GREEN TEA AMELIORATES RENAL OXIDATIVE DAMAGE INDUCED BY GENTAMICIN IN RATS

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ABSTRACT

Recent studies indicate that free radicals are important mediators of renal damage induced by gentamicin (GM), an aminoglycoside antibiotic widely used in treating severe gram-negative infections. Green tea extract (GTE) was reported to have antioxidant and free radical scavenging activities. Therefore, the aim of this work was to investigate the possible protective effect of GTE against gentamicin-induced nephrotoxicity. For this purpose, rats were divided into four groups. Group-1 (control) received normal saline. Group-2 received GTE (300 mg/kg/d, orally). Group-3 received gentamicin (80 mg/kg/d, intraperitoneally). Group-4 was injected with GTE plus gentamicin simultaneously. Daily urinary total protein levels were estimated to assess kidney dysfunction. The rats were sacrificed on the seventh day and kidneys were collected for histopathological studies. Blood urea nitrogen (BUN) and creatinine levels were measured in the blood. Moreover, glutathione (GSH), lipid peroxide expressed as thiobarbituric acid reactive substance (TBARS) levels, superoxide dismutase (SOD) and catalase (CAT) activities were determined in renal tissues. GM produced elevation in urinary total protein, BUN, serum creatinine and TBARS levels. On the other hand, GM reduced the GSH level and SOD, CAT activities. The simultaneous administration of GTE plus gentamicin protected kidney tissues against nephrotoxic effect of gentamicin as evidenced from amelioration of histopathological alterations and normalization of kidney biochemical parameters.

Keywords: Gentamicin, green tea extract, nephrotoxicity, antioxidant, free radicals, glutathione.

INTRODUCTION

Green tea derived from the leaves of the Camellia Sinensis plant. Originally cultivated in East Asia, this plant grows as large as a shrub or tree. Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called polyphenols also known as green tea catechins (Alic, 1999). The important catechins contents of green tea are (−)-epicatechin (EC), (−)-epicatechin-3-gallate (EGC), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) (McKenna et al., 2000). Green tea polyphenolic compounds present in the green tea extract have demonstrated significant antioxidant, anti-carcinogenic, anti-inflammatory and anti-microbial properties in human, animal, and in vitro studies (Zhong et al., 2001; Ahmad et al., 1997; Smith and Dou, 2001). Interestingly, the antioxidant effects of polyphenols appear to be more potent than vitamin C and E (Rice-Evans et al., 1995). These polyphenols in green tea can neutralize free radicals and may reduce or even help to prevent some of the damage caused by reactive oxygen species (ROS) (Dulloo et al., 1999). Accordingly GTE may have the capacity to protect the kidney against the deleterious effects of drugs known to enhance the generation of ROS like gentamicin.

Gentamicin (GM) is an aminoglycoside antibiotic that is very effective in treating life-threatening gram-negative Infections (Ho and Barza, 1987). Unfortunately, 30% of patients treated with GM for more than seven days show some signs of nephrotoxicity (Cuzzocrea et al., 2002). It has been reported that GM-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule (Pedraza-Chaverri et al., 2003). The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (50 to 100 times greater than serum) (Humes and Weinberg, 1986). The exact mechanism of GM-induced nephrotoxicity is unknown. However, GM has been shown to enhance the generation of ROS (Yanagida et al., 2004) causing deficiency in intrinsic antioxidant enzymes (Maldonado et al., 2003). ROS have been suggested as a cause of death for many cells in different pathological states including various models of renal and cardiac diseases (McCord et al., 1985; Baliga et al., 1999). Several compounds with antioxidant activity have been successfully used to prevent or ameliorate GM-induced nephrotoxicity (Pedraza-Chaverri et al., 2003; Yanagida et al., 2004; Maldonado et al., 2003). However, until now, the ameliorative effect of GTE against GM-induced nephrotoxicity has not been fully investigated.

Based on the reported antioxidant properties of polyphenolic compounds that present in the green tea extract, the hypothesis was made that GTE could
ameliore GM-induced oxidative stress and renal damage. Therefore, the major objective of this study was to investigate the possible protective effect of GTE against GM-induced renal damage, which was evaluated by measuring kidney biochemical parameters such as urinary excretion of total protein, BUN and serum creatinine as well as histological analysis of the renal tissues. Moreover, ROS scavenging properties of GTE was investigated by measuring oxidative stress biomarkers such as glutathione (GSH), thiobarbituric acid reactive substances (TBARS) levels, superoxide dismutase (SOD) and catalase (CAT) activities.

MATERIALS AND METHODS

Drugs and chemicals
Gentamicin sulfate was obtained from Memphis Co. for Pharm. and Chem. Ind. (Cairo, Egypt). Green tea extract was prepared from the plant *Camellia Sinensis* as described below. All other chemicals used were of good quality and analytical grade.

Preparation of Green tea extract (GTE)
GTE was prepared as described by Babu et al. (2006). Briefly, green tea leaves were ground in a miller as temperature of the container maintained at less than 50°C. Tea powder was extracted with 95% methanol (1:10 w/v) for 2 days with constant stirring. Suspensions were filtered through Whatman No. 1 filter paper to retain the clear solution. The residue was extracted again. The pooled tea extract was vacuum evaporated below 50°C. The dried extracts were stored at 4°C.

Animals
Forty adult female Wister albino rats weighing 150-200 g were selected for this study. The animals were obtained from animal house, faculty of Medicine, Assiut University (Assiut, Egypt), which were fed standard diet from animal house, faculty of Medicine, Assiut. The animals were obtained for 2 days with constant stirring. Suspensions were filtered through Whatman No. 1 filter paper to retain the clear solution. The residue was extracted again. The pooled tea extract was vacuum evaporated below 50°C. The dried extracts were stored at 4°C.

Experimental protocol
The animals were divided into 4 groups each of 10 rats: 
Group 1: Rats in this group were injected with normal saline, intraperitoneally and served as a control. 
Group 2: Rats in this group were orally treated with 300 mg/kg/d of GTE for seven consecutive days (Babu et al., 2006). 
Group 3: Rats in this group were injected intraperitoneally with 80 mg/kg/d of gentamicin sulfate for 7 d (Silan et al., 2007; Soliman et al., 2007). 
Group 4: Rats in this group were simultaneously treated with the same previous doses of GTE and gentamicin for 7 d. Total protein levels were estimated in 24-h urine samples every other day. The rats were sacrificed on the seventh day; blood samples were collected into tubes and allowed to clot at room temperature. Thereafter, serum was separated by centrifugation at 1,200 Xg for 15 min at 4°C for determination of blood urea nitrogen (BUN) and serum creatinine. Both of the kidneys were collected and fixed with 10% buffered formalin solution in the room temperature for histopathological evaluation. Tissue samples from the kidney were stored at -70°C liquid nitrogen for enzymatic analysis. Kidney samples were thawed and homogenized (10% w/v) in 0.15 M KCl at 4°C then centrifuged at 10000 g for 90 min. The supernatant was used as the source of experimental product for determination of oxidative stress biomarkers.

Biochemical analysis

Determination of total protein contents in 24-h urine samples
Rats were placed in metabolic cages; urine was collected for 24 hours every other day. Protein concentrations were measured using rat urinary protein assay kit (Chondrex, USA) by precipitation with 3% sulfosalicylic acid, and the resultant turbidity (Nishi and Elin, 1985) was determined by measurement of absorbance at 580 nm.

Determination of blood urea nitrogen (BUN)
BUN level was measured using Urea Enzymatic Colorimetric Kit (Linear Chemicals, S.L., Spain) according to the method of Fawcett and Scott (1960). Principle: Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia ions react with hypohlorite and salicylate to give green dye (2,2 dicarboxyindingophenol). The color of the dye was measured at 580 nm.

Determination of serum creatinine level
Serum creatinine level was determined using Creatinine Colorimetric Kit (BioMerieux, France) according to the method of Henry (1974). Principle: Creatinine in alkaline solution reacts with picrate to form a colored complex. The color of the complex was measured at 492 nm.

Determination of oxidative stress biomarkers in renal tissues
Glutathione (GSH) content of kidney tissues homogenate was determined using Ellman’s reagent according to the method described by Ellman (1959). Rat kidney homogenate lipid peroxide levels were measured by colorimetric determination of TBARS is based on the reaction of one molecule of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA) at low pH (2-3) according to the method of Mihara and Uchiyama (1978). The enzymatic activity of renal superoxide dismutase (SOD) was assessed according to the method of Marklund (1985). In brief, SOD activity was determined by computing the difference between auto-oxidation of pyrogallol alone and in presence of SOD enzyme. The catalase activity was
estimated in the rat kidney tissue depending on the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide by catalase according to the method of Clairborne (1985). All the measurements were carried out by using a spectrophotometer (Shimadzu spectrophotometer, UV, 1201, Japan).

**Histopathological evaluations**

The kidneys of each animal were dissected out then fixed in buffered formalin for 12 hours and processed for histopathological examination. Four µm-thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol (Allen, 1992). Other paraffin sections were stained with periodic acid-Schiff (PAS) (Bancroft and Stevens, 1982) to detect some pathological alterations clearly. A minimum of 8 fields for each kidney section were examined and assigned for severity of changes by an observer blinded to the treatments of the animals.

**STATISTICAL ANALYSIS**

Results were expressed as the means ± SEM. Statistical significant difference was determined by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test for multiple comparison. Probability values ($P$) less than 0.05 were considered to be statistically significant.

### RESULTS

#### Kidney function tests

GM caused an elevation in urinary excretion of total protein levels (mg/24h) from $12 \pm 1.73$ to $70 \pm 5.7$ after 7 days of its injection. In the animal group simultaneously treated with GTE, the elevated urinary content of total protein was significantly reduced by 47% to $37 \pm 3.4$ (mg/24h) (table 1). Moreover, GM produced a significant elevation in blood urea nitrogen levels (BUN, mg/dl) from $26.6 \pm 2.3$ to $53 \pm 2.8$ (table 1) and serum creatinine levels (mg/dl) from $0.24 \pm 0.023$ to $2.07 \pm 0.28$ (table 1) after 7 d of its injection when compared to untreated control rats. On the other hand, simultaneous administration of GTE plus GM significantly caused 36% reduction in the elevated BUN of GM-treated rats to give a value of $34 \pm 2.89$ mg/dl and 60% reduction in the elevated serum creatinine levels to give a value of $0.82 \pm 0.75$ mg/dl (table 1). Administration of GTE alone had no effect on urinary excretion of total protein, BUN and serum creatinine levels (table 1).

#### Effects of GTE on renal oxidative stress biomarkers: GSH, TBARS levels, CAT and SOD activities

Glutathione (GSH) has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes; GSH is also a scavenger.

### Table 1: Effects of gentamicin (GM), green tea extract (GTE) and their combination (GM+GTE) on urinary excretion of total protein, blood urea nitrogen (BUN) and serum creatinine, compared to control group (CO).

<table>
<thead>
<tr>
<th>Urine and Serum Renal Biochemical Parameters</th>
<th>CO</th>
<th>GTE</th>
<th>GM</th>
<th>GM + GTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Protein Excretion Levels (mg/24h)</td>
<td>$12^\dagger \pm 1.73$</td>
<td>$13^\dagger \pm 0.73$</td>
<td>$70^{** \dagger \ddagger} \pm 5.7$</td>
<td>$37^* \pm 3.4$</td>
</tr>
<tr>
<td>Blood Urea Nitrogen Levels (BUN, mg/dl)</td>
<td>$26.6 \pm 2.3$</td>
<td>$26.1 \pm 3.1$</td>
<td>$53^{** \dagger \ddagger} \pm 2.8$</td>
<td>$34 \pm 2.89$</td>
</tr>
<tr>
<td>Serum Creatinine Levels (mg/dl)</td>
<td>$0.24^\dagger \pm 0.023$</td>
<td>$0.25^\dagger \pm 0.021$</td>
<td>$2.07^{** \dagger \ddagger} \pm 0.28$</td>
<td>$0.82^* \pm 0.75$</td>
</tr>
</tbody>
</table>

Data expressed as means ± SEM, (n=10/group). The significant difference between two groups was determined by ANOVA followed by Dunnett’s multiple comparison tests. $^*P<0.05$, $^{**}P<0.01$, statistically significant difference from control group; $^\dagger P<0.05$; $^{\dagger \ddagger} P<0.01$, statistically significant difference from GM+GTE group.

### Table 2: Effects of gentamicin (GM), green tea extract (GTE) and their combination (GM+GTE) on renal tissue contents of glutathione (GSH), lipid peroxide (TBARS), superoxide dismutase (SOD) and catalase (CAT) activities compared to control group (CO).

<table>
<thead>
<tr>
<th>Oxidative Stress Biomarkers</th>
<th>CO</th>
<th>GTE</th>
<th>GM</th>
<th>GM + GTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH renal tissue contents (nmol/mg protein)</td>
<td>$20.9 \pm 1.15$</td>
<td>$21.9 \pm 2.3$</td>
<td>$9.4^{** \dagger \ddagger} \pm 1.7$</td>
<td>$17.9 \pm 1.9$</td>
</tr>
<tr>
<td>TBARS renal tissue contents (nmol/mg protein)</td>
<td>$0.024 \pm 0.0023$</td>
<td>$0.025 \pm 0.0032$</td>
<td>$0.073^{** \dagger \ddagger} \pm 0.004$</td>
<td>$0.025 \pm 0.0043$</td>
</tr>
<tr>
<td>SOD activity in renal tissue (U/mg protein)</td>
<td>$18 \pm 1.73$</td>
<td>$16 \pm 2.1$</td>
<td>$8.66^{** \dagger} \pm 1.45$</td>
<td>$15 \pm 1.48$</td>
</tr>
<tr>
<td>CAT activity in renal tissue (K/mg protein)</td>
<td>$0.45 \pm 0.034$</td>
<td>$0.44 \pm 0.024$</td>
<td>$0.23^{** \dagger} \pm 0.017$</td>
<td>$0.41 \pm 0.034$</td>
</tr>
</tbody>
</table>

Data expressed as means ± SEM, (n=10/group). The significant difference between two groups was determined by ANOVA followed by Dunnett’s multiple comparison test. $^{**}P<0.01$, statistically significant difference from control group; $^\dagger P<0.05$, $^{\dagger \ddagger} P<0.01$, statistically significant difference from GM+GTE group.
Green tea ameliorates renal oxidative damage

of hydroxyl radicals and singlet oxygen (Diplock, 1994). In this study, GM produced a decrease in GSH levels (nmol/mg protein) from 20.9±1.15 to 9.4±1.7. GTE prevented the GM-induced decline in GSH level causing 90% increase in GSH content compared to GM treated group and restored its normal level to 17.9±1.9 (table 2). Free oxygen radicals can induce lipid peroxidation in cells, malONDialdehyde (MAD) is formed during oxidative degeneration and accepted as an indicator of lipid peroxidation. In this study, GM caused an elevation in lipid peroxide (TBARS) levels (nmol/mg protein) in renal tissues from 0.024 ± 0.0023 to 0.073 ± 0.004. GTE was able to normalize the elevated TBARS levels to 0.025 ± 0.0043, producing 66% reduction in the elevated TBARS levels (table 2). SOD (U/mg protein) and CAT activities (k/mg protein) were decreased in renal tissues of GM-treated rats from 18 ± 1.73 to 8.66 ± 1.45 (for SOD) and from 0.45±0.034 to 0.23±0.017 (for CAT). However, the reduced SOD and CAT activities were increased by 73% and 78% to give values of 15 ± 1.48 and 0.41 ±0.034 respectively after GTE administration (table 2). Administration of GTE did not show any significant effects on GSH, TBARS levels or SOD and CAT activities (table 2).

**Histopathological analysis**

Sections from control group showed normal histological structure of the glomeruli and renal tubules in the cortex (fig. 1a) and normal tubules in the medulla (fig. 1b). In renal sections from GM-treated rats, the glomeruli showed atrophy in some of them and hypertrophy in others (fig. 1c). There were degeneration and necrobiosis in the epithelial cells lining the renal tubules with cystic luminal dilatation in others at the cortex (fig. 1e). The endothelial cells lining the glomerular tufts showed swelling and there was intacytoplasmic vacuolation as detected by PAS (fig. 1f). Mononuclear leucocytes inflammatory cells infiltration was observed in focal manner between the tubules in the corticomedullary junction as well as in the perivascular area of the dilated blood vessels associated with edema (fig. 1d). GTE reversed most of the histopathological alterations induced by gentamicin as seen from sections from GM-GTE treated rats (table 3). Photomicrographs from GM-GTE group revealed mostly normal glomeruli with absence of glomerular atrophy and hypertrophy (fig. 1g) and (table 3). GTE also alleviated tubular degeneration and necrobiosis at the cortex usually seen with GM (fig. 1h) and (table 3). In addition, GTE reduced the mononuclear leucocytes inflammatory cells infiltration and alleviated the perivascular edema in the corticomedullary junction (fig. 1h) and (table 3). The rest of histopathological changes produced by GM were completely prevented by GTE treatment (table 3). In addition to its protective effects, GTE alone was found to be safe and did not induce any histopathological changes in the kidney (table 3). The histological alterations produced by GM that are ameliorated by GTE are summarized in table 3.

**DISCUSSION**

Recently, green tea is being widely studied for its beneficial effect in the treatment and prevention of human diseases. It is considered to be anti-inflammatory, anti-oxidative, anti-mutagenic and anti-carcinogenic (Liao et al., 2001; Crespy and Williamson, 2004). It was reported that green tea catechins can act as scavengers of free radicals caused by reactive oxygen species and prevent

<table>
<thead>
<tr>
<th>Histopathological Alterations</th>
<th>CO</th>
<th>GTE</th>
<th>GM</th>
<th>GM + GTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular atrophy</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>Glomerular hypertrophy</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Tubular degeneration</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Swelling of endothelium</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Vacuolation of endothelium</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Leucocytes cells infiltration</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Perivascular edema</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Data were obtained from investigation of 4 histological sections from kidneys of control group (CO), Green Tea extract-treated group (GTE), gentamicin-treated group (GM) and the combination of gentamicin plus GTE (GM+GTE). A minimum of 8 fields for each kidney section were examined and assigned for severity of changes by an observer blinded to the treatments of the animals and assigned for severity of changes using Scores of 0: absent. +: (Mild level) less than 25% of the total fields examined revealed histopathological alterations. ++: (Moderate level): less than 50% of the total fields examined revealed histopathological alterations. +++: (Severe level): less than 75% of the total fields examined revealed histopathological alterations.

Table 3: The ameliorative effects of GTE against histopathological alterations induced by gentamicin (GM) in the kidney tissues of different experimental groups.
free radical damage (Liao et al., 2001). Babu et al. (2006) have reported that oral GTE administration impedes dyslipidemia, lipid peroxidation and protein glycation in the heart of streptozotocin-diabetic rats. These effects could be attributed to the antioxidant and free radical scavenging properties of GTE. It has been shown that GM exerts its adverse renal effect by generation of reactive oxygen species (ROS) (Kadkhodae et al., 2005) which results in severe tissue damage. Under normal conditions, ROS, which are generated during cellular functions, are eliminated by intrinsic antioxidant enzyme systems like superoxide dismutase, catalase and glutathione peroxidase (Kaul et al., 1993). Therefore, GTE with its antioxidant and reactive oxygen species scavenger properties may have the capacity to partially reduce or eliminate the deleterious effects caused by GM.
Results from many studies (Silan et al., 2007; Soliman et al., 2007) have shown that GM produced an elevation in the concentrations of biochemical indicators of kidney function such as BUN, serum creatinine and total protein excretion in urine. Consistent with the data from the study of Pedraza-Chaverrí et al. (2004) we observed in our study that urinary excretion of total protein was increased after GM injection indicating tubular damage. On the other hand, BUN and serum creatinine levels were augmented indicating glomerular damage. However, the combined administration of GTE plus GM to rats resulted in significant reduction in the elevated levels of urinary total protein concentrations, BUN and serum creatinine. These results could be in accord with several other researches, which reported that, compounds with antioxidant properties like s-allylmercaptocysteine (Pedraza-Chaverrí et al., 2004) and diallyl sulfide (Pedraza-Chaverrí et al., 2003) inhibited the increased urinary excretion of total protein induced by GM in rats. Other compounds like resveratrol (Silan et al., 2007), carnosine (Soliman et al., 2007) or garlic extract (Maldonado et al., 2003), partially prevented the increase in BUN and serum creatinine levels induced by GM.

In the present study, the role of ROS in gentamicin nephrotoxicity was assessed by evaluation of alterations in the biochemical indicators of oxidative stress induced by GM or combination of GM plus GTE. These oxidative stress parameters include GSH, TBARS levels, SOD and CAT activities beside histological changes. In the present study, the levels of GSH in rat kidney tissues were significantly reduced after GM injection compared with control group. This result is confirmed by other studies, which have pointed to reduction of GSH levels after GM administration (Silan et al., 2007; Soliman et al., 2007). An explanation to GSH depletion after GM treatment is increased consumption of GSH in non-enzymatic removal of oxygen-radicals. In addition, oxidation of GSH to GSSG by the oxidant stress, with efflux of GSSG being the major factor responsible for maintenance of the redox ratio (Eberle et al., 1981). Sinha et al. (2007) have reported that galactosamine, an established experimental toxin, decreases the reduced glutathione (GSH) and enhances the renal tissue content of the oxidized form (GSSG). The conversion of GSSG to GSH is mediated through the enzyme glutathione reductase. Therefore, GM may act like S-(1,2-dichlorovinyl)-L-cysteine a known nephrotoxicant and interferes with the recycling of GSSG into GSH by inhibition of the enzyme glutathione reductase (Van de Water et al., 1996). It has been reported that, in addition to directly quenching reactive oxygen species, tea polyphenols have the ability to participate in vitamin E recycling (Zhu et al., 1999) and thus complementing some of the functions of glutathione (GSH). Babu et al. (2006) have reported that green tea by scavenging the free radicals directly in rats may reduce the utilization of GSH and thereby exhibiting an increase in the GSH content in diabetic rats treated with green tea extract. Our results were consistent with this finding as simultaneous administration of GTE and GM significantly increased the GSH levels compared to that of GM-treated group only.

Moreover, GM causes rapid changes in membrane lipid composition. These changes of membrane lipid composition may be induced by free radical-initiated lipid peroxidation (Sandhya and Varalakshmi, 1997). This view is supported by increased MDA levels, one of the products of lipid peroxidation, in GM treated rats kidney (Parlakpinar et al., 2005). We have found elevated lipid peroxide levels (TBARS) in the GM treated group, consistent with previous studies mentioned. On the other hand, the activities of superoxide dismutase (SOD) and catalase (CAT) enzymes were greatly reduced in GM-treated rats compared with control group. The scavenging of superoxide radicals is achieved through an upstream enzyme, SOD, which catalyses the dismutation of superoxide to H2O2. This reduction in SOD and CAT activities after GM injection has been previously recorded (Nitha and Janardhanan, 2008; Farombi and Ekor, 2006) respectively, suggesting that oxidative stress is one of the causes of GM-induced renal damage. Interestingly, the combined administration of GTE plus GM to rats reversed all of these alterations. Consistent with the results obtained from the study of Upaganlawar et al. (2006) GTE markedly, enhanced the activities of SOD and CAT enzymes and reduced the elevated levels of TBARS indicating that GTE treatment decreases oxidative stress through its antioxidant properties.

Previous studies have shown that agents including gentamicin that enhance the generation of hydrogen peroxide and superoxide anion by mitochondria also enhances the generation of hydroxyl radical (Doroshow and Davies, 1986). Walker and Shah (1988) have examined the biological processes that may be affected by the hydroxyl radical generated from GM-treatment leading to acute renal failure. The damage in plasma membrane following lipid peroxidation results in loss of osmotic balance and intracellular calcium levels increase. Cellular swelling is the first manifestation of these reversible changes (Silan et al., 2007). Swelling of endothelium lining the glomerular tufts and tubular vacuolization is the reflection of these reversible changes in kidneys (Kumar et al., 1999). In gentamicin group, we have observed swelling of endothelium lining the glomerular tufts and vacuolization in all samples. In GM-GTE group, most of the glomeruli are normal in size and shape. GTE blocked cellular inflammatory process as indicated from alleviation of perivascular edema and reduction in mononuclear leukocytes inflammatory cells infiltration. Biochemical data were concordant with pathological findings since GTE was able to normalize the elevated lipid peroxide (TBARS) levels and completely block lipid peroxidation.
If intracellular free oxygen radicals increase, irreversible cellular injury process begins (Silan et al., 2007). Lysosomal enzymes activated and irreversible cell injury microscopically observed as tubular necrosis and tubular degeneration of kidney occurs (Kumar et al., 1999). Scavenging of free oxygen radicals prevent irreversible renal cell injury and necrosis (Silan et al., 2007). We have observed tubular necrosis as a sign of irreversible injury in most sections examined from gentamicin group. GTE as an antioxidant inhibits lipid peroxidation and prevents renal cell injury manifested as tubular necrosis an irreversible cell damage. We have observed that GTE treatment affected biochemical values and pathological findings, in accordance. GTE prevented the decrease in GSH levels, SOD and CAT activities and the increase in TBARS levels. In the same way, GTE prevented reversible cell damage such as swelling of endothelium and vacuolization and reduced irreversible cell damage incidence such as tubular necrosis and degeneration.

In conclusion, the present study revealed the nephrotoxic effects of GM. The use of GTE in combination with GM minimized its toxicity as evidenced from decreasing urinary excretion of protein, BUN and serum creatinine levels. The correction of oxidative stress biomarkers by GTE was consistent with amelioration of the histopathological changes induced by GM. Thus, ameliorative effect of GTE against GM-induced renal damage may be at least in part due to its antioxidant and free radicals scavenger properties of GTE.

ACKNOWLEDGMENTS

We are grateful to Prof. Dr. Adel M. Bakeer, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University for his kind help in performing histopathological studies and interpretation of the results. We are also thankful to Dr. Ehab S. Elkhayat, Department of Pharmaco-cognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt for his kind help in preparation of green tea extract.

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