Cyclosporine-Induced Oxidative Stress and renal Dysfunction in Rat kidneys: A Possible Ameliorated Effect by Curcumin as an Antioxidant

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ABSTRACT

Curcumin (CMN) has been well studied due to its economic and medical importance. Traditional Egyptian Medicine claims the use of its powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis. The current study was designed to examine the possible beneficial effect of CMN in preventing the acute renal failure and related oxidative stress caused by chronic administration of cyclosporine (CsA) in rats. The study included two experiments, the first one was carried out to follow up the changes that could occur in kidney function as a result of cyclosporine (CsA) administration. Cyclosporine administration exerted significant (P< 0.01) elevation of serum urea, creatinine, potassium (K), parathormone (PTH), malondialdehyde (MDA) and asymmetrical dimethylarginine (ADMA). Meanwhile, cyclosporine treatment exerted significant (P< 0.01) decline in the level of serum sodium (Na) and total nitric oxide (NO), the content of kidney reduced glutathione (GSH) and the activities of glutathione peroxidase (G_{px}), catalase (CAT) and superoxide dismutase (SOD) as compared with their corresponding normal rats. In the second experiment, the nephritic rats were treated with curcumin and remarkable corrections were occurred in all previous parameters. Thus, the current investigation was designed to examine the possible beneficial effect of CMN in preventing the renal failure and related oxidative stress caused by administration of CsA in rats.

Key words: Cyclosporine - Curcumin - kidney function tests - Rats

INTRODUCTION

The aminoglycosides antibiotics are so named because they consist of two or more amino sugars joined in a glycosidic linkage to a central hexose nucleus. The incidence of renal dysfunction following aminoglycoside administration ranges from 5 to 26%[1,2] .

Cyclosporine A has been shown to enhance generation of hydrogen peroxide *in vitro* and enhances lipid peroxidation *in vitro* and *in vivo*. Antioxidants have been shown to be protective in cyclosporine A nephrotoxicity. Such collective body

of evidence suggests an important role for reactive oxygen metabolites in toxic acute renal failure and may provide therapeutic opportunities of preventing or treating acute renal failure in humans[2-5].

Cyclosporine (CsA) (formerly called cyclosporine A), a hydrophobic cyclic un decapeptide produced by the fungus *Tolypocladium inflatum*, can be considered the prototype of immunosuppressant that has revolutionized the management of allotransplantation[6]. CsA combines low myelotoxicity with effectiveness in preventing allograft rejection and graft versus host disease as well as in the treatment of various autoimmune and ocular inflammatory diseases [7]. Nephrotoxicity and hypertension are the major adverse effects that often limit CsA treatment following solid organ transplantation and autoimmune diseases[8]. The functional changes caused by CsA are dose dependant and are usually reversible after short-term CsA treatment[9].

Cumulative data suggest a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CsA nephrotoxicity. CsA results in enhanced generation of hydrogen peroxide in cultured hepatocytes [10] and mesangial cells[11]. *In vitro* and *in vivo* studies indicate that CsA enhances lipid peroxidation, reduces renal microsomal NADPH cytochrome P_{450}, and renal reduced/oxidized glutathione ratio (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria[5,12]. Antioxidants such as α-tocopherol, ascorbate, silibinin, lazaroid, propionyl carnitine and superoxide dismutase/catalase, have been shown to ameliorate cyclosporine-induced renal toxicity [10, 13].

Current traditional Indian medicine claims the use of Curcuma longa L. ***(Zingiberaceae)*** powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis [14]. Curcumin (CMN) is a major component in curcuma/turmeric, being responsible for its biological actions. More and more studies now show that CMN exhibit anti-inflammatory [17], anti-human immunodeficiency virus[16,17], anti-bacterial [18] and nematocidal activities [19]. Various *in-vitro* and *in-vivo* studies increasingly establish the antioxidant properties of CMN [20, 21]. It is well documented that CMN scavenges superoxide anions [22], peroxynitrite radicals[23,24] and quenches singlet oxygen[25]. CMN has also been shown to inhibit hydrogen-peroxide-induced cell damage [21].

Thus, the current study was designed to examine the possible beneficial effect of CMN in preventing the acute renal failure and related oxidative stress caused by chronic administration of CsA in rats.

MATERIAL & METHODS

Fifty adult male albino rats ***Rattus rattus*** (140±l0g) were employed in the present study. They were housed in a well ventilated animal house and kept under the same managerial and environmental conditions. They were fed to appetite on a standard laboratory animal diet and fresh tap water was available at all times[26]. The animals were caged

in wire bottom galvanized metal wall boxes.

The study included two experiments; the first one was carried out to follow up the changes that could occur in kidney function tests as a result of cyclosporine (CsA) treatment. To achieve that purpose, a comparison was carried out between a group of five normal control rats injected subcutaneous daily with olive oil for 21 days and other five animals were daily injected subcutaneous (s.c.) with cyclosporine (CsA) at a dose of 20 mg/kg/day dissolved in olive oil for the same period[2].

In the second experiment, four comparisons were made between three groups of rats with renal toxicity induced by cyclosporine (CsA) administration, where the first group of animals (ten rats) was left without further treatment (recovery nephrotoxicated group). The second group of cyclosporine (CsA) toxicated rat (ten rats) was treated orally with 15 mg curcumin (CMN) / kg b.wt. The third group of cyclosporine (CsA) toxicated rat (ten rats) was CsA treated orally with 30 mg curcumin (CMN) / kg b.wt. The fourth group animal was non-treated and served as control animal group. All animal groups were divided into two intervals (two and four weeks and five rats in each interval).

Cyclosporine (CsA) was purchased from Sigma Chem. Co., St Louis, Mo. U.S.A. Commercial curcumin (CMN) was used. Biochemical analyses have been made in order to evaluate the possible ameliorating effect of curcumin (CMN) on the kidney function at various intervals as a result of cyclosporine administration.

At the end of each experimental period, blood samples were collected from each group by decapitation killing. The contents of serum urea and creatinine, were assayed colorimetrically using commercial kits (Randox Ltd., Co. UK)[27,28]. Sodium (Na) and potassium (K) analysis were accomplished by emission flame photometry after suitable dilutions[29]. Serum parathormone (PTH) was assayed by radioimmunoassay kit using solid phase component (ICN Pharmaceuticals Co., USA) [30]. Plasma ADMA [31] and serum total nitric oxide [32] and malondialdehyde (MDA) [33] were assayed by ELISA technique using commercial kits (Oxis, Inc., USA).

After sacrifice, kidneys were obtained at the end of each experimental period and washed with saline solution (0.9% Na Cl). After washing, the kidneys were homogenized in ice-cold 0.25 M sucrose containing 1mM diethylenetriamine penta-acetic acid (1:1 w/v). Each sample was then centrifuged for 20 min at 20,000 g and 4°C. The supernatant was aspirated for measuring the content of reduced GSH [34] and the activities of glutathione peroxidase (G_{PX})[35], catalase (CAT)[36] and superoxide dismutase (SOD)[37] by ELISA technique using commercial kits (IBL Gesellschaft, Hamburg, Germany).

Data were statistically analyzed using Student "t" test in the first experimental[38]. Moreover, two way analysis of variance (ANOVA) followed by Duncan's multiple range test in the second experiment[39].

RESULTS & DISCUSSION

In the current study, rat was used as an animal model for induction of acute renal failure by cyclosporine injection at a dose equivalent to that used clinically in man[2]. Tirkey et al., found that cyclosporine injection (20 mg/kg b.wt for 21 days) developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure. Injection of rats with cyclosporine (30 mg / kg b.wt for 14 days) created degenerative nephropathy[40].

Cyclosporine is used for the prevention of rejection of kidney[41], liver[42] or heart allografts[43]. So, cyclosporine is used to prolong graft survival of allogenic renal transplants[44,45]. Therapy with cyclosporine alone has achieved graft survival rates ranging from 71–91% 1 year after renal transplantation [46]. In a retrospective study, patients and graft survival rates were 86 and 70%, respectively, 4 years after transplantation in cyclosporine-treated patients[45].

The most frequent and clinically important adverse effect of cyclosporine is nephrotoxicity[41]. Nephrotoxic effects (usually manifested as increased BUN and serum creatinine concentrations) of cyclosporine have been observed in 25–32, 38, or 37% of patients receiving the drug for kidney, heart, or liver allografts, respectively[45]. Elevations of BUN and serum creatinine concentrations resulting from cyclosporine therapy appears to be dose related, may be associated with high concentrations of the drug, and are usually reversible upon discontinuance of the drug [44, 45]. Clinical manifestations of cyclosporine-induced nephrotoxicity may include fluid retention, dependent edema, and, in some cases, a hyperchloremic, hyperkalemic metabolic acidosis[47,48]. The risk of cyclosporine-induced nephrotoxicity may be increased in patients receiving other potentially nephrotoxic agents. Mild cyclosporine-induced nephrotoxicity generally occurs within 2 to 3 months after transplantation. Although some decline from preoperative levels generally occurs in patients with mild nephrotoxicity, the BUN and serum creatinine concentrations reportedly become stabilized in the range of 35–45 mg/dL and 2–2.5 mg/dL, respectively, in these patients; however, these elevations often respond to dosage reduction. In some patients, more severe nephrotoxic effects have been observed early after transplantation and have been characterized by rapid increases in BUN and serum creatinine concentrations; these elevations usually respond to dosage reduction[47, 48].

In the current investigation, acute renal failure was characterized by disorders in some biochemical parameters in cyclosporine (CsA) treated rats. Serum urea and creatinine increased to about 210 % and 230 % respectively over their corresponding values in control group (Table 1). Similar greater serum urea over control rats was obtained[2] in a dose-related fashion in rats treated with graded doses of cyclosporine (CsA). Accordingly, these changes reflected the severity of renal insufficiency. It is possible that all these biochemical

alterations occurred in association with the sudden fall in glomerular filtration rates because of the majority of administered cyclosporine (CsA) dose enters specifically the proximal tubular epithelial cells, binds to anionic phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and orgranelles[49,50,51]. Animals with cyclosporine (CsA) nephrotoxicity showed increased lipid peroxidation in the kidney cortex[2, 51,52,53].

Table (1): Effects of cyclosporine administration on some physiological and biochemical parameters in rats

Parameters	Control group n = 5 rats	cyclosporone group n = 5 rats
Urea (mg/dL)	16.347±0.496	34.112 ± 0.925*
Creatinine (mg/dL)	0.482 ± 0.007	1.104 ± 0.016*
Sodium (Na) (meq/L)	132.913 ± 1.965	124.627 ± 1.783*
Potassium (K) (meq/L)	4.037 ± 0.184	5.114 ± 0.261*
Parathormone (PTH) (ng/ml)	10.516 ± 0.592	17.815 ± 0.883*
ADMA (µmol/L)	1.137±0.079	2.842 ± 0.096*
TNO (µmol/L)	59.41 ± 1.217	30.916 ± 1.031*
MDA (nmol /dL)	0.537± 0.009	0.921 ± 0.018*
GSH (mg/g protein)	11.142 ± 0.683	7.056 ± 0.497*
Gpx (µmol GSH utilized/min/g protein)	23.351 ± 0.947	16.682 ± 0.782*
CAT (nmol/60 min/ mg protein)	42.573± 1.108	35.011±0.926*
SOD (Nu/60 min/mg protein)	5.104±0.213	3.825±0.187*

- *Values are expressed as mean ± SE.* *– n = number of rats.*
- * *Means a significant (P< 0.001).*

Electrolytes in serum was disturbed remarkably (P<0.05) in cyclosporine (CsA) treated rats as compared with untreated animals. As shown in table (1), lower value of serum Na^+ in cyclosporine (CsA) treated rats than controls indicates inability of kidney to conserve sodium and chloride. Hemodilution too may be involved in the fall of Na^+ value *via* excess of water intake and/or increased production of endogenous water. In turn, the reversed increase of K^+ appeared to be due to reduced excretion of K^+ aggravated by leakage of intracellular K^+ into blood stream as a result of cyclosporine (CsA) induced lesions in renal tubular epithelium[54- 57].

Moreover, the exact mechanism of CsA-induced hypertension and nephrotoxicity remain obscure but several studies suggest that a defect in intracellular calcium handling[57], magnesium deficiency[56], oxidative stress[58,59] and nitric oxide (NO) system[60] are involved. So, acute renal failure due to CsA is widely attributed

to the generation of reactive oxygen species (ROS) by CsA.

The current study revealed that chronic administration of CsA for 21 days caused a marked impairment of renal function along with significant oxidative stress in the kidneys causing a significant decline in the content of kidney GSH and the activities of kidney G_{px}, CAT and SOD (Table 1)[2,21,61]. These finding could be attributed these results to the increase of lipid peroxidation and free radicals production.

It has been reported that binding of pimonidazole, a hypoxia marker in the kidneys, was increased nearly three fold by CsA, indicating marked tissue hypoxia[61]. Moreover, free radicals in the urine were increased dramatically after CsA treatment[62,63]. It is also known that CsA increases renal nerve activity resulting in vasoconstriction in the kidney[40]. In addition, CsA causes vasoconstriction directly in isolated renal arterioles[64,65]. It has been demonstrated that CsA blocks mitochondrial calcium (Ca^{+2}) release, inducing a drastic enhancement in intracellular free Ca^{+2}, which could account for the vasoconstrictive effect of CsA [66, 67]. These alterations could theoretically lead to a classical hypoxia-reoxygenation injury involving oxygen free radicals. In addition, ROS could be derived directly from CsA or during its metabolism by the CYP_{450} system [11]. It has been demonstrated that cyclosporine increased level of superoxide (O_2^-) in endothelial and mesangial cells [5]. Studies show that CsA-induced local production of hydroxyl radical, a highly active and detrimental radical that plays an important role in CsA nephrotoxicity[68].

Couple of studies suggested that CsA induces apoptosis characterized by internucleosomal DNA cleavage due to endonuclease activation, chromatin condensation, and apoptotic bodies in hematopoietic cells [69]. Because oxidants are capable of inducing apoptosis in various types of cells[70], including renal tubular epithelial cells[71], it is conceivable that reactive oxygen metabolites may play a role in apoptotic mechanism of CsA-induced nephrotoxicity.

The current investigation revealed that chronic administration of CsA for 21 days caused a marked impairment of renal function along with significant oxidative stress in the kidneys. Curcumin significantly and dose-dependently improved creatinine and urea levels, and decreased the elevated levels of serum creatinine and urea. Earlier studies have also shown that CMN pretreatment decreases ischemia-reperfusion induced rise in serum creatinine levels in kidney[72]. Chronic administration of CsA also produced oxidative stress and increased the lipid peroxidation in kidneys as is seen by the serum MDA level. That effect of CsA was again ameliorated by CMN treatment and is in line with various previous reports, which showed that CMN decreases lipid peroxidation possibly by its antioxidant mechanism [73]. Oxidative stress could promote the formation of a variety of vasoactive mediators that can affect renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient; and thus

reducing glomerular filtration rate [74]. Thus, the attenuation of lipid peroxidation in CsA-treated rats by CMN provides a convincing evidence for the involvement of ROS in CsA-induced lipid peroxidation. **Rukkumani et al.**[75] reported protective effect of CMN on circulating lipids in plasma and lipid peroxidation products in alcohol and polyunsaturated fatty acid-induced toxicity. *In-vitro*, **Somasundaram et al.**[76] supported the hypothesis that CMN inhibits free radical induced apoptosis in cell lines.

From the data presented in table (2), it is obvious that late CMN administration proved to have some ameliorating effects against undesirable changes in kidney function following cyclosporine (CsA) injection for 21 days. With the progress of time after the drug was discontinued, serum urea, creatinine, K, PTH, ADMA and MDA decreased ($P< 0.05$) significantly during the treatment with CMN as compared with nephrotoxic group, although the levels of all those variables were still significantly ($P<0.05$) higher than saline injected rats (controls). On other hand, serum Na and total nitric oxide (NO) increased ($P<0.05$) significantly during the treatment with CMN as compared with nephrotoxic animal group (Table 2). These increments were still not reached to that corresponding normal control rat group.

More and more studies now established the ability of CMN to mainly eliminate the hydroxyl radical, superoxide radical[77], singlet oxygen[79], nitrogen dioxide[80] and NO[78].

It has also been demonstrated that CMN inhibits the generation of the superoxide radical [82]. In the current study, CsA administration caused marked deterioration of endogenous antioxidant profile as evidenced by decrease in SOD and CAT activities, an effect which was effectively reversed by CMN treatment [22]. CMN manganese complex and acetylcurcumin manganese complex, low molecular weight synthetic compounds, showed much greater SOD activity and an inhibitory effect on lipid peroxidation.

Further, GSH a major non-protein thiol in living organisms plays a crucial role in coordinating the body's antioxidant defense processes. Results in the current study indicated that CsA administration drastically lowered the levels of GSH in the kidney. Improvement of renal GSH levels in CMN treated rats in comparison to CsA administered rats further demonstrates the antioxidative effect of CMN. CMN has been shown to increase the levels of glutathione reductase in ischemic brains of rats as well as alveolar and human leukemia cell[2,21,57]. The prolonged treatment of CMN also improved the levels of two key antioxidant enzymes SOD and catalase (CAT) in CsA administered rats.

Table (2): Effect of curcumin (15 & 30 mg/B Wt) treatment on kidney function tests in cyclosporine-nephrotoxic rats

Groups / Parameters		Control	Recovery	Cyclosporine +Curcumin 15 mg	Cyclosporine +Curcumin 30 mg
Urea (mg/dL)	**15 days n = 5**	16.421±0.489[a]	33.102±0.917[b]	30.561±0.832[d]	28.473±0.792[e]
	30 days n = 5	16.407±0.489[a]	31.354±٠.869[c]	28.162±0.764[e]	21.958±0.711[f]
Creatinine (mg/dL)	**15 days n = 5**	0.484±0.007[a]	1.073±0.017[b]	0.892±0.013[c]	0.802±0.012[d]
	30 days n = 5	0.484±0.006[a]	1.008±0.014[b]	0.795±0.012[d]	0.653±0.011[e]
Sodium (Na) (meq/L)	**15 days n = 5**	132.871±1.894[a]	125.712±1.692[b]	126.983±1.749[c]	129.237±1.814[d]
	30 days n = 5	133.145±1.971[a]	125.946±1.571[b]	129.052±1.802[d]	132.152±1.853[a]
Potassium (K) (meq/L)	**15 days n = 5**	4.112±0.187[a]	5.092±0.257[b]	4.639±0.237[c]	4.412±0.213[d]
	30 days n = 5	4.103±0.185[a]	5.011±0.258[b]	4.405±0.219[d]	4.121±0.197[a]
Parathormone (PTH)	**15 days n = 5**	10.547±0.587[a]	17.564±0.856[b]	15.932±0.749[c]	14.567±0.712[d]
	30 days n = 5	10.621±0.590[a]	17.439±0.868[b]	13.037±0.663[e]	12.108±0.641[f]
ADMA (µmol/L)	**15 days n = 5**	1.142±0.082[a]	2.715±0.093[b]	2.441±0.085[c]	2.011±0.081[d]
	30 days n = 5	1.139±0.082[a]	2.436±0.087[c]	1.983±0.079[d]	1.502±0.083[e]
TNO (µmol/L)	**15 days n = 5**	58.971±1.188[a]	34.042±1.141[b]	38.899±1.127[c]	42.004±1.159[d]
	30 days n = 5	59.156±1.197[a]	39.117±1.032[c]	44.769±1.162[e]	49.537±1.194[f]

- Values are expressed as mean ± SE. *- n= number of rats*

-[a, b, c ,d ,e ,f] Means with a common subscript within an each parameter are not significantly different (P>0.05).

Peroxynitrite anions have been generated by the reaction of nitric oxide with superoxide anion. These peroxynitrite anions oxidize biomolecules, which finally leads to lipid peroxidation and tubular cell damage **(77, 78).** Large amounts of nitric oxide can lead to the depletion of cellular ATP which can inactivate enzymes that contain iron-sulfur clusters, such enzymes involved in mitochondrial electron transport**(83)**. Nitrosylation of sulfhydryl groups or tyrosine residues in proteins may impair the functional properties of these proteins. Nitric oxide damages DNA, and this in turn, stimulates the DNA repair enzyme poly-ADP-ribose synthetase**(85).** Studies done by **Amore et al.(84)** demonstrated that CsA

induces apoptosis in various renal cell lines, and this effect is mediated by the induction of iNOS. In line with studies where CMN is reported to inhibit iNOS gene expression in isolated BALB/c mouse peritoneal macrophages and also in the livers of lipopolysaccharide injected mice[85]. The current study showed that CsA-induced nitrosative stress was significantly and dose dependently attenuated by CMN. **Sumanont et al.,** studied the effect of CMN and its analogues on peroxynitrite anions scavenging activity *in vitro* using sodium nitroprusside (SNP) generating nitric oxide system. All compounds effectively reduced the generation of NO radicals in a dose dependent manner. It is also known that ROS mediates peroxidation of lipid structures of the tissue, resulting in subcellular damage, as observed in histopathological examination[24,85].

Table (3): Effect of curcumin (15 & 30 mg/B Wt) treatment on serum malondialdehyde and kidney GSH content & G_{px}, CAT and SOD activities in cyclosporine-nephrotoxic rats

Groups / Parameters		Control	Recovery	Cyclosporine +Curcumin 15 mg	Cyclosporine +Curcumin 30 mg
MDA (nmol /dL)	**15 days n = 5**	0.539±0.009[a]	0.862±0.017[b]	0.761±0.016[c]	٠.652±0.012[d]
	30 days n = 5	0.537±0.009 [a]	0.759±0.015[c]	0.703±0.014[e]	0.589±0.013[f]
GSH (mg/g protein)	**15 days n = 5**	11.211±0.702 [a]	7.911±0.512[b]	8.802±0.519[c]	9.401±0.557[d]
	30 days n = 5	11.189±0.694 [a]	8.795±0.527[c]	9.392±0.568[d]	10.378±0.632[e]
Gpx (µmol GSH utilized/min/g protein)	**15 days n = 5**	23.671±0.918 [a]	17.549±0.803[b]	19.562±0.856[d]	20.493±0.917[e]
	30 days n = 5	23.958±0.932 [a]	18.827±0.798[c]	21.917±0.902[f]	23.714±0.898[a]
CAT (nmol/60 min/ mg protein)	**15 days n = 5**	42.398±1.112 [a]	36.817±1.032[c]	38.117±1.105[c]	39.627±1.176[d]
	30 days n = 5	42.711±1.136[a]	38.032±1.032[b]	40.718±1.148[e]	42.451±1.213[a]
SOD (Nu/60 min/mg protein)	**15 days n = 5**	5.113±0.221[a]	3.917±0.192[b]	4.539±0.201[d]	4.792±0.198[e]
	30 days n = 5	5.122±0.218 [a]	4.202±0.189[c]	4.917±0.211[f]	5.117±0.226[a]

- Values are expressed as mean ± SE. *- n= number of rats.*

- [a, b, c ,d ,e ,f] Means with a common subscript within an each parameter are not significantly different (P>0.05).

In conclusion, this study demonstrated that CMN through its marked antioxidant activity coupled with favorable hemodynamic effects salvages CsA nephrotoxicity depending on the dose and time of treatment.

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أحداث الفشل الكلوي والعبأ التأكسدي في كلي الجرذان بواسطة السيكلوسبورون

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** المعهد القومي للكبد والأمراض المتوطنة –جامعة القاهرة

يعتبر الكركوم احد النباتات التي تمتاز بأهمية طبية وأقتصادية عالية. ولقد أوضح لنا الطب القديم الدور الفعال الذي يلعبه الكركوم في علاج أضطرابات المرارة والكحة و البول السكري وأضطرابات الكبد و الألتهاب المفصلي. وقد تم تصميم هذا البحث لدراسة الدور الفعال للكركوم للتقليل وكذلك منع الفشل الكلوي والعبأ التأكسدي المحدث نتيجة المعالجة بالسيكلوسبورن في الجرذان.

وقد تم تقسيم الدراسة الي تجربتين ، الأولي نفذت لتتبع التغير في وظائف الكلي الناجم عن المعالجة بالسيكلوسبورون. ولقد أظهرت المعالجة بالسيكلوسبورون أرتفاعا ذا قيمة أحصائية في مستوي البولينا والكرياتنيين والبوتاسيوم و والباراثهرمون و ثنائي ألدهيد المالون والدايميثيل أرجينين . بينما أحدث السيكلوسبورون أنخفاضا في مستوي الصوديوم والنيتريك أكسيد ومستوي الجلوتاثيون ونشاط أنزيم الجلوتاثيون فائق الأكسدة والكتاليز والسبروكسد ديسميتويز بالمقارنة بالمجموعة الضابطة.

وبالنسبة للتجربة الثانية تم معالجة الجرذان المصابة بالفشل الكلوي بالكركوم ولقد حدث تحسن ملحوظ في كل القياسات السابقة.

ولذلك فقد أوضحت الدراسة مدي فائدة المعالجة بالكركوم لمنع الفشل الكلوي والعبأالتأكسدي المسبب نتيجة المعالجة بعقار السيكلوسبورون في الجرذان.