AMELIORATIVE EFFECT OF GREEN TEA EXTRACT ON ALCOHOL INDUCED RENAL DAMAGE IN RATS

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ABSTRACT: This study investigates the protective effect of Green tea extract (GTE) against alcohol-induced renal damage. Studies were conducted with male albino wistar rats treated with 20% alcohol (5g/kg b.wt/day) and GTE (300 mg/kg b.wt/day) for 60 days. Results showed that significantly increased plasma creatinine, urea levels with a decrease in uric acid levels in alcohol administered rats compared with other experimental groups. Alcohol administration also decreased plasma electrolytes and mineral levels compared with other experimental groups. Upon GTE supplementation to the alcoholic rats all these abnormalities were brought to normal. Further, alcohol administration decreased activities of renal enzymes, such as SOD, CAT, GPx and GSH content in comparison with other experimental groups. Moreover, increased TBARS, protein carbonyls and peroxynitrite levels in kidney homogenates were observed in alcohol administered rats. Our results showed that GTE administration significantly protected kidney against alcohol-induced oxidative and nitrosative stress. Further, these renoprotective effects of GTE against alcohol induced damage are confirmed by histopathological studies these effects are attributable to GTE constituents, in particular the abundantly available GTE flavonoids - EGCG, ECG, EGC, EC. In conclusion, the flavonoids present in GTE are capable of scavenging free radicals thereby reduced alcohol induced renal damage.

Key words: Alcohol; Green Tea Extract; Kidney; Nitrosative stress; Oxidative stress

INTRODUCTION
Chronic alcohol consumption affects all body tissues and organs including renal system (Wang et al., 2015). The increased oxidative stress and nitrosative stress is responsible for alcohol-induced toxicological and pathological condition for which therapeutic approaches are sought (Maturu et al., 2012a; Hebbani et al., 2015). Kidney is an important organ in excretory function, maintenance and counter regulation of electrolyte disturbances like phosphate and potassium hypoglycemia etc. Reports revealed that excessive intake of ethanol generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) are considered as the key risk factors for alcohol-induced renal damage (Kumar et al., 2008; Kadir et al., 2013). The alcohol-induced signaling pathways and underlying molecular mechanisms in the development of diseases are well known. Now the researchers are focusing their work towards remedies using herbal medicines.
Now a day’s majority of the world population turned towards herbal medicines to treat various ailments. These herbal medicines are safe and do not possess side effects. Herbal medicines are rich in flavonoids, tannins, alkaloids etc. The protective effects or health beneficial effects of herbal medicines attributed to its bioactive compounds. The uses of herbal medicines are greatly mentioned for the treatment of various diseases in the classical Indian traditional medicine, Ayurveda (Patwardhan et al., 2004). Recent reports revealed that the antioxidant activity of flavonoids present in herbal extracts against various ailments. Green tea is one of the most popular, health promoting and extensively used dietary supplement in daily life, belonging to the Theaceae family. Reports revealed that green tea has protective effects against a broad range of pathologies, including cancer (Kavanagh et al., 2001), inflammation (Dona et al., 2003), diabetes (Waltner-law et al., 2002) and cardiovascular diseases (Sueoka et al., 2001). These protective effects are due to the presence of many bioactive components particularly catechins viz., Epigallocatechingallate (EGCG), Epigallocatechin (EGC), Epicatechingallate (ECG), and Epicatechin (EC). An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage. Catechins protect cells against the damaging effects of ROS and RNS (Senanayake et al., 2013). Though many studies are carried out on alcohol induced kidney damage, there is a paucity of information on ameliorative effect of GTE against alcohol induced renal damage. Hence, an attempt has been made to investigate protective effect of aqueous green tea extract against alcohol induced nephrotoxicity.

MATERIALS AND METHODS
The chemicals used in the present study were procured from Sigma-Aldrich chemical Co. (St. Louis, MO, USA) and SRL chemicals (Mumbai, India). Aqueous green tea leaf extract dry powder (Extract contains 75% catechins in that 50% EGCG) was obtained from Guardian Biosciences, Phoenix, Arizona, USA (Manufacturers and exporters of herbal extracts).

Animals
Two month-old male albino wistar rats weighing 120 to 140 g were procured from Sri Venkateswara Agencies, Bangalore, India. Animals were maintained on a standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and water ad libitum with 24 h light-dark cycle in the university animal house. After acclimatization for a week, animals were divided into four groups (n=8) viz., group-I control, group-II alcohol, group-III GTE alone and group-IV alcohol and GTE. Alcohol (20%) was administered at a dose of 5g/kg b.wt/day based on earlier studies (Reddy et al., 2012) and GTE was administered at a dose of 300 mg/kg b.wt/day based on earlier studies (Arteel et al., 2002). Control rats received iso-caloric glucose solution. All treatments were given orally using intubation tube for a period of 60 days. Experimentation and animal maintenance were done with prior approval of institutional animal ethical committee. At the end of experimental period the animals were fasted overnight and sacrificed by cervical dislocation. Immediately blood was collected into heparinized tubes by cardiac puncture and plasma was separated by centrifugation at 3000 rpm for 10 min. Kidney samples were collected and stored at -80°C until assays were carried out. A part of the kidney tissue was fixed in 4% formalin buffer solution for histopathological analysis.

Preparation of tissue homogenate
10% kidney homogenate was prepared by homogenizing the tissue in 50mM phosphate buffer (pH7.4). The homogenates were then centrifuged at 12,000 rpm for 10 min at 4°C and the supernatant was used for the biochemical assays.

Measurement of nitrogenous compounds, electrolytes and minerals
Plasma nitrogenous compounds were measured by using commercially available kits (Span Diagnostics, Surat, India). Plasma creatinine, urea, uric acid, sodium, potassium, chloride, calcium, and magnesium were determined as described previously (Brito et al., 2012; Padmavathi et al., 2009).

Measurement of MDA, nitric oxide and protein carbonyls levels
The extent of lipid peroxidation in kidney was measured as described previously (Ohkawa et al., 1979). Total nitric oxide (NOx) in the form of nitrite and nitrate levels in kidney was measured by the method of Sastry et al., (2002). The concentration of protein carbonyls was determined using 2, 4-dinitrophenylhydrazine (DNPH) assay according to the method of Reznick and Packer (1994).

Measurement of peroxynitrite content
The sample containing peroxynitrite was added to phenol in 50 mM sodium phosphate buffer (pH 7.4) mediated nitration of phenol, after incubation for 2 h at 37°C; NaOH was added to produce the salt nitrophenol, which has a maximum absorbance at 412 nm (Beckman et al., 1992). The yield of nitrophenol was calculated from 4400 M−1 cm−1 as an index of peroxynitrite concentrations.
Histopathological studies
A portion of kidney was dissected and fixed in 4% neutral buffered formalin solution for 24 h as described by Reddy et al., (2010). The fixed tissue was processed routinely, and then embedded in paraffin, sectioned to 3-5 µm thickness, deparaffinised and rehydrated using standard techniques. Morphological changes in kidney sections were recorded by staining with hematoxylin and eosin.

Statistical analysis
Data, subjected to statistical analyses, are mean ± SD of 6 rats in each group. Student t-test followed by Duncan’s Multiple Range (DMR) test was performed to find out significant differences between groups. A p<0.05 was considered statistically significant.

RESULTS
Green tea extract contains multiple poly phenolic compounds mainly flavonoids. Some of the important flavonoid compounds are presented in figure 1. The flavonoid compounds including Epigallocatechingallate (EGCG), Epigallocatechin (EGC), Epicatechingallate (ECG), and Epicatechin (EC) are mainly present in the GTE.

Fig. 1: Major bioactive components of GTE; (a) Epicatechin, (EC), (b) Epigallocatechin (EGC), (c) Epicatechingallate (ECG), (d) Epigallocatechingallate (EGCG).

The data presented in the Table-1 shows the significant increase in plasma creatinine, urea with a significant decrease in uric acid in alcohol administered rats compared with other experimental groups. Administration of GTE to alcohol administered rats restored the above said parameters to normal. This indicates that administration of green tea ameliorates glomerular function.
Data presented in Table-2 significant decrease in plasma electrolytes and minerals of alcohol administered rats when compared with other groups. GTE supplementation to alcoholic rats restores these abnormalities to the normal levels.

Table 1. Effect of GTE on plasma creatinine, urea, uric acid of alcohol administered rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>ALC</th>
<th>GTE</th>
<th>ALC+GTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>1.31 ± 0.07</td>
<td>2.41 ± 0.13*</td>
<td>0.37 ± 0.09</td>
<td>1.11 ± 0.06</td>
</tr>
<tr>
<td>Urea</td>
<td>24.5 ± 0.71</td>
<td>38.5 ± 1.12*</td>
<td>24.9 ± 1.78</td>
<td>23.5 ± 0.82</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.72 ± 0.26</td>
<td>3.02 ± 0.14*</td>
<td>1.6 ± 0.2</td>
<td>1.44 ± 0.23</td>
</tr>
</tbody>
</table>

Values are expressed as mg/dL and represented as mean ± SD. Asterisk (*) indicates significantly from control.

Data presented in Table-2 significant decrease in plasma electrolytes and minerals of alcohol administered rats when compared with other groups. GTE supplementation to alcoholic rats restores these abnormalities to the normal levels.

Table 2. Effect of GTE on plasma electrolytes and minerals in alcohol administered rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>ALC</th>
<th>GTE</th>
<th>ALC+GTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>122 ± 1.9</td>
<td>66 ± 1.7*</td>
<td>120 ± 0.9</td>
<td>108 ± 1.3</td>
</tr>
<tr>
<td>Potassium</td>
<td>6.1 ± 0.7</td>
<td>3.3 ± 0.2*</td>
<td>5.9 ± 0.6</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Chloride</td>
<td>89.6 ± 0.6</td>
<td>44.5 ± 0.2*</td>
<td>88.3 ± 0.5</td>
<td>79.2 ± 0.3</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.91 ± 0.2</td>
<td>0.54 ± 0.1*</td>
<td>0.96 ± 0.2</td>
<td>0.90 ± 0.2</td>
</tr>
<tr>
<td>Calcium</td>
<td>6.9 ± 0.3</td>
<td>3.5 ± 0.4*</td>
<td>6.2 ± 0.4</td>
<td>6.7 ± 0.3</td>
</tr>
</tbody>
</table>

Values of electrolytes are expressed as mM/L and minerals as mg/dL. Values are represented as mean ± SD. Asterisk (*) indicates significantly from control.

Data depicted in Table-3 indicates that chronic alcohol administration led to a significant decrease in antioxidant activities such as SOD, Catalase, GPx and GSH content in kidney homogenate. However, administration of GTE to alcohol receiving rats restored these abnormalities to normal.
Fig-2 shows increased MDA levels (a), protein carbonyls (b), nitrite/nitrate and peroxynitrites and levels in kidney of alcohol administered rats, upon GTE supplementation to alcoholic rats these abnormalities are brought to normal levels.

Fig-2: Effect of GTE administration on (a) MDA levels (b) protein carbonyls (c) nitrite/nitrates (d) peroxynitrites in alcohol administered rats. Values are represented as the mean ± SD (n=8). A p < 0.05 is considered as significantly different between groups. Asterisk (*) indicates significant from controls, “ns” indicates not significant from controls and GTE alone administered rats.
Fig-3 the alcohol-induced biochemical alterations further confirmed by the histopathological studies, where ethanol group show necrosis of proximal tubules, vacuolization of cytoplasm and massive mononuclear inflammatory infiltrates in interstitium. Administration of green tea to alcholic rats had renoprotective effect and showed only mild infiltrations, normal glomeruli and alleviated tubular degeneration.

Fig 3

**DISCUSSION**

Chronic and excessive alcohol intake results renal dysfunction (Kumar and Vasudevan, 2008). Metabolism of alcohol in kidney takes place through renal alcohol dehydrogenase, CYP2E1 and CYP24A1, which causes the production of ROS/RNS, eliciting the oxidative stress in the kidney, increase the risk of acute renal failure in unobstructed acute pyelonephritis and lead to the development of renal papillary necrosis (Deng et al., 2007; Sarada et al., 2016). In the present study, we observed significantly increased level of creatinine, urea with decreased uric acid in plasma of alcohol administered rats, which indicates renal dysfunction. Urea, creatinine and uric acid are the catabolites of ammonia, creatine and purine nucleotides respectively released into blood and are eliminated by the kidney (Damodar et al., 2007). Due to a higher sensitivity of the glomerular region to oxidative damage induced by alcohol would have decreased the filtration rate and clearance of substances in alcohol administered rats. Oxidative stress alters the structure and function of the glomerulus because of the effect of ROS on mesangial and endothelial cells and increased glomerular damage. ROS induces tubular necrosis, decrease of glomerular filtration rate and inflammation by activating nuclear factor kappa B (Veljković et al., 2015). Our in vitro studies showed significant free radical scavenging activity. Moreover, flavonoids present in GTE are capable of quenching these free radicals generated during ethanol metabolism.
The kidney is involved in the development, maintenance and counter regulation of complex electrolyte disturbances like phosphate, potassium and hypoglycemia etc. Chronic alcohol consumption reduce plasma electrolytes (Na⁺, K⁺, Cl⁻) and minerals (Ca²⁺, Mg²⁺) concentrations in several animal studies (Adewale et al., 2014; Reddy et al., 2007). These electrolyte disturbances may be associated with the alcohol induced hyperparathyroidism and parathyroid hormone resistance of the skeletal muscle as well as with the decrease of serum osteocalcin (Das et al., 2008). In the present study, there is significant decrease in plasma electrolytes and minerals in alcohol administered rats, which are restored to normal levels upon GTE supplementation.

Oxidative/nitrosative stress mediates a wide range of renal impairments, ranging from acute renal failure, obstructive nephropathy and glomerular damage to chronic renal failure associated with inflammation (González et al., 2011). Malondialdehyde (MDA) an index of lipid peroxidation, protein carbonyl and NOX and peroxynitrite levels were markedly increased in kidney. Upon GTE supplementation all these abnormalities are brought to normal levels. Chronic ethanol administration decreases the renal tubular reabsorption and reduces renal function. Multiple functional abnormalities of renal tubules may be associated with ethanol-induced changes in membrane composition and lipid peroxidation (Okbi et al., 2014). Strong NOx scavenging activity, metal chelating activity and antioxidant activity of GTE preserve membrane integrity offering protection against alcohol induced renal damage. In addition, GTE mediated modulation of the production of NOx might be playing a role in the observed ameliorative response of GTE. The vulnerability of the kidney to oxidative damage has been partly attributed to its high content of long-chain polyunsaturated fatty acids (Nasri et al., 2015). Renal ultra structural abnormalities due to ethanol exposure may be important in the genesis of functional disturbances (Das et al., 2008). Our results suggest that GTE prevent the formation of radicals and reduced oxidative stress, thereby resist the extent of lipid peroxidation, protein carbonyls, NOx and peroxynitrite levels.

Antioxidant enzymes, SOD, CAT and GPx stand in the first line of defense against oxidative damage (Maturu et al., 2012b). These antioxidants play an important role in scavenging ROS, reduction of hydrogen peroxide and maintaining redox balances in biological system. GSH, an important non-enzymatic antioxidant biomolecule in tissues, is the substrate for GPx, and also involved in the removal of free oxygen species, such as H₂O₂, superoxide anions, alkoxy radicals, and maintenance of membrane protein thiols (Padmavathi et al., 2009). In this study, the activities of SOD, CAT and GPx and the content of GSH decreased in alcohol administered rats, and the decrease in GPx activity may be due to the low availability of GSH. Decreased catalase activity accounts for less hydrogen peroxide decomposition, consequently the possible overproduction of hydroxyl radicals via fenton reaction (Reddy et al., 2010). Restoration of antioxidant status in terms of GSH content and activities of defense enzymes to normal level in alcoholic rats receiving GTE supplementation is evident from the results of the study. Antioxidants, particularly polyphenols are expected to decrease the vulnerability of the kidney to oxidative challenges (Rodrigo et al., 2002). EGCG and EGC with the latter being the main polyphenol in green tea, acting protective against renal injury (Salem et al., 2010). It is suggested that EGC participates in the elimination of uremic toxins and prevention of renal failure (Nakagawa et al., 2004). Moreover different individual phytocompounds of GTE might have also acted synergistically to exert full beneficial effect to ameliorate the alcohol induced renal toxicity in this study. Antioxidant activity of flavonoids depends substantially on the number and position of hydroxyl groups in the molecule. In addition, several structural elements such as o-dihydroxylation catechol structure in the B-ring, the presence of unsaturation and 4-oxo group in the C-ring are also presumed to increase the antioxidant activity of flavonoids. The 2, 3- double bond in the C-ring along with 4-oxo function in the C-ring facilitates electron delocalization from the B-ring. Moreover, hydroxyl groups at positions 3 and 5 providing hydrogen bonding to the 4-oxo group in the C-ring is another structural feature attributed to the antioxidant activity of flavonoids in this study.

Alcohol-induced nephro-toxicity was further evidenced from histopathological studies. Photomicrographs represent the glomerular tubules, interstitium and blood vessels in alcohol-administered rats showing degenerative changes, constriction of blood vessels and inflammatory infiltration compared to normal cellularity and blood vessel architecture of control rats. Alcohol and GTE administered rats showed a marked reduction in the degenerative changes of glomerular as evidenced by the normal cellularity of the interstitium and reduction in the constriction of blood vessels. GTE alone administered rats showed a normal physiology of glomerular and blood vessels comparable to that of control rats. The inhibition of the deleterious effects produced by free radicals, enhanced supply of antioxidants and regeneration of glomerular region in the kidney might result from the potential therapeutic phytocompounds like EGCG, EGC, ECG and EC present in GTE.

In conclusion, the GTE used in the present study is rich in multiple phytocompounds, especially flavonoids like EGCG, EGC, ECG and EC. These compounds present in GTE might act at different levels of altered biochemical, pathological and morphological changes in the kidney and finally protect against alcohol-induced toxicity.
Conflict of interest statement
The authors declare that there are no conflicts of interest.

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REFERENCES


