Effect of Green Tea Extract on Lipid Peroxidation and Antioxidant Activity on Mercuric Chloride Induced Toxicity in Rats

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ABSTRACT
Lipidperoxidative and antioxidative efficacy of Camellia sinensis was investigated against mercuric chloride induced toxicity in Wistar rats. Toxicity was induced in Wistar rats by daily intraperitoneal injections of a freshly prepared solution of mercuric chloride at a dose of 1.25mg/kg body weight for 45 days. Levels of lipid peroxidation were assessed by estimating TBARS and lipid hydroperoxide and the antioxidant levels were assessed by estimating the levels of GSH, SOD, CAT and GPx. Significant increase was observed in the levels of TBARS, hydroperoxide in HgCl2 treated rats. These levels were significantly decreased in HgCl2 and Green Tea Extract treated rats. The biochemical alteration during GTE treated in HgCl2 treated rats may be due to presence of natural antioxidants and free radical scavenging activity, antioxidant property and health protecting potential. From the results it can be concluded that pretreatment of Green Tea Extract appears to exhibit protective effect in Hgcl2 treated rats by reducing oxidant and antioxidant imbalance.

Keywords: Oxidative stress, Camellia sinensis, mercuric chloride.

INTRODUCTION
Mercury a highly toxic metal, which induces oxidative stress in the body and results in a variety of adverse health effects including renal, neurological, respiratory, immune, dermatologic, reproductive and developmental sequelae. It is well known that inorganic mercury causes severe kidney damage after acute and chronic exposure. Chronic exposure to inorganic mercury salts primarily affects the renal cortex and may manifest as renal failure (dysuria, proteinuria, hematuria, oliguria and uremia) or gastrointestinal problems (colitis, gingivitis, stomatitis and excessive salivation). Irritability and occasionally acrodynia can occur.

Inorganic mercury has a non-uniform distribution after absorption being accumulated mainly in the kidneys causing acute renal failure. Once absorbed into the bloodstream, inorganic mercury combines with proteins in the plasma or enters the red blood cells. It does not readily pass into the brain or fetus but may enter into other body organs. It accumulates in the kidneys and may cause severe damage. Poisoning can result from inhalation, ingestion or absorption through the skin. Mercury can cause biochemical damage to tissues and genes through diverse mechanisms, such as interrupting intracellular calcium homeostasis, disrupting membrane potential, altering protein synthesis and interrupting excitatory amino acid pathways in the central nervous system.

Mitochondrial damage from oxidative stress may be the earliest sign of neurotoxicity with methyl mercury. A study in neural tissue indicates the electron transport chain appears to be the site where free radicals are generated, leading to oxidative damage induced by methylmercury⁶.

Mercuric chloride toxicity and lipid peroxidation
Mercury also promotes the formation of reactive oxygen species by Fenton transition equation, such as hydrogen peroxides and enhances the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical⁷⁻¹⁰. Lipid peroxides alter membrane structure and are highly disruptive of mitochondrial structure. Mercury also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase and perhaps the GSH peroxidase¹¹. From the present investigation it can be suggested that HgCl2 treatment significantly reduces the GSH content and the antioxidant potential and thus accelerates the lipid peroxidation resulting in cellular toxicity.

Mercury bound to SH groups may result in decreased gluthathione levels, leading to an increase of reactive oxygen species, like superoxide anion radical, hydrogen peroxide and hydroxyl radical¹², which induce lipid, protein and DNA oxidation¹³. Besides, it has been shown that in vitro Hg²⁺ both hinders the antioxidant potential of glutathione and yields reactive species via thiol complexation¹⁴. Accordingly, mercury exposure has been demonstrated to induce lipid peroxidation detected by increased thiobarbituric acid-reactive substances (TBARS) in kidney and other tissues. Thus, it is believed that antioxidants should be one of the important components of effective treatment for mercury poisoning.

Scavenging systems to limit free radical damage
The possible mechanisms by which antioxidants may protect against ROS toxicity are
1. Chelating the transition metal catalysts.
2. Chain-breaking reactions.
3. Reducing the concentration of reactive radicals.
4. Scavenging initiating radical.

Antioxidants are of two types, preventive antioxidants and chain breaking antioxidants[16]. Preventive antioxidants act by reducing peroxides to molecular products without the production of radicals (e.g., GPx, catalase), removing (or) decreasing oxygen, removing key reactive catalytic metal ions, scavenging initiating free radicals such as hydroxyl, alkoxyl, peroxy species, breaking the chain of initiated sequence (or) by quenching singlet oxygen. Antioxidants that can trap radicals directly, thereby shortening the chain length are classified as chain breaking antioxidants. This class includes vitamin E (peroxy radical trap), SOD (superoxide trap).

**Herbal Medicines**

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease[17]. More attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially whenever free radical generation is involved[18]. Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress[19,20], especially in case of cancer. Flavonoids are a group of polyphenolic compounds that occur widely in fruit, vegetables, tea, cocoas and red wine[21,22]. 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[23].

Tea, a product made up from leaf and bud of the plant Camellia sinensis, is the second most consumed beverage in the world, well ahead of coffee, beer, wine and carbonated soft drinks[24].

Tea has been found to exhibit various bioregulatory activities, such as anti-carcinogenic[25-27], anti-metastatic[28], anti-oxidative[29,30], anti-hypertensive[31], anti-hypercholesterolemic[32,33], anti-dental caries[34,35], anti-bacterial[36], and to contribute to intestinal flora amelioration activity[37]. Catechins, a group of polyphenolic compounds, have been shown to be largely responsible for these activities.

However, no investigation has been carried out on effect of Green tea extract on mercuric chloride induced toxicity in rats.

Therefore, the present study deals with the influence of GTE on mercuric chloride induced toxicity in rats by evaluating the changes in the levels of lipid peroxidation (TBARS) and Lipidhydroperoxide and changes in the levels of non-enzymatic antioxidants and enzymatic antioxidants.

**MATERIALS AND METHODS**

**Maintenance of Animals**

Adult male albino rats (Wistar strain of body weight 180-200 g) bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India were used in this study. The animals were housed in polypropylene cages and provided with food and water ad libitum. They were maintained in a controlled environment under standard conditions of temperature and humidity with alternating light/dark (LD 12:12) cycle. All animals were fed standard pellet diet (Hindustan Lever Ltd., Bangalore, India). Composed of 21% protein, 81% ash, 5% lipids, 4% crude, 3.4% glucose, 2% vitamins, 1% calcium, 0.6% phosphorus and 55% nitrogen free extract (carbohydrates). It provides a metabolisable energy of 3600 Kcal.

**Chemicals**

Green Tea Extract was purchased from Sigma Aldrich, U.S.A. Mercuric chloride, Other chemicals and biochemicals used in this study were of analytical grade.

**Experimental Design**

Animals were divided into 4 groups, contained 6 animals (n = 6) each. Toxicity was induced in Wistar rats by daily intraperitoneal injections of a freshly prepared solution of mercuric chloride at a dose of 1.25mg/kg body weight for 45 days (Rao and Sharma, 2001).

**Group I**: Normal untreated rats.

**Group II**: The GTE was made according to Maity, 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 for 45 days.

**Group III**: Rats were injected with mercuric chloride (i.p)(1.25mg/kg body weight) (Sharma, 2007).

**Group IV**: Rats were treated with mercuric chloride (1.25mg/kg body weight) as in group III and Green Tea Extract (1.5%) as in group II rats.

After the end of the experimental period (45 days) the animals were sacrificed by cervical dislocation, the blood was collected in heparinised tubes and plasma was separated for TBARS and GSH.

**Preparation of plasma**

Blood was collected in heparinised tubes and plasma was separated by centrifugation at 2000 rpm for 15 min for various biochemical estimations. Hemolysate was obtained for the estimation of SOD, catalase and GPx.
Preparation of hemolysate

After separating the plasma, the packed cells were washed thrice with physiological saline. 0.5 ml of erythrocyte was lysed with 2.5 mL hypotonic phosphate buffer, pH 7.4. The hemolysate was separated by centrifugation at 2500 rpm for 15 min at 2 °C for the estimation of enzymic antioxidants.

TBARS were estimated by thiobarbituric acid assay method of (Niehaus and Samuelsson, (1968)38. Lipid hydroperoxides in the plasma, erythrocytes and tissues were estimated by the method of Jiang (1992)39. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar (1984)40. The catalase activity was determined by the method of Sinha (1972)41. glutathione peroxidase was assayed by the method of Rotruck (1973)42. Reduced glutathione was determined by the method of Ellman, (1959)43.

Statistical Analysis

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT). The level of statistical significance was set at p ≤ 0.05.

RESULTS

The fresh leaves of camellian sinensis have been found to contain epigallocatechin gallate. The antioxidant activity of green tea extract from camellian sinensis was found to be increased in group III rats. Group IV rats showed no significant changes in GSH levels when compared with the corresponding group III rats. Group IV rats showed significantly increased levels of GSH when compared with group I rats.

TBARS and Hydroperoxides

The levels of TBARS and hydroperoxides in plasma, was found to be increased in group III rats. Group IV rats showed significant reduction in the levels of TBARS and hydroperoxides when compared with group III rats. Group II rats showed no significant changes in the levels of TBARS and hydroperoxides when compared with group I (Table 1).

Reduced Glutathione (GSH)

Administration of HgCl₂ caused a significant decrease in the GSH concentration of plasma in group III rats. Group IV rats showed significantly increased levels of GSH when compared with corresponding HgCl₂ treated rats. Group II rats showed no significant changes in GSH levels when compared with control (Table 1).

Superoxide Dismutase (SOD), Catalase and Glutathione Peroxidase (GPx)

SOD, Catalase and GPx activities were found to be significantly decreased in hemolysate of group III rats when compared with group I rats. A significant increase in SOD, Catalase and GPx activities were observed in group IV rats when compared with the corresponding group III rats. Group II rats showed no significant change in SOD, Catalase and GPx activities when compared with group I rats (Table 2).

Table 1: Changes in the levels of TBARS and Lipid hydroperoxides in circulation of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (mmoles/dl)</th>
<th>Lipid Hydroperoxides (x 10⁻⁵ mM/100ml)</th>
<th>GSH mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.163 ± 0.016⁴</td>
<td>11.55 ± 0.76⁴</td>
<td>19.83 ± 1.20⁴</td>
</tr>
<tr>
<td>Group II</td>
<td>0.154 ± 0.011¹</td>
<td>10.77 ± 0.60⁴</td>
<td>21.33 ± 1.56⁴</td>
</tr>
<tr>
<td>Group III</td>
<td>0.236 ± 0.019⁵</td>
<td>14.70 ± 0.98⁵</td>
<td>15.06 ± 1.21³</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.184 ± 0.016⁶</td>
<td>12.62 ± 0.67⁷</td>
<td>18.30 ± 1.01¹</td>
</tr>
</tbody>
</table>

Values are mean ± SD for 6 rats in each group. Values not sharing a common superscript letters (a-c) differ significantly at p < 0.05 (DMRT).

Table 2: Changes in the activities of circulatory SOD, CAT and GPx of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units⁵/mg Hb)</th>
<th>CAT (Units⁶/mg Hb)</th>
<th>GPx (Units⁷/mg Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2.85 ± 0.11⁴</td>
<td>2.20 ± 0.14⁴</td>
<td>22.75 ± 1.58⁴</td>
</tr>
<tr>
<td>Group II</td>
<td>2.90 ± 0.17²</td>
<td>2.03 ± 0.16²</td>
<td>25.00 ± 2.4³</td>
</tr>
<tr>
<td>Group III</td>
<td>1.79 ± 0.15⁴</td>
<td>1.45 ± 0.24⁴</td>
<td>16.67 ± 1.14⁴</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.43 ± 0.27</td>
<td>1.77 ± 0.31²</td>
<td>20.33 ± 1.06²</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letters (a-c) differ significantly at p < 0.05 (DMRT).

* Units of enzyme activities are expressed as: SOD - One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute. CAT - μ moles of hydrogen peroxide consumed / minute. GPx - μg of glutathione consumed / minute.

DISCUSSION

Lipid peroxidation Changes

Enhanced levels of TBARS in plasma of HgCl₂ treated rats indicated the increased levels of lipid peroxidation. Reports have shown that mercury promotes the formation of ROS by fenton transition equation, such as hydrogen peroxides and enhances the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radicals⁵,⁷,¹⁰,⁴⁴.

Lipid peroxides alters the membrane structure and are highly disruptive of mitochondrial structure. Mercury also inhibits free radical quenching enzymes such as CAT, SOD and perhaps the GSH peroxidase and thus resulting in cellular toxicity⁴⁵. Simultaneous administration of GTE decreased the formation of lipid peroxidation products, and it possesses antioxidant activity⁴⁶. Thus, this agent might provide more medical benefit because the use of this agent could simultaneously alleviate oxidative damage⁴⁷.

Nevertheless, a substantial number of human intervention studies with green tea demonstrate a significant increase in plasma antioxidant capacity in
humans after consumption of moderate amounts (1–6 cups/day); there are also initial indications which show that the enhanced blood antioxidant potential leads to a reduced oxidative damage in macromolecules such as DNA and lipids\textsuperscript{48}. McKay and Blumberg, (2002)\textsuperscript{49} reported that the repeated consumption of green tea and encapsulated green tea extracts for one to four weeks has been demonstrated to decrease biomarkers of oxidative status. Erba (2005)\textsuperscript{50} suggested the ability of green tea, consumed within a balanced controlled diet, to improve overall the antioxidative status and to protect against oxidative damage in humans.

**Changes in the levels of Non-Enzymatic Antioxidants**

**Glutathione (GSH)**

GSH is a major thiol, which binds electrophilic molecular species and free radical intermediates. It plays a central role in the antioxidant defense system, metabolism and detoxification of exogenous and endogenous substances. Mercury has a high affinity on GSH and causes the irreversible excretion of, upto two GSH tripeptides\textsuperscript{51}. The metal-GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. However, it depletes the GSH from the cell and thus decreases the antioxidant potential\textsuperscript{45}.

**Changes in the levels of Enzymatic Antioxidants**

Superoxide dismutase catalyses the dismutation of superoxide anion to \(H_2O_2\), which in turn can be destroyed by catalase or glutathione peroxidase reactions. Catalase, which is present virtually in all mammalian cells, is responsible for the removal of \(H_2O_2\)\textsuperscript{52}. It plays an important role in the acquisition of tolerance to oxidative stress in adaptive response of cells\textsuperscript{53}.

Glutathione peroxidase is the most important cellular antioxidant defense mechanism. It catalytically removes \(H_2O_2\) and lipid hydroperoxides from the cell, thereby reducing the generation of the OH\(^-\). In addition, GPx converts GSH to its oxidized product, GSH disulfide (GSSG). GRd reduces GSSG to GSH; it has been important role in the recycling of GSH and thereby reducing free radical damage. Besides functioning in the removal of \(H_2O_2\) from cells, GPx also reduces peroxynitrite anion, thus having an additional catalytic function to lower oxidative stress\textsuperscript{34}.

The inactivation in CAT and GPx induced by HgCl\(_2\) may be probably due to their inactivation during the catalytic cycle\textsuperscript{54}. Interestingly, SOD activity was found to be depressed in animals treated with HgCl\(_2\). SOD inhibition may be related to a covalent attachment of mercury ions to its reactive cysteine residues\textsuperscript{55} which are involved in the detoxification of metals like mercury. Alternatively, SOD inhibiton might also be a consequence of an excess of ROS, which would affect enzyme structure\textsuperscript{56}. Mercury induced oxidative stress is an important mechanism for the inhibition of antioxidant enzymes in HgCl\(_2\) induced kidney, liver and brain injury\textsuperscript{54,57}.

Green tea is considered a dietary source of antioxidant nutrients: green tea is rich in polyphenols (catechins and gallic acid, particularly), but it also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as Cr, Mn, Se or Zn, and certain phytochemical compounds. These compounds could increase the Green Tea Extract antioxidant potential. GTP present antioxidant activity in vitro by scavenging reactive oxygen and nitrogen species and chelating redoxactive transition metal ions; GTP can chelate metal ions like iron and copper to prevent their participation in Fenton and Haber-Weiss reactions\textsuperscript{59,58}.

They may also function indirectly as antioxidants through

1. Inhibition of the redoxsensitive transcription factors;

2. Inhibition of ‘pro-oxidant’ enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase; and

3. Induction of antioxidant enzymes, such as glutathione-S-transferases and superoxide dismutases.

From the results it is concluded that pretreatment of Green Tea Extract appears to exhibit protective effect in HgCl\(_2\) treated rats by reducing oxidant and antioxidant imbalance.

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