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Research Article
Evaluation of Antioxidative Effect of Green Tea Catechins Against Isoniazid-induced Biochemical Alterations in Rats

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Abstract

Background and Objectives: Different studies indicated association of isoniazid (INH) treatment with different adverse reactions. Green tea catechins (GTC) showed antioxidative activities in different reports. The aim of this study was to assess the antioxidative activity of GTC against the INH-induced alterations in certain serum lipids and liver function biomarkers in addition to certain endogenous antioxidants. **Materials and Methods:** One hundred male albino rats were randomly divided into four groups: control, INH-treated, (INH+GTC)-treated and GTC-treated groups. The INH and GTC were orally administered at dose levels of 27 and 50 mg kg^{-1} b.wt., respectively, daily for 5 weeks. Statistical analysis was performed using t-test and one-way analysis of variance (ANOVA). **Results:** In the INH-treated animals, the lipid profile showed high levels of most serum lipids. Liver function biomarkers were elevated indicating hepatic affection. Oxidative stress was reflected in the increased liver malondialdehyde (MDA) content and decreased hepatic glutathione (GSH) level and superoxide dismutase (SOD) and catalase (CAT) activities, in addition to the reduced activity of erythrocyte G6PD. Yet, the administration of GTC 1 h prior to the INH resulted in the alleviation of these alterations. **Conclusion:** The co-administration of GTC with the INH ameliorated the INH-induced alterations in serum lipids, liver function biomarkers and antioxidant systems.

Key words: Anti-TB drugs, INH-induced adverse reactions, reactive metabolites, oxidative stress, flavonoid benefits, green tea catechins, antioxidative activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB), but can also affect other sites (extrapulmonary TB)[1]. It is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS)[2]. Both streptomycin and para-aminosalicylic acid showed activity against *Mycobacterium tuberculosis* and were followed in rapid succession by isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol, cycloserine and ethionamide among others[3,4]. Due to these advances, TB was transformed from a predictably fatal disease to a curable disease[5].

The currently recommended treatment for cases of drug-susceptible TB is a 6-month regimen of four first-line drugs: INH, RIF, PZA and ethambutol[2]. This includes the administration of INH, RIF, PZA and ethambutol for 2 months followed by INH and RIF for 4 months[6]. Since 2000, more than 60 million people have been documented as treated and cured and case and death rates have fallen steadily[2]. However, anti-TB drugs have unfortunately been reported to be associated with a significant number of adverse reactions[7-9]. Among these adverse reactions was hepatotoxicity[10-12]. Yet, most studies of anti-TB drugs did not attribute the adverse reactions to a specific drug.

Although INH is considered one of the most important first-line anti-TB drugs, used in combinations with other anti-TB drugs[13,14] or alone as a prophylactic drug[15,16], it was associated with different adverse reactions including bilateral optic neuritis[17], lupus erythematosus[18], gynecomastia[19], eosinophilic pneumonia[20], motor-dominant neuropathy[21] and rhabdomyolysis[22]. Yet, the most serious adverse reaction of isoniazid was hepatotoxicity[23-25]. The INH metabolism is thought to be associated with INH-induced liver injury[26]. Acetylhydrazine (AcHz), hydrazine (Hz) and acetylisoniazid (AcINH) are the major metabolites of INH[24]. In certain studies, the INH-induced hepatotoxicity has been attributed to Hz[27,28]. Cytochrome P450 isoenzymes were proposed to be involved in the oxidization of INH metabolites, Hz and AcHz, to reactive metabolites which are thought to be involved in INH hepatotoxicity[24]. Moreover, it has been reported that INH itself is oxidized to a reactive metabolite that can bind to liver proteins of mice *in vivo* and to human liver microsomes *in vitro* causing immune-mediated hepatotoxicity[26]. Application of gas chromatography-mass spectrometry (GC-MS)-based metabolomics revealed that oxidative stress and GSH consumption play important roles in the etiology of Hz-induced hepatotoxicity[27]. Hence, as the INH-induced hepatotoxicity was attributed to oxidative stress, it seemed necessary to suggest an antioxidative agent to prevent or at least alleviate this effect.

Tea (*Camellia sinensis*) is one of the most popular beverages in the world[29,30]. Flavonoids are the most important polyphenols in tea leaves and catechins are the main flavonoids found in green tea beverage[31]. The polyphenols of the green tea leaves determined by high performance liquid chromatography (HPLC) are composed of (+) catechin (C), (−) epicatechin (EC), (+) gallocatechin (GC), (−) epigallocatechin (EGC), (+) catechin gallate (CG), (−) epicatechin gallate (ECG), (+) gallocatechin gallate (GCG) and (−) epigallocatechin gallate (EGCG)[31,32]. Many biological functions of green tea catechins (GTC) have been studied including anti-obesity[33], antidiabetic[34] and anticarcinogenic activities[35]. In an *in vitro* study the EGCG showed antioxidative effect against D-galactosamine-induced injury in primary culture of rat hepatocytes[36]. Moreover, it has been reported that GTC have the ability to prevent atherosclerosis, hypertension, endothelial dysfunction, ischemic heart diseases, cardiomyopathy, cardiac hypertrophy and congestive heart failure by decreasing oxidative stress[37].

In the light of the afore-cited reports about the adverse reactions of INH and the antioxidative activity of GTC, the present study was suggested. The objective of this study was to evaluate the possible protective role of GTC against the adverse reactions and oxidative stress of INH at a therapeutic dose level throughout 5 weeks of daily administration to rats.

MATERIALS AND METHODS

This study was carried out in 2014-2016, in the labs of Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt.

Chemicals: The INH was obtained from Memphis Co. for Pharmaceutical and Chemical Industries (8 Sawah St., Amyria, Cairo, Egypt), as a highly purified white crystalline powder and it was suspended in 1% carboxymethyl cellulose (CMC). Leaves of green tea (*Camellia sinensis*) were subjected to extraction procedures using hot water (95°C) followed by ethyl acetate in order to obtain their catechins according to Ninomiya *et al*.[38]. The biochemical assays were carried out by using commercial reagent kits purchased from Stanbio Co. (San Antonio, Texas, USA), Spectrum Co. (Hannover, Germany) and Biodiagnostics Co. (Dokki, Giza, Egypt), using Perkin-Elmer Lambda UV/VIS spectrophotometer for measurements.

Animal model: Adult healthy male Sprague Dawley rats, *Rattus norvegicus* weighting 140-180 g were obtained from the animal house of National Organization for Drug Control and Research (NODCAR), Agouza, Giza, Egypt. Animals were housed in clean polyacrylic cages and maintained under standard animal house conditions: Room temperature (22-25°C), free air circulation and 12 h alternate light/dark cycle, in the animal house of Zoology Department, Faculty of Science, Cairo University. Animals were allowed free access to standard rodent pellet diet and drinking water. All the animals were acclimatized under standard conditions for 7 days before the start of the experiment.

Experimental design: Randomly, 100 rats were divided into four equal groups (control, INH-treated, (INH+GTC)-treated and GTC-treated with 25 individuals per group. Rats of the control group received 1% CMC. The INH-administered group was orally administered a daily dose of 27 mg kg^{-1} b.wt., of INH (in 1% CMC), which is equivalent to the therapeutic dose of human (300 mg/day, WHO[13]) and measured according to the body surface area ratio between man and rat[39] for 5 weeks. This dose was also used in other studies[40,41]. The animals of the third group were orally administered a dose of 50 mg kg^{-1} b.wt. of GTC, 1 h prior to the administration of INH (27 mg kg^{-1} b.wt.) for 5 weeks. The fourth group orally received a daily dose of 50 mg kg^{-1} b.wt., of GTC (in 1% of CMC) for 5 weeks. The dose of GTC were assigned according to certain studies[42,43].

Blood and liver sampling: After each week of experiment, five rats from each group were taken out and sacrificed. Blood samples were divided into two parts. The first part was collected on EDTA for red cell enzyme assay. The second part was collected into dry test tubes and then centrifuged at 3000 rpm in order to separate serum. The sera were kept at -80°C for further biochemical analysis. In order to collect liver samples, rats were immediately dissected. The liver was homogenized with 10% (w/v) ratio in ice-cold 50 mM phosphate buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was collected and kept at -80°C for further analyses.

Estimation of serum biochemical parameters: In the serum of all the experimental groups, the levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (ALP), were measured colorimetrically using the reagent kits purchased from Spectrum (Hannover, Germany) and Stanbio (San Antonio, Texas, USA). Both atherosclerotic indices, namely TC/HDL-C and LDL/HDL-C were calculated.

Estimation of liver biochemical parameters

Lipid peroxide assay: Using the reagent kit of the Biodiagnostics Co. (Dokki, Giza, Egypt), the level of malondialdehyde (MDA) in the liver homogenate was estimated by following its instruction pamphlet. The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation with thiobarbituric acid (TBA) in acidic medium at temperature of 95°C for 30 min to form pink TBA reactive product, the absorbance of which can be measured at 534 nm.

Non-enzymatic and enzymatic antioxidant assay: The concentration of non-enzymatic (glutathione, GSH) as well as the activities of enzymatic (catalase, CAT, superoxide dismutase, SOD) antioxidants were estimated in the homogenate of the liver of experimental animals by using the commercial reagent kits of Biodiagnostics Co. (Dokki, Giza, Egypt). The method, by which GSH content was measured, was based on the reduction of 5,5'dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound which is directly proportional to the concentration of GSH and its absorbance can be measured at 405 nm. The SOD activity determination was based on the ability of SOD to inhibit the reduction reaction of nitroblue tetrazolium dye mediated by phenazine methosulphate. The assay of CAT depends on its reaction with a known quantity of H_2O_2 then the reaction is stopped by a CAT-inhibitor after exactly 1 min. The remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) in the presence of peroxidase to form a chromophore with a color intensity inversely proportional to the amount of CAT in the original sample.

Estimation of erythrocyte glucose-6-phosphate dehydrogenase (G6PD): The reagent kit of the Biodiagnostics Co. (Dokki, Giza, Egypt) was used to determine the activity of the erythrocyte G6PD following its instruction manual. A volume of 0.2 mL of blood was washed with 2 mL aliquots of 0.9% NaCl solution followed by centrifugation at 3000 rpm for 10 min and this step was repeated 3 times. The washed centrifuged erythrocytes were suspended in 0.5 mL of digitonin and let stand for

15 min at +4°C and then centrifuged again. The supernatant was used in the assay within 2 h. The enzyme activity was estimated by measuring the rate absorbance change at 340 nm due to the reduction of $NADP^+$ into NADPH in the presence of G6P which converts into gluconate-6-P.

Statistical analysis: To reveal the effect of INH treatment, the data of INH-treated group were compared with those of vehicle-treated control group. To clarify the ameliorative effect of GTC, the data of the animal group treated with GTC prior to INH administration were compared with those of INH-treated group. To show the side effects of GTC, the data of GTC-treated group were compared with those of vehicle-treated control group. Data are presented as mean±standard error of mean (M±SEM) of five animals. The levels of statistical significance ($p<0.05$, $p<0.01$ and $p<0.001$) of results were determined by using t-test and one-way ANOVA according the data analysis software of Microsoft Excel (version 14.0).

RESULTS

The results of the biochemical parameters investigated in the present study are graphically presented in the Fig. 1-6. The significance levels according to the t-test are shown in the figures revealing the differences between animal groups at different weeks of administration period. The main key findings in the results are described according to the ANOVA test as follows:

Effect of INH and GTC on serum TC and TG concentrations: Regarding the concentrations of serum TC (Fig. 1a) and TG (Fig. 1b), data showed that the administration of INH caused significant increase ($p<0.001$) in their mean values. However, by the administration of GTC prior to the INH treatment, the levels of both TC and TG were reduced significantly ($p<0.001$) as compared with those of animal group treated with INH alone. No significant alteration was observed in any of the TC and TG levels of the animal group treated with GTC alone as compared to the controls ($p>0.05$).

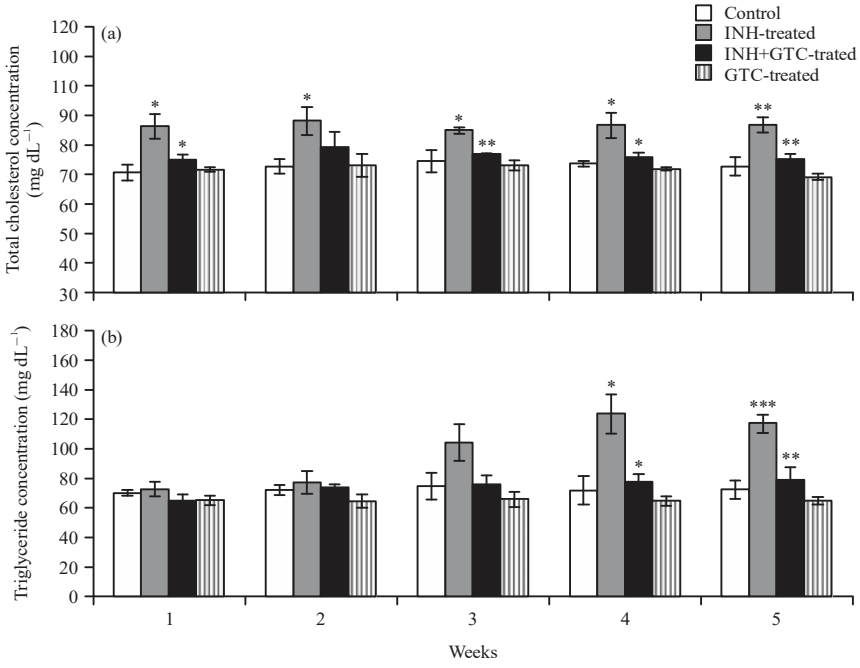

Fig. 1(a-b): Effect of daily oral administration of isoniazid (INH) (27 mg kg^{-1} b.wt.) and green tea catechins (GTC) (50 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone on the concentrations of (a) Serum total cholesterol (TC) and (b) Triglyceride (TG) of male albino rats. Both TC and TG concentrations were significantly increased in INH-treated group as compared with those of controls. Yet, they were significantly reduced in INH+GTC-treated group when compared with those of rats treated with INH alone. In GTC-treated group, no significant change was observed in TC or TG as compared with controls

Data are illustrated as mean±standard error of mean (M±SEM) of five animals in each group. According to t-test: *Significant ($p<0.05$), **Highly significant ($p<0.01$), ***Very highly significant ($p<0.001$)

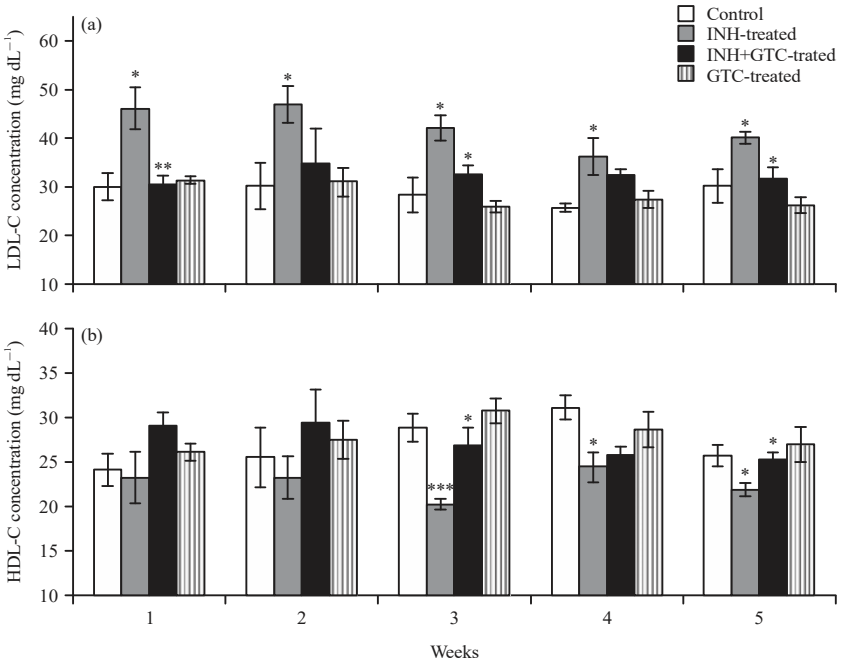

Fig. 2(a-b): Effect of daily oral administration of isoniazid (INH) (27 mg kg^{-1} b.wt.) and green tea catechins (GTC) (50 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the concentrations of (a) Serum low density lipoprotein cholesterol (LDL-C) and (b) High density lipoprotein cholesterol (HDL-C) of male albino rats. While LDL-C concentration was significantly increased, HDL-C concentration was significantly reduced in INH-treated group as compared with those of controls. Yet, the changes of both parameters were reversed in INH+GTC-treated group when compared with those of rats treated with INH alone. In GTC-treated group, neither LDL-C nor HDL-C concentration was changed as compared with those of controls

Data are illustrated as mean±standard error of mean (M±SEM) of five animals in each group. According to t-test: *Significant ($p<0.05$), **Highly significant ($p<0.01$), ***Very highly significant ($p<0.001$)

Effect of INH and GTC on serum LDL-C and HDL-C concentrations: While the concentration of LDL-C was significantly elevated in INH-treated group as compared with control group (Fig. 2a), the level of HDL-C was remarkably decreased ($p<0.001$) at most time intervals of treatment (Fig. 2b). These alterations in both LDL-C and HDL-C concentrations were significantly ($p<0.001$) reversed in animal group administered INH+GTC as compared with INH-treated animals. No significant change ($p>0.05$) was observed in both lipoproteins of animal group administered GTC alone.

Effect of INH and GTC on atherosclerotic indices: Both TC/HDL-C ratio (Fig. 3a) and LDL-C/HDL-C ratio (Fig. 3b) showed significant ($p<0.001$) increase in their mean values recorded for INH-treated rats as compared with those of controls. Yet, this increase was significantly reduced ($p<0.001$) in animal group administered GTC before INH. Neither TC/HDL-C ratio nor LDL-C/HDL-C ratio was significantly altered in GTC-treated group as compared with controls.

Effect of INH and GTC on liver function biomarkers: The administration of INH resulted in significant elevation ($p<0.001$) in the activity of each of the serum ASAT (Fig. 4a), ALAT (Fig. 4b) and ALP (Fig. 4c). Still, in the animal group treated with GTC before INH, the activities of serum ASAT, ALAT and ALP were significantly ($p<0.001$) lower than those of animal group treated with INH alone. On the other hand, the administration of GTC alone did not significantly alter the activity of any of the liver function biomarkers ($p>0.05$).

Effect of INH and GTC on MDA and GSH: Significant increase ($p<0.001$) was recorded in the concentration of liver MDA (Fig. 5a) and decrease ($p<0.001$) in the level of hepatic GSH (Fig. 5b) of INH-treated rats as compared with those of controls. In the animal group treated with both INH and GTC, the level of hepatic MDA was significantly decreased ($p<0.01$),

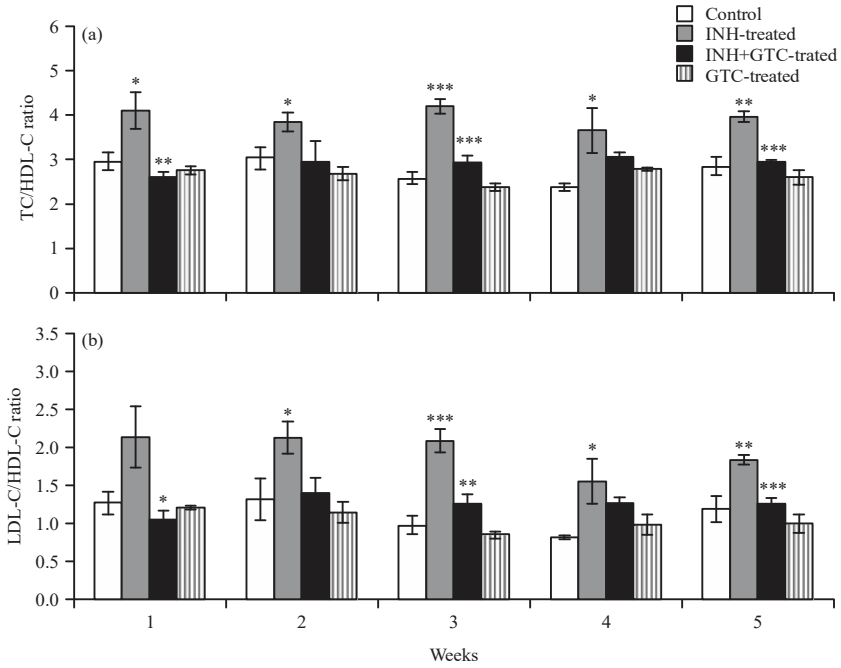

Fig. 3(a-b): Effect of daily oral administration of isoniazid (INH) (27 mg kg^{-1} b.wt.) and green tea catechins (GTC) (50 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the concentrations of (a) Serum TC/HDL-C and (b) LDL-C/HDL-C ratios of male albino rats. Both TC/HDL-C and LDL-C/HDL-C ratios were significantly increased in INH-treated group as compared with those of controls. Yet, they were significantly reduced in INH+GTC-treated group when compared with those of animal group treated with INH alone. Neither TC/HDL-C ratio nor LDL-C/HDL-C ratio was altered in GTC-treated group as compared with controls

Data are illustrated as mean±standard error of mean (M±SEM) of five animals in each group. According to t-test: *Significant ($p<0.05$), **Highly significant ($p<0.01$), ***Very highly significant ($p<0.001$)

while the hepatic GSH content was significantly increased ($p<0.001$) as compared with the corresponding levels of INH-treated rats. Yet, the administration of GTC alone did not significantly alter MDA or GSH concentrations ($p>0.05$).

Effect of INH and GTC on enzymatic antioxidants: The administration of INH resulted in significant decrease ($p<0.001$) in the activity of each of the liver SOD (Fig. 6a) and CAT (Fig. 6b) and erythrocyte G6PD (Fig. 6c). Nonetheless, in the animal group administered GTC before INH, significant rise was observed in the activities of SOD ($p<0.01$), CAT ($p<0.001$) and G6PD ($p<0.01$) as compared with those of INH-treated group. The administration of GTC alone did not significantly alter the activity of SOD, CAT or G6PD ($p>0.05$).

DISCUSSION

In general, the majority of the biochemical parameters herein studied were found to be significantly altered in INH-treated rats. Regarding serum lipids, the administration of INH at its chosen dose significantly caused an increase in the concentrations of serum TC, TG and LDL-C concurrent with decreased level of HDL-C. Consequently, the atherosclerotic indices (*vis.* TC/HDL-C and LDL-C/HDL-C ratios) markedly increased. In agreement with these findings, it was found that the administration of INH to the wild-type mice resulted in increased serum TC and TG[44]. Also, Usmani *et al.*[45] recorded the elevated cholesterol level in rats administered INH plus RIF (50 mg kg^{-1}/day p.o., for each) for 28 days.

The alterations herewith observed in serum lipids could be attributed to the INH-induced oxidative stress and lipid peroxidation (LPO) in rat tissues particularly liver. The LPO indicated by elevated MDA level was recorded in association with the INH treatment in the present study (Fig. 5a) and in combination with other anti-TB drugs[46-48]. Dyslipidemia was found to be associated with increased oxidative stress, diminished overall antioxidative protection and increased risk for atherosclerosis[49]. The enhanced lipid production may be due to the ability of INH to alter the gene expression of some enzymes that were involved in the lipid synthesis[50].

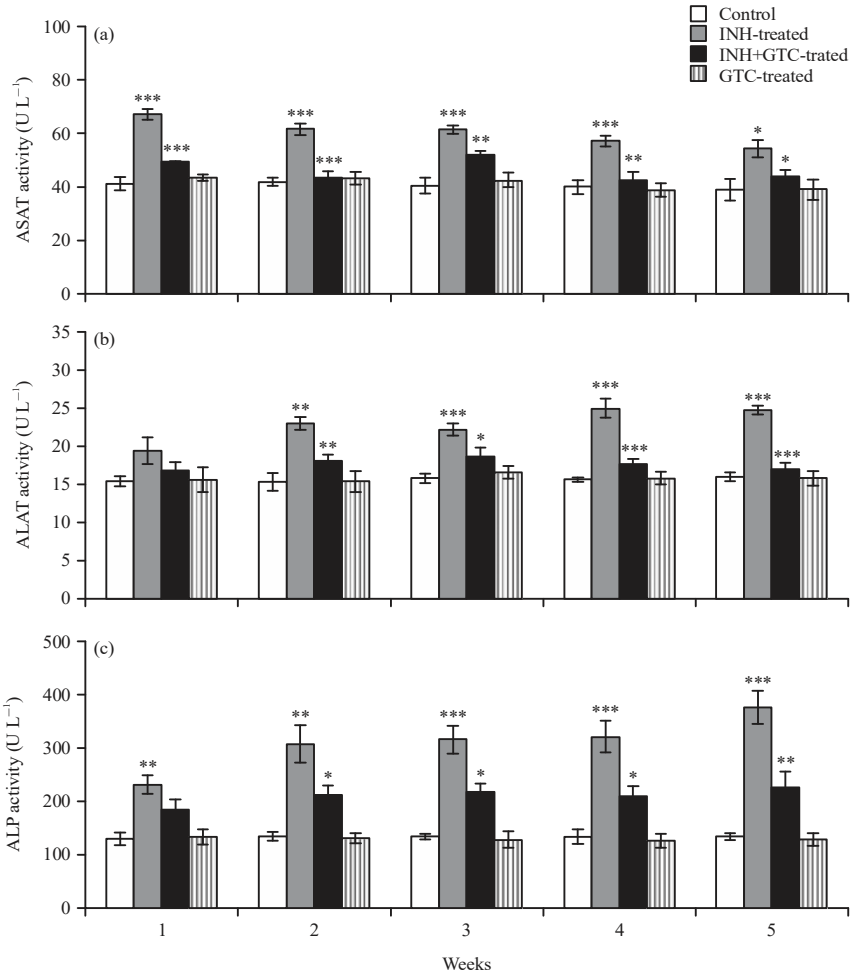

Fig. 4(a-c): Effect of daily oral administration of isoniazid (INH) (27 mg kg^{-1} b.wt.) and green tea catechins (GTC) (50 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the activities of (a) Serum aspartate aminotransferase (ASAT), (b) Alanine aminotransferase (ALAT) and (c) Alkaline phosphatase (ALP) of male albino rats. The activities of serum ASAT, ALAT and ALP were significantly elevated in INH-treated group as compared with those of controls. Yet, they were significantly reduced in INH+GTC-treated group as compared with those of rats treated with INH alone. In GTC-treated group, no significant change was observed in ASAT, ALAT or ALP as compared with controls

Data are illustrated as mean±standard error of mean (M±SEM) of five animals in each group. According to t-test: *Significant ($p<0.05$), **Highly significant ($p<0.01$), ***Very highly significant ($p<0.001$)

The present data showed a marked alleviation in all the studied lipid profile parameters in the serum of rats administered GTC before the treatment with INH. This beneficial effect of GTC on INH-induced dyslipidemia seems a consequence of the antioxidative effect of GTC through scavenging of free radicals, reducing LPO and improving of the endogenous antioxidants. In this regard, it has been reported that the streptozotocin (STZ)-induced diabetes complications, reflected by elevation in TG, TC, LDL-C and reduction in HDL-C were ameliorated in the catechin-treated diabetic rats[51]. The latter authors indicated that catechin adjusted oxygen radical generation, which may be responsible at least in part for the improved hyperglycemia, hyperlipidemia and oxidative stress in STZ-diabetic rats. Regarding the direct beneficial hypolipidemic effect of GTC, it has been reported that (-)-epicatechin significantly reduced TC, LDL-C and TG and alleviated liver fat accumulation, while it increased HDL-C, in hyperlipidemic rats[52].

As regards the biomarkers of the hepatic function, the results obtained in the present study revealed that the activities of each of the serum ASAT, ALAT and ALP were remarkably elevated in INH-treated rats. In the light

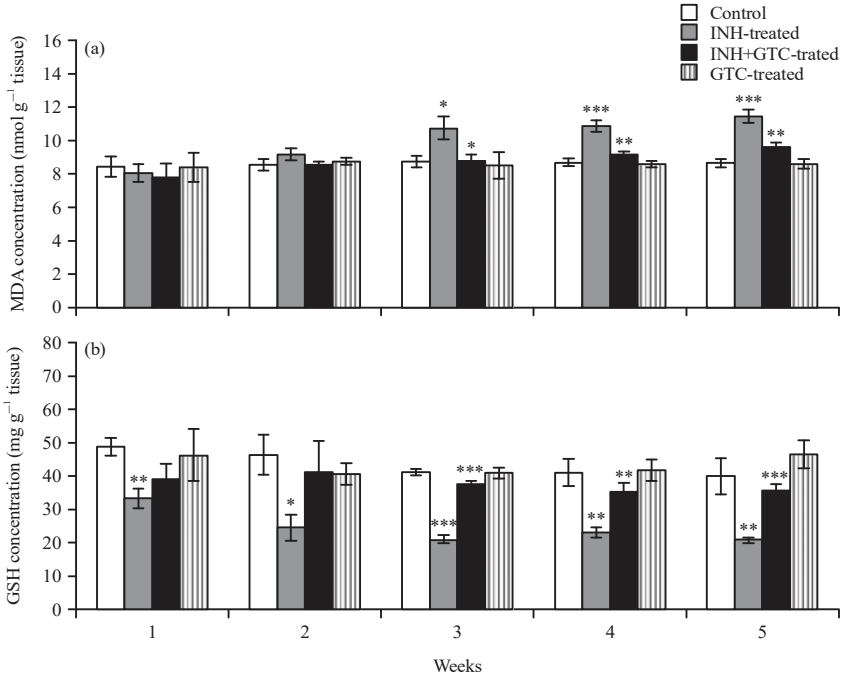

Fig. 5(a-b): Effect of daily oral administration of isoniazid (INH) (27 mg kg^{-1} b.wt.) and green tea catechins (GTC) (50 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the concentrations of (a) Liver malondialdehyde (MDA) and (b) Reduced glutathione (GSH) of male albino rats. While hepatic MDA concentration was significantly increased, liver GSH content was decreased in INH-treated group as compared with controls. In rats administered GTC before INH, the MDA level was decreased while GTC content was increased as compared with INH-treated group. Both parameters were not altered in rats treated with GTC alone

Data are illustrated as mean±standard error of mean (M±SEM) of five animals in each group. According to t-test: *Significant (p<0.05), **Highly significant (p<0.01), ***Very highly significant (p<0.001)

of this elevation in liver function markers, the hepatic tissue seemed to be affected to a certain extent. In accordance with these results, different reports have indicated the increased activities of serum ASAT, ALAT and ALP in association with the INH treatment, either alone or in combination with other anti-TB drugs, in patients[53], rats[54,55] and mice[56,57]. Yet, other studies indicated that INH in particular stands behind hepatotoxicity[58-60].

It seemed evident that INH-induced liver injury or hepatotoxicity is due to oxidative stress[56,61,62] supported by the result of INH-induced lipid LPO in this study (Fig. 5a) and in other studies[63,64]. An *in vitro* study reported that when human hepatoma HepG2 cells were exposed to different concentrations of INH, cytotoxicity, oxidative stress and apoptosis were evidenced[65]. The same study indicated that INH exposure causes increased ROS generation along with the alteration in the levels of enzymatic antioxidants[65]. There is evidence that Hz, the metabolite formed by amidase-catalyzed hydrolysis of INH, plays an important role in the mechanism of INH-induced hepatotoxicity[66]. So, Lee and Boelsterli[67] stated that the acylamidase inhibitor bis-p-nitrophenyl phosphate prevented cell injury, suggesting that Hz greatly contributed to the toxicity. In addition, it was also indicated that the INH hepatotoxicity occurred alongside the down-regulation of the bile salt export pump (BSEP) and multidrug resistance protein 2 (MRP2) *in vivo* and *in vitro*, leading to the accumulation of toxic substrates in the hepatocytes[60].

In the animal group herein treated with GTC prior to INH administration, all the increments in serum ASAT, ALAT and ALP were reduced. These results could indicate the hepatoprotective role of GTC against the INH toxic effect. In addition, the administration of GTC alone did not significantly change any of the aforementioned criteria of liver function. As the hepatoprotective role of GTC was reviewed in the literature, it has been found that the administration of a polyphenols-enriched extract from green tea in mice prior to CCl_4 injury significantly decreased the CCl_4-induced

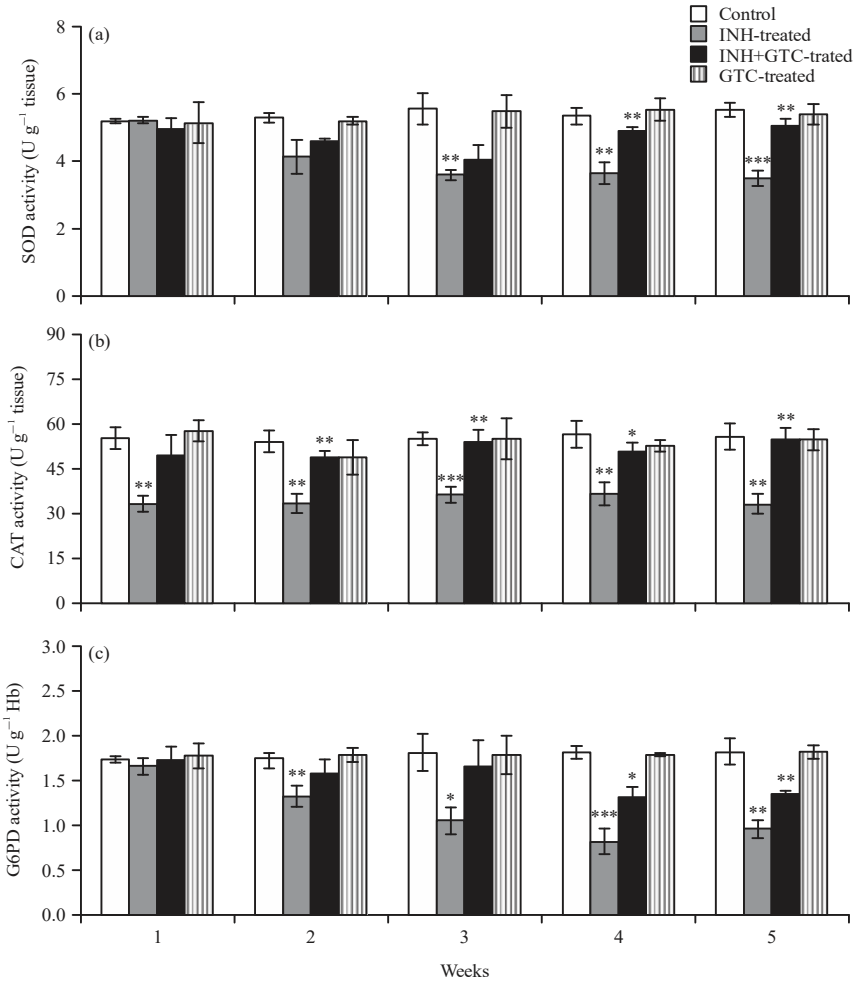

Fig. 6(a-c): Effect of daily oral administration of isoniazid (INH) (27 mg kg^{-1} b.wt.) and green tea catechin (GTC) (50 mg kg^{-1} b. wt.), 1 h prior to INH administration and alone, on the activities of (a) Liver superoxide dismutase (SOD) and (b) Catalase (CAT) and (c) Erythrocyte glucose-6-phosphate dehydrogenase (G6PD) of male albino rats. In INH-treated rats, the activities of hepatic SOD and CAT and erythrocyte G6PD were significantly reduced as compared with those of controls. Yet, in GTC+INH-treated rats, the activities of these enzymes were significantly higher than those of INH-treated group. No change was observed in the activity of these enzymes in case of GTC-treated group

Data are illustrated as mean±standard error of mean (M±SEM) of five animals in each group. According to t-test: *Significant ($p<0.05$), **Highly significant ($p<0.01$), ***Very highly significant ($p<0.001$)

elevation of serum ALAT, ASAT and ALP activities[68]. In addition, the administration of green tea polyphenols (GTP) to Zucker fatty (ZF) rats could attenuate the increase in serum ASAT and ALAT activities induced by feeding high-fat diet (HFD) for 2 weeks[69]. More specifically, it has been reported that EGCG effectively improved hepatic pathological damage and decreased serum levels of ALAT in concanavalin A-challenged mice[70]. To evaluate safety of GTC, some investigators found that the daily intake of a standardized, decaffeinated, catechin mixture containing 200 mg EGCG (twice daily) for 1 year was well tolerated and did not produce adverse effects in men with baseline high-grade prostatic intraepithelial neoplasia[71].

As previously mentioned in the present study, the INH-induced hepatotoxicity seemed a consequence of the oxidative stress caused by INH or its metabolites. This oxidative stress was obvious through the increased level of hepatic MDA, indicating LPO, concurrent with decreased level of liver GSH and the activities of hepatic SOD and CAT. In addition, erythrocyte G6PD activity was reduced in INH-treated rats. In the same sense, INH (alone or in combination with other anti-TB drugs) was reported to induce

oxidative stress and hepatotoxicity via the increase in the hepatic MDA content and the decrease in the level of GSH and the activities of SOD and CAT in rats[41,59,72,73]. Regarding the *in vitro* studies, it has been found that the exposure of human hepatoma HepG2 cells to INH caused increased ROS generation along with alteration in levels of enzymatic antioxidants such as SOD, CAT and G6PD[65]. Also, by the exposure of isolated rat hepatocytes to INH and its metabolite Hz, Heidari *et al.*[28] found that INH caused considerable ROS formation in GSH-depleted cells while Hz caused ROS formation and LPO in both intact and GSH-depleted cells. They added that Hz lowered cellular GSH reserve and increased GSSG.

In the animal group pretreated with GTC before INH, the hepatic level of MDA was lower than that of INH-treated rats while the GSH content and SOD, CAT and G6PD activities were higher than the corresponding ones of rats administered INH alone. This could indicate the effective antioxidative role of GTC against oxidative stress induced by INH and/or its metabolites. In this regard, different studies indicated that the increase in the levels of tissue MDA and the decrease in GSH content in response to oxidative stress induced by cisplatin[74], concanavalin A[70] and homocysteine[75] were markedly reversed by the co-treatment with GTC. In addition, the reduction in the activities of tissue SOD and CAT due to the oxidative effect of doxorubicin[76], cyclosporine[77] and ammonium metavanadate[78] were alleviated in association with GTC administration[79].

CONCLUSION

The administration of INH to rats at a dose level equivalent to a human therapeutic dose, resulted in remarkable alterations in certain serum lipid parameters, liver function biomarkers and endogenous antioxidant systems. However, the pre-treatment of GTC before INH administration markedly alleviated the INH-induced alterations. In addition, the administration of GTC alone did not alter any of the parameters studied.

This study suggested further studies to evaluate the co-administration of GTC with other medications known with oxidative stress-induced side effects. In addition, to make a clear vision of the exact mechanisms of the oxidant-antioxidant interaction, different recent assays and sophisticated techniques are required.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the mechanisms accounting for GTC ameliorating effect against drugs-induced metabolic alterations. It may pave the way for incorporating the GTC with the anti-TB drugs during the treatment regimens, in the future. In addition, a new trend on the drug-flavonoid combination may be followed and developed using various recent techniques in different scientific scopes.

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