Hepatoprotective Effect of Green Tea (Camellia sinensis) Extract against Tamoxifen-induced Liver Injury in Rats

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Tamoxifen citrate (TAM), is widely used for treatment of breast cancer. It showed a degree of hepatic carcinogenesis. The purpose of this study was to elucidate the antioxidant capacity of green tea (Camellia sinensis) extract (GTE) against TAM-induced liver injury. A model of liver injury in female rats was done by intraperitoneal injection of TAM in a dose of 45 mg Kg⁻¹ day⁻¹, i.p. for 7 successive days. GTE in the concentration of 1.5%, was orally administered 4 days prior and 14 days after TAM-intoxication as a sole source of drinking water. The antioxidant flavonoid; epicatechin (a component of green tea) was not detectable in liver and blood of rats in either normal control or TAM-intoxicated group, however, TAM intoxication resulted in a significant decrease of its level in liver homogenate of tamoxifen-intoxicated rats. The model of TAM-intoxication elicited significant declines in the antioxidant enzymes (glutathione-S-transferase, glutathione peroxidase, superoxide dismutase and catalase) and reduced glutathione concomitant with significant elevations in TBARS (thiobarbituric acid reactive substance) and liver transaminases; sGPT (serum glutamate pyruvate transaminase) and sGOT (serum glutamate oxaloacetate transaminase) levels. The oral administration of 1.5% GTE to TAM-intoxicated rats, produced significant increments in the antioxidant enzymes and reduced glutathione concomitant with significant decrements in TBARS and liver transaminases levels. The data obtained from this study speculated that 1.5% GTE has the capacity to scavenge free radical and can protect against oxidative stress induced by TAM intoxication. Supplementation of GTE could be useful in alleviating tamoxifen-induced liver injury in rats.

Keywords: Catalase, Epicatechins, Green tea extract, Glutathione peroxidase, Glutathione-S-transferase, Oxidative stress and liver injury, Reduced glutathione, Tamoxifen, Thiobarbituric acid reactive substance, Superoxide dismutase

Introduction

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease (Gupta et al., 2004). More attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially whenever free radical generation is involved (Frei and Higdon, 2003). Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress (Okada et al., 2001; Britich et al., 2005), especially in case of cancer. Flavonoids are group of polyphenolic compounds that occur widely in fruit, vegetables, tea, cocoas and red wine (Arts et al., 1999; Bearden et al., 2000; Matito et al., 2003). Tea is second only to water in popularity as a beverage. Green tea (Camellia sinensis) extract is fast becoming ubiquitous in consumer products supplemented with green tea such as shampoos, creams, soaps, cosmetics, vitamins, drinks, lollipops and ice creams (Mukhtar and Ahmad, 2000). Fresh tea leaves are rich in flavanol monomers known as catechins such as epicatechins (Graham, 1992), which are 13.6 g/100 g in green tea and 4.2 g/100 gm dry weight in black tea (Peterson, et al., 2005). Catechins have beneficial effects in prevention of cardiovascular diseases including LDL oxidative susceptibility, serum lipids and lipoprotein concentrations (Wan et al., 2001). In animal studies, Defeudis et al., 2003; Banskota et al., 2000; Ostrowska et al., 2004, revealed that green tea may protect liver and brain cells against sequelae of oxidative stress induced by ethanol intoxication. In a recent study, supplementation of green tea extract (GTE) attenuates cytochrome A-induced oxidative stress in rats (Mohamadin et al., 2005). Also, it can be reduce the risk of colorectal, pancreatic cancers (Ji et al., 1998; Brown, 1999), inflammatory disease and muscle necrosis (Benelli et al., 2002). On the other hand, Chantre and...
Lairon, 2002, revealed GTE can be effective for treatment of obesity. Lee et al., 2004, suggested that the development of chewing gum fortified with green tea extract will be effective to oral cavity and periodontal disease (Hirasawa et al., 2002). Fujiki et al., 2002, recommended daily consumption of 10 cups of GTE (about 25 g green tea).

Tamoxifen citrate (TAM), 1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-butene), is a nonsteroidal antiestrogen drug that is used in the treatment and prevention of all stages of hormone-dependent breast cancer (Desai et al., 2002; Jordan, 2003). It reduces level of estrogen and estrogen receptor with no change in progesterone contents (Liu et al., 2004). This effect is mediated by the inhibition of the action of both 17 β estradiol and esterone (Stefano et al., 2003) and competition with estrogen for binding to its receptor (Ali and Coombes, 2002). It was revealed that, TAM in high dose is a known liver carcinogen in rats (Ahotupa et al., 1993; Calbhallero et al., 2001) which is due to oxygen radical overproduction which occurs during TAM metabolism. Lipid peroxidation via subtraction of hydrogen from unsaturated fatty acids forms carbon-centered lipid radicals (McCay et al., 1984). This molecule adds molecular oxygen to form lipid peroxy radicals, thereby initiating the process of lipid peroxidation (Letteron et al., 1990). Also, TAM has been shown to potentiate nitrous oxide production in breast cancer patients through enhancement of nitric oxide synthase II expression (Simoane et al., 2002). This strong hepatocarcinogenic effect of TAM in rats raises issues bearing on the prophylactic chronic administration to healthy women (Williams et al., 1993).

The aim of this work is to study and gain insight the biochemical effects and antioxidative profile exerted upon green tea extract (GTE) supplementation to the tamoxifen-induced liver injured rats.

Materials and Methods

Chemicals Tamoxifen citrate (TAM), was a kind gift obtained from medical union pharmaceutical drug company (MUP), Egypt. All the other chemicals were of analytical grade and purchased from Sigma (St. Louis, USA) and Fluka (Buchs, Switzerland).

Green tea extract The GTE was made according to Maity et al., 1998, by soaking 15 g of instant green tea powder in 1 L of boiling distilled water for 5 minutes. The solution was filtered to make 1.5% green tea extract (GTE). This solution was provided to rats as their sole source of drinking water.

Animals Forty adult female albino rats, weighing 120-170 gm were used as experimental animals in this study. The animals were housed in the animal care centre of faculty of Pharmacy, Al-Azhar University. They were kept in wire-floored cages under standard laboratory conditions of 12 h/12 h light/dark, 25±2°C with free access to food and water.

Experimental protocol The rats were randomly divided into 4 groups of 10 animals each as follows:

- **Group 1**: Normal control untreated rats.
- **Group 2**: TAM-intoxicated rats: Rats were treated with TAM in a dose of 45 mg Kg⁻¹ day⁻¹, i.p., for 7 successive days (Hard et al., 1993).
- **Group 3**: GTE rats: Rats were orally administered 1.5% green tea extract (GTE) as their sole source of drinking water for 18 days.
- **Group 4**: TAM-GTE rats: Rats were orally administered 1.5% GTE as their sole source of drinking water 4 days before and 14 days after TAM intoxication (in a dose identical to group 2) as protection against liver injury induced by TAM.

Biochemical parameters Epicatechin (ng/ml) was determined in liver homogenate and serum (Malani et al., 1997; Wan et al., 2001) using a Shimadzu LC-6AD HPLC instrument (Kyoto, Japan). 250 ml sample, 25 ml of ascorbic acid (2%) and 10 ml EDTA were combined in a 1.5 ml polypropylene centrifuge tube. Then, 7.50 ml of 1.2 mol HCl in methanol was added to the tube and the solution was mixed by vortex for 2 minutes. The resulting mixture was then hydrolyzed in the dark at room temperature for 18 hours, mixed by vortex for 1 minute and centrifuged for 10 minutes at 2,300 rpm. A 25 ml of the supernatant was injected into a LC-DP (25 cm × 4.6 mm) column. Solvent A consisted of 4% acetic acid in water and solvent B consisted of acetic acid/methanol/water (1 : 25 : 25). These solutions were eluted over the HPLC column as follows:- 0-1.5 min, 100%A; 1.5-10 min, 100%A to A: B (50 : 50); 10-12 min, A : B (50 : 50) to 100% B. The retention time for epicatechin was 10.76 minutes at 276 nm.

Serum was used to measure the level of sGPT and sGOT liver transaminases (White et al., 1970). The determination of sGOT and sGPT transaminases based on the fact that phenylhydrazine which produced after incubation the substrate with the enzyme, was measured spectrophotometrically. The amount of phenyl hydrazine formed was directly proportional to the enzyme quantity.

The GST was determined spectrophotometrically using the aromatic substrate (1-chloro-2,4 dinitrobenzene) and monitor the change in absorbance due to thiocystere formation. One unit of the enzyme is defined as the amount of glutathione-S-transferase which needed to catalyze the formation of 1 mmol of thiocystere per minute and the specific activity is expressed as nmol/min/mg protein. The
GPX activity that is expressed as U/mg protein was measured by NADPH oxidation in a coupled reaction system containing t-butyl hydroperoxide and oxidized glutathione. The CAT activity was measured using H$_2$O$_2$ as substrate that can be decomposed by CAT enzyme. One unit of CAT is defined as the amount needed to decompose 1 mmol H$_2$O$_2$ per minute and the specific activity is expressed as U/mg protein. The SOD activity of liver tissue was analyzed spectrophotometrically, using nitroblue tetrazolium as a substrate and phenazine methosulphate. The colour intensity of the chromogen produced was measured at 560 nm. The concentration of SOD was expressed as U/mg protein.

The GSH was measured spectrophotometrically using 5,5′-dithiobis(2-nitrobenzoic acid), that was converted to 2-nitro-S-mercaptobenzoic acid per one mole of glutathione. The amount of GSH is expressed as nmol/g protein. The lipid peroxides were measured spectrophotometrically using 1,1,3,3-tetraethoxypropane.

Data analysis All data were expressed as mean ± S.E. The standard error was calculated by dividing the standard deviation by the square root of the number of observations. Paired t-test was carried out to compare populations using GraphPad Prism software (San Diego, USA). A 0.001 level of probability was used as the criterion for significance.

Results

Epicatechin was not detectable in liver and blood of rats in either the control or the tamoxifen-intoxicated group. Epicatechin concentration in blood (serum) of rats is less than its level in liver homogenate of groups receiving GTE. The data revealed that, the epicatechin level in the TAM-GTE group was significantly lesser than those in the GTE group by 10%, p < 0.001 (Fig. 1). The administration of high dose of TAM to rats (45 mg Kg$^{-1}$ day$^{-1}$ i.p. for 4 days), resulted in a significant decline in body weight by −43%, p < 0.001, compared to normal control group. This loss in body weight was compensated after GTE administration (+37.5%, p < 0.001), compared to TAM-intoxicated group (Fig. 2). Also, administration of TAM elicited a dramatic increase in the activity of sGPT (+202%, p < 0.001) and sGOT (+133%, p < 0.001) liver transaminases compared to normal control rats. The liver damage was evaluated by the measurement of serum level of GPT and GOT transaminases. Rats that orally administered GTE 4 days before and 14 days after TAM intoxication, showed significant decrease of sGPT and sGOT levels in comparing to tamoxifen-intoxicated group. This was observed by significant reduction of sGPT and sGOT levels in comparing to the TAM-induced liver injury group. The sGPT was significantly decreased by 56%, p < 0.001. While, the sGOT was significantly decreased by 36%, p < 0.001 (Fig. 3), as compared to TAM-intoxicated rats.

The influence of TAM intoxication and GTE on the antioxidant enzyme activities (GST, GPX, SOD and CAT) and GSH of rat liver are showed in Table 1. The TAM-intoxication resulted in a state of liver injury in rats as manifested by the significant declines in GST, GPX, CAT, SOD and GSH enzyme levels by −26%, −39%, −39%, −38% and −27%, p < 0.001, respectively compared to the normal
control. However, TBARS level showed a huge significant increase in TAM-intoxicated rats by +2223% in comparing to the normal control group (Fig. 4). The level of TBARS is an indicative for lipid peroxidation of hepatic cells. Administration of GTE to normal rats showed non significant changes in GST, GPX, CAT, SOD, GSH enzyme levels and TBARS, compared to the normal rats (Table 1 and Fig. 4).

Moreover, GTE supplementation to TAM-intoxicated rats (Table 1), improved the activities of antioxidant enzymes such as GST, GPX, SOD and CAT as well as the level of GSH and TBARS, compared to TAM-intoxicated liver injured rats. The results showed significant increments in the levels of GST, GPX, CAT, SOD and GSH enzymes by +25%, +45%, +48% and +60 and +18%, respectively compared to TAM-

Table 1. The influence of oral administration of green tea extract (1.5%) to tamoxifen (TAM)-treated rats on antioxidant enzyme activities [glutathione-S-transferase, GST; glutathione peroxidase, GPX; superoxide dismutase, (SOD) and catalase, CAT] and reduced glutathione (GSH) of tamoxifen-intoxicated rat liver homogenate. Data are expressed as mean ± S.E. *Significance difference from TAM-intoxicated group at p < 0.001. #Significance difference from normal control group at p < 0.001. Number of rats per group n = 10.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Parameters</th>
<th>GST nmol/min/ mg protein</th>
<th>GPX U/mg protein</th>
<th>CAT nmol/g protein</th>
<th>SOD U/mg protein</th>
<th>GSH nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal Control rats</td>
<td></td>
<td>270 ±7</td>
<td>18 ± 1</td>
<td>65 ± 3</td>
<td>70 ±2</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>2. GTE Control rats</td>
<td>M ± S.E.</td>
<td>289 ±8</td>
<td>18 ± 2</td>
<td>68 ± 3</td>
<td>75 ±2</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>% Change from group 1</td>
<td>+7%</td>
<td>0%</td>
<td>+4.6%</td>
<td>+7%</td>
<td>+16.6%</td>
<td></td>
</tr>
<tr>
<td>3. TAM-intoxicated rats</td>
<td>M ± S.E.</td>
<td>200±4*</td>
<td>11 ± 1*</td>
<td>40 ±2*</td>
<td>45± 2*</td>
<td>22 ± 1*</td>
</tr>
<tr>
<td>% Change from group 1</td>
<td>- 26%</td>
<td>-39%</td>
<td>-39%</td>
<td>-36%</td>
<td>-2.7%</td>
<td></td>
</tr>
<tr>
<td>4. TAM-GTE rats</td>
<td>M ± S.E.</td>
<td>280±5*</td>
<td>16±1*</td>
<td>59 ± 2*</td>
<td>72 ±3*</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>% Change from group 3</td>
<td>+25%</td>
<td>+45%</td>
<td>+48%</td>
<td>+60%</td>
<td>+18%</td>
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GTE: green tea extract; TAM: tamoxifen citrate.
intoxicated rats, \( p < 0.001 \) (Table 1). The administration of GFE to TAM-intoxicated rats resulted in highly significant decrease in TBARS level by \(-86\%\), as compared to TAM-intoxicated rats, \( p < 0.001 \) (Fig. 4).

**Discussion**

It was obvious that tamoxifen in toxic doses lead to oxidative liver damage (Hard et al., 1993), as it has been elucidated to be a hepatocarcinogen in rats (Ahotupa et al., 1994; Karki et al., 2000; El-Beshbishy, 2005). It may be more toxic to liver because it has much higher affinity for hepatic tissue than for any other tissues (Desai et al., 2002). It was found to produce five DNA adducts in rat liver that appeared to be responsible for carcinogenesis (Hemminki et al., 1995; Hellmann-Blumberg et al., 2000).

This study was conducted in order to investigate the role of GFE in alleviating the oxidative stress status produced after TAM-intoxication in female rats that resulted in liver injury due to involvement of oxidant mechanisms (Akcay et al., 2000). The oxidation process resulted as a result of TAM-intoxication leads to release of iron ions. These ions, therefore, become more reactive in liver. Free iron ions participate in generation of hydroxyl radicals which are the most active reactive oxygen species (ROS) and they react readily with most cellular components (Ostrowska et al., 2004).

In accordance with the data obtained from this study, Stanley et al., 2001; El-Beshbishy, 2005, reported that TAM administration resulted in significant increase in TBARS production. The lipid peroxidation may be attributed to the fact that hexose monophosphate shunt (HMP) in rat liver is strongly inhibited by high dose of TAM, so that the NADPH levels inside cells is decreased. The state of oxidative stress observed during TAM administration in high dose was accompanied by decreased hepatic glutathione content and increased peroxidation (Ahotupa et al., 1994). The activities of some intracellular antioxidant enzymes decreased with the increase of lipid peroxidation levels (Diplock et al., 1994; El-Beshbishy, 2005), which were concomitant with the results achieved from this study. Hepatic peroxides are reported to be increased during oxidative stress (Sut et al., 1999). Also, it was reported that, due to liver damage, there was an observed decrease in antioxidant defenses in the liver (Seven et al., 2004). The impaired regeneration of protective and antioxidants such as reduced glutathione also contribute to oxidative stress (Sun et al., 1999).

Epicatechins (antioxidant present in green tea) scavenge a wide range of free radicals including the most active hydroxyl radical, which may initiate lipid peroxidation. It prevents the loss of lipophilic antioxidant a-tocopherol, by repairing tocopherol radicals and protection of the hydrophilic antioxidant ascorbate (Skrzyllewska et al., 2002). Therefore, it may decrease the concentration of lipid free radicals and terminate initiation and propagation of lipid peroxidation (Guo et al., 1999). Epicatechins are effective scavengers of physiologically active reactive oxygen and nitrogen species including superoxide (Nakagawa and Yokozawa, 2002; Cui et al., 2005), peroxyl radical (Guo et al., 1999), peroxynitrite (Paquay et al., 2000) and hypochlorous acid (Scott et al., 1993). It was repoted that, epicatechines can react with superoxide radical via one electron transfer mechanism or by a hydrogen abstraction mechanism to form the corresponding semiquinone (Wang et al., 1996). Epicatechins may chelate metal ions, especially iron and copper, which, in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides which causes reactive aldehyde formation (Azam et al., 2004). In this study, the epicatechins level was significantly decreased in the liver homogenate (not blood) of TAM-intoxicated rats received GFE (Fig. 1).

The decrease in antioxidant defence systems of TAM-intoxicated rats render them more susceptible to hepatotoxicity (Palomero et al., 2001). The liver damage was determined by measurement of sGOT and sGP T while level of TBARS in liver was used as an indicative of lipid peroxidation. The levels of antioxidant enzyme activities in liver homogenates (GST, GPX, SOD and CAT) and GSH were significantly improved upon treatment of TAM-intoxicated rats with 1.5% GFE which inhibited TAM-induced hepatic injury and thereby the level of oxidative stress as it can decrease lipid peroxidation and enhance antioxidant enzyme activities (Table 1), whereas the level of TBARS were significantly decreased comparable to TAM-intoxicated group (Fig. 4). On the contrary, Oge et al., 2003, showed that CAT did not show any significant difference after TAM intoxication. In agreement with the results obtained in this study, Ostrowska et al., 2004, stated that the administration of green tea to ethanol-intoxicated rats (that resulted in a state of liver injury), resulted in the normalization of lipid peroxidation as well as glutathione concentration and GPX activity in liver. The increase in GPX and SOD activities after GFE administration to TAM-intoxicated rats, was attributed to induction or mutually protective interaction especially superoxide dismutase, which present at low level only but highly inducible under oxidative stress (Shull et al., 1991; Gonzalez et al., 2000). The destruction and degradation of phospholipids hydroperoxides are carried out by GPX and this suggested to be a pathway of cytoprotection against the deleterious effects of phospholipids hydroperoxides. The decreased GPX level of TAM-intoxicated rats, leads to an increase of toxic level to the cells (Ostrowska et al., 2004). The decreased activities of GPX and SOD in liver homogenate of TAM-intoxicated rats may be due to oxidative stress induced inactivation and/or exhaustion (Wohlieb and Godin, 1987). Also, it was reported that, the decreased GPX activity, leads to H2O2 accumulation in the liver which in turns inactivates SOD (Godin et al., 1988; Kakkar et al., 1997). Liver is the major site for synthesis of GSH. The detoxification of different drugs and xenobiotics in the liver involves GSH (Seven et al., 2004). GSH plays a
common role in cellular resistance to oxidative damage as a free radical scavenger or protein-bound glutathione and by generation of ascorbate or tocopherol in liver (Mark et al., 1996). The decreased hepatic GSH in TAM-intoxicated rats could be as a result of hexose monophosphate (HMP) shunt impairment due to TAM-intoxication and thereby NADPH availability is reduced and the ability to recycle GSSG to GSH is decreased (Lu, 1999). By blocking oxidative damage through lipid peroxidation and protein oxidation, green tea extract prevent the loss of membrane permeability and dysfunction of cellular proteins and decreases the endogenous level of hydroxyl radical and GSH (Seven et al., 2004).

Conclusions

The data achieved from this study revealed that, the oral supplementation of 1.5% GTE to TAM-intoxicated rats, exerted an improvement against TAM-induced liver injury as it have beneficent effects on damaged liver cells to prevent lipid peroxidation and improve antioxidant enzyme activities. Information on potential synergistic or antagonistic interactions between the constituents of green tea and other chemicals, particularly metals, will lead to a clearer and better understanding of the potential health effects of green tea.

References


