Protective effects of (-)-epigallocatechin-3-gallate against acetaminophen-induced liver injury in rats

Hsien-Tsung Yao*, Yu-Chi Yang, Chen-Hui Chang, Hui-Ting Yang, Mei-Chin Yin**

Department of Nutrition, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan

Received 4th of May 2015  Accepted 30th of June 2015
© Author(s) 2015. This article is published with open access by China Medical University

ABSTRACT

(-)-Epigallocatechin-3-gallate (EGCG) is the most abundant catechin with various biological activities found in tea. In this study, the effects of EGCG on the metabolism and toxicity of acetaminophen in rat liver were investigated. Male Sprague-Dawley rats were fed a controlled diet without or with EGCG (0.54%, w/w) for 1 week and were then intraperitoneally injected with acetaminophen (1 g/kg body weight) and killed after 12 h. Concentrations of acetaminophen and its conjugates in plasma and liver were then determined. The cytochrome P450 (CYP) and phase II enzymes activities were also evaluated. Rats fed the EGCG diet had lower plasma alanine aminotransferase and aspartate aminotransferase activities, as indices of hepatotoxicity, after acetaminophen treatment. Morphological damage by acetaminophen was lower in rats fed the EGCG diet. In addition, EGCG significantly reduced hepatic activities of midazolam 1-hydroxylation (CYP3A), nitrophenol 6-hydroxylation (CYP2E1), UDP-glucuronosyltransferase, and sulfotransferase. Finally, EGCG feeding reduced acetaminophen-glucuronate and acetaminophen-glutathione contents in plasma and liver. These results indicate that EGCG feeding may reduce the metabolism and toxicity of acetaminophen in rats.

1. Introduction

Phytochemicals are found in plant-based foods such as fruits, vegetables, beans, and grains, and they may reduce the risk of a number of chronic diseases including cancer, cardiovascular disease, and diabetes [1]. It is known that phytochemicals can also influence the pharmacological activity of drugs and their toxicities by modifying the drug metabolism system, including drug-metabolizing enzymes and transporters [2, 3].

Acetaminophen (N-acetyl-p-aminophenol, APAP) is an antipyretic and analgesic drug. When an overdose is taken, it can induce severe hepatotoxicity in both humans and experimental animals [4]. APAP is primarily metabolized in the liver by phase II conjugating enzymes, mainly UDP-glucurosyltransferase (UGT) and sulfotransferase (ST), to generate the nontoxic metabolites APAP-glucuronate and APAP-sulfate [4]. The initiation of APAP-induced liver injury results from the cytochrome P450 (CYP)-mediated metabolism of APAP into a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which exerts its toxicity by covalently binding to cellular macromolecules such as proteins, lipids, and DNA [5]. NAPQI also reacts with glutathione (GSH), leading to cellular GSH depletion and the production of reactive oxygen species in the liver. Studies have shown that natural products that decrease CYP enzyme activity, increase antioxidant enzyme activity or GSH levels may attenuate APAP-induced liver toxicity [6, 7].

(-)-Epigallocatechin-3-gallate (EGCG) is the most abundant and active polyphenol in green tea. Studies suggest that EGCG reduces the development and progress of various diseases such as cancer and cardiovascular disease [8, 9]. The principal hypothesis associated with the putative benefits of tea polyphenols or EGCG is linked to its strong free radical scavenging and antioxidant and anti-inflammation properties, as well as its modulating effects on drug-metabolizing enzymes, which reduce the bioactivation of carcinogens [10, 11]. Studies have shown that EGCG reduces hepatic CYP3A activity and increases the oral bioavailability of nicardipine and diltiazem in rats [12, 13]. Also, in vitro studies have indicated that EGCG reduces UGT and SUT activities [14, 15]. However, there is currently a lack of information about the effect of EGCG on the phase II detoxifying enzymes in vivo.

Recently, EGCG has been shown to have hepatoprotective activity against chemically induced liver injuries [16, 17]. However, the mechanism of action remains unknown. In this study, we investigated whether EGCG feeding could change the metabolism and toxicity of APAP in rats.

2. Materials and methods

2.1. Materials

Acetaminophen, methoxyresorufin, resorufin, p-nitrophenol, 4-nitrocatechol, NADPH, glutathione, 1-chloro-2,4-dinitrobenzene,
and heparin, were obtained from Sigma (St. Louis, MO, USA). Midazolam and 1-hydroxymidazolam were purchased from Ultrafine Chemicals (Manchester, UK). All other chemicals and reagents were of analytical grade and were obtained commercially. EGCG was purchased from Huzhou Ruzhou Rongkai Foliage Extract Co. LTD (Huzhou, China). The purity of the EGCG used was >99% as determined by high performance liquid chromatography (HPLC).

2.2. Animal study

First, in the preliminary study, we investigated the effect of EGCG on the drug-metabolizing enzymes in rat livers. Male Sprague-Dawley rats (aged 6 weeks) obtained from BioLASCO in Ilan, Taiwan were used. Rats were fed a laboratory chow diet with or without 0.15% and 0.54% of EGCG for 1 week. Second, to investigate the effect of EGCG on the metabolism and toxicity of APAP, the male Sprague-Dawley rats weighing 210 ± 10 g (6 weeks old) were randomly divided into three groups with six rats in each group. The animals in Group 1 (control group) and 2 were fed a laboratory chow diet. The animals in Group 3 were fed the same diet fortified with 0.54% EGCG. The daily dose of EGCG was about 460 mg/kg in rats, which was equivalent to the dose used in a previous study that found that EGCG did not change the liver function [18]. The rats were all housed in plastic cages in a room kept at 23 ± 1°C with 60 ± 5% relative humidity and a 12-h light-dark cycle. Food and drinking water were available ad libitum for 1 week. At the end of the 1-week feeding period, food was withdrawn for 12 h and a single 1000-mg/kg dose of APAP, as a solution in polyethylene glycol 400/water (50/50, v/v), was intraperitoneally injected into each animal in Group 2 and 3. At 12 h after the APAP dose, the animals in all three groups were killed by exsanguination via the abdominal aorta while under carbon dioxide (70:30, CO2/O2) anesthesia. Heparin was used as the anticoagulant, and the plasma was separated from the blood by centrifugation (1750 × g) for 20 min. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were measured immediately by use of commercial kits (Randox Laboratories, Antrum, UK). The liver samples of the three groups were excised and fixed in 10% neutral formalin followed by dehydration in ascending grades of alcohol, clearing in xylene, and embedding in paraffin wax. Liver sections (5 µm thickness) were stained with hematoxylin and eosin (H&E) for the histological examination [19]. The other liver samples from each animal were stored at -80°C. Microsome preparation and enzyme assays were performed within 2 weeks of liver collection. The separated plasma was used for the determination of APAP, APAP-sulfate, APAP-glucuronate, and APAP-glutathione concentrations. This study was approved by the Animal Center Management Committee of China Medical University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the Ministry of Science and Technology, Taiwan.

2.3. Determination of APAP and APAP conjugates in plasma and liver

Liver homogenates were prepared by homogenizing each gram of liver with 4 mL of ice-cold phosphate-buffered saline (pH 7.4). For determining APAP, APAP-sulfate, and APAP-glucuronate, plasma samples and liver homogenates were diluted 10-fold with control plasma and control liver homogenates, respectively. For determining APAP-glutathione, plasma samples and liver homogenates were not diluted. An aliquot (50 µL) of plasma or liver homogenate was then extracted with 100 µL of acetonitrile and centrifuged at 10,000 × g for 15 min at 4°C. The acetonitrile extract thusly obtained was then analyzed by an HPLC--mass spectrometry (HPLC/MS) method. Calibration standards of APAP, APAP-sulfate, APAP-glucuronate, and APAP-glutathione were prepared by serial dilution of the stock solution of each compound with control plasma or liver homogenate yielding final concentrations of APAP, APAP-sulfate, APAP-glucuronate, or APAP-glutathione that ranged from 1 to 200 µg/mL of plasma or liver homogenate. An aliquot (50 µL) of the spiked plasma or liver homogenate was then extracted with 100 µL of acetonitrile as described above.

To determine hepatic APAP protein adducts, liver homogenate was filtered through Nanosep centrifugal devices (Pall Life Sciences, Ann Arbor, MI, USA) with a membrane molecular weight cutoff of 30 kDa to remove low molecular weight compounds with the potential to interfere in the assay. The filtrate was then digested for 16 hours with proteases to free the APAP-cysteine from APAP protein adducts [7].

The HPLC/MS system consisted of an Agilent 1100 series LC System (Palo Alto, CA, USA). A Mightyil RP-18 GP column (5 µm, 250 × 4.6 mm i.d., Kanto Chemical) was used for the determination of APAP. An Agilent Zorbax Eclipse XDB-C18 column (5 µm, 250 × 3.0 mm i.d., Agilent) was used for the determination of APAP conjugates. The HPLC system was interfaced to an Agilent MSD mass spectrometer equipped with an electro-spray ionization source. The column temperature was set to 25 °C. Mobile phase A was 10 mM ammonium acetate containing 0.5% formic acid. Mobile phase B was acetonitrile containing 0.5% formic acid. An isocratic system containing 20%A/80%B was used to determine APAP. The flow rate was 0.5 mL/min. The retention time of APAP was 5.0 min. A gradient system with the following composition was used to determine the APAP conjugates: 90% A (0-2 min), 90% A to 10% A (2-3 min), 10% A (3-5 min), 10% A to 90% A (5-6 min), 90% A (6-12 min). The retention times of the analytes were 2.5 min (APAP-glucuronate), 2.4 min (APAP-sulfate), 2.5 min (APAP-glutathione), and 2.7 min (APAP-cysteine), respectively. The flow rate was 0.5 mL/min. The injection volume was 10 µL. The MS data acquisition was via selected ion monitoring. Ions representing the positive of the testing compound were selected and the peak was measured.

2.4. Preparation of liver microsomes

The frozen liver was thawed and then homogenized (1:4, w/v) in an ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 10,000 × g for 15 min at 4°C. The supernatants were then centrifuged at 105,000 × g for 60 min. The resulting microsomal pellets were suspended in a 0.25 M sucrose solution containing 1 mM EDTA and were stored at -80°C until they were used. The microsomal protein concentration was determined by using a BCA protein assay kit (Pierce, Rockford, IL, USA).

2.5. Drug-metabolizing enzyme activity assays

The CYP enzyme activities were determined by the previously reported HPLC/MS methods [20]. Methoxyresorufin (5 µM), p-nitrophenol (50 µM), and midazolam (2.5 µM) were respectively used as the probe substrates for methoxyresorufin O-demethyla-
Table 1 − Drug-metabolizing enzymes in the liver of rats fed the EGCG diet for 1 week.

| Drug Metabolizing Enzyme                                      | Control      | EGCG 0.18%   | EGCG 0.54%   |
|---------------------------------------------------------------|--------------|--------------|--------------|
| Testosterone 6β-hydroxylase (CYP3A) (pmol/min/mg protein)    | 798.9 ± 43.8 | 599.7 ± 121.6 | 452.2 ± 109.4 |
| p-Nitrophenol 6-hydroxylase (CYP2E1) (pmol/min/mg protein)   | 325.5 ± 27.1 | 371.0 ± 34.0  | 357.3 ± 16.6  |
| Methoxyresorufin O-demethylase (CYP1A2) (pmol/min/mg protein) | 31.0 ± 4.5   | 34.0 ± 5.6   | 29.5 ± 4.5    |
| UDP-glucuronosyltransferase (nmol/min/mg protein)             | 53.9 ± 22.2  | 34.0 ± 3.4   | 24.5 ± 4.3    |
| Sulphotransferase (pmol/min/mg protein)                       | 1,575.6 ± 47.5 | 1,386.0 ± 26.0 | 1,397.1 ± 38.6 |
| Glutathione S-transferase (nmol/min/mg protein)               | 148.2 ± 4.4  | 108.9 ± 3.5* | 116.4 ± 10.1* |

Results are expressed as the mean ± S.D. of three rats in each dietary group. *Significantly different from control group, $P < 0.05$.

2.6. Determination of GSH and glutathione S-transferase (GST) activity

Liver homogenate was prepared by homogenizing each gram of liver with 10 ml of ice-cold 1.15% KCl and centrifuging the homogenate at 10,000 × g for 15 min at 4°C. The resulting supernatant was used to determine the GSH content and GST activity. The GSH content in liver homogenates was determined by HPLC/MS [21]. The cytosolic ST activity was determined by using phosphoadenosine 5-phosphosulphate as the substrate and $p$-nitrophenol as the acceptor of the sulfate, and the rate of formation of adenosine 3,5-diphosphate was measured by HPLC/MS [22].

2.7. Statistical analysis

Statistical differences among groups were calculated by using a one-way ANOVA (SAS Institute, Cary, NC, USA). The differences were considered to be significant at $P < 0.05$ as determined by independent-sample $t$-tests.

3. Results

Table 1 shows the effect of EGCG feeding on drug-metabolizing enzyme activity in the liver. Rats fed on the 0.18% and 0.54% EGCG diets for 1 week had significantly reduced ($P < 0.05$) testosterone 6β-hydroxylase (CYP3A) in their livers. In addition, lower ST and GST activities was found in rats that were fed the EGCG diets ($P < 0.05$). The UGT activity was reduced only in 0.54% EGCG group ($P < 0.05$). No significant difference in plasma ALT and AST activities was observed, indicating EGCG caused no hepatotoxicity (data not shown).

After a single dose of the APAP treatment, there were no significant differences in body weight and liver weight among the three groups (data not shown). APAP treatment increased plasma ALT and AST activities compared with those same activities in control animals ($P < 0.05$) (Figure 1A, 1B). However, rats fed the EGCG diets had lower ($P < 0.05$) plasma ALT and AST activities after APAP treatment.

Histological examination of H&E stained liver sections was conducted 12 h after APAP administration to confirm the pattern of hepatotoxicity and compare the extent of liver injury between the control and the EGCG fed animals (Figure 2). Morphological findings were consistent with plasma transaminase observations. The APAP-induced histopathological changes in the liver came with significant degeneration and necrosis of hepatocytes in the centrilobular region and with perivenular inflammatory infiltrates. These APAP-induced histopathological changes were significantly reduced by EGCG treatment. These results indicate that the hepatotoxicity induced by APAP treatment in rats was reduced by EGCG.
BioMedicine | http://biomedicine.cmu.edu.tw/ 19

September 2015 | Volume 5 | Issue 3 | e42

APAP-glucuronide, and APAP-glutathione contents in the livers were noted in the EGCG group after APAP treatment. EGCG, however, had no effect on APAP-sulfate and APAP protein adducts contents in rat livers ($P > 0.05$).

The effect of EGCG on drug-metabolizing enzymes after APAP treatment is shown in Table 3. After APAP treatment, there was no significant difference ($P > 0.05$) on the activities of methoxyresorufin O-demethylase (CYP1A2), nitrophenol 6-hydroxylase (CYP2E1), and midazolam 1-hydroxylation (CYP3A); however, GST, UGT, and ST activities were lower than in the control group ($P < 0.05$). Among the APAP-treated groups, CYP3A and CYP2E1 activities were significantly lowered ($P < 0.05$) by EGCG. A significant decrease ($P < 0.05$) in ST activity was observed in rats fed a diet containing EGCG. EGCG had no effect ($P > 0.05$) on UGT activity in rats treated with APAP. In addition, EGCG feeding reversed the reduction of GST activity that was induced by APAP ($P < 0.05$).

A dramatic decrease ($P < 0.05$) in the hepatic GSH level was found in the APAP-treated groups. EGCG had no significant effect ($P > 0.05$) on hepatic GSH content after APAP treatment (Figure 3).

4. Discussion

The results of the present study show that EGCG feeding significantly reduced the elevation of plasma ALT and AST activities that were first induced by APAP. Morphological damage by APAP was lower in rats fed the EGCG diet. In addition, EGCG feeding reduced CYP3A and CYP2E1 activities and lowered APAP-glutathione content in rat livers ($P < 0.05$). A lower ($P < 0.05$) APAP, APAP-glucuronide, and APAP-glutathione contents in the livers were noted in the EGCG group after APAP treatment. EGCG, however, had no effect on APAP-sulfate and APAP protein adducts contents in rat livers ($P > 0.05$).

Table 2 – APAP and its related conjugates in the plasma and the liver of the rats.

|                | APAP               | APAP+EGCG          |
|----------------|--------------------|--------------------|
| Plasma (μg/ml) |                    |                    |
| APAP           | 371.2 ± 126.2      | 387.5 ± 62.9       |
| APAP-glucuronate| 135.9 ± 10.0       | 83.5 ± 24.9*       |
| APAP-sulfate   | 42.2 ± 3.4         | 36.9 ± 3.0*        |
| APAP-glutathione| 18.3 ± 2.2         | 10.7 ± 2.9*        |
| Liver (μg/g liver) |                |                    |
| APAP           | 116.4 ± 58.1       | 45.9 ± 18.6*       |
| APAP-glucuronate| 55.5 ± 3.5         | 32.2 ± 12.4*       |
| APAP-sulfate   | 11.0 ± 0.8         | 10.3 ± 0.6         |
| APAP-glutathione| 58.2 ± 28.4        | 17.6 ± 4.1*        |
| APAP protein adducts | 40.5 ± 3.1 | 39.4 ± 2.7         |

Results are expressed as the mean ± S.D. of six rats in each dietary group. The amount of EGCG in the diet was 0.54% (w/w).

*Significantly different from APAP group, $P < 0.05$.

After the intraperitoneal injection of a single dose of APAP, plasma APAP concentration remained unchanged in EGCG-treated rats (Table 2). However, EGCG feeding significantly reduced plasma APAP-glucuronide, APAP-sulfate, and APAP-glutathione concentrations in rat livers ($P < 0.05$). Lower ($P < 0.05$) APAP, APAP-glucuronide, and APAP-glutathione contents in the livers were noted in the EGCG group after APAP treatment. EGCG, however, had no effect on APAP-sulfate and APAP protein adducts contents in rat livers ($P > 0.05$).

The effect of EGCG on drug-metabolizing enzymes after APAP treatment is shown in Table 3. After APAP treatment, there was no significant difference ($P > 0.05$) on the activities of methoxyresorufin O-demethylase (CYP1A2), nitrophenol 6-hydroxylase (CYP2E1), and midazolam 1-hydroxylation (CYP3A); however, GST, UGT, and ST activities were lower than in the control group ($P < 0.05$). Among the APAP-treated groups, CYP3A and CYP2E1 activities were significantly lowered ($P < 0.05$) by EGCG. A significant decrease ($P < 0.05$) in ST activity was observed in rats fed a diet containing EGCG. EGCG had no effect ($P > 0.05$) on UGT activity in rats treated with APAP. In addition, EGCG feeding reversed the reduction of GST activity that was induced by APAP ($P < 0.05$).

A dramatic decrease ($P < 0.05$) in the hepatic GSH level was found in the APAP-treated groups. EGCG had no significant effect ($P > 0.05$) on hepatic GSH content after APAP treatment (Figure 3).

4. Discussion

The results of the present study show that EGCG feeding significantly reduced the elevation of plasma ALT and AST activities that were first induced by APAP. Morphological damage by APAP was lower in rats fed the EGCG diet. In addition, EGCG feeding reduced CYP3A and CYP2E1 activities and lowered APAP-glutathione content in rat livers. These results suggest that EGCG feeding may reduce CYP-mediated APAP bioactivation in liver and, at least in part, contribute to its ability to lower hepatotoxicity.

In addition to lower plasma ALT and AST activities, we also observed that morphological damage by APAP was lower in rats fed the EGCG diet. In this study, hepatic CYP2E1 and CYP3A activities after APAP treatment were reduced in rats fed the EGCG diet. CYP3A and CYP2E1 are two major enzymes that catalyze the oxidative metabolism of APAP and that may generate the toxic
In addition, rats fed tea polyphenols have been shown to have reduced microbial β-glucuronidase activity in the cecum [30]. Therefore, it is possible that EGCG feeding may have reduced enzymatic deconjugation of APAP-glucuronate in the intestine and decreased the re-absorption of APAP via the entero-hepatic circulation pathway. The lowered re-absorption of APAP may result in the lower ($P < 0.05$) APAP content in the liver and thus reduce the CYP-mediated bioactivation of APAP.

In summary, EGCG may act as a hepatoprotective agent against APAP-induced liver injury. Although the exact mechanism is still not clear, our study is the first to demonstrate that feeding rats a diet containing EGCG for 1 week reduces the metabolism and toxicity of APAP.

### Table 3 – Effect of EGCG feeding on drug-metabolizing enzyme activities in rat livers.

| Enzyme Activity                                      | Control           | APAP      | APAP+EGCG   |
|------------------------------------------------------|-------------------|-----------|-------------|
| Midazolam 1-hydroxylation (CYP3A4) (pmol/min/mg protein) | 213.1 ± 26.4      | 181.8 ± 29.0 | 126.2 ± 26.5* |
| Nitrophenol 6-hydroxylase (CYP2E1) (pmol/min/mg protein) | 395.1 ± 83.5      | 318.5 ± 48.5 | 221.7 ± 81.2* |
| Methoxyresorufin O-demethylase (CYP1A2) (pmol/min/mg protein) | 47.5 ± 9.4        | 45.6 ± 5.8   | 46.1 ± 4.1   |
| UDP-glucuronosyltransferase (nmol/min/mg protein)     | 36.2 ± 3.4        | 30.4 ± 2.3*  | 28.6 ± 5.5   |
| Sulphotransferase (pmol/min/mg protein)               | 339.8 ± 57.0      | 223.0 ± 56.8* | 137.9 ± 43.7* |
| Glutathione S-transferase (nmol/min/mg protein)       | 192.9 ± 5.3       | 177.9 ± 8.0* | 192.2 ± 9.8* |

Results are expressed as the mean ± S.D. of six rats in each dietary group. The amount of EGCG in the diet was 0.54% (w/w).

*Significantly different from control group; *Significantly different from APAP group, $P < 0.05$.

![Glutathione levels in rats after APAP treatment](image_url)

**Fig. 3** - Effect of EGCG feeding (0.54%, w/w) on GSH levels in rats after APAP treatment. *Significantly different from control ($P < 0.05$). Values are the mean ± SD of n = 6.

### References

1. Nishino H, Murakoshi M, Mou XY, Wada S, Masuda M, Ohsaka Y, et al. Cancer prevention by phytochemicals. Oncology 2005; 69: 38-40.
2. Gurley BJ. Pharmacokinetic herb-drug interactions (part 1): origins, mechanisms, and the impact of botanical dietary supplements. Planta Med 2012; 78: 1478-89.
3. Rodriguez-Fragoso L, Martinez-Arismendi JL, Orozco-Bustos D, Reyes-Esparza J, Torres E, Burchiel SW. Potential risks resulting from fruit/vegetable-drug interactions: effects on drug-metabolizing enzymes and drug transporters. J Food Sci 2011; 76: 112-4.
4. Thomas SHL. Paracetamol (acetaminophen) poisoning. Pharmacol Ther 1993; 60: 91-120.
5. James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatotoxicity. Drug Metab Dispos 2003; 31: 1499-506.
6. Murtil AE, Goosen TC, Bauman JN, Williams JA, Kulkarni S, Kostubsky S. Kinetics of acetaminophen glucuronidation by UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential implications in acetaminophen-induced hepatotoxicity. Chem Res Toxicol

### Acknowledgments

This study was financially supported by the grant-aid (NSC 102-2313-B-039-007) of the National Science Council, Taiwan.

Open Access This article is distributed under terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided original author(s) and source are credited.
[7] Acharya M, Lau Cam CA. Comparison of the protective actions of N-acetylcysteine, hypotaurine and taurine against acetaminophen-induced hepatotoxicity in the rat. J Biomed Sci 2010; 17: 35-46.

[8] Zhang L, Wei Y, Zhang J. Novel mechanisms of anticancer activities of green tea component epigallocatechin-3-gallate. Anticancer Agents Med Chem 2014; 14: 779-86.

[9] Mak JC. Potential role of green tea catechins in various disease therapies: progress and promise. Clin Exp Pharmacol Physiol 2012; 39: 265-73.

[10] Tipoe GL, Leung TM, Hung MW, Fung ML. Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. Cardiovasc Hematol Disord Drug Targets 2007; 7: 135-44.

[11] Muto S, Fujita K, Yamazaki Y, Kamataki T. Inhibition by green tea catechins of metabolic activation of procarcinogens by human cytochrome P450. Mutat Res 2001; 479: 197-206.

[12] Choi JS, Burm JP. Effects of oral epigallocatechin gallate on the pharmacokinetics of nicardipine in rats. Arch Pharm Res 2009; 32: 1721-5.

[13] Li C, Choi JS. Effects of epigallocatechin gallate on the bioavailability and pharmacokinetics of diltiazem in rats. Pharmazie 2008; 63: 815-8.

[14] Mohamed MF, Tseng T, Frye RF. Inhibitory effects of commonly used herbal extracts on UGT1A1 enzyme activity. Xenobiotica 2010; 40: 663-9.

[15] Isozaki T, Tamura H. Epigallocatechin gallate (EGCG) inhibits the sulfation of 1-naphthol in a human colon carcinoma cell line, Caco-2. Biol Pharm Bull 2001; 24: 1076-8.

[16] Thangapandiyan S, Miltonprabu S. Epigallocatechin gallate effect-ively ameliorates fluoride-induced oxidative stress and DNA damage in the liver of rats. Can J Physiol Pharmacol 2013; 91: 528-37.

[17] Moravcová A, Cervinkova Z, Kucera O, Lotkova H. Anti-oxidative effect of epigallocatechin gallate against D-galactosamine-induced injury in primary culture of rat hepatocytes. Acta Medica (Hradec Kraľove) 2014; 57: 3-8.

[18] Liu TT, Liang NS, Li Y, Yang F, Lu Y, Meng ZQ, et al. Effects of long-term tea polyphenols consumption on hepatic microsomal drug-metabolizing enzymes and liver function in Wistar rats. World J Gastroenterol 2003; 9: 2742-4.