and triglycerides. This vouches the hypolipidemic and hypocholesterolemic effects of GTE.

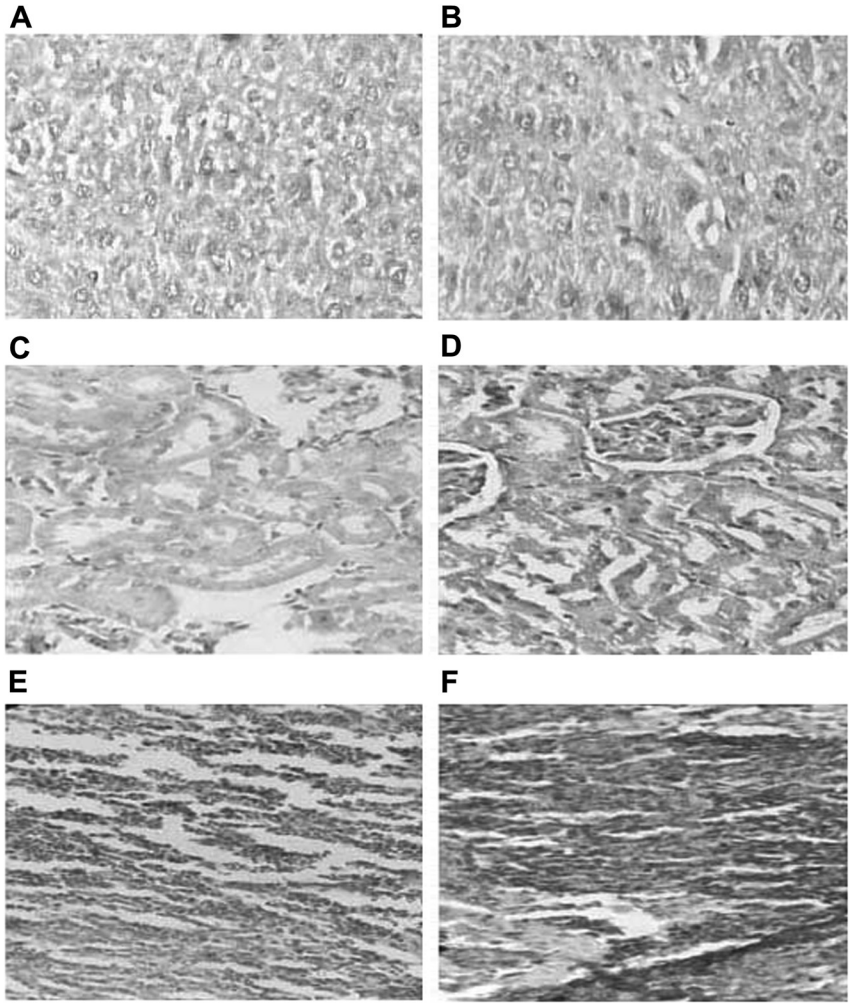

Fig. 3. Effect of 28 day repeated dosing of GTE on liver, kidney, and spleen. A: Photomicrograph of liver showing hepatocytes (H&E, × 10) of rats administered with GTE; B: photomicrograph of liver showing hepatocytes (H&E, × 10) of control rats; C: photomicrograph of kidney showing mild degenerative changes of proximal and distal convoluted tubule (H&E, × 40) of rats administered with GTE; D: photomicrograph of kidney of control rats showing no pathological changes (H&E, × 40); E: photomicrograph of spleen of GTE treated rats, showing mild congestion (H&E, × 40); F: photomicrograph of spleen of control rats, showing no pathological changes (H&E, × 40)

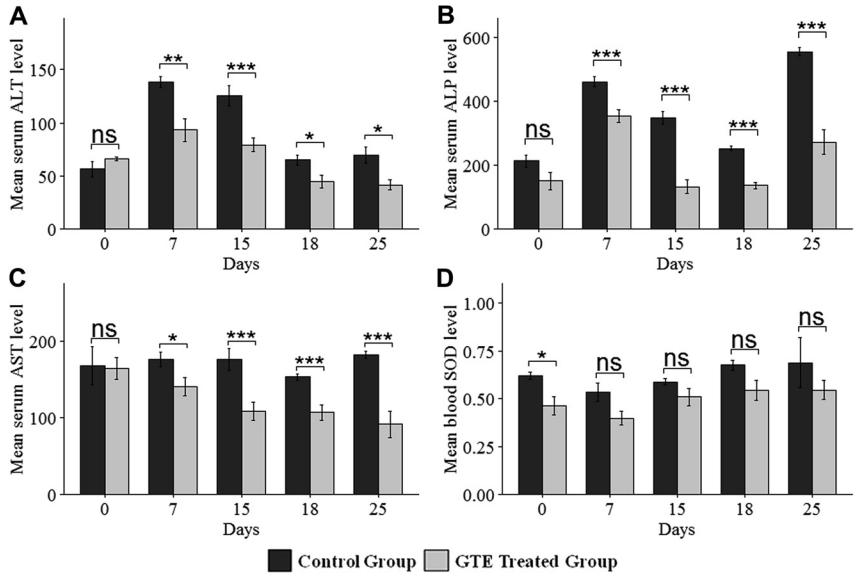

Fig. 4. Influence of GTE supplementation on the serum biochemical parameters of CCl_4-intoxicated rats. A: Serum ALT level (U L^{-1}); B: serum ALP level (U L^{-1}); C: serum AST level (U L^{-1}); D: SOD level (U mg^{-1} protein). (con = Control Group, trt = GTE treated group. (***$P < 0.005$, **$P < 0.01$, * $P < 0.05$, ^{ns} $P > 0.05$)

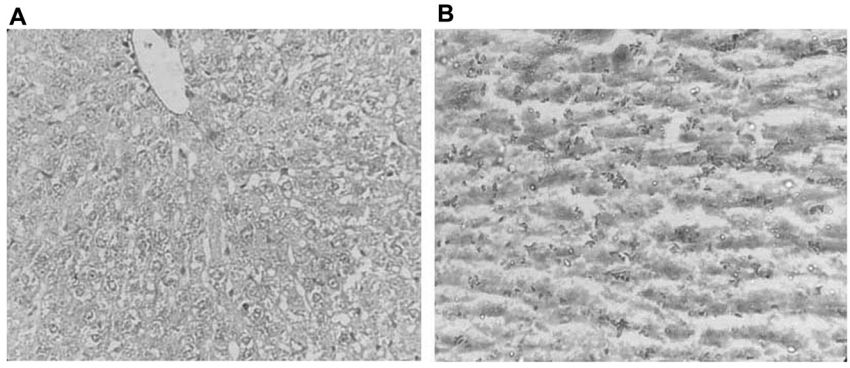

Fig. 5. A: Photomicrograph of liver sample of control group administered CCl_4 (H&E, × 10) showing hepatic necrosis; B: photomicrograph of liver sample of GTE treated group administered CCl_4 (H&E, × 40) showing slight sinusoidal congestion

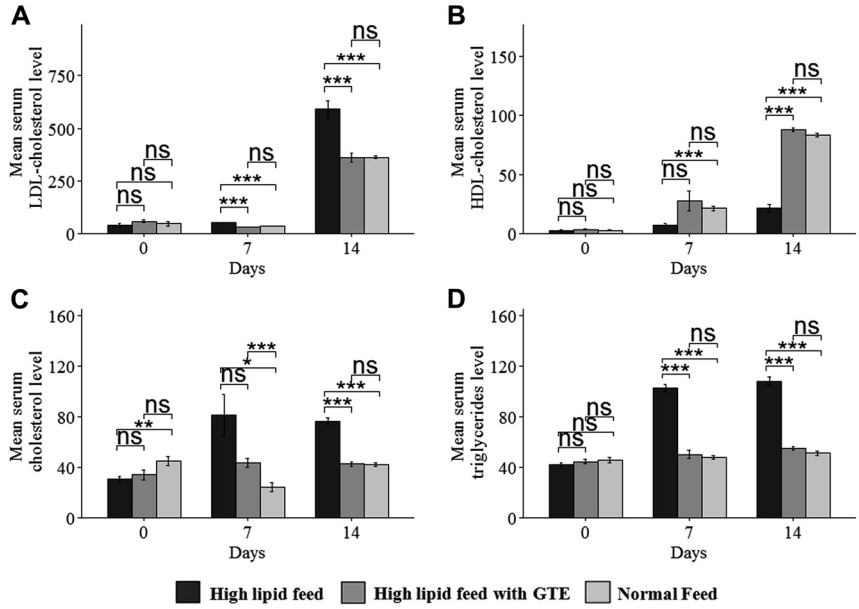

Fig. 6. Hypocholesterolemic and hylipidemic effect of GTE in rats maintained in HL, NF and HLTE diet. A: serum LDL-cholesterol level (mg dL^{-1}); B: serum HDL-cholesterol level (mg dL^{-1}); C: serum total cholesterol level (mg dL^{-1}) and D: serum triglyceride level (mg dL^{-1}). (***$P < 0.005$, **$P < 0.01$, * $P < 0.05$, ^{ns} $P > 0.05$)

4. CONCLUSIONS

The presence of (-)-EGCG and (+)-catechin as the principal flavan phenolics was confirmed by chromatographic analysis, the occurrence of which validated the strong radical scavenging activity shown by GTE. In acute toxicity study, GTE was found to be safe when dosed @ 2000 mg kg^{-1} BW, as it did not indicate any mortality, weight loss, or anomalous clinical signs in rats. GTE administered orally to rats did not cause any detrimental effect on liver, as discernible from either biochemical or histopathological assays performed for subacute toxicity. Histopathological analyses indicated moderate spleen congestion and minor changes in the kidney; nonetheless, these alterations were not sufficient to stimulate elevated serum creatinine levels, a prime indicator of renal degeneration. Unlike few contradictory records of induction of hepatic toxicity by GTE, the same was shown to be hepatoprotective against oral CCl_4 challenges in the present study. GTE was also indicative of causing positive impact on cholesterols and triglycerides of rats. While GTE appears to be a promising candidate for manufacturing hypolipidemic or hypocholesterolemic drugs or drinks, caution must be exercised with respect to its potential for latent degenerative changes at higher dosage, which further necessitates long-term human trials.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1556/066.2021.00007.

REFERENCES

Babu, P.V.A., Sabitha, K.E., and Shyamaladevi, C.S. (2006). Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. *Chemico-Biological Interactions*, 162(2): 114–120.

Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1): 70–76.

Brand-Williams, W., Cuvelier, M.E., and Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28(1): 25–30.

Chan, P.C., Ramot, Y., Malarkey, D.E., Blackshear, P., Kissling, G.E., Travlos, G., and Nyska, A. (2010). Fourteen-week toxicity study of green tea extract in rats and mice. *Toxicologic Pathology*, 38(7): 1070–1084.

Chen, Z.Y. and Chan, P.T. (1996). Antioxidative activity of green tea catechins in canola oil. *Chemistry and Physics of Lipids*, 82(2): 163–172.

Dahiya, S., Rani, R., Dhingra, D., Kumar, S., and Dilbaghi, N. (2018). Conjugation of epigallocatechin gallate and piperine into a zein nanocarrier: implication on antioxidant and anticancer potential. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 9(3): 035011.

Das Purkayastha, M., Das, S., Manhar, A.K., Deka, D., Mandal, M., and Mahanta, C.L. (2013). Removing antinutrients from rapeseed press-cake and their benevolent role in waste cooking oil-derived biodiesel: conjoining the valorization of two disparate industrial wastes. *Journal of Agricultural and Food Chemistry*, 61(45): 10746–10756.

Essex, K., Mehdi, A., and Shibeeb, S. (2019). Green tea consumption does not adversely affect kidney function and haematological parameters. *Food and Public Health*, 9(3): 79–83.

Galati, G., Lin, A., Sultan, A.M., and O'Brien, P.J. (2006). Cellular and in vivo hepatotoxicity caused by green tea phenolic acids and catechins. *Free Radical Biology and Medicine*, 40(4): 570–580.

He, J., Xu, L., and Le Yang, X.W. (2018). Epigallocatechin gallate is the most effective catechin against antioxidant stress via hydrogen peroxide and radical scavenging activity. *Medical Science Monitor*, 24: 8198–8206.

Hotta, H., Nagano, S., Ueda, M., Tsujino, Y., Koyama, J., and Osakai, T. (2002). Higher radical scavenging activities of polyphenolic antioxidants can be ascribed to chemical reactions following their oxidation. *Biochimica et Biophysica Acta (BBA) – General Subjects*, 1572(1): 123–132.

Hu, J., Webster, D., Cao, J., and Shao, A. (2018). The safety of green tea and green tea extract consumption in adults – results of a systematic review. *Regulatory Toxicology and Pharmacology*, 95: 412–433.

Kakkar, P., Das, B., and Viswanathan, P.N. (1984): A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biophysics and Biochemistry,* 21(2): 130–132.

Lambert, J.D., Kennett, M.J., Sang, S., Reuhl, K.R., Ju, J., and Yang, C.S. (2010). Hepatotoxicity of high oral dose (−)-epigallocatechin-3-gallate in mice. *Food and Chemical Toxicology*, 48(1): 409–416.

Muramatsu, K., Fukuyo, M., and Hara, Y. (1986). Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *Journal of Nutritional Science and Vitaminology*, 32(6): 613–622.

Oketch-Rabah, H.A., Roe, A.L., Rider, C.V., Bonkovsky, H.L., Giancaspro, G.I., Navarro, V., Paine, M.F., Betz, J.M., Marles, R.J., . . . and Ko, R. (2020). United States Pharmacopeia (USP) comprehensive review of the hepatotoxicity of green tea extracts. *Toxicology Reports*, 7: 386–402.

Park, K.S. (2018). Nonclinical and clinical safety studies on green tea extracts. *Journal of Natural and Ayurvedic Medicine*. 2(4): 000136.

Salminen, W.F., Yang, X., Shi, Q., Greenhaw, J., Davis, K., and Ali, A.A. (2012). Green tea extract can potentiate acetaminophen-induced hepatotoxicity in mice. *Food and Chemical Toxicology*, 50(5): 1439–1446.

Schönthal, A.H. (2011). Adverse effects of concentrated green tea extracts. *Molecular Nutrition & Food Research,* 55(6): 874–885.

Senthilkumar, S.R., Sivakumar, T., Arulmozhi, K.T., and Mythili, N. (2017). FT-IR analysis and correlation studies on the antioxidant activity, total phenolics and total flavonoids of Indian commercial teas (*Camellia sinensis* L.) – a novel approach. *International Journal of Scientific Research in Biological Sciences*, 6(3): 1–7.

Shimamura, T., Sumikura, Y., Yamazaki, T., Tada, A., Kashiwagi, T., Ishikawa, H., Matsui, T., Sugimoto, N., Akiyama, H., and Ukeda, H. (2014). Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives – inter-laboratory evaluation study. *Analytical Sciences*, 30(7): 717–721.

Wang, D., Wei, Y., Wang, T., Wan, X., Yang, C.S., Reiter, R.J., and Zhang, J. (2015). Melatonin attenuates (–)-epigallocatehin-3-gallate-triggered hepatotoxicity without compromising its downregulation of hepatic gluconeogenic and lipogenic genes in mice. *Journal of Pineal Research*, 59(4): 497–507.

Wang, H., Chen, L., Weng, L.L., Zhang, M.Y., and Shen, Q. (2014). Surface properties and dissolution kinetics of tea polyphenols. *Journal of Adhesion Science and Technology*, 28(24): 2416–2423.

Wolfram, S. (2007). Effects of green tea and EGCG on cardiovascular and metabolic health. *Journal of the American College of Nutrition*, 26(4): 373S–388S.