Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: informahealthcare.com/journals/irnf20

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To cite this article: Muragundla Anjaneyulu, Naveen Tirkey & Dr. Kanwaljit Chopra (2003) Attenuation of Cyclosporine-Induced Renal Dysfunction by Catechin: Possible Antioxidant Mechanism, Renal Failure, 25:5, 691-707, DOI: 10.1081/JDI-120024285

To link to this article: https://doi.org/10.1081/JDI-120024285

Published online: 07 Jul 2009.

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RENAL FAILURE
Vol. 25, No. 5, pp. 691–707, 2003

CLINICAL STUDY

Attenuation of Cyclosporine-Induced Renal Dysfunction by Catechin: Possible Antioxidant Mechanism

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ABSTRACT

One of great use of immunosuppressant, Cyclosporine-A (CsA) is in the solid organ transplantation; however the extensive use of this is cautionable due to its toxic effect in renal tissue, characterized by the tubular atrophy, interstitial fibrosis, and progressive renal impairment. However, there are many mediators are associated with pathogenesis of nephrotoxicity of CsA, the exact mechanism is still in debate. Recent studies indicate that Reactive Oxygen Species (ROS) induced oxidative stress and lipid peroxidations are the important mechanisms implicated in the pathophysiology of nephrotoxicity with CsA. In the present study we examined effect of dietary flavonoid catechin on oxidative damage in cyclosporine-A induced nephrotoxicity. Chronic administration of CsA (20 mg/kg/day) subcutaneously for 21 days significantly decreased the body weight as compared with vehicle treated rats. CsA (20 mg/kg/day) administration for 21 days significantly decreased the renal function by increase in the serum creatinine, blood urea nitrogen, and decrease in the creatinine and urea clearance as compared with vehicle treated rats. Catechin (100 mg/kg/day) for 21 days along with CsA significantly reversed the changed renal parameters, however lower dose of catechin (50 mg/kg/day) restored only increased serum creatinine levels as compared with CsA alone treated

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DOI: 10.1081/JDI-120024285
0886-022X (Print); 1525-6049 (Online)

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group. Biochemical analysis revealed that chronic administration of CsA (20 mg/kg/day) for 21 days significantly induced lipid peroxidation and decreased the glutathione levels in the kidney homogenate of rats. It is also observed that chronic CsA administered rats showed decrease in antioxidant defense enzyme superoxide dismutase and increase in the catalase activity as compared with vehicle treated rats. Co-administration of catechin (100 mg/kg/day) orally along with CsA for 21 days significantly reduced the lipid peroxidation and restored the decreased glutathione levels as compared with CsA alone group, but lower dose of catechin (50 mg/kg/day) restored only decreased glutathione levels induced by CsA. Co-administration of only higher dose of catechin (100 mg/kg/day) along with CsA significantly increased the superoxide dismutase (SOD) levels as compared with CsA alone treated group. It is also observed that catechin (100 mg/kg/day) along with CsA further increased the catalase levels as compared with CsA alone treated group, but not with lower dose of catechin. Animals administered with catechin (100 mg/kg/day) alone for 21 days showed significant increase in the catalase levels as compared with vehicle treated group. The major findings of the present study suggest that oxidative stress might play a significant role in CsA-induced nephrotoxicity. In conclusion, dietary administration of flavonoid catechin could be a useful component for the prevention/treatment of CsA-induced nephrotoxicity.

Key Words: Cyclosporine; Nephrotoxicity; Oxidative stress; Catechin.

INTRODUCTION

Cyclosporine, a lipophilic undecapeptide of fungal origin is a potent immunosuppressant agent, widely used to prevent rejection of transplanted organs.[1] The immunosuppressive effect of CsA is due to binding to its protein receptors, Immunophilins.[2] Even though it is extensively used in the organ transplantation and autoimmune diseases, the use of CsA is limited due to its side effects on cardiovascular and renal tissues.[3–8] CsA-induced nephrotoxicity remains an important clinical challenge. CsA nephrotoxicity has been characterized by a 20–30% reduction in glomerular filtration rate (GFR) and up to 40% reduction in renal blood flow (RBF), which results in an increase in the serum creatinine levels, a decrease in creatinine, and urea clearance and reduction in both sodium and potassium excretion.[9]

Different forms of CsA nephrotoxicity have been described, among those acute form is usually reversible and dose dependent is catheterized by reduction of GFR and RBF that appears to be related to afferent arteriolar vasoconstriction, possibly mediated by imbalance between renal vasoconstrictors (catecholamines, angiotensin II, endothelin, thromboxane, platelet activating factor), and vasodilators (prostaglandins, nitric oxide).[7,10] The chronic form of nephrotoxity is characterized by structural changes in the kidney and appears as tubulointerstitial fibrosis of a striped pattern in arteriolopathy of the afferent arterioles, tubular atrophy, and glomerulosclerosis.[11] The pathophysiology of CsA nephrotoxicity still remains unclear. Clinical and experimental studies have revealed that several factors may be involved.[12–14] Recently oxidative stress has been proposed as major causative

factors in CsA induced nephrotoxicity.[15,16] An increase in renal excretion of lipid peroxides has been observed in patients with renal transplant treated with CsA.[17] It has been suggested that reactive oxygen species could also be derived directly from CsA or during its metabolism by the cytochrome p450 system.[18] Both in vivo and in vitro experiments revealed that CsA directly increases levels of superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2).[15,19] It is reported that CsA-induced renal nerve activity results in vasoconstriction in the kidney.[20] It has been demonstrated that CsA blocks the mitochondrial calcium (Ca^{2+}) release, including an increase in intracellular free calcium that could accounts for the CsA vasoconstrictive effect.[21]

Flavonoids are a group of low molecular weight polyphenolic compounds that are widely distributed in fruits and vegetables.[22] Renewed interest has been observed in recent years on the novel and multiple activities of flavonoids. Flavonoids have been reported to exhibit potent antioxidative and free radical scavenging abilities in various disease conditions. They may scavenge reactive oxygen species, chelate metal ions, act as a chain-breaking antioxidant by scavenging lipid peroxyl radicals, or portion into the lipid bilayer to prevent lipid damage.[23,24] (+)-Catechin, which is a type of flavonoid from the group of catechins such as (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechingallate, (−)-epigallocatechingallate, and (+)-gallocatechin are known to be present in green tea, block tea, and other plant foods.[22] Both epidemiological and in vitro studies suggest that catechins have effects on human health, serving to protect against congestive heart failure and cancer due to their antioxidative activity.[25–27]

With this in mind, the present study was designed to demonstrate the role of CsA-induced oxidative stress, its relation to renal dysfunction, and to investigate the effect of catechin in salvaging CsA-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Animals

Wistar rats (150–200 g) of either sex bred in Central Animal House facility of Panjab University were used. The animals were housed under optimal conditions, maintained on a 12-h light and dark cycle and free access to food and water ad libitum. All the experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals.

Drugs and Reagents

Following drugs were used in the present study. Cyclosporine-A (Gift sample from Panacea Biotec Ltd., Lalru, India) was dissolved in olive oil, administered subcutaneously, and Catechin (Sigma, St. Louis, MO, USA) suspension was prepared in 0.5% Sod. CMC solution, administered orally. All the drugs were administered in a constant volume (0.1 mL in case of olive oil and CsA, 0.5 mL in case of catechin) per 100 g of body weight of rat and the solutions were made freshly at the beginning of each experiment.

Treatment Schedule

Rats were divided in five groups each consisting of 5 to 6 animals. (1) A control group received vehicle of CsA i.e., olive oil, subcutaneously (s.c.) and 0.5 % sodium carboxy methylcellulose of catechin, orally for 21 days. (2) A second group received CsA (20 mg/kg/day, s.c.) dissolved in olive oil for 21 days. (3) A third group received both the CsA (20 mg/kg/day, s.c.) and catechin (50 mg/kg/day, p.o.) for 21 days. (4) A fourth group received both the CsA (20 mg/kg/day, s.c.) and catechin (100 mg/kg/day, p.o) for 21 days. (5) A fifth group received only catechin (100 mg/kg/day, p.o.) for 21 days so as see its per se effect. Cyclosporine dose was selected from the previous experiments in our laboratory.[28,29]

The body weights of animals were recorded on day 0 and day 21st. At the end of the experiment, food intake, water intake, and urine output were measured. Kidney weights also measured after immediately sacrificing the rats.

Assessment of Renal Functions

At the end of 21st day, rats were kept individually in metabolic cages for 24 h to collect urine for estimation of renal function. Renal function was assessed by measuring serum and urine levels of creatinine and urea by colorimetric estimation using semi auto-analyzer (Erba Chem-5 plus, Transasia, India). Both serum creatinine and urea as blood urea nitrogen (BUN) were assessed. Creatinine and urea clearance were measured as an index of glomerular filtration rate.

Assessment of Renal Oxidative Stress

Preparation of renal homogenate: immediately after sacrificing the rats, left kidney was dissected and rinsed with isotonic saline and weighed. After weighing, kidney tissue was minced properly and the homogenate was prepared with 10% (w/v) phosphate buffer saline (0.1 M, pH 7.4) with the help of homogenizer and used to estimate MDA and reduced glutathione. Post mitochondrial supernatant was obtained by centrifugation at $10{,}000 \times g$ for 20 min at 4°C. This preparation was further used to estimate SOD and catalase.[30]

Determination of lipid peroxides: lipid peroxides were measured as malonaldehyde (MDA), an index of lipid peroxidation produced by acid heating using its reaction to thiobarbituric acid (TBA), was estimated according to the method of Wills.[31] The amount of MDA formed was measured spectrophotometrically at 532 nm by using Erba Chem 5 Plus (Transasia, India) semi auto-analyzer. The results were expressed as nmol of malonaldehyde per mg protein using the molar extinction coefficient of chromophore ($1.56 \times 10^5\,M^{-1}\,cm^{-1}$).

Estimation of reduced glutathione (GSH): reduced glutathione in the kidney was estimated according to the method of Ellman.[32] A, 0.75 mL of homogenate was precipitated with 0.75 mL of 4% sulphosalicylic acid. The samples were centrifuged at $1200 \times g$ for 15 min at 4°C. The assay mixture contained 0.5 mL of supernatant and 4.5 mL of 0.001 M (in 0.1 M phosphate buffer, pH 8.0) DTNB (5-5′-dithio

bis-(2-nitrobenzoic acid)). The yellow color developed was read immediately at 412 nm using Erba Chem 5 Plus (Transasia, India) semi auto-analyzer. The results were expressed as nmol of GSH per mg protein.

ENZYME ASSAY

Superoxide Dismutase (SOD) activity: superoxide dismutase activity was assayed according to the method of Kono.[33] Wherein the reduction of nitrazoblue tetrazolium (NBT) was inhibited by the superoxide dismutase is measured at 560 nm using Erba Chem 5 Plus (Transasia, India) semi auto-analyzer. Briefly the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of kidney homogenate. The results were expressed as units mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 100%.

Catalase activity: catalase activity was assayed by the method of Luck.[34] Where the breakdown of hydrogen peroxide (H_2O_2) being measured at 240 nm. Briefly, the assay mixture consisted of 3 mL of H_2O_2 phosphate buffer (1.25×10^{-2} H_2O_2 m) and 0.05 mL of supernatant of kidney homogenate (10%) and the change in absorbance were recorded at 240 nm using Erba Chem 5 Plus (Transasia, India) semi auto-analyzer. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The results were expressed as μmol H_2O_2 decomposed/min/mg protein.

Protein Estimation

The protein content was measured according to the method of Folin-Lowry[35] using bovine serum albumin as standard.

Statistical Analysis

All the data are expressed as mean ± S.E.M. The significant difference was analyzed using the ANOVA followed by Turkey test. $P < 0.05$ was considered as significant.

RESULTS

Effect of Catechin on Body Weight, Water Intake, Urine Output, and Kidney Weight in CsA Treated Rats

Chronic treatment of rats with CsA for 21 days failed to gain body weight significantly as compared with vehicle treated group. Further CsA increased food intake, water intake, and decreased the urine output and kidney weight as compared

Table 1. Effect of catechin on body weight, kidney weight, food intake, water intake, or urine output in CsA and catechin treated animals. Values are in mean ± S.E.M. Veh: Vehicle, CsA: cyclosporine-A, Cat (50): catechin (50 mg/kg/day), Cat (100): catechin (100 mg/kg/day).

Group	Veh	CsA (20)	CsA (20) + Cat (50)	CsA (20) + Cat (100)	Cat (100)
% increase in body weight	14.58 ± 1.56	6.35 ± 1.38[a]	7.81 ± 5.56	5.95 ± 2.58	13.28 ± 0.90
Kidney weight (g)	0.59 ± 0.03	0.53 ± 0.03	0.59 ± 0.02	0.55 ± 0.03	0.55 ± 0.03
Food intake (g)	26 ± 3.37	31.4 ± 2.67	26.9 ± 3.13	26.9 ± 3.12	24.42 ± 3.08
Water intake (mL)	31.7 ± 4.51	36.2 ± 1.88	33.89 ± 1.03	32.9 ± 2.60	36.22 ± 3.45
Urine output (mL)	10 ± 2.00	6.67 ± 0.67	5.4 ± 0.35	9.45 ± 1.54	8.38 ± 0.41

[a] $P < 0.05$, significantly different from corresponding vehicle treated rats.

with vehicle treated rats, but these effects do not significant. Treatment with catechin and combination with CsA do not significantly affect any of the changed parameters by CsA (Table 1).

Effect of Catechin on CsA induced renal functions: cyclosporine treatment for 21 days significantly increased the serum creatinine and blood urea nitrogen as compared with the vehicle treated group, while treatment with catechin (100 mg/kg) along with CsA significantly reversed the elevated serum creatine (Fig. 1, upper panel) and blood urea nitrogen (Fig. 1, lower panel) as compared with CsA treated group. The lower dose of catechin (50 mg/kg) restored only elevated serum creatinine induced by CsA, but not BUN. Further it is also observed that CsA treatment for the 21 days significantly decreased the creatinine and urea clearance as compared with vehicle treated group. Catechin (100 mg/kg) along with cyclosporine treatment for 21 days significantly reversed the decreased creatine clearance (Fig. 2, upper panel) and urea clearance (Fig. 2, lower panel) as compare with CsA treated group, but not with lower dose of catechin. Catechin (100 mg/kg/21 days) alone did not induce any changes in renal functions as compared with vehicle treated group.

Effect of Catechin on CsA Induced Oxidative Stress Markers

Effect of Catechin on the Renal MDA Levels in CsA Treated Rats

Chronic treatment group of CsA for 21 days induced lipid peroxidation as indicted by a significant raise in the kidney tissue homogenate MDA levels as compared to vehicle treated rats. The treatment group of co-administration of catechin (100 mg/kg) along with CsA for 21 days significantly reversed the increased lipid peroxidation as compared with CsA alone treatment (Fig. 3, upper panel). However, a lower dose of catechin (50 mg/kg) along with CsA failed to reverse CsA induced increase in MDA levels in kidney homogenate as compared with CsA alone treated group. Catechin per se treated group did not alter the MDA levels in kidney homogenate as compared with vehicle treated group.

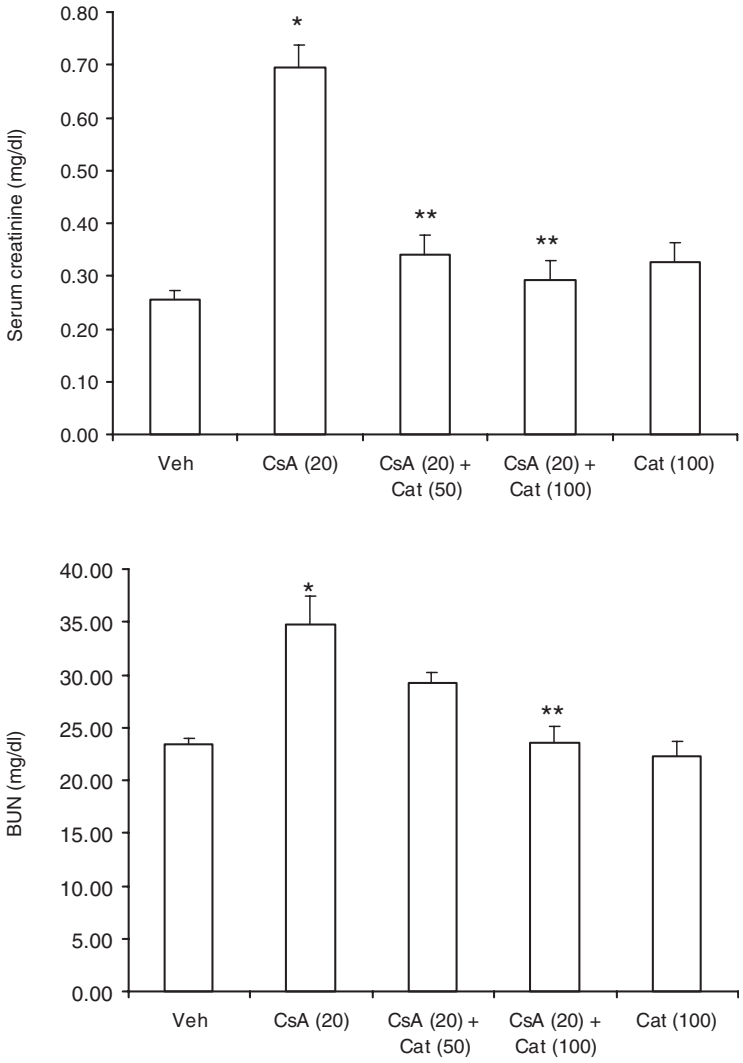

Figure 1. Effect of catechin on serum creatinine (upper panel) and blood urea nitrogen (lower panel) in CsA treated rats. Values are in mean ± S.E.M. Veh: Vehicle, CsA: Cyclosporine-A, Cat (50): Catechin (50 mg/kg/day), Cat (100): Catechin (100 mg/kg/day). $*P < 0.05$, as compared to vehicle, $**P < 0.05$, as compared to CsA alone treated rats.

Effect of Catechin on the Renal Glutathione Levels in CsA Treated Rats

Chronic treatment group of CsA for 21 days significantly decreased the kidney GSH levels. Co-administration of catechin (50 and 100 mg/kg) along with CsA for 21 days significantly reversed the CsA induced decrease in the kidney GSH levels (Fig. 3, lower panel). Catechin per se did not alter the renal GSH levels.

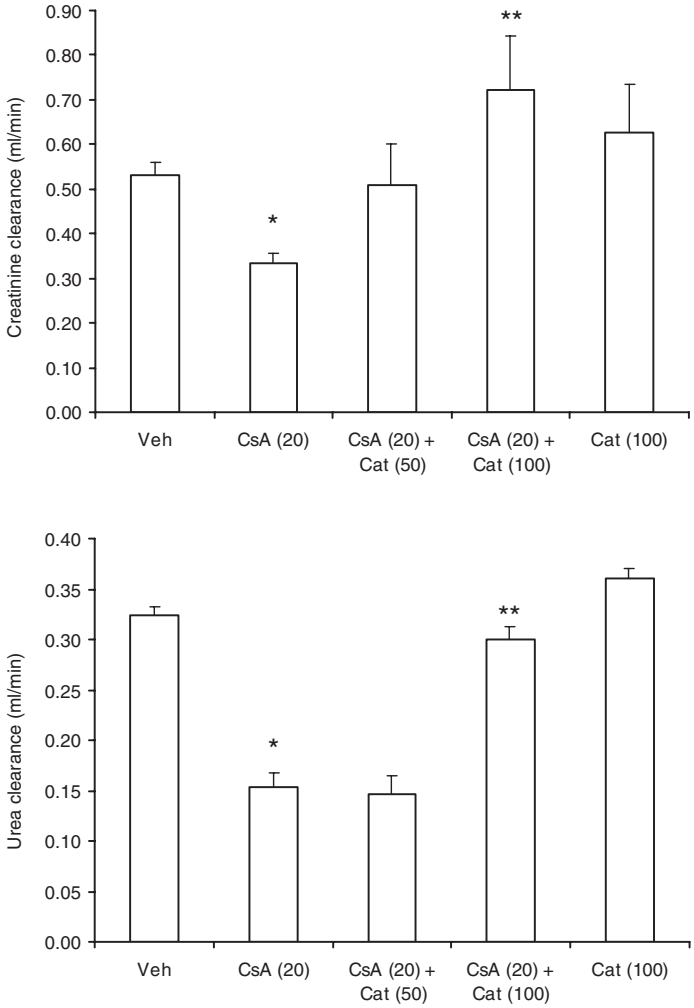

Figure 2. Effect of catechin on creatinine clearance (upper panel) and urea clearance (lower panel) in CsA treated rats. Values are in mean ± S.E.M. Veh: vehicle, CsA: cyclosporine-A, Cat (50): catechin (50 mg/kg/day), Cat (100): catechin (100 mg/kg/day). $*P < 0.05$, as compared to vehicle, $**P < 0.05$, as compared to CsA alone treated rats.

Effect of Catechin on the Renal Antioxidant Enzyme, SOD Levels in CsA Treated Rats

Chronic treatment group of CsA for 21 days significantly decreased the kidney SOD levels. Co-administration of catechin (100 mg/kg) along with CsA for 21 days significantly reversed the CsA induced decrease in the kidney SOD levels (Fig. 4, upper panel). However, a lower dose of catechin (50 mg/kg) failed to reverse CsA induced decrease in SOD levels in kidney homogenate. Catechins per se not alter the SOD levels in kidney homogenate as compared with vehicle treated group.

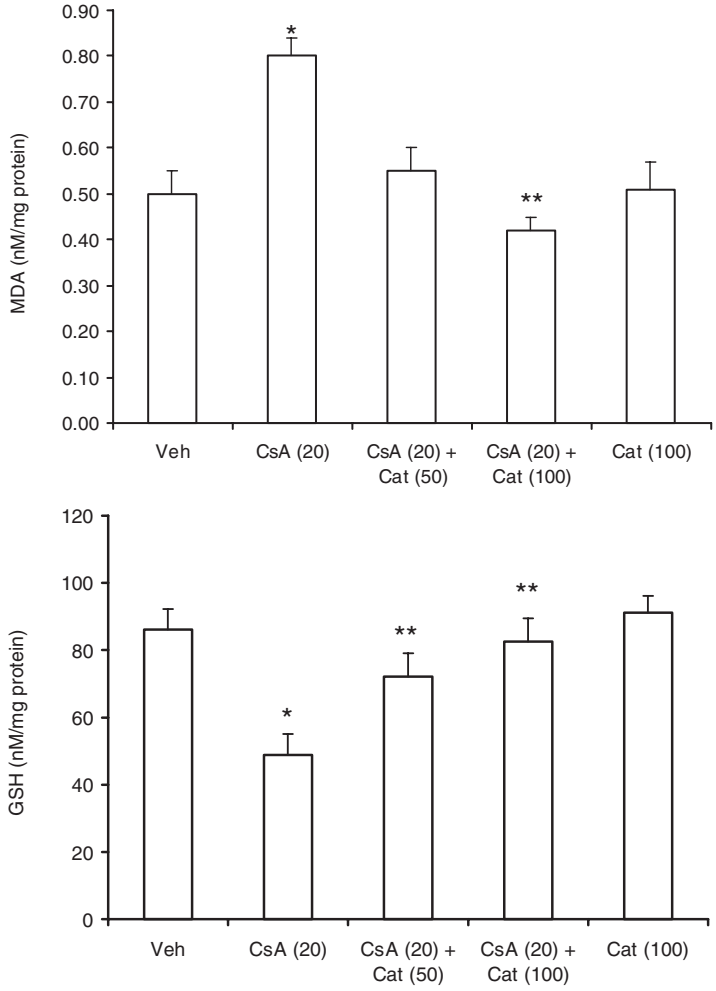

Figure 3. Effect of catechin on malonaldehyde (upper panel) and glutathione (lower panel) levels in CsA treated rats. Values are in mean ± S.E.M. Veh: vehicle, CsA: cyclosporine-A, Cat (50): catechin (50 mg/kg/day), Cat (100): catechin (100 mg/kg/day). $*P < 0.05$, as compared to vehicle, $**P < 0.05$, as compared to CsA alone treated rats.

Effect of Catechin on the Renal Antioxidant Enzyme, Catalase Levels in CsA Treated Rats

Chronic treatment group of CsA for 21 days significantly increased the kidney catalase levels. Co-administration of catechin (100 mg/kg) along with CsA for 21 days further raise the CsA induced increase in the kidney catalase levels (Fig. 4, lower panel). However, a lower dose of catechin (50 mg/kg) did not affect CsA induced increase in catalase levels in kidney homogenate. Catechin per se treated group also significantly increased the catalase levels in kidney homogenate as compared with vehicle treated group.

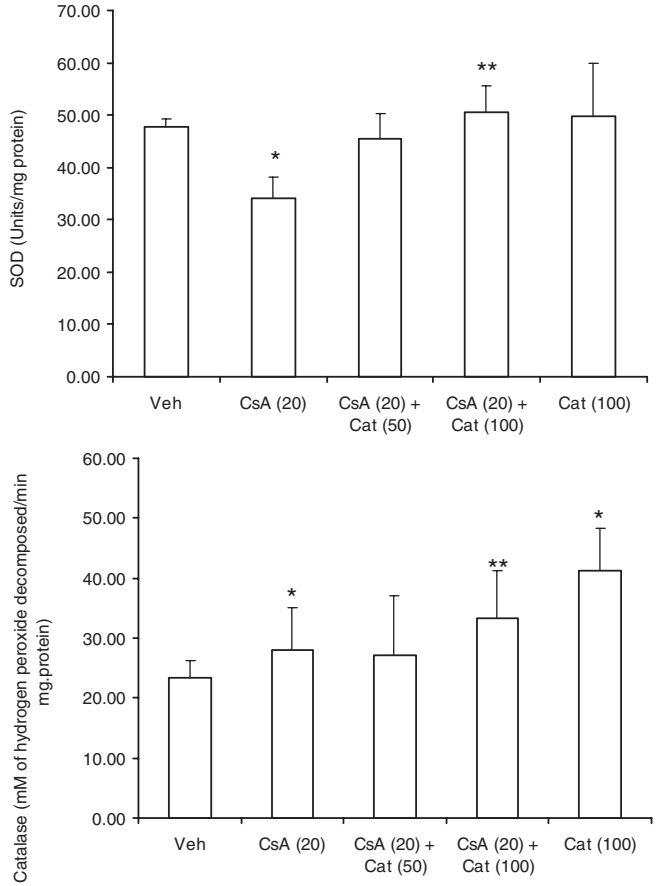

Figure 4. Effect of catechin on superoxide dismutase (upper panel) and catalase (lower panel) activity in CsA treated rats. Values are in mean ± S.E.M. Veh: vehicle, CsA: cyclosporine-A, Cat (50): catechin (50 mg/kg/day), Cat (100): catechin (100 mg/kg/day). $*P < 0.05$, as compared to vehicle, $**P < 0.05$, as compared to CsA alone treated rats.

DISCUSSION

Cyclosporine remains the first line immunosuppressant in the human solid organ transplantation, because it improves graft survival and is not associated with myelosuppression. Although it is extensively used as immunosuppressant, adverse effects such as cardiovascular and chronic renal failure[4,7] limit the usage of it. Both the structural and functional changes in the kidney of transplant patients and experimental animals have been reported with use of CsA.[36] The exact mechanism of CsA nephrotoxicity remains unclear; however increasing evidence suggests that oxidative stress is responsible for CsA-induced cardiac and renal toxicity.[37,38] A recent study has shown that CsA-induced local production of hydroxyl radical, a highly active and detrimental radical, plays an important role in CsA nephrotoxicity.[39]

In the present study we found that chronic cyclosporine treated animals showed significant decrease in body weight as compared with vehicle treated rats. The decrease in the body weight gain in CsA treated animals has seen in other studies.[40,41] The decreases in the body weights are the factor of high dose, however others have reported decrease in body weights with cyclosporine even with lower dose.[42]

Cyclosporine treatment for a longer time deteriorated the renal functions in experimental animals and produced a marked renal oxidative stress. This is in support with previous experimental reports in our laboratory that CsA (20 mg/kg) for 21 days exerted the significant oxidative stress in correlation with renal functional changes and treatment with antioxidants poses the beneficial effects in CsA-induced nephrotoxicity.[28,29] In the present study daily administration of CsA for 21 days produced a significant decrease in creatinine and urea clearance. Increased blood urea nitrogen and plasma creatinine in the present study with chronic cyclosporine treatment for 21 days indicates that a progressive renal damage, an index of altered renal glomerular filtration rate.[9] It has been reported that treatment of rats with CsA decreased the glomerular filtration and this decreased filtration is associated with in vivo formation of reactive oxygen intermediates.[39,43] We also found a significant correlation between lipid peroxidation and renal dysfunction. In the present study, co-treatment of catechin, a natural antioxidant along with CsA, reversed the glomerular filtration rate by decreasing the increased serum creatine and blood urea nitrogen and thus increased the decreased creatinine and urea clearance induced by CsA further support this hypothesis. It is clear that decreased GFR microcirculation, both at preglomerular and post-glomerular sites caused by CsA might contribute to these alterations.[44,45] In addition, oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal functions directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient and thus reduce the glomerular filtration rate.[46,47] One of the possibilities is that CsA causes an increase in intracellular calcium, which enhances the contractility of both mesangial cells and vascular smooth muscle cells and leads to intrarenal vasoconstriction.[21,48] Moreover, this increased intracellular Ca^{2+} results in the release of vasoconstrictions such as Angiotensin-II,[49] thromboxane,[50] and endothelins.[51] Cyclosporine has been reported to inhibit iNOS, which leads to impair NO activity. The decrease in the NO activity is responsible for vasoconstriction and this could be another reason to cause unwanted side effect of CsA on cardiovascular and renal tissues.[5,6]

The molecular mechanisms responsible for the free radical damage in the renal tissue treated with CsA have not yet been clarified. The arachidonate metabolite and potent vasoconstrictor thromboxane A_2 was implicated in the CsA toxicity.[52] Another mechanism by witch CsA induces free radical generation is eicosanoids through cyclooxygenase and /or free radical catalyzed noncyclooxygenase mechanisms.[53] The increased malonaldehyde levels with treatment of CsA in the isolate hepatic microsomes suggest that metabolism of CsA by cytochrome P450 could directly lead to free radicals.[54] In addition, cyclosporine P450 could be a source of iron that could be involved in conversion of hydrogen peroxide to hydroxyl radicals.[55] Previous studies showed that CsA increase the activity of renal nerves[20] and causes vasoconstriction. Hydroxy radical production caused by CsA can be minimized by renal denervation,[39] suggesting that CsA causes oxidative

stress by inducing vasoconstriction, which leads to hypoxia reoxygenation. In addition, CsA causes vasoconstriction directly in isolated renal arterioles.[44,45] It has been demonstrated that CsA blocks mitochondrial calcium (Ca^{2+}) release, indicating a drastic enhancement in intracellular free Ca^{2+}, witch could account for the vasoconstriction effect of CsA.[21,48] These alterations could theoretically leads to a classical hypoxia-reoxygenation injury involving oxygen free radicals generation. In support of this hypothesis, glycine, which decreases renal nerve activity, and blocks free radical production, minimizes kidney injury caused by CsA.[39,56]

Unbalanced productions of free radicals are related, at least in part to increased lipid peroxidation and reduction in specific endogenous antioxidants such as GSH and antioxidant enzymes SOD and catalase. In the present study, chronic treatment with CsA for 21 days significantly increased the MDA levels an index of lipid peroxidation and decreased the antioxidant glutathione levels in the renal tissue. This is in support with other study that CsA treatment increases the oxidized glutathione/glutathione sulfhydryl (GSSG/GSH) ratio, malonaldehyde, and conjugated dienes in the kidney.[38,40] Treatment of catechin along with CsA significantly reversed the increased MDA levels and decreased glutathione levels as compared with CsA alone treated group further supporting the oxidative stress role is the primary mechanism of CsA induced nephrotoxicity. This is further supported by other experimental data stating that CsA-induced functional and structural deterioration of the kidney was accompanied by renal lipid peroxidation and that concurrent administration of an antioxidants, lazaroid, *N*-acetylcysteine, vitamin E, and melatonin afforded renal protection and attenuated renal injury.[40,57–60] This renal protection afforded by treatment with antioxidants indicating the important role of oxidative stress against CsA nephrotoxicity.

In the present study it is also observed that antioxidant enzyme, SOD was decreased in 21 days CsA treated group as compared with vehicle treated and this decreased SOD levels were attenuated by the catechin treatment. This is indicating that cyclosporine treatment decreases the antioxidant enzymes thus cause the oxidative stress to further leads to nephrotoxicity. However, in the present study, it is observed that CsA treated animals showed increase in antioxidant enzyme catalase and co-administration of the catechin along with CsA further increased the levels of catalase as compared with CsA alone treated group. The further increase in the catalase levels, may one of the reason that catechin per se significantly increased the catalase as compared with vehicle treated control rats in the present study. It is reported that CsA increases the levels of superoxide in endothelial[19] and mesangial cells. Oxidative stress caused by the formation of superoxide radicals, which in turn are converted to highly reactive hydroxyl radicals through H_2O_2 in the presence of transition metals.[23] In the biological system, the antioxidants are activated in order to decrease this free radical formation and further decrease the deleterious effect of free radicals. This could be one of the reasons to increase catalase activity in the rats kidney as a compensatory mechanism against increased free radical generation by CsA.

The molecular mechanism of antioxidant properties of the flavonoids are due to direct scavenging of some radical species by acting as chain breaking antioxidants.[61] In addition, they may recycle other chain-breaking antioxidants such as α-tocopherol by donating a hydrogen atom to the tocopheryl radical.[62]

Transition metals such as iron and copper are important pro-oxidants, and some flavonoids can chelate divalent metal ions, hence preventing free radical formation. Dietary supplementation of tea catechins has been shown to posses antioxidative activity both in vivo[63,64] and in vitro.[65] Our data showed that catechin significantly improved CsA-induced renal dysfunction and changed oxidative markers; these could be the many potential health benefits of catechins, which also reported by others[66,67] in oxidative stress induced complications. The antioxidant action of catechin has been speculated to be due to having the Angiotensin Converting Enzyme (ACE) inhibition[68] properties, along with hypotensive[69] activity. This could be one of the reason of which catechin having its beneficial effect by inhibiting angiotensin formation and direct vasorelaxation effect along with antioxidant properties. Because, this enzyme (ACE) is having an important contribution of converting Angiotensin-I (Ag-I) to Ag-II and further leads to vasoconstriction, which is observed in the CsA-induced nephrotoxicity. Another mechanism that poses the antioxidant properties of catechins is administration of cocoa powder, which contains catechins in rats, significantly reduced the accumulation of lipid peroxides in plasma, and significantly reduced the consumption of alpha-tocopherol.[70] In the present study also treatment with catechin alone significantly increased the one of the antioxidant, catalase levels.

In conclusion the findings of the present study strongly suggest that role of oxidative stress in the pathophysiology of CsA-induced nephrotoxicity and a bioflavonoid, catechin or group of catechins could be substituted as a dietary supplement to prevent or treatment of CsA-induced nephrotoxicity.

ACKNOWLEDGMENT

Authors greatly acknowledge the University Grant Commission (UGC), New Delhi, India for supporting present work and Ms. Saraswatigupta for technical assistance.

REFERENCES

1. Borel, J.F.; Bauman, G.; Ghapman, I.; Donatsh, P.; Fhr, A.; Mueller, E.A.; Vigouret, J.M. In-vivo pharmacological effect of cyclosporine analogues. Adv. Pharmacol. **1996**, *35*, 115–246.
2. Schreiber, S.L.; Crabtree, G.R. The mechanism of cyclosporine-A and FK-506. Immunol. Today **1992**, *13*, 136–142.
3. Khan, B.D. Cyclosporine. New. Engl. J. Med. **1989**, *321*, 1725–1738.
4. Diederich, D.; Yang, Z.; Lusher, T.F. Chronic cyclosporine therapy impairs endothelium dependent relaxation in the renal artery of the rat. J. Am. Soc. Nephrol. **1992**, *2*, 1291–1297.
5. Marumo, T.; Nakaki, T.; Hishikawa, K.; Suzuki, H.; Kato, R.; Saruta, T. Cyclosporine-A inhibits nitric oxide synthase induction in vascular smooth muscle cells. Hypertension **1995**, *25*, 764–768.

6. Stephan, D.; Billing, A.; Krieger, J.P.; Grima, M.; Fabre, M.; Hofner, M.; Lmbs, J.L.; Barthelmcbs, M. Endothelium-dependent relaxation in the isolated rat kidney: impairment by cyclosporine-A. J. Cardi. Pharmacol. **1995**, *26*, 859–868.
7. Campistol, J.M. Mechanism of nephrotoxicity. Transplantation **2000**, *69*, SS5–SS10.
8. Tariq, M.; Morais, C.; Sobki, S.; Al Sulaiman, M.; Al Khader, A. Effect of lithium on cyclosporine-induced nephrotoxicity in rats. Renal Failure **2000**, *22*, 545–560.
9. Weir, M.R.; Klasen, D.K.; Shen, S.Y. Acute effect of cyclosporine on renal function in healthy humans. Transplant Proc. **1989**, *21*, 915–917.
10. Bennett, W.M. Mechanism of acute and chronic nephrotoxicity from immunosuppressive drugs. Renal Failure **1996**, *18*, 453–460.
11. Andoh, T.F.; Burdmann, E.A.; Fransechini, N.; Houghton, D.C.; Benett, W.M. Comparison of acute rapamycin nephrotoxicity with cyclosporine and FK-506. Kidney Int. **1998**, *50*, 1110–1117.
12. Wang, C.; Salahudeen, A.K. Cyclosporine nephrotoxicity: attenuation by an antioxidant-inhibitor of lipid peroxidation in vitro and in vivo. Transplantation **1994**, *58*, 940–946.
13. Sturrock, N.D.; Lang, C.C.; MacFarlane, L.J.; Dockrell, M.E.; Ryan, M.; Webb, D.J.; Struthers, A.D. Serial changes in the blood pressure, renal function, endothelin and lipoprotein (A) during the first 9 days of cyclosporine therapy in males. J. Hypertens. **1995**, *13*, 667–673.
14. Abassi, Z.A.; Pieruzzi, F.; Nakhout, F.; Keiser, Hr. Effect of cyclosporine-A on the synthesis, excretion and metabolism of endothelin in the rat. Hypertension **1996**, *27*, 1140–1148.
15. Wolf, A.; Clemann, N.; Frieauff, W.; Ryffel, B.; Cordier, A. Role of reactive oxygen formation in the cyclosporine-A-mediated impairment of renal functions. Transplant Proc. **1994**, *26*, 2902–2907.
16. Longoni, B.; Boschi, E.; Demontis, G.C.; Ratto, G.M.; Mosca, F. Apoptosis and adaptive responses to oxidative stress in human endothelial cells exposed to cyclosporine-A correlate with BCL-2 expression levels. FASEB J. **2001**, *15*, 731–740.
17. Knight, J.A.; Cheung, A.K.; Servilla, K. Increase urinary excretion of lipid peroxidation levels in renal transplant patients. Ann. Clin. Lab. Sci. **1989**, *19*, 238–243.
18. Ahmed, S.S.; Napoli, K.L.; Strobel, H.W. Oxygen radical formation during cytochrome P450-catalyzed cyclosporine metabolism in heart and human liver microsomes at varying hydrogen ion concentrations. Mol. Cell Biochem. **1995**, *15*, 131–140.
19. Diederich, D.; Skopec, J.; Diederich, A.; Dai, F.X. Cyclosporine produces endothelial dysfunction by increased production of superoxide. Hypertension **1994**, *23*, 957–961.
20. Moss, N.G.; Powell, S.L.; Falk, S.L. Intravenous cyclosporine-A activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. Proc. Natl. Acad. Sci. USA **1985**, *82*, 8222–8226.
21. Lo Russo, A.; Passaquin, A.C.; Andre, P.; Skutella, M.; Ruegg, U.T. Effect of cyclosporine-A and analogues on cytosolic calcium and

vasoconstriction: passible lack of relationship to immunosuppressive activity. Br. J. Pharmacol. **1996**, *118*, 888–892.
22. Cook, N.C.; Samman, S. Flavonoids: chemistry, metabolism, cardio protective effects and dietary source. J. Nutritional Biochemistry **1996**, *7*, 66–77.
23. Halliwell, B.; Gutterridge, J.M.C. *Free Radicals in Biology and Medicin*e, 3rd Ed.; Oxford University Press: Oxford, 1999; Vol. 1.
24. Plumb, W.; Price, K.R.; Williamson, G. Antioxidant properties of flavonol glycosides from green beans. Redox. Rep. **1999**, *4*, 123–127.
25. Ishikawa, T.; Suzukawa, M.; Ito, T.; Yoshida, H.; Ayaori, M.; Nishiwaki, M.; Yonemura, A.; Hara, Y.; Nakamura, H. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. American J. Of Clinical Nutrition **1997**, *66*, 261–266.
26. Jankun, J.; Selman, S.H.; Swiercz, R.; Skrzypczak-Jankun, E. Why drinking green tea could prevent cancer. Nature **1997**, *387*, 561.
27. Yamanaka, N.; Oda, O.; Nagao, S. Green tea catechins such as (−)-epicatechin and (−)-epigallocatechin accelerate Cu^{2+}-induced low density lipoproteins oxidation in propagation phase. FEBS Letters **1997**, *401*, 230–234.
28. Satyanarayana, P.S.V.; Singh, D.; Chopra, K. Quercetin, A. Bioflavonoid, protects against oxidative stress-related renal dysfunction by cyclosporine in rats. Methods Find Exp. Clin. Pharmacol. **2001**, *24* (4), 175–181.
29. Satyanarayana, P.S.V.; Chopra, K. Oxidative stress-mediated renal dysfunction by cyclosporine-A in rats: attenuation by trimetazidine. Renal Failure **2002**, *24* (3), 259–274.
30. Naidu, P.S.; Singh, A.; Kulkarni, S.K. Carvedilol attenuates neuroleptic-induced orofacial dyskinesia: possible antioxidant mechanisms. Br. J. Pharmacol. **2002**, *136*, 193–200.
31. Wills, E.D. Mechanism of lipid peroxide formation in animal tissues. Biochem. J. **1996**, *99*, 667–676.
32. Ellman, G.L. Tissue sulfhydryl group. Arch. Biochem. Biophys. **1959**, *82*, 70–77.
33. Kono, Y. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophy. **1978**, *186*, 189–195.
34. Luck, H. Catalase. In *Methods of Enzymatic Analysis*; Bergmeyer, H.U., Ed.; Academic Press: New York, 1971; 885–893.
35. Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the folin-phenol reagent. J. Biol. Chem. **1951**, *193*, 265–275.
36. Andoh, T.F.; Bennett, W.M. Chronic cyclosporine nephrotoxicity. Curr. Opin. Nephrol. Hypertens. **1996**, *7*, 260–270.
37. Baliga, R.; Ueda, N.; Walker, P.D.; Shah, S.V. Oxidative mechanism in toxic acute renal failure. Am. J. Kidney Dis. **1997**, *29*, 465–477.
38. Haberland, A.; Hence, W.; Grune, T.; Siems, W.; Jung, K.; Schimke, I. Differential response of oxygen radical metabolism in rat heart, liver and kidneyc to cyclosporine-A treatment. Inflamm. Res. **1997**, *46*, 452–454.
39. Zhong, Z.; Arteel, G.E.; Cornor, H.D.; In, M.; Frankenberg, M.V.; Stachlewitz, R.F.; Raleigh, J.A.; Mason, R.P. Cyclosporine-A increases hypoxia and free radicals production in rat kidneys: prevention by dietary glycine. Am. J. Physiology **1999**, *275*, F595–F604.

40. Wang, C.; Salahudeen, A.K. Lipid peroxidation accompanies cyclosporine nephrotoxicity: effect of vitamin E. Kidney Int. **1995**, *47*, 927–934.
41. Clarke, H.; Ryan, M.P. Cyclosporine A-induced alteration in magnesium homeostasis in rat. Life Sci. **1999**, *64*, 1295–1306.
42. Ferguson, C.J.; Ruhland, C.; Parry-Jones, D.J. Low-dose cyclosporine-A nephrotoxicity in the rat. Nephrol. Dial. Transplant. **1993**, *8*, 1258–1263.
43. Zhong, Z.; Connor, H.D.; Yin, M.; Moss. N.; Mason, R.P.; Bunzendahl, H.; Forman, D.T.; Thurman, R.G. Dietary glycine and renal denervation prevents cyclosporine A-induced hydroxyl radical production in rat kidney. Mol. Pharmacol. **1998**, *56*, 455–463.
44. Lanese, D.M.; Conger, J.D. Effect of endothelin receptor antagonist on cyclosporine induced vasoconstriction in isolated rat renal arterioles. J. Clin. Investigation **1993**, *91*, 2144–2149.
45. Lanese, D.M.; Falk, S.A.; Conger, J.D. Sequential agonist activation and site-specific mediation of acute cyclosporine constriction in rat renal arterioles. Transplantation **1994**, *58*, 1371–1378.
46. Baud, L.; Ardaillou, R. Involvement of reactive oxygen species in kidney damage. Br. Med. Bull. **1993**, *49*, 621–629.
47. Bomzon, A.; Holt, S.; Moore, K. Bile acids, oxidative stress and renal function in bilary obstruction. Semin. Nephrol. **1997**, *17*, 549–562.
48. Avdonin, P.V.; Cottet-Maire, F.; Afanasjeva, G.V.; Loktionova, S.A.; Lhote, P.; Ruegg, U.T. Cyclosporine-A up-regulates angiotensin-II receptors and calcium responses in human vascular smooth muscle cells. Kidney Int. **1999**, *55*, 2407–2414.
49. Murray, B.M.; Paller, M.S.; Ferris, T.F. Effect of cyclosporine-A administration on renal hemodynamics in conscious rats. Kidney Int. **1985**, *28*, 767–774.
50. Perico, N.; Benigni, A.; Zoja, C.; Delani, F.; Remuzzi, G. Functional significance of exaggerated renal thromboxane A2 synthesis induced by cyclosporine-A. Am. J. Physiol. **1986**, *251*, F581–F587.
51. Fogo, A.; Hellings, S.E.; Inagami, T.; Kon, V. Endothelin receptor antagonism is protective in in vivo acute cyclosporine toxicity. Kidney Int. **1992**, *42*, 774–779.
52. Benighi, A.; Chlabrando, C.; Piccinelli, A.; Perico, N.; Gavinelli, M.; Furei, L.; Patino, O.; Abbate, M.; Bertani, T.; Remuzzi, G. Increased urinary excretion of thromboxane B_2 and 2,3-dinor-Txb_2 in cyclosporine-A nephrotoxicity. Kidney Int. **1988**, *34*, 164–174.
53. Takahashi, K.; Nammour, T.M.; Fukunaga, M.; Ebert, J.; Murrow, J.D.; Robert, L.J 2nd.; Hoover, R.L.; Badr, K.F. Glomerular actions of a free radical generated novel prostaglandin, 8-epiprostaglandin F_2 A, in the rat: evidence for interaction with thromboxane A_2 receptors. J. Clin. Invest. **1992**, *90*, 136–141.
54. Inselmann, G.; Hannemann, J.; Baumann, K. Cyclosporine-A induced lipid peroxidation and influence of glucose-6-phospahtase in rat hepatic and renal microsomes. Res. Commun. Chem. Pathol. Pharmacol. **1990**, *68*, 189–203.
55. Freeman, B.A.; Crapho, J.D. Biology of disease: free radicals and tissue injury. Lab. Invest. **1982**, *47*, 412–426.

56. Thurman, R.G.; Zhong, Z.; Frankenberg, M.V. Prevention of cyclosporine-induced nephrotoxicity with dietary glycine. Transplantation **1997**, 63, 1661–1667.
57. Krysztopik, R.J.; Benttley, F.R.; Spin, D.A.; Wilson, M.A.; Garrison, R.N. Lazaroids prevent acute cyclosporine A-induced renal vasoconstriction. Transplantation **1997**, *63*, 1215–1220.
58. Tariq, M.; Morais, C.; Sobki, S.; Al Sulaiman, M.; Al Khader, A. *N*-acetylcysteine attenuates cyclosporine-induced nephrotoxicity in rats. Nephrol. Dial. Transplant. **1999**, *14*, 923–929.
59. Kanji, V.K.; Wang, C.; Salahudeen, A.K. Vitamin E suppresses cyclosporine A-induced increase in the urinary excretion of arachidonic acid metabolites including F2-isoprostanes in the rat model. Transplant Proc. **1999**, *31*, 1724–1728.
60. Vijay Kumar, K.; Naidu, M.U.R.; Shifow, A.A.; Prayag, A.; Ratnakar, K.S. Melatonin: an antioxidant protective cyclosporine-induced nephrotoxicity. Transplantation **1999**, *7*, 1065–1083.
61. De-Whalley, C.; Rankin, S.M.; Houct, J.R.S.; Jessup, W.; Leake, D.S. Flavonoids inhibits the oxidative modification of low-density lipoproteins by macrophages. Biochem. Pharmacol. **1990**, *39*, 1743–1750.
62. Francel, E.N.; Kanner, J.; German, J.B.; Packs, E.; Kinsella, J.E. Inhibition of oxidation of human low-density lipoprotein by phenols substances in red wine. Lancet **1993**, *341*, 454–457.
63. Rice-Evans, C.A.; Miller, N.J.; Bolwell, P.G.; Bramley, P.M.; Pridham, J.B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res. **1995**, *22*, 375–383.
64. Nanjo, F.; Mori, M.; Goto, K, Hara, Y. Radical scavenging activity of tea catechins and their related compounds. Biosci. Biotechnol. Biochem. **1999**, *63*, 1621–1623.
65. Sano, M.; Takahashi, Y.; Yoshino, K.; Shimoi, K.; Nakamura, Y.; Tomita, I.; Oguni, I.; Konomoto, H. Effect of tea (*Camellia sinensis* L.) on lipid peroxidation in rat liver and kidney: a comparison of green and black tea feeding. Biol. Pharm. Bull. **1995**, *18*, 1006–1008.
66. Gupta, S.; Ahmad, N.; Mohan, R.R.N.; Husain, M.M.; Mukhtar, H. Prostate cancer chemoprevention by green tea: in vivo and in vitro inhibition of testosterone-mediated induction of ornithin decarboxylase. Cancer Res. **1999**, *59*, 2115–2120.
67. Vanhet Hof, K.H.; Wiseman, S.A.; Yang, C.S.; Tijburg, L.B. Plasma and lipoprotein levels of tea catechins following repeated tea consumption. Proc. Soc. Exp. Biol. Med. **1999**, *220*, 203–209.
68. Hara, Y.; Matsuzaki, T.; Suzuki, T. Angiotensin-I converting enzyme inhibiting activity of tea components. Nippon Nogeikagaku Kaishi **1987**, *61*, 803–808.
69. Hara, Y.; Tono-oka, F. Hypotensive effect of tea catechins on blood pressure of rats. J. of the Japanese Society of Nutr. and Food Sci. **1990**, *43*, 345–348.
70. Baba, S.; Osakabe, N.; Natsume, M.; Yassuda, A.; Takizawa, T.; Nakamura, T.; Tearao, J. Coca powder enhances the levels of antioxidative activity in rat plasma. Br. J. of Nutr. **2000**, *84*, 673–680.

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