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# Protective Effect of Erdosteine on Cyclosporine Induced Chronic Nephrotoxicity in Rats

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**ABSTRACT**

*Background:* Cyclosporine (CsA) is a clinically used immunosuppressive agent; but nephrotoxicity is a serious side effect of this drug. Some antioxidants may be used to diminish oxidative stress related to cyclosporine.
*Objectives:* The aim of this study was to determine the protective effect of erdosteine on CsA induced chronic nephrotoxicity.
*Materials and Methods:* We assessed oxidative stress enzymes (SOD, CAT, GSHPx, MDA, NO and PC levels) and light microscopic changes before and after erdosteine treatment in damaged kidney. The rats were assigned randomly to one to four groups. These were: control group (n = 8), CsA group (15 mg/kg day, n = 8), erdosteine treated group (10 mg/kg day orally, n = 8) and CsA + erdosteine group (n = 8). CsA nephrotoxicity was induced by administrating oral dose of 15 mg/kg CsA daily for 21 days.
*Results:* We observed that the activities of glutathione peroxidase (GSHPx) were higher and MDA, NO activities were lower in CsA plus erdosteine group than in CsA group. Histological parameters significantly improved after erdosteine treatment.
*Conclusions:* Erdosteine does seem to have a protective effect on CsA nephrotoxicity by reducing oxidative stress.



*Implication for health policy/practice/research/medical education:*
As an exogenous antioxidant agent; erdosteine may return CsA nephrotoxicity by reducing its effect on lipid peroxidation and glomerular ROS production.

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## 1. Background

Calcineurin inhibitors are widely used as immunosuppressive therapy at solid organ transplantation. Cyclosporine is one of them which have some dose-related side effects including nephrotoxicity. Acute nephrotoxicity is frequently associated with high blood levels of the drugs and manifests with sudden increase in renal function tests related to vasospasm of afferent arteriole. Generally, acute toxicity can be reversed by dose reduction. On the contrary, chronic nephrotoxicity occurs in prolonged exposure to lower drug levels. Because of underlying morphologic changes, chronic type of nephrotoxicity doesn't improve after dose reduction. Animal studies have shown that functional abnormalities of kidney after CsA therapy were accompanied by

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increased renal tissue content of malondialdehyde, lipid hydroperoxides, conjugated dienes and radical oxygen species with decreased glutathione levels. This excessive free radical production has been attributed to inadequate renal perfusion and hypoxia-reoxygenation injury or direct cellular membrane lesion (1). Effect of antioxidants on free radical production provides improvement in renal function (2-4).

Erdosteine, a mucolytic and anti-inflammatory agent, is used in treatment of chronic pulmonary diseases. This molecule has two blocked sulfhidryl groups which becomes free only after hepatic metabolism. The reducing potential of these sulfhidryl groups accounts for free radical scavenging and antioxidant activity of erdosteine (5).

## 2. Objectives

The major purpose of this study is to observe the possible protective effect of erdosteine on cyclosporine nephrotoxicity by assessing changes in antioxidant enzymes (glutathione peroxidase (GSHPx), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and nitric oxide (NO) levels and histopathologic feautures in tissue before and after erdosteine treatment.

## 3. Materials and Methods

Thirty-two male Wistar albino rats (180 – 200 gm) were used in this study. The study was performed according to Principles of Laboratory animal care' NIH publication revised 1996; as well as instructions of local Ethical Committee. The rats were assigned randomly into following four groups: The control group: 2 ml sunflower oil is given daily, group 2: 15 mg/kg/day CsA is given for twenty-one days by oral gavage, group 3: animals were treated with 10 mg/kg/day erdosteine orally and group 4: animals received oral CsA (15 mg/kg) and erdosteine (10 mg/kg) per day. After 21 day period, the animals in all groups were anesthesized and bilateral nephrectomies were performed. Left kidneys were separated for enzymatic analysis at -80 °C, right kidneys were fixed in %10 formaline for histological analysis. After weighing, these samples were homogenized in a 10 volumes of tris HCl buffer (0.2 Mm, pH = 7.5, at 16000 rpm) in 2 minutes. A part of homogenate separated to ependorf tubes for MDA, NO and protein analysis. The remaining part was centrifugated at + 6C at 3220 rpm to obtain supernatant. CAT and protein levels were assessed from this supernatant. Protein levels were detected with Lowry method in all compartments (6).

Total (Cu-Zn, Mn) SOD activities were determined by Sun method modified by Durak *et al.* via NBT reduction (7, 8). Glutathione peroxidase activity was measured by Paglia method associated with hydrogen peroxide activity (9). CAT activity was determined according to Aebi's spectrophotometric method (10). MDA level is the indicator of free radical production and the end product of lipid peroxidation. MDA levels were determined by the method based on the reaction of MDA with thiobarbituric acid at 532 nm with the method of Draper and Hadley's double heating method (11). Most of the studies express NO level as nitrite and nitrate levels at tissue and organ fluids. Because NO is converted to firstly nitrite and then nitrate within seconds. Firstly samples are deproteinized and total nitrite levels detected with the Griess reaction.

Rat kidney tissues were fixed in 10% buffered formalin solution. After the routine tissue protocols, embedded in wax and from this paraffin blocks, 5 μm sections were processed. These sections were stained with hemotoxylene eosin (bHE), periodic acid-Schiff (PAS) and Masson's trichrome staining methods and evaluated with the light microscope. Histologic evaluation was done by a blinded way. At least one hundred glomerules were assessed from each preparate. Tubular damage was evaluated with the regard to tubular dilatation, tubular cast, vacuolization, degeneration and thickening of tubular basement membrane semiquantitively and this scoring is calculated: 1 = 10% tubular damage, 2 = 10-25% , 3 = 26-50% , 4 = 51-75%, 5 = 75% tubular damage. At least 250 tubules were assessed. Interstitial fibrosis is evaluated with Trichrpme stainig. 0 = normal interstitium, 1 = mild fibrosis expanding minimal intertubular space,

**Table 1.** Grading of Tubular Damage,Interstitial Fibrosis and Arterioleopathy at Control, Csa Taking Group and Csa + Erdosteine Treated Group

| | **Tubular Damage** Mean [Min-Max] | **Anterstitial Fibrosis** Mean [Min-Max] | **Arterioleopathy** [a] Mean [Min-Max] |
|---|---|---|---|
| Groups | | | |
| I-Control, n = 8 | 0.500 [0.00-1.00] | 0.000 [0.00-0.00] | 3.875 [2.00-5.00] |
| II-CsA [b] , n = 8 | 2.500 [2.00-3.00] | 1.750 [1.00-3.00] | 24.750[23.00-29.00] |
| III- Erd [b] , n = 8 | 0.500 [0.00-1.00] | 0.000 [0.00-0.00] | 5.500 [4.00-7.00] |
| IV- CsA + Erd , n = 8 | 1.375 [1.00-2.00] | 1.125 [0.00-2.00] | 15.750[10.00-21.00] |
| *P* values | | | |
| I-II | 0.0001 | 0.0001 | 0.0001 |
| I-III | NS [b] | NS | NS |
| I-IV | 0.003 | 0.0001 | 0.0001 |
| II-III | 0.0001 | 0.0001 | 0.0001 |
| II-IV | 0.0001 | 0.03 | 0.0001 |
| III-IV | 0.003 | 0.0001 | 0.0001 |

[a] Number of affected glomerule = 100
[b] Abbreviations: CsA, Cyclosporine; Erd, Erdosteine; NS, Not significant

3 = moderate fibrosis expanding intertubular space, 4 = severe fibrosis expanding apparently intertubular space. SPSS for Windows 15.0 statistical program was used for evaluating the data. Median (confidence interval) ANOVA and post-hoc multiple comparison tests (LSD) were performed on the basis of biochemical and histopathologic parameters to detect differences among the groups. $P$ value < 0.05 was accepted as statistically significant. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a prior approval by the institution's animal research committee. The local ethics committee approved the study.

## 4. Results

There was no significant histopathologic abnormality at control group and erdosteine-treated group rats *(Figure1)*. CsA- treated rats showed typical histopathologic features of chronic CsA nephrotoxicity. Arteriolopathy was more apparent in CsA group than the control group ($P$ = 0.0001). All CsA treated rats showed degenerative changes at afferent arteriole such as widening and vacuolization of cytoplasm and also at the terminal arteriole smooth muscle eosinophilic, PAS positive circular or pearl-like hyaline deposits were present *(Figure 1)*. Proximal tubule degenerative changes, interstitial fibrosis, peritubular capillary congestion and mononuclear cell accumulation were markedly decreased after erdosteine treatment *(Figure 2)*. There was significant difference about arteriolopathy ($P$ = 0.0001), tubular damage and interstitial fibrosis ($P$ = 0.03, $P$ = 0.0001) between CsA and CsA + erdosteine group. The differences between the groups about this three subject expressed in *Table 1*.

The activities of kidney SOD, CAT, GSHPx, NO and MDA levels are presented in *Table 2*. There was increase in SOD enzyme levels but not statistically significant at CsA treated rats. CsA caused a decrease in CAT content of kidney compared with the control and erdosteine group ($P$ = 0.0001). Also there was clear decline at GSHPx activity at CsA-treated group; this antioxidant enzyme activity increased to statistically significant levels after CsA + erdosteine treatment ($P$ = 0.0001). This increased value by erdosteine treatment after CsA toxicity provided GSHPx levels near to control group's values. MDA and NO levels in the rat kidneys were increased by the CsA treated, erdosteine management prevented these increments at a statistically significant level ($P$ = 0.0001).

## 5. Discussion

Mechanism of cyclosporine nephrotoxicity is not completely defined; reactive oxygen species (ROS), thromboxane and lipid peroxidation products may be associated factors (12). CsA is very lipophilic agent; this facilitates its attachment to cellular membranes. Organelle membranes especially endoplasmic reticulum has large amounts of unsaturated fatty acids and also they have very large total surface area. Because of this features they are very susceptible to oxidative stress related to CsA (13). There is some hypothesis about relationship between CsA therapy and ROS over-production: up regulation of cytochrome p-450, tubular ischemia- reperfusion related to vasoconstriction vasodilatation, increase in renal TXA2 production and changes in NO levels (14, 15). These studies have shown that CsA therapy causes dose and time related deterioration of kidney functions; its mechanism of effect is associated with increase in ROS, thromboxane secretion and also decrease of glomerular filtration rate with ROS, thromboxane and lipid peroxidates overproduction.

Phospolipase A2 is a membrane enzyme; initiates the synthesis of arachidonic acids and eicosanoids via metabolizing oxidized fatty acids. Degradation of arachidonic acid may result in increased free oxygen radicals mostly malondialdehyde (MDA). MDA leads to cross linking of compounds by acting on ion exchange at cell membranes and by this way; it may cause negative consequences such as change in enzyme activity and ion permeability (11). The implication of oxidative stress in kidney damage related to CsA therapy was strengthened by Rabl and colleague's study (16). They include kidney

**Table 2**. Kidney Tissue Enzyme Activities at Control, CsA, CsA + Erdosteine Groups

| | SOD [a], U/mg protein, Mean ± SD | CAT [a], k/g protein, Mean ± SD | GSHPx [a], U/g protein, Mean ± SD | MDA [a], nmol/g protein, Mean ± SD | NO [a], μmol/g protein, Mean ± SD |
|---|---|---|---|---|---|
| Groups | | | | | |
| I-Control, n = 8 | 0.377 ± 0.012 | 22.89 ± 0.70 | 1.716 ± 0.029 | 11.49 ,± 0.30 | 0.134 ± 0.004 |
| II-CsA, n = 8 | 0.406 ± 0.009 | 12.99 ± 0.43 | 1.294 ± 0.019 | 16.00 ± 0.17 | 0.213 ± 0.005 |
| III-Erd, n = 8 | 0.376 ± 0.010 | 23.52 ± 0.81 | 1.627 ± 0.026 | 11.77 ± 0.06 | 0.145 ± 0.003 |
| IV-CsA + Erd, n = 8 | 0.376 ± 0.012 | 15.98 ± 0.85 | 1.699 ± 0.028 | 12.30 ± 0.19 | 0.139 ± 0.004 |
| *P* values | | | | | |
| I-II | NS [a] | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| I-III | NS | NS | 0.023 | NS | NS |
| I-IV | NS | 0.0001 | NS | 0.007 | NS |
| II-III | NS | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| II-IV | NS | 0.006 | 0.0001 | 0.0001 | 0.0001 |
| III-IV | NS | 0.0001 | NS | NS | NS |

[a] Abbreviations: CAT, Catalase; GSHPx , Glutathione peroxidase; MDA, Malondialdehyde; NO, Nitric oxide; NS, Not significant; SOD, Superoxide dismutase

**Figure 1.** Afferent Arteriolopathy Under Light Microscope (A), Normal Glomerulus. Pas X 1000 (B), Pas Positive Material Deposition at Cyclosporine Group. Pas X 1000 (C), Decreasing Arterioleopathy at Erdosteine + Cyclosporine Taking Group. Pas X 1000.

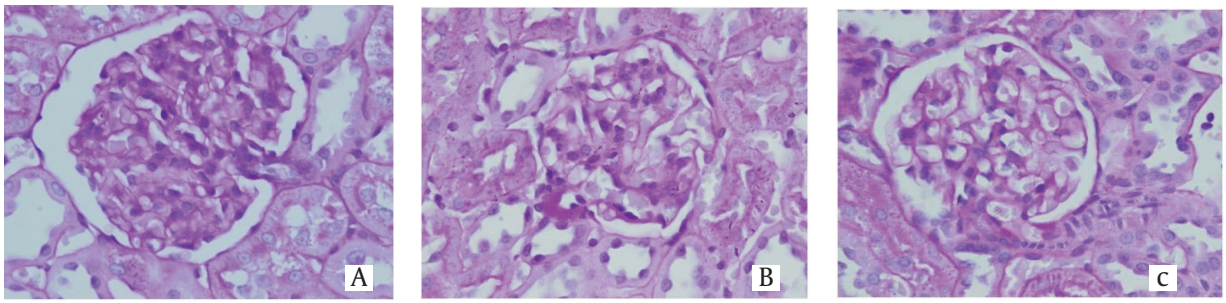
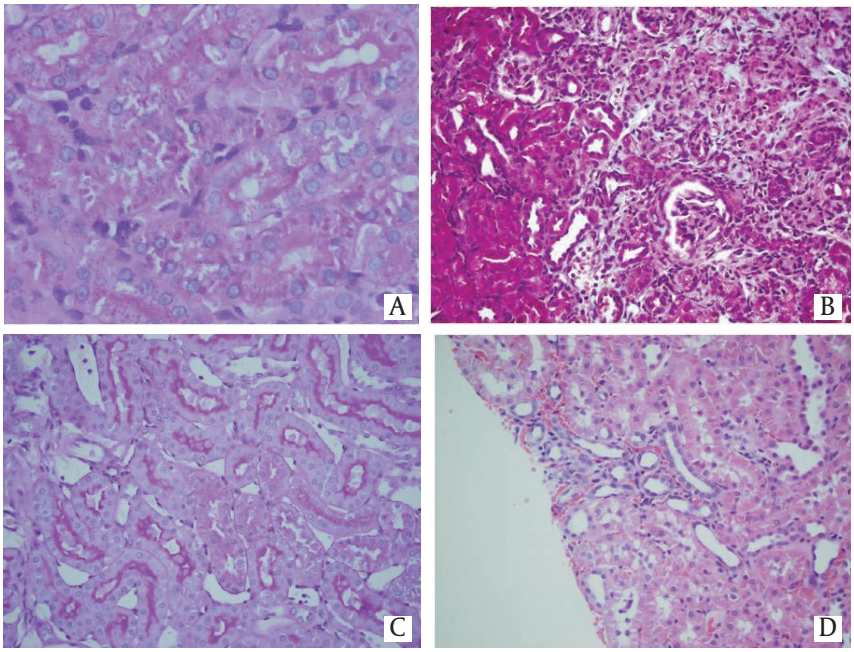



**Figure 2.** Comparison of Proximal Tubule Damage and Tubular Atrophy Between Cyclosporine and Cyclosporine + Erdostein Groups.




(A), Proximal tubule damage with cytoplasmic vacuolization PAS X 1000 in cyclosporine group (B), Typical interstitial fibrosis and tubular atrophy Masson trichrome X400 (C), Median proximal tubular damage in cyclosporine + erdosteine group PAS X 400 (D), Minimal subcapsular interstitial fibrosis and atrophic tubules in cyclosporine + erdosteine group HE X 400.

recipients and have found that intravenous application of various antioxidants (vit C, vit E, pro-vit A, vit B1, vit B2, niacine and panthetonate) half an hour before reperfusion reduce MDA levels and lead to early fall in serum creatinine. In our study, MDA levels in the rat kidneys were increased by the CsA treated, erdosteine management prevented these increments at a statistically significant level ($P = 0.0001$).

Endothelial NO production is the basis of vasodilatation. However, recent studies discuss the role of NO in the pathophysiology of acute renal failure. Peresleni *et al.* showed that oxidative stress in epithelial cells reduces cell life by the way of increased NO and nitrite levels (17, 18). In our study, increase in NO levels in CsA treated rats was determined and this increase was significantly less observed in the rats with erdosteine added to the treatment. Antioxidants have healing effect on renal functions and histological damage related to CsA. Several studies on other antioxidants showed beneficial effects of these molecules on toxic renal disease (2, 19, 20). Melatonin is an antioxidant molecule and may cause improvement of renal failure due to CsA treatment and prevention of micro calcifications (3). Durak and colleagues performed a study of vitamin E and vitamin C improves histological damage related to oxidative stress and lipid peroxidation products; and also TBARS (thiobarbituric acid reactive substances) and antioxidant enzymes CAT and GSHPx activity was found to increase by this therapy (21). When CsA is given together with N-acetyl sistine, reduction in histological changes and improvement of oxidative stress markers such as lipid hydroperoxide can be observed (22). In our study, rat kidney tissue sections in CsA treated group was compared with the control group, histological changes were typical of chronic nephrotoxicity. These changes were afferent arteriolopathy, band-like fibrosis in interstitium, tubular

atrophy and focal inflammatory cell growth, respectively. Expansion of afferent arteriole cell cytoplasm and eosinophilic, granular, PAS-positive circular and pearl-like hyaline deposition at terminal arteriolar smooth muscle cells were seen. In rats with CsA + erdosteine treatment, structural changes caused by CsA application was found to decrease at histopathologic examination. Proximal tubule degeneration and interstitial fibrosis infiltration areas were decreased, peritubular capillary congestion was limited and mononuclear cell infiltration was also significantly reduced.

Erdosteine(N-(carboxymehylthioacetyl)-homocysteine thiolactone) is a mucolytic, expectorant and anti-inflammatory agent, effective at chronic pulmonary disease. Except for increasing the mucus viscosity and tracheobronchial clearance; this agent gains the antioxidant and free radical scavenger activity with sulphidril group blockade after hepatic metabolism. It is proven that erdosteine reduces cardiotoxicity and hepatotoxicity in rat models (23, 24). We proved nephro-protective effect of this molecule. For the first time, we have demonstrated in vivo that erdosteine causes improvement in cyclosporine related nephrotoxicity by increasing GSHPx, CAT, MDA and NO levels. Also, histological clues of renal damage can be diminished by erdosteine treatment. As an exogenous antioxidant agent, erdosteine may return CsA nephrotoxicity by reducing its effect on lipid peroxidation and glomerular ROS production. However, more animal and human studies must be done to further elucidate the possible mechanisms.

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