Research Article

Green Tea Consumption Ameliorates Intestinal and Hepatic -Toxicity induced by Long-Term administration of Cisplatin -

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ABSTRACT

Background: Cisplatin (CP) is one of the most effective anti-cancer agents but its use is limited due to renal, hepatic, and intestinal toxicity**.** Various strategies were made to ameliorate CP - induced damage but were not found suitable for clinical practice. Green tea (*Camellia sinensis*) has been used for centuries as a medicinal drink. Green Tea (GT) polyphenols have shown strong chemo preventive and chemotherapeutic effects against various pathologies including cancer, diabetes, depression, cardiovascular, and neurological disorders and renal failure. Recently we have shown that GT consumption prevents CP induced nephrotoxicity.

Aim: We now hypothesized that GT would protect against CP-induced intestinal and hepatic toxicity by virtue of its antioxidant properties.

Methods: CP was co - administered to control and GT - fed male Wistar rats over a period of 25 days. Enzymes of oxidative stress, Brush Border Membrane (BBM), and carbohydrate metabolism were analyzed.

Results: CP Suppressed Superoxide Dismutase (SOD) and catalase activities in both intestine and liver. The activity of glucose-6-phosphate dehydrogenase increased whereas that of malic enzyme decreased. However, GT given to CP rats improved the overall metabolism, enhanced antioxidant defense and energy metabolism. The BBM enzymes were differentially altered by GT.

Conclusion: In conclusion, Green Tea Ameliorates CP elicited toxicity and oxidative damage by improving antioxidant defense, tissue integrity and energy metabolism.

Keywords: Antioxidants; Green Tea; Cisplatin; Carbohydrate Metabolism; Intestine; Liver

ABBREVIATIONS

Acid Phosphatase; an enzyme (ACPase); Alkaline Phosphatase; an enzyme (ALP); Analysis of Variance; statistical tool (ANOVA); Adenosine 5'-Triphosphate; energy currency (ATP); Brush Border Membrane; intestinal membrane (BBM); Brush Border Membrane Vesicles (BBMV); Blood Urea Nitrogen; blood parameter (BUN); Cisplatin(CP); Green Tea (GT); γ-Glutamyl Transferase; an enzyme (GGTase); Glucose-6-Phosphatase; an enzyme (G6Pase); Glucose-6-Phosphate Dehydrogenase; an enzyme (G6PDH); Hexokinase; an enzyme (HK); Hexose Monophosphate; an enzyme (HMP); Leucine Aminopeptidase; an enzyme (LAP); Lactate Dehydrogenase; an enzyme (LDH); Lipid Peroxidation; an enzyme (LPO); Malate Dehydrogenase; an enzyme (MDH); Malic Enzyme; an enzyme (ME); Nicotinamide Adenine Dinucleotide Phosphate (reduced); reducing equivalent (NADPH); Nicotinamide Adenine Dinucleotide Phosphate (NADP$^+$); Nicotinamide Adenine Dinucleotide Reduced (NADH); Inorganic Phosphate (Pi); Reactive Oxygen Species (ROS); Superoxide Dismutase; an enzyme (SOD); Sulfhydryls Groups (SH); Tricarboxylic Acid Cycle (TCA cycle).

INTRODUCTION

We are exposed daily to numerous environmental toxins including certain drugs and chemicals that dramatically alter the structure and functions of many organs including the kidney, liver and intestine [1-12]. Cisplatin (cis-diaminedichloroplatinum (II), (CP) is one of the most effective and widely used chemotherapeutic agents in the treatment of various human solid tumors [13-15]. However, CP accumulates in the kidney and other tissues and causes nephrotoxicity, neurotoxicity, ototoxicity, Gastro Intestinal (GI) toxicity, and hepatic toxicity [16-18] that limits the long term clinical use of CP in cancer therapy. Although nephrotoxicity is the major side effect, CP administration also caused profound GI symptoms and morphological and biochemical alterations in the small intestinal mucosa and liver [16,19]. Histological analysis revealed that CP damages the mucosal structure from jejunum to ileum by causing acute epithelial necrosis [20-21] along with certain untoward adverse complications including nausea, vomiting, diarrhea and myelosuppression [17,22-23]. Cisplatin has also been found to induce mitochondrial oxidative stress with impairment of energetic

metabolism, membrane revivification and apoptosis in rat liver [24]. Several agents including antioxidants, modulators of nitric oxide, diuretics and cytoprotective and apoptotic agents etc., have been tested over the last two decades to reduce CP - induced toxicity for effective cancer therapy [25-28]. However, none of them were found safe for clinical practice. In the past decade much interest has been focused on disease prevention via naturally occurring substances for the control and management of various chronic diseases. Green tea (*Camelia sinensis*) has been used for centuries as a medicinal drink that showed extensive health benefits and physiological effects [29]. Green Tea (GT) consumption has been linked to retard various forms of cancer, diabetes, depression, cardiovascular and neurological disorders and renal failure due to natural and chemical toxins [29-30]. It is an excellent source of polyphenols. In particular, GT catechins have been held responsible for most of the beneficial health effects ascribed to GT [31] by their antioxidant, ant mutagenic and ant carcinogenic properties [29]. GT polyphenols were found to inhibit the growth of stomach cancer and gastritis and modulate intestinal functions [32]. GT catechins were also found to exert a protective effect on the gastrointestinal mucosa and prevent intestinal atrophy [33]. GT catechins improve gut flora by selectively increasing the growth of bifid bacteria and lactobacilli in the gut wall while decreasing levels of potential pathogens [34]. GT have been shown to reduce inflammation associated with Crohn's disease and ulcerative colitis, a type of Inflammatory Bowel Disease (IBD) [33]. It also appears to protect liver from damaging effects of toxic substances and also against the development of liver tumors**.** Although CP nephropathy has been extensively studied but CP induced GI - and hepatic-toxicity are not vigorously pursued. Most of the studies on CP - induced nephropathy or other toxic effects were carried out by using a single injection; the present research determined the effect of CP and its protection by using multiple injections over a period of 25 days with or without GT consumption as may be required in cancer therapy. Recently we have shown that GT prevented GM - induced GI and hepatic toxicity and oxidative damage [35], we now hypothesize that GT would prevent CP - induced GI - and hepatic toxicity. The results obtained indicate that CP administration caused selective alterations in the activities of various enzymes of carbohydrate metabolism, BBM and oxidative stress and increased lipid peroxidation. However, GT consumption markedly reversed CP-induced alterations by

improving energy metabolism, BBM integrity and by enhancing antioxidant defense mechanism as shown earlier. The present results suggest that GT consumption can be an option for long-term clinical use of CP without causing harmful side effects.

MATERIALS AND METHODS

Chemicals and drugs

Green tea (Lipton / Kangra - brand) was purchased from commercial sources (Jain Pan House, New Delhi, India). All other chemicals used were of analytical grade and were purchased either from Sigma Chemical Co. (St Louis, MO, USA) or Sisco Research Laboratory, Mumbai, India.

Green Tea Extract

Green Tea Extract (GTE) was prepared by adding green tea (30 g) to 500 ml of boiling water, steeped for 15-20 min. Infusion was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 500 ml of boiling water and filtered, and the two filtrates were combined to obtain 3% green tea extract (3 g tea leaves/100 ml H_2O). The resulting clear solution is similar to tea brews consumed by humans. A known amount of green tea extract (2 X 125 mL= 250 mL/day) was provided in two servings to GT consuming rats for 25 days and was found to be sufficient. According to the manufacturer's information, the antioxidant content was 95 mg/g of GT.

Experimental design

The animal experiments were conducted according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Adult male Wistar rats (8 rats/ group) weighing 150-180 g fed with standard rat chow (Aashirwaad Industries, Chandigarh, India) and water *ad* libitum were conditioned for one week before the start of the experiment. Initially two groups of rats entered the study after acclimatization (Figure 1). They were fed on a normal rat chow diet with one group consuming water (control) and the other consuming GT extract (3% w/v) in drinking water for 25 days as reported earlier [2-3]. CP (3 mg/kg bwt/ day), in 0.9% saline was administered every fifth day for 25 days to one of the sub-group designated as CP and TCP. The other sub-group from each group received an equivalent volume of normal saline for the same period. The rats were sacrificed on the fifth day after the last injection under light ether anesthesia. Blood samples were collected and the liver and intestine were extracted and processed for the preparation of homogenates and BBMVs. All the preparations and

analyses of various parameters were carried out simultaneously under similar experimental conditions to avoid any day-to-day variations. Body weights of rats were recorded at the start and completion of the experimental procedure.

Preparation of homogenates and Brush Border Membranes (BBM)

After the completion of the experiment, liver and intestine were extracted. The intestines were washed by flushing them with ice cold buffered saline (1mM Tris-HCl, 9g/L NaCl, pH 7.4). The liver was put in Tris Buffered Saline (TBS). A 10% liver homogenate was prepared in 10mM Tris-HCl buffer, pH 7.5. The homogenate was centrifuged at 3000g at 4˚C for 15 min to remove cell debris and the supernatant was saved in aliquots and stored at -20˚C for assaying the enzymes of carbohydrate metabolism, free-radical scavenging enzymes and for estimation of total-SH and lipid per oxidation. The intestinal BBMV was prepared as described by Farooq et al. [36], using differential precipitation by $CaCl_2$. Mucosa scraped from 4-5 washed intestines was used for each BBM preparation. Briefly, the mucosal scrapings were collected in a beaker containing 50 mM mannitol, 5 mM Tris-HCl, and pH 7.5. The mucosal homogenate was diluted with the above mentioned Tris-mannitol buffer (15 ml/g tissue) and further homogenized using Ultra-Turrex T25 homogenizer with three pulses of 30s each with 30s interval between each pulse. Aliquots of mucosal homogenate were saved and quickly frozen for further analysis. $CaCl_2$ was added to the filtrate, to a final concentration of 10 mM and was kept for 20 min on ice, with intermittent stirring. The homogenate were then centrifuged at 2000 g (5000 rpm) for 10 min in a Beckman J2-M1 refrigerated centrifuge using a JA-17 rotor. The pellet was discarded and the supernatant was recentrifuged at 35000 g (17000 rpm) for 30 min. The pellet was resuspended in a small volume (1-2 ml) of 50 mM sodium maleate buffer, pH 6.8, with four complete passes by a loose fitting Dounce homogenizer (Wheaton, USA) and centrifuged at 35,000 g (17,000 rpm) for 30 min in 15 ml corex glass tube using JA-20 rotor. The white outer fluffy portion of the pellet was resuspended carefully in a small volume of the above mentioned buffer. The BBM suspension was quickly frozen in small aliquots and used for enzyme analysis. All the steps involved were strictly carried out at 0-4˚C unless otherwise specified.

Enzyme assays

The activities of BBM biomarkers enzymes, Alkaline Phosphatase (ALP), Leucine Amino Peptidase (LAP), γ- glutamyl transferase (GGTase) in the homogenates and BBM preparations and lysosomes marker enzyme, Acid Phosphatase (ACPase) in the homogenates were determined as described earlier [37]. The enzymes of carbohydrate metabolism, e.g., Lactate (LDH), Malate (MDH), Glucose-6-Phosphate (G6PDH) Dehydrogenase and NADP-Malic Enzyme (ME), involved in oxidation of NADH or reduction of NADP were determined by measuring the extinction changes at 340 nm in a spectrophotometer (Cintra 5; GBC Scientific Equipment, Pty., Victoria Australia) as described elsewhere [38-39]. The other enzymes, Glucose-6-Phosphatase (G6Pase), Fructose-1, 6-Bisphospatase (FBPase) and Hexokinase (HK) were determined as described in our previous studies [39]. The activities of Superoxide Dismutase (SOD), Catalase and Glutathione Peroxidase (GSH-Px) were determined as described by Priyamvada et al. [8]. Lipid Peroxidation (LPO) and total SH-groups were estimated as described earlier [8]. Protein concentration was determined by the modified method of Lowry et al [40] as described by Yusufi et al [41].
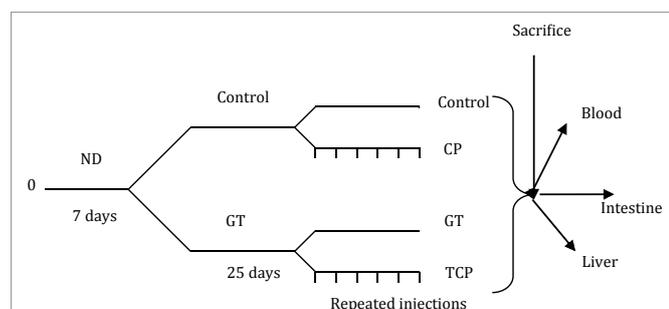

Figure 1: Experimental Design (ND = normal diet; GT = green tea; TCP = green tea + CP; CP = Cisplatin, =   intraperitoneal administration of CP every fifth day for 25 days)

Statistical analyses

All data are expressed as Mean ± SEM for at least 4-5 different preparations. Statistical evaluation was conducted by one-way ANOVA and by unpaired student's t test using SPSS 7.5 software. A probability level of $p < 0.05$ was selected as indicating statistical significance. Most of the changes between various groups were compared with control values for better understanding and clarity. However, specific differences and statistical significance between other groups were evaluated separately e.g. CP vs. TCP.

RESULTS

The effect of Green Tea (GT) consumption and Cisplatin (CP) was determined on various parameters in rat serum, intestine and liver. In general the rats remained active and alert throughout the study. The daily food and fluid intake was similar in various experimental groups of rats. There was a slight loss in body weight in GT, CP and GT + CP rat (data not shown).

Effect of Green Tea (GT) on Cisplatin(CP) - induced toxicity parameters in serum

As reported earlier [3], the administration of Cisplatin (CP) to control rats caused significant increase in serum creatinine (+ 38%), Blood Urea Nitrogen (BUN) (+ 47%), cholesterol (+ 22%) and phospholipids (+ 36%) but decrease in serum glucose (- 28%) and inorganic phosphate (- 21%) (Table 1). GT alone had no effect on serum creatinine whereas BUN conspicuously decreased. GT significantly decreased serum glucose, cholesterol and Pi whereas phospholipids significantly increased. When GT was given to CP rats, CP elicited nephrotoxic alterations appeared to be ameliorated. CP induced increase in serum creatinine and BUN were prevented by prolonged GT consumption. CP induced increase in serum cholesterol was also lowered by GT. However, serum glucose and Pi remained depressed whereas phospholipids further increased by GT consumption to CP rats compared to control rats.

Effect of Green Tea (GT) on Cisplatin(CP) induced alterations on biomarker enzymes of BBM and lysosomes

To address the hypothesis whether Green Tea (GT) extract would prevent CP induced intestinal and hepatic toxicity and oxidative damage, the effect of GT was determined on the biomarker enzymes of Brush Border Membrane (BBM), carbohydrate metabolism, and lysosomes and on the parameter/enzymes of oxidative stress in the intestinal and liver homogenates as well as in the isolated intestinal BBMV preparations from rats.

Effect of cisplatin (CP) and green tea (GT) on biomarkers of BBM and lysosomes in the homogenates: The activities of Alkaline Phosphatase (ALP), γ-Glutamyl Transferase (GGTase), Leucine Aminopeptidase (LAP), Sucrose and Acid Phosphatase (ACPase)

Table 1: Effect of Green Tea (GT) consumption on several serum parameters with/without Cisplatin (CP) treatment.

Groups	Creatinine (mg/ dL)	BUN (mg/ dL)	Glucose (mg/ dL)	Cholesterol (mg/ dL)	Phosphate (µmol/ mL)	Phospholipid (µg/ mL)
Control	1.07 ± 0.4	22.60 ± 1.89	32.9 ± 1.6	119.88 ± 1.3	3.72 ± 0.26	97.2 ± 8.8
CP	1.48 ± 1.11* (+ 38%)	33.12 ± 0.4* (+ 47%)	23.8 ± 2.86* (- 28%)	146.24 ± 3.8* (+ 22%)	2.95 ± 0.25 (- 21%)	132.05 ± 40.05* (+ 36%)
GT	0. 96 ± 0.33 (- 10%)	12.6 ± 0.23* (- 44%)	26.3 ± 2.47 (- 20%)	98.4 ± 0.6 (- 18%)	2.6 ± 0.35* (- 30%)	142.15 ± 2.35* (+ 46%)
TCP	1.07 ± 0.28	19.1 ± 0.48 + (- 16%)	20.1 ± 1.25* (- 39%)	102.12 ± 1.7† (- 15%)	2.5 ± 0.2* (- 33%)	149 ± 3.3* (+ 53%)

Results are Mean ± SEM of six different samples
* Significantly different from control, + significantly different from CP at $p < 0.05$ by one way ANOVA.
Values in parentheses represent percent change from control.

Table 2: Effect of Green Tea (GT) consumption on brush border membrane enzymes in intestinal and liver homogenates with/without Cisplatin (CP) treatment.

Tissue	ALP (µmol/mg protein/h)	GGTase (µmol/mg protein/h)	LAP (µmol/mg protein/h)	ACPase (µmol/mg protein/h)	Sucrase (µmol/mg protein/h)
Intestine					
Control	1.38 ± 0.06	2.96 ± 0.07	3.2 ± 0.05	2.5 ± 0.05	33.9 ± 0.56
CP	1.10 ± 0.01* (- 20%)	2.3 ± 0.04* (- 22%)	2.4 ± 0.07* (- 25%)	1.98 ± 0.02* (- 21%)	27.96 ± 0.7* (- 18%)
GT	1.64 ± 0.01* (+ 19%)	3.98 ± 0.17* (+ 34%)	4.5 ± 0.12* (+ 41%)	3.13 ± 0.15* (+ 25%)	38.4 ± 0.64* (+ 13%)
TCP	1.48 ± 0.02† (+ 7%)	3.64 ± 0.17*† (+ 23%)	3.6 ± 0.1† (+ 13%)	3.1 ± 0.11*† (+ 25%)	34.2 ± 1.13† (+ 1%)
Liver					
Control	1.28 ± 0.03	1.57 ± 0.04	1.38 ± 0.04	2.6 ± 0.06	
CP	1.6 ± 0.09* (+ 25%)	1.99 ± 0.02* (+ 27%)	1.23 ± 0.04 - 11%)	2.95 ± 0.09* (+ 13%)	
GT	1.15 ± 0.05* (- 10%)	1.22 ± 0.1* (- 22%)	1.58 ± 0.04 (+ 14%)	2.54 ± 0.05 (- 2%)	
TCP	1.33 ± 0.05 (+ 4%)	1.56 ± 0.07†	1.47 ± 0.05† (+ 7%)	2.3 ± 0.21† (- 12%)	

Results are Mean ± SEM of 3-4 different preparations.
*Significantly different from control, † significantly different at $p < 0.05$ from CP by one way ANOVA.
Values in parentheses represent percent change from control.

were determined under different experimental conditions in mucosal and liver homogenates (Table 2). Administration of CP to control rats caused a significant decrease in the activities of all BBM and lysosomal enzymes in the intestine. However, CP significantly increased the activity of ALP (+ 25%), GGTase (+ 27%) and ACPase (+ 13%) but decreased the activity of LAP (- 11%) in the liver homogenate. On the other hand GT alone significantly increased the activities of ALP, GGTase, LAP and ACPase in intestinal homogenate as reported earlier [35]. Moreover, the activity of ALP and GGTase significantly increased but that of LAP and ACPase decreased in liver homogenates by GT consumption. The co-administration of GT to CP treated rats caused a significant reversal in CP induced alterations in the activities of BBM and lysosomal enzymes (Table 2). The decrease caused by CP treatment in the activities of ALP, GGTase, LAP, sucrase and ACPase was significantly prevented by simultaneous GT consumption in the intestine and the activities of these enzymes remained significantly higher in TCP compared to control or CP - treated rats. In the liver homogenates, CP induced increase in ALP and GGTase and decrease in LAP activities tended to be back to near control values whereas ACPase activity was decreased by GT given to CP treated rats.

Effect of Green Tea (GT) on Cisplatin (CP) induced alterations on Brush Border Membrane (BBM) enzymes in isolated BBM preparations: The effect of CP, GT and their combination was further analyzed on the activities of BBM marker enzymes in BBMV isolated from intestinal mucosa. The data summarized in Table 3 and Figure 2, showed a similar activity pattern of BBM enzymes in BBM preparations as observed in the homogenates; however the magnitude of effects was more apparent. The increase or decrease in the activities of ALP, GGTase, LAP or sucrase (intestinal marker) by CP or GT was significantly much higher in BBMVs as compared to intestinal homogenates. The damaging effect of CP was clearly ameliorated by GT, as decrease in the activity of GGTase, LAP and sucrase by CP was not only prevented but increased significantly by GT consumption as compared to respective control values. Thus, it appears that co-administration of GT to CP-treated rats, to a larger extent reduced CP induced structural alterations and improved BBM's integrity and the activities of their components.

Effect of Green Tea (GT) on Cisplatin(CP) induced alterations on the enzymes of carbohydrate metabolism in rat intestine and liver

The effect of CP, GT and CP + GT was determined on the activities of various enzymes of carbohydrate metabolism in the intestine and liver (Table 4). CP treatment to control rats significantly increased
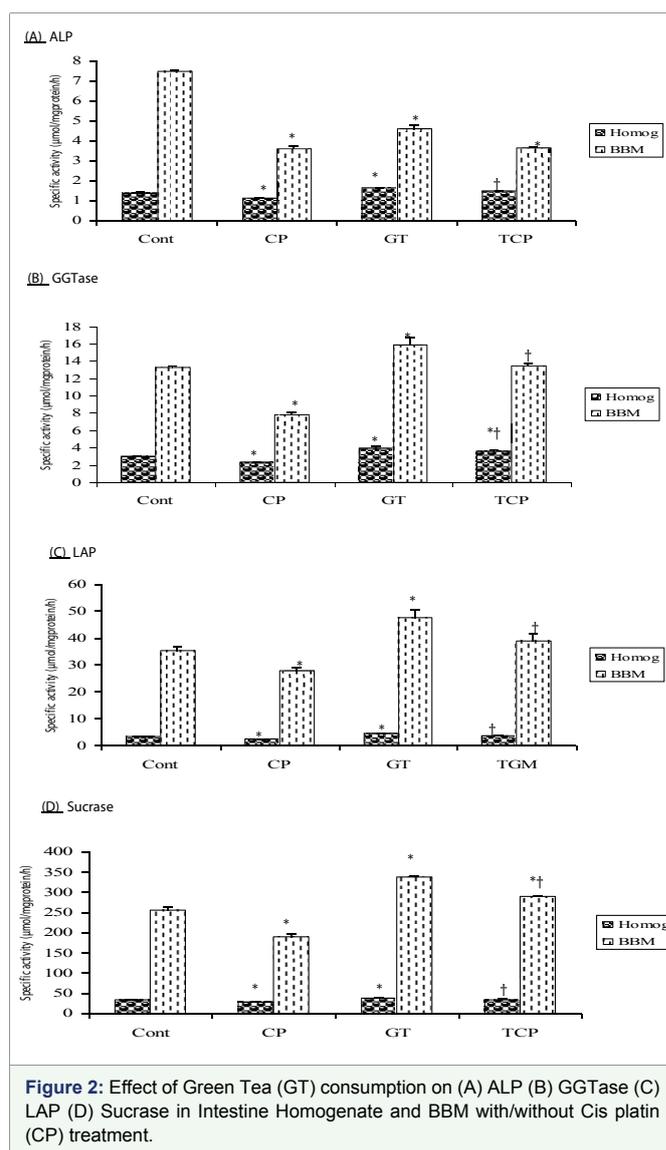

Figure 2: Effect of Green Tea (GT) consumption on (A) ALP (B) GGTase (C) LAP (D) Sucrase in Intestine Homogenate and BBM with/without Cis platin (CP) treatment.

hexokinase activity in the intestine (+ 14%) and liver (+ 30%). The activity of LDH (a marker of anaerobic glycolysis), however profoundly increased in the intestine (+ 50%) whereas decreased in the liver (- 30%). However, the effect of CP was differentially observed on MDH activity (an enzyme of TCA cycle). CP caused significant decline in MDH activity in the intestine (- 11%) whereas increase in the liver (+ 66%). As previously reported [35], GT consumption significantly increased the activity of HK, LDH and MDH in the intestine and liver. When CP treatment was extended to GT drinking rats, CP induced decrease in MDH activity was not only prevented but the activity remained significantly higher in TCP compared to either control or CP treated rats in the intestine (Table 4). The activity of LDH was especially found to be profoundly enhanced in the intestine and CP induced decrease in LDH activity was significantly reversed by GT consumption in the liver. The effect of CP and GT on the activity of glucose-6-phosphatase (G6Pase), a marker enzyme of gluconeogenesis was also observed in the intestine and liver. CP significantly increased the activity of G6Pase in the intestine but caused marked reduction in the liver (Table 5). In contrast, GT given to control rats enhanced the activity of G6Pase in the intestine whereas lowered in the liver as reported by Khan et al [35]. As a result, simultaneous GT co- administration with CP treatment resulted

Table 3: Effect of green tea (GT) consumption on brush border membrane enzymes in BBMV isolated from small intestine with/without Cisplatin (CP) treatment.

Groups	ALP (µmol/mg protein/h)	GGTase (µmol/mg protein/h)	LAP (µmol/mg protein/h)	Sucrase (µmol/mg protein/h)
Control	7.5 ± 0.04	13.35 ± 0.12	35.5 ± 1.3	255.98 ± 8.5
CP	3.6 ± 0.11* (- 52%)	7.85 ± 0.22* (- 41%)	27.9 ± 1.28* (- 21%)	190.5 ± 6.5* (- 26%)
GT	4.6 ± 0.2* (- 39%)	15.9 ± 0.86* (+ 19%)	47.8 ± 2.88* (+ 35%)	337.5 ± 3.11* (+ 32%)
TCP	3.64 ± 0.05* (- 51%)	13.5 ± 0.3† (+ 1%)	38.9 ± 2.78† (+ 10%)	289.01 ± 2.9*† (+ 13%)

Results are Mean ± SEM of 3-4 different preparations
*Significantly different from control, † significantly different from CP at $p < 0.05$ by one way ANOVA.
Values in parentheses represent percent change from control.

in significant increase of G6Pase not only in the intestine but CP induced decrease in G-6-Pase activity was also markedly prevented in the liver. The effect of GT-extract alone and with CP was also determined on the activities of glucose-6-phosphate dehydrogenase (G6PDH, HMP-shunt) and malic enzyme (ME), source of NADPH needed in reductive anabolic reactions. CP treatment to control rats significantly decreased G6PDH activity both in the intestine (- 14%) and liver (- 23%) whereas the activity of ME significantly increased in the intestine (+ 12%), but remained unchanged in liver (Table 5). In contrast, the activity of both G6PDH and ME markedly decreased in the intestine (- 63%) and liver (- 23%) by GT given alone to control rats. GT consumption by CP treated rats; however prevented CP-induced decrease in the activity of both G6PDH and ME.

Effect of Green Tea (GT) on Cisplatin(CP) induced alterations in antioxidant defense parameters in different rat tissues

The effect of CP, GT and CP + GT was determined on the parameters of antioxidant defense system such as Lipid Peroxidation (LPO), total SH, SOD and catalase (Table 6). Antioxidant status is a potential biomarker to determine the physiological state of a cell, tissue or organ. The drug including GM or CP and chemical induced nephrotoxicity and tissue injury has been suggested to be mediated in part by perturbation in the balance of antioxidant defense system [42-43]. CP administration to control rats resulted in a profound increase in the production of Malondialdehyde (MDA), an end product of lipid per-oxidation in the intestine and liver. However, the increase in LPO was much more pronounced in the intestine than liver. CP caused marked reduction in total SH levels in the intestine and liver. These changes in LPO and total SH were associated with marked decrease in the activity of both SOD and catalase in the intestine. However, in the liver the activity of SOD slightly decreased whereas catalase increased by CP. In contrast, the effect of GT consumption significantly decreased the activity of SOD in the intestine and liver whereas the activity of catalase significantly increased in the intestine and liver. These alterations in the antioxidant enzymes were associated

Table 4: Effect of green tea (GT) consumption on the activity of metabolic enzymes in intestinal and liver homogenates with/without Cisplatin (CP) treatment.

Tissue	Hexokinase (µmol/mgprotein/h)	LDH (µmol/mg protein/h)	MDH (µmol/mgprotein/h)
Intestine			
Control	161.4 ± 2.2	5.2 ± 0.95	12.65 ± 0.54
CP	183.5 ± 6.1* (+ 14%)	7.78 ± 1.24* (+ 50%)	11.24 ± 1.15 (- 11%)
GT	189.4 ± 4.8* (+ 17%)	9.74 ± 0.72* (+ 87%)	14.75 ± 1.26* (+ 17%)
TCP	187.5 ± 2.4* (+ 16%)	10.8 ± 0.2* (+ 108%)	12.13 ± 0.28 (- 4%)
Liver			
Control	28.2 ± 0.7	10.6 ± 0.8	3.2 ± 0.25
CP	36.6 ± 0.3* (+ 30%)	7.4 ± 1.2* (- 30%)	5.3 ± 1.3* (+ 66%)
GT	32.5 ± 0.72* (+ 15%)	12.2 ± 0.88* (+ 15%)	3.42 ± 0.28 (+ 7%)
TCP	32.5 ± 1.22* (+ 15%)	10.68 ± 0.28† (+ 0.8%)	3.75 ± 0.62† (+ 17%)

Results are Mean ± SEM of 3-4 different preparations.
* Significantly different from control, † significantly different from CP at $p < 0.05$ by one way ANOVA.
Values in parentheses represent percent change from control.

Table 5: Effect of Green Tea (GT) consumption on the activity of metabolic enzymes in intestinal and liver homogenates with/without Cisplatin (CP) treatment.

Tissue	G6Pase (µmol/ mgprotein/h)	G6PDH (µmol/ mgprotein/h)	ME (µmol/mgprotein/h)
Intestine			
Control	0.97 ± 0.08	0.7 ± 0.05	1.2 ± 0.07
CP	1.5 ± 0.17* (+ 55%)	0.6 ± 0.09 (- 14%)	1.34 ± 0.05 (+ 12%)
GT	1.2 ± 0.03 (+ 24%)	0.26 ± 0.01* (- 63%)	0.46 ± 0.07* (- 62%)
TCP	1.1 ± 0.05† (+ 13%)	0.53 ± 0.05 (- 24%)	1.02 ± 0.01† (- 15%)
Liver			
Control	0.14 ± 0.02	0.82 ± 0.07	1.4 ± 0.1
CP	0.11 ± 0.03* (- 21%)	0.63 ± 0.15 (- 23%)	1.4 ± 0.15
GT	0.12 ± 0.03* (- 14%)	0.63 ± 0.13 (- 23%)	0.89 ± 0.03* (- 36%)
TCP	0.11 ± 0.03* (- 21%)	0.56 ± 0.1* (- 32%)	1.15 ± 0.15 (- 18%)

Results are Mean ± SEM of 3-4 different preparations.
* Significantly different from control, † significantly different from CP at $p < 0.05$ by one way ANOVA.
Values in parentheses represent percent change from control.

Table 6: Effect of Green Tea (GT) consumption on enzymic and non-enzymic antioxidant parameters in intestinal and liver homogenates with/without Cisplatin (CP) treatment.

Tissue	LPO (nmol/ g tissue)	Total-SH (µmol/ g tissue)	SOD (Units/ mg protein)	Catalase (µmol/ mg protein/ min)
Intestine				
Control	18.6 ± 0.46	3.78 ± 0.06	4.0 ± 0.7	5.1 ± 0.40
CP	30.4 ± 1.3* (+ 63%)	2.69 ± 0.15* (- 29%)	2.1 ± 0.16* (- 48%)	2.83 ± 0.33* (- 45%)
GT	24.9 ± 1.8† (+ 34%)	4.3 ± 0.22* (+ 14%)	1.5 ± 0.12* (- 63%)	10.17 ± 1.3* (+ 99%)
TCP	23.5 ± 1.5† (+ 26%)	4.22 ± 0.15*† (+ 12%)	3.11 ± 0.82* (-22%)	10.9 ± 4.9*† (+ 114%)
Liver				
Control	244.2 ± 10.5	13.88 ± 1.33	32.4 ± 0.23	9.2 ± 0.9
CP	321.4 ± 29.14* (+ 32%)	11.68 ± 0.54* (- 16%)	29.03 ± 1.5 (- 10%)	10.96 ± 1.4 (+ 19%)
GT	224.0 ± 12.51 (- 8%)	12.65 ± 1.15 (- 9%)	22.6 ± 2.58* (- 30%)	13.14 ± 1.2* (+ 43%)
TCP	196.3 ± 18.1† (- 20%)	11.7 ± 0.125 (- 16%)	24.6 ± 0.74* (- 24%)	11.15 ± 1.49 (+ 21%)

Results are Mean ± SEM of 3-4 different preparations.
* Significantly different from control, † significantly different from CP at $p < 0.05$ by one way ANOVA.
Values in parentheses represent percent change from control.

with marked decrease of LPO in the liver but remained significantly higher in the intestine in GT compared to control rats. The total SH levels were increased in the intestine but did not change in the liver. Consumption of GT along with CP treatment significantly prevented CP induced increase in LPO both in the intestine and liver and CP induced decrease in total SH was also prevented in the intestine. GT consumption showed marked protection against CP induced decrease in SOD activity in the intestine and liver. CP induced decrease in catalase activity was also prevented by GT consumption in the intestine whereas catalase activity remained higher in the liver after

GT consumption by CP treated rats. In general, the present results demonstrate marked protection by GT against CP induced alterations in oxidative stress parameters albeit differentially in different tissues.

DISCUSSION

The small intestine epithelium plays a crucial role in the terminal digestion/modification of food components, absorption of nutrients and recognition of food-derived signals. These intestinal functions are influenced by pharmaceuticals and other environmental contaminants including cisplatin [44-45]. Despite successful against many solid tumors including gastric cancer, cisplatin causes significant side effects such as severe nausea and vomiting and nephrotoxicity, hepatic and intestinal toxicity [3,10,46] that limits its long-term clinical use [22,23]. Much evidence indicates that polyphenolic compounds from plants, including Green Tea (GT) have various biological activities including antifungal, antimutagenic, anti- oxidative, anti-carcinogenic, antitumor; anti-diabetic, and nephroprotective effects besides other health benefits [29,3,12]. Rameshrad et al. [30] demonstrated that green tea and its constituents; mainly catechins have protective effects against various natural and chemical toxins due to their anti-oxidative, radical scavenging, chelating, anti-apoptotic properties and modulating inflammatory responses. We have shown that Green Tea (GT) consumption prevented Genta Micin (GM) and CP-induced nephrotoxic and GM-induced intestinal and hepatic toxic effects [2,3,12]. The present studies were carried out to study detailed mechanism of CP-induced alterations and possible action mechanism of GT in preventing those deleterious changes in rat intestine and liver. As shown earlier [3], long-term CP administration produced a typical pattern of nephrotoxicity and hepatic toxicity as indicated by increased serum creatinine and BUN, serum cholesterol and phospholipids along with significant decrease in serum glucose and inorganic Phosphate (Pi). These changes were accompanied by marked reductions in the activities of ALP, GGTase, LAP and sucrase (BBM marker enzymes) in the intestinal homogenate and purified BBMV that indicate that long-term CP administration caused severe damage to the structural architecture of intestinal BBM. Since these enzymes are BBM components which are involved in the terminal digestion and absorption of nutrients might have released from the damaged BBM as reported by morphological and some biochemical studies [20,45,46,47]. An increase in the activity of ALP and GGTase and decrease of LAP activity in the liver also indicate CP-induced mal-functioning of liver. The activity of lysosomal enzyme, ACPase was also specifically altered in the liver and intestine. GT alone significantly increased the activities of BBM and lysosomal enzymes in the homogenate and BBM of the intestine indicating an overall improvement in mucosal BBM integrity [12,35]. Thus when GT was given to CP treated rats; it caused reversal of CP-induced alterations in serum parameters as well as in the activities of BBM enzymes in the intestinal membranes. CP elicited increased levels of serum creatinine and BUN and serum glucose and cholesterol were all decreased showing protective effects of GT on CP-induced nephrotoxicity and hepatic toxicity. CP induced decrease in the activities of GGTase, LAP and sucrase but ALP was also markedly prevented by GT in intestinal BBM. Alterations in ACPase activity demonstrate CP induced loss of lysosomal function [42,48]. In contrast, GT consumption prevented this loss by lessening the damage caused by CP or by increasing the regeneration process or both. However, in the case of the intestine a different adaptive mechanism might be initiated where ACPase activities were increased both by CP and GT. Taken

together the results demonstrate that CP treatment indeed significantly altered the structure of intestinal mucosal membrane and lysosomes whereas GT consumption appears to improve integrity of the mucosal membranes due to its biochemical and anti-oxidant properties as mentioned above. To assess the functional aspects, the activities of various enzymes of carbohydrate metabolism were determined under different experimental conditions. As shown in the Results, the activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway were differentially altered in the intestine and liver by CP treatment and/ or by GT consumption. CP caused significant increase in LDH but decrease in MDH activity in the intestine which was associated with simultaneous increase in hexokinase activity in the intestine. However, in the liver, an opposite CP effect was observed where the decrease in LDH activity was associated with increased MDH and hexokinase activity. Although the actual rates of glycolysis or TCA cycle were not determined, marked decrease in MDH activity at least in the intestine appears to be due to CP induced damage to mitochondria [3,42]. An impaired oxidative metabolism of glucose/ fatty acid due to mitochondrial dysfunction would result in lower ATP production by oxidative metabolism and hence other ATP-dependent processes. A marked increase in LDH and to some extent hexokinase activity with simultaneous decline in TCA cycle enzyme i.e., MDH appear to be an adaptive cellular effect in energy metabolism from aerobic metabolism alternatively to anaerobic glycolysis due to CP induced mitochondrial dysfunction. CP also caused differential effect on the enzymes of gluconeogenesis and HMP-shunt pathway in different tissues. The activity of G6Pase significantly increased in the intestine but decreased in the liver upon CP treatment. The activity of G6PDH profoundly decreased in all the tissues studied. However, the activity of NADP – malic enzyme (ME) variably increased in the intestine but decreased in the liver. The present data indicate that CP caused variable effects on different enzymes of carbohydrate metabolism in different tissues. In contrast to CP, GT consumption alone caused selective alterations in the activities of various enzymes involved in carbohydrate metabolism in different rat tissues. The activity of HK, LDH (glycolysis), MDH (TCA cycle) and G6Pase significantly increased in the intestine but decreased in the liver as reported earlier [35]. GT administration to CP-treated rats resulted in overall improvement of carbohydrate metabolism in various tissues as evident by higher activities of LDH; MDH and G6Pase in the intestine in GT + CP compared to CP alone or control rats. GT might have lowered number of damaged mitochondria or affected macromolecules or may have increased number of normally active organelles or macromolecules. The decrease/ increase in G6Pase by CP/ GT respectively can be attributed to the availability of oxaloacetate produced from malate by MDH. Thus decrease/increase in MDH activity may affect both the TCA cycle and gluconeogenic activity as oxaloacetate is required by both pathways. In turn, ATP production would be influenced accordingly. It has been reported that most toxicants including certain drugs [1,43,49] and heavy metals [27,50,51] exert their toxic effects by inducing the generation of Reactive Oxygen Species (ROS). CP has been found to increase mitochondrial generation of oxidative stress and ROS and subsequent cell death [52]. Several other studies have shown that a single nephrotoxicity dose of CP caused increase in Lipid Per-Oxidation (LPO) and decrease in GSH, protein thiols and activities of ant-oxidant enzymes in several tissues including the kidney, liver and intestine [2,3,10,12,45,46]. Cisplatin induced toxicity in human and experimental animals has been shown to be protected by prior treatment with various antioxidants such as

ebseleen [53], Vitamin C [25,27] and selenium [54,55]. However, none of these strategies were found to be safe for clinical practice. Recently, Rameshrad et al [30]. demonstrated that green tea and its constituents; mainly catechins have protective effects against various natural and chemical toxins due to their anti-oxidative, radical scavenging, chelating, anti-apoptotic properties and modulating inflammatory responses. We have shown that GT consumption enhanced cellular energy metabolism and antioxidant defense mechanism in the liver, kidney, and small intestine [56] and prevented GM and CP-induced nephrotoxicity and oxidative damage in the rat kidney [2,4,35]. The present result showed that long-term multiple injections of CP over a period of 25d as may be required for cancer therapy significantly decreased the activities of SOD, and catalase and enhanced LPO in the intestine and liver indicating CP induced oxidative damage. The severity of the damage appeared to be more prominent in the intestine than in the liver. In contrast, GT by virtue of its antioxidant properties, significantly enhanced antioxidant defense mechanism albeit differentially in different tissues. The activity of SOD significantly decreased in the intestine and liver but catalase activity profoundly increased in both the tissues. These alterations were associated with lowering of LPO in the liver. GT consumption thus prevented CP induced increase in LPO and decrease in the activities of SOD and catalase in the intestine. CP induced increase in LPO in the liver were also prevented by GT consumption. The results suggest that GT provide protection against CP-induced oxidative damage either by SOD-mediated or catalase mediated mechanism or involving both mechanisms simultaneously in certain tissues. In conclusion, the results of present study show that CP induces generation of free radicals that causes oxidative damage to cellular organelles, macromolecules and especially to mitochondria and plasma membrane. In contrast, GT reduces oxidative stress by virtue of its antioxidant properties thus improving the structural integrity of various organelles/ macromolecules and eventually enhances metabolic capabilities.

ACKNOWLEDGEMENT

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