Phytotherapeutic Effect of Green Tea (*Camellia sinensis*) to Induced Renal Ischemia in Rats

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**Abstract:** Evidence suggests that reactive oxygen species play a role in the pathogenesis of renal ischemia/reperfusion (I/R) injury. This study was designed to investigate the effect of pre-treatment with Green tea (*Camellia sinensis*) extract (GTE) on kidney antioxidant status in renal ischemia/reperfusion (IR) induced injury in the rats. A total of 40 male Wistar rats were randomly divided into 5 groups: sham, IR model and three IR+GTE (0.5, 1.0 and 1.5%)-treated groups (n = 8 per group). I/R groups’ kidneys were subjected to 60 min of global ischemia at 37 °C followed by 30 min of reperfusion. At the end of reperfusion period, the rats were sacrificed. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPX), reduced glutathione and renal malondialdehyde (MDA) content were determined in the renal tissues. Results were compared with a group of rats with sham operation. Results showed high malondialdehyde (MDA) level and low antioxidant enzyme activities in I/R rats compared to the sham rats. Pre-treatment of GTE extracts for 30 days prior to IR operation, significantly reduced lipid peroxidation product (MDA) in kidney tissue and significantly increased renal antioxidant enzymes activities. The study showed that GTE improved renal antioxidant status in I/R injury.

**Key words:** Green Tea (*Camellia sinensis*) · Ischemia-Reperfusion · Kidney · Oxidative Stress · Rat

**INTRODUCTION**

Renal ischemia/reperfusion (I/R) injury is a major cause of acute renal failure (ARF) [1], associated in many clinical cases with kidney transplantation, partial nephrectomy, hemorrhagic shock, hypotension, renal artery angioplasty and aortic aneurysm surgery [2-6]. Renal ischemia induces oxidative stress, which results in aggravated and prolonged systemic inflammatory response and the eventual death of renal cells. [4,5]. Ischemia injury is one of the main causes of acute kidney injury, which can manifest histologically as acute tubular necrosis [7]. Reactive oxygen species (ROS) which are generated in high concentration in ischemic organs have diverse cytotoxic effects, including DNA damage, protein oxidation, lipid peroxidation and induction of apoptosis [8]. Increased reactive oxygen species during renal ischemia, directly compromises glomerular and tubular epithelium integrity, one of the factors in the development of acute tubular necrosis [9]. Free radical ablation for the treatment of ischemia injury implicated its first clinical application in the prevention of post-ischemic tissue injury after organ transplantation [10,11]. Thus, agents proposed to be useful in the clinical settings of I/R damage include free radical scavengers.

Recently, antioxidant therapy has been well documented to help in the improvement of organ functions. Many studies have been focused on the role of naturally occurring dietary substances for the control and management of various diseases [12]. Herbs and spices have been used for generations by humans as food and to treat ailments. Scientific evidence is that many of these herbs and spices do have medicinal properties [13].
Since ancient times, green tea (GT) consumption has been considered as Nature's gift for promoting human health. Due to the presence of wide range of well defined phytochemicals that are digested, absorbed and metabolized by the body and exhibit antinflammatory, anti carcinogenic, anti atherosclerotic and antibacterial properties [14]. In particular green tea catechins and their derivatives have been recognized as antioxidants that scavenge free radicals to protect cells in normal and pathological states [15,16]. Among all tea polyphenols, epigallocatechin-3-gallate has been shown to be responsible for much of the health promoting ability of GT. Also GTE has been shown to improve kidney function in diabetic rats [17], reduce the risk of cardiovascular diseases, cancer [18], obesity and oral health problems [19]. It has been shown that GTE consumption involved in specific adaptive alterations in the improvement of cellular/energy metabolism and antioxidant defense mechanism that was associated with lower lipid peroxidation in intestine, liver and kidney of normal rats [20]. In a preliminary report, GTE has been shown to mitigate gentamicin-nephrotoxicity by lowering the level of serum urea, creatinine and tissue LPO content [21].

The current study was designed to determine the possible protective effect of GTE extract against oxidative stress during I/R injury of the kidney, by determining biochemical parameters.

**MATERIALS AND METHODS**

**Chemicals:** All chemicals used in this study were of analytical grade and obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

**Plant:** The Green tea was identified by the Department of Cultivation and Development of Institute of Medicinal Plants, Tehran, Iran. Scientific Classification is Kingdom: Plantae, Order: Ericales, Family: Theaceae, Genus: *Camellia*, Species: *C. sinensis*, Binomial name: *Camellia sinensis* [22].

**Green Tea Extract:** The leaves of Green tea were collected from a local herb shop, Tabriz City, Iran in February 2012. The green tea extract was made according to Maity, Vadasiromoni and Gangulydso [23]. Briefly, 10 g of instant green tea lyophilized water extract were soaked in 1 l of boiling distilled water for 5 min and filtered to make 1% instant green tea solution. The filtrate, designated as green tea extract (GTE), was provided to rats as their sole source of drinking water.

**Animals:** Forty male Wistar rats (220-250 g) were selected for the study and randomly divided into five equal groups: sham operation group, IR group and three I/R+GTE (0.5, 1.0 and 1.5%)-treatment groups. Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23 ± 1 °C, with 55 ± 5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Three weeks before the experiment, the drinking water of I/R+GTE-treatment animals were replaced with green tea solutions and these three groups of rats received different concentrations (0.5, 1.0 and 1.5%) of GTE, as their sole source of drinking water for 14 days. The experiments and surgical procedures performed in the different groups of animals are described below.

**Surgery and Experimental Design:** Animals were anesthetized by intraperitoneal injection of ketamine-xylazine (50 mg/kg and 10 mg/kg, respectively). The abdominal area was prepared with povidone iodine. Autoclave-sterilized surgical instruments were used for the procedure. A laparotomy was performed with a vertical midline incision. Both renal arteries were exposed by blunt dissection. Hemostatic micro clamps were applied on the renal arteries of the kidney for 60 min to create complete renal ischemia. The clamps were removed later to allow restoration of blood flow to the kidneys for 30 min of reperfusion. Additionally, sham-operated rats underwent a simple laparotomy under identical conditions and served as the operation controls. After removing the clamps, the abdomen was closed in 2 layers. In all groups, the animals were kept in metabolic cages for 24 hours. At the end of the 24 hours, the rats were killed by decapitation. The left kidneys were quickly removed, perfused immediately with ice cold hypertonic saline solution and homogenate 10% prepared in 1.15% w/v of potassium chloride for measurement of antioxidant activity.
Estimation of Antioxidant Activity: The kidney homogenate was centrifuged at 7000 ×g for 10 minutes at 4°C and supernatant was used for measurement of oxidative stress by estimation of reduced glutathione (GSH) and determination of malondialdehyde (MDA) as well as antioxidant enzymes (AOE) such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). GSH, MDA, SOD, CAT and GSH-Px were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Reduced glutathione (GSH) content was determined according to Sedlak and Lindsay [24]. GSH reacts with 5,5'-dithiobis-2-nitrobenzoic acid and the absorbance spectra of the product have a maximum absorbance at 410 nm. The results were expressed as μmol/gwt protein. Kidney homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein. Degree of lipid peroxidation in kidney tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARSs) formation by following the protocol of Esterbauer and Cheesman [25]. SOD activity was measured by Nishikimi method [26] and was modified by Kakkar method [27]. Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was measured by Claiborne method [28] and was based on hydrogen peroxide breakdown. GSH-Px activity was measured by Rotruck method [29] and was expressed as micromole of GSSG /minute/milligram of protein, based on below reaction:

$$2\text{H}_2\text{O} + \text{GSSG} \rightarrow \text{H}_2\text{O}_2 + 2\text{GSH}$$

Statistical Analysis: The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) was used. All data are presented as mean ± SE. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey’s post hoc multiple comparison test. The Kruskal-Wallis test, followed by Mann-Whitney U post test, was used for the analysis of degree of histopathological kidney injury. P < 0.05 was considered statistically significant.

RESULTS

Effects of GTE pretreatment on renal MDA and GSH levels and SOD, CAT and GSH-Px in experimental rats are depicted in Table 1. In I/R group, ischemia and reperfusion caused significant decreases in tissue GSH levels and antioxidant enzymes (SOD, CAT and GSH-Px) activities (P<0.001) when compared with the sham control group. In the I/R+ GTE (1.0 and 1.5%) groups, GSH levels and these antioxidant enzymes activities were found to be dose-dependently significantly increased (P<0.05 and P<0.01, respectively) compared to IR model group. Pretreatment with GTE (0.5%) led to a non-significantly increases in the levels of mentioned renal antioxidant agents (Table 3). As expected, the level of renal MDA was significantly increased in IR model rats compared with sham rats as shown in Table 3. In the I/R+ GTE (1.0 and 1.5%) groups, levels of renal MDA were dose-dependently significantly decreased (P<0.05 and P<0.01, respectively) in comparison with IR model rats. Pretreatment with GTE (0.5%) led to a non-significantly increase in the renal MDA level.

DISCUSSION

In this study pretreatment with GTE protected the kidneys from elevated ROS products and depletion of superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione in rats exposed to the renal I/R.
Acute renal failure produced by ischemia and reperfusion is characterized by inflammation of renal tissue, extensive tubular damage, tubular cell necrosis, glomerular injury and signs of tubular obstruction with cell debris [30-32]. Much of this tubular and glomerular dysfunction has been postulated to occur during the reperfusion period following anoxia and generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury. In renal I/R injury, ROS are responsible for lipid peroxidation of biological membranes, which it will ultimately results in cell death [33-37]. The protection provided by free radical scavengers against ROS produced during I/R supports the hypothesis that free radical species are involved in the cellular pathogenesis of I/R [38].

We assessed the potential of GTE by studying its effect on lipid peroxidation, which was measured in terms of MDA, a stable metabolite of the free radical-mediated lipid peroxidation cascade. In our study, animals subjected to renal I/R demonstrated an increase in the renal MDA and attenuated antioxidant enzymes pool. GTE reversed the increase of MDA levels to a considerable extent, thereby confirming its antioxidant role in I/R, indicating that GTE prevented lipid peroxidation and protein oxidation in the renal I/R process. In our study, GSH which is known to be depleted following an ischemic insult [39] was decreased with renal ischemia reperfusion process. GTE-pretreated rats exhibited higher GSH contents than their respective controls, indicating that GTE helped in replenishing the GSH pool. The fact that GTE causes a significant increase in CAT, GSH-Px and SOD activities in comparison with I/R group, suggesting that it might have an antioxidant effect through the increase in SOD, GSH-Px and CAT enzyme activities.

Green tea extract contains polyphenols (e.g., catechin, epicatechin, epigallocatechin and their gallates), teain and caffeine. The extract also includes pyrroloquinoline quinone, a newly discovered vitamin [40]. It has been suggested that catechins, antioxidant compounds present in green tea, may improve the defence system of the organism as demonstrated in several in vitro and ex vivo models [41-43]. Green tea extracts are more stable than pure epigallocatechin gallate, the major constituents of green tea, because of the presence of other antioxidant constituents in the extract [44]. In general, herbal medicines are complex mixtures of different compounds that often act in a synergistic fashion and exert their full beneficial effect as total extracts [45].

Altogether, the mechanism of the protective effect of GTE on renal I/R injury can be explained by its antioxidant activity. The rennin-angiotensin system plays a pivotal role in regulation of blood pressure. Renin acts on angiotensinogen to form angiotensin-I, which is converted to angiotensin-II with the help of angiotensin-converting enzyme [46]. Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage [47]. Generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury. Oxidative stress can result from increased ROS production and/or from decreased ROS scavenging capability. The ROS attach to the polyunsaturated fatty acids in the membrane lipids and result in peroxidation, which may lead to disorganization of cell structure and function. After reperfusion and reoxygenation, the imbalance between restoration of oxygen supply and mitochondrial respiratory function results in massive generation of superoxide anion in mitochondria [48].

Under these conditions, the defensive system, which is known as antioxidant or antioxidant enzymes, cannot prevent the escape of ROS, especially in mitochondria and their effects on other intracellular sites. This cascade of events is known as reperfusion injury [49]. In this study, renal I/R increased oxidative stress products including tissue MDA and depleted the antioxidant enzymes pool, as is evident from the declined activity of superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione. It has been reported that GT may ultimately prove to be a useful dietary supplement in patients with CRF to attenuate the symptoms of chronic renal diseases and protect against its related complications [50].

CONCLUSION

The results of this study showed that Green tea extract pre-treatment significantly improves antioxidant status in renal reperfusion injury in the rats.

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REFERENCES