Protective Effect of Lycopene against Nephrotoxicity Induced by Cyclosporine in Rats

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Abstract: Effects of lycopene (Lyc) on the nephrotoxicity induced by cyclosporine (CsA) were investigated. After injection of CsA (15 mg kg^{-1} day^{-1} i.p for 10 days), it induced nephrotoxicity, manifested biochemically by a significant elevation of serum urea and creatinine. In addition, a marked increase in lipid peroxides measured as malondialdehyde (MDA) as well as a significant decrease in enzyme activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). As well as reduction of glutathione content (GSH) in kidney tissues homogenate were observed. Nephrotoxicity was further confirmed by histopathological investigation. Oral administration of Lyc (40 mg kg^{-1} day^{-1} p.o) for 5 days before and 10 days concomitant with CsA injection produced a significant protection against nephrotoxity induced by CsA. The amelioration of nephrotoxicity was evidenced by significant reductions in serum urea and creatinine concentrations. In addition, Lyc prevented the rise of MDA as well as reduction in the enzyme acitivities of GSH-Px and SOD. Also, It restored reduced GSH content in kidney tissue. The protective effects of Lyc against CsA-induced nephrotoxicity were further confirmed by histopathological examination. These results suggest that Lyc can ameliorate kidney dysfunction induced by CsA via oxidative stress mechanisms which involve the production of reactive oxygen species. Lyc may therefore be a beneficial remedy for CsA nephrotoxicity and can be used to improve the therapeutic index of CsA.
[Ali M. Gado, Abdel N. Ismail Adam and Badr A. Aldahmash. **Protective Effect of Lycopene against Nephrotoxicity Induced by Cyclosporine in Rats.** *Life Sci J* 2013;10(2):1850-1856] (ISSN: 1097-8135). http://www.lifesciencesite.com. 260

Key Words: lycopene, cyclosporine, oxidative stress, nephrotoxicity

1. Introduction

Cyclosporine is an immunosuppressive of choice in human organ transplantation. The clinical usage of CsA is however restricted due to both functional and structural changes in the kidney of transplant patients and experimental animals.[1,2,3] The renal damage consequent to CsA administration ranges from haemodynamics alteration to irreversible chronic lesion.[3] Consequently, there is a much great interest to increase its clinical usefulness by developing new agents in order to reduce its toxicity.[2]

The mechanism of nephrotoxicity induced by CsA is thought to be due to afferent arteriolar vasoconstrictor effect and consequently decreased extremely glomerular filtration rate.[4] CsA administration produces an acute decrease in renal cortical and medullary blood flow.[5] Chronic CsA administration leads to loss of proximal tubular epithelial integrity with secondary fibrosis.[6] In addition, Cyclosporine induces structural changes due to direct vasoconstrictive effects with subsequent ischemia. The early medullary changes such as tubule-interstitial proliferation and macrophage infiltration correlate with the decreased urinary concentrating ability and elevated creatinine level.[2] The initial vasoconstriction induced by CsA depends on the imbalance between the various modulators of the renal vascular tone, among which, the most powerful; endothelins and nitric oxide.[7] However, lipid peroxidation and free radical generation in the tubular cells have been suggested to be responsible for the kidney damage.[8,9]

Lycopene is one of the most potent carotenoid antioxidants . It is naturally found in many plant foods, being especially abundant in tomatoes.[10,11] Lycopene is not synthesized by humans and thus must be obtained through dietary consumption. According to recent reports, lycopene is associated with decreased incidence of many important diseases including cancer, atherosclerosis, age-related macular degeneration and multiple sclerosis, probably via prevention of lipid peroxidation.[12] Lycopene is most likely involved in the scavenging of two reactive oxygen species, singlet molecular oxygen and peroxyl radicals, contributing to the defence against lipid peroxidation.[13] The data have shown that this carotenoid is able to protect against cisplatine-induced oxidative damage in kidney of rats [14] and iron-induced oxidative damage in bowel and prostate of rats.[115,16]

Therefore the present work was conducted to

investigate the possible protective effect of Lyc on nephrotoxicity induced by CsA. This aim will be achieved by study the effect of CsA on some biochemical parameters related to renal functions. Furthermore, the possible effect of Lyc on CsA-induced kidney injury will be investigated.

2.Materials and Methods:

Chemicals:

Cyclosporine was purchased from Sandoz Pharma Ltd, (Basle, Switzerland), Lyc was supplied from Shanxi Sciphar Co, Ltd, and thiobarbituric acid (TBA) was a product of Fluka (Buchs, Switzerland). All the remaining chemicals were of the highest grade commercially available.

***Animals*:**

Male Swiss albino rats weighing 150-200 g were used in all experiments. Animals were maintained under standard conditions of temperature & humidity with regular light/dark cycle and allowed free access to food (Purina Chow) and water. All animal experiments were conducted according to the regulations of the Committee on Bioethics for Animal Experiments of Riyadh colleges of dentistry and pharmacy.

Animal Treatments:

The animals were divided at random into four groups of 10 animals each. The first group (control) received vehicles used for CsA (physiological saline solution, i.p). The second group, Lyc received by oral gavage (40 mg kg^{-1} day^{-1}) (1 ml/kg body weight) .[11] The third group, was injected with CsA (15 mg kg^{-1} day^{-1} i p) for 10 days.[17] The fourth group, received Lyc by oral gavage (40 mg kg^{-1} day^{-1}) for five days then injected with CSA(15 mg kg^{-1} day^{-1} i.p) for 10 days concomitantly with L-yc by oral gavage (40 mg kg^{-1} day^{-1}).

One day later, blood samples were taken by cardiac puncture, under light ether anesthesia, into non-heparinized tubes. Serum was separated by centrifugation for 5 min at 4000 rpm and stored at -20°C until analysis. Animals were sacrificed by cervical dislocation and the kidneys were quickly isolated, washed with saline, blotted dry on filter paper, weighed, and then 10% (w/v) homogenate of the left kidney was made in ice cold saline.

Measurement of serum biochemical parameters:

Serum creatinine and urea concentrations were determined colorimetrically as described by Bartles *et al.,* 1972 and Patton & Crouch,1977 respectively, using commercially available diagnostic kits (bioMérieux-RCS Lyon-France).[18,19]

Determination of lipid peroxides, glutathione content and enzyme activities of Glutathione peroxidase and superoxide dismutase in kidney homogenate:

Glutathione contents and lipid peroxidation (Malondialdhyde (MDA) production) in the kidney tissues were determined according Ellman,1959 and Ohkawa *et al.,* 1979 respectively.[20,21] The *enzyme activity of* Glutathione peroxidase (GSH-Px) and superoxide dismutase were measured in the kidney homogenates according Kraus and Ganther ,1980, Misra and Fridovich, 1972 respectively .[22,23]

Histopathology:

Histopathological examination was performed on the animals of each group. Right kidney samples were taken. The tissues were fixed for at least 48 hours in 10% formalin in phosphate buffer (pH 7). The samples were then embedded in paraffin wax, cut into 5 µm sections, and stained with hematoxylin-eosin. The slides were coded and were examined by histopathologist who was unaware about the treated groups. The nephrotoxic histological features were graded as follows:

I. Low grade tubular necrosis- small predominantly subcapsular foci of the tubular necrosis occupying and seen in up to 2 low power light microscopic fields-mark given 1+
II. High grade tubular necrosis-multiple and larger cortical medullary foci of tubular necrosis occupying more than 2 low power light microscopic field-Mark given 2+
III Interstitial nephritis 1+

The marking system used was according to the following:-

A. No nephrotoxic effect: 0+ only
B. Mild nephrotoxic effect:1+ only
C. Moderate nephrotoxic effect: 2+ only
D. Severe nephrotoxicity effect:3+ only

Different sections from kidney tissues were taken and examined, since the necrosis of the tubular cells is coagulative and the damage was unevenly distributed. The sections which are selected can show the renal damage for examination by light microscope.

Statistical Analysis

Data are expressed as (means±SEM). Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test to judge the difference between various groups. Significance was accepted at $p< 0.05$.

3.Results:

Effects of Lyc on CsA-induced changes in serum biochemical parameters:

Serum urea and creatinine were significantly increased in comparison with the control group

(P<0.001) after injection of CsA reaching 102 ± 6.5 and1.14 ±. 0 1mg% respectively as compared with the control group. . Pretreatment of animals with Lyc (40 mg kg-1 day-1 p.o) five days before and concomitantly with CsA markedly reduce significantly the rise in the level of serum urea and creatinine. (Figs.1,2).

Oxidative Stress Biomarkers

Figs. 3 and 4 show the effects of CsA , Lyc and their combination on oxidative stress biomarkers namely thiobarbituric acid reactive substance (MDA) and GSH in renal tissues respectively. CsA resulted in a significant 25% decrease in GSH and a significant 35% increase in TBARS as compared to the control group. Combined CsA treatment with Lyc decreased significantly MDA (P<0.001) and restore GSH level in renal tissues compared to the control values.

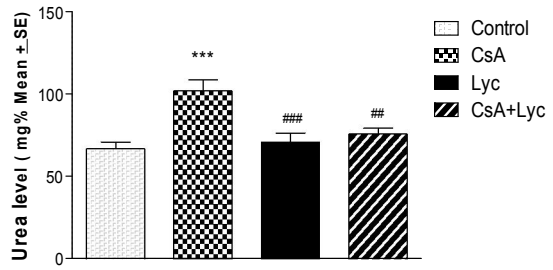
ANTIOXIDANT ENZYME ACTIVITIES

Figs. 5 and 6 show the effects of CsA , Lyc and their combination on the activity of antioxidant enzymes Gpx and SOD in renal tissues respectively. CsA resulted in a significant decrease in both Gpx and SOD enzyme activities as compared to the control gro (P<0.001 and P<0.001 respectively). Combined CsA treatment with Lyc improve significantly both enzymes activity (P<0.001) in renal tissues compared to the control values.

Kidney Pathology

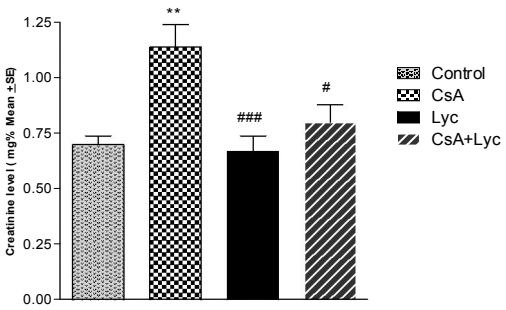
Pathological examination of the kidneys of control, and Lyc groups showed non-significant changes (Figure H1). However, animals treated with CsA showed vacuolation and focal necrosis of tubular lining and interstitial cell infiltration (Figures H2,H3). On the other hand, consistent with the biochemical results tubular injury was markedly reduced in Lyc plus CsA group. Co-administration of Lyc and CsA produced minimal interstitial nephritis and slightly vacuolated but otherwise normal tubular cells (Fig. H4).

Fig (1) Effects of Lyc on elevated leve of serum urea induced by CsA



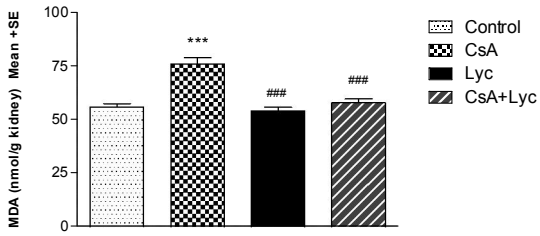
Lyc (40 mg/kg/day p.o) was given for 5 day before and concomitant with CsA (150mg/kg i.p.) .
· Significantly different from control group # Significantly different from CsA
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (2) Effects of Lyc on elevated leve of serum creatinine induced by CsA



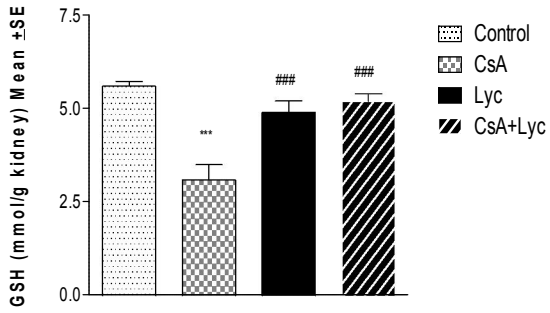
Lyc (40 mg/kg/day p.o) was given for 5 day before and concomitant with CsA (150mg/kg i.p.) .
· Significantly different from control group # Significantly different from CsA
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (3) Effect of CsA, Lyc and their combination on the levels of thiobarbituric acid reactive substance (MDA) in rat renal tissues



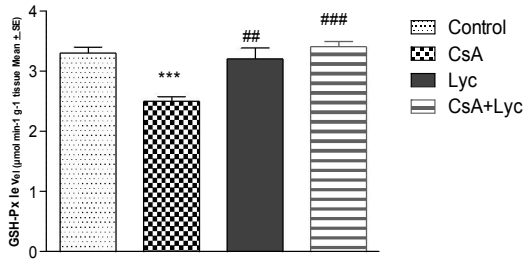
Lyc (40 mg/kg/day p.o) was given for 5 day before and concomitant with CsA (150mg/kg i.p.) .
· Significantly different from control group # Significantly different from CsA
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (4) Effect of CsA, Lyc and their combination on the levels of reduced glutathione in rat renal tissues.



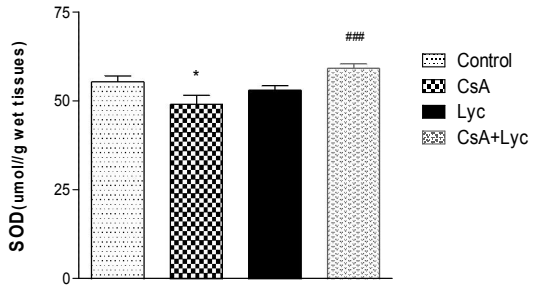
Lyc (40 mg/kg/day p.o) was given for 5 day before and concomitant with CsA (150mg/kg i.p.) .
· Significantly different from control group # Significantly different from CsA
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (5) Effects of Lyc on changes in GSH-Px enzyme activities induced by CsA



Lyc (40 mg/kg/day p.o) was given for 5 day before and concomitant with CsA (150mg/kg i.p.) .
· Significantly different from control group # Significantly different from CsA
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (6) Effect of CsA, Lyc and their combination on the level of superoxide dismutase (SOD) activity in rat renal tissues.



Lyc (40 mg/kg/day p.o) was given for 5 day before and concomitant with CsA (150mg/kg i.p.) .
· Significantly different from control group # Significantly different from CsA
#* P<0.05 ##** P<0.01 ###*** P<0.001

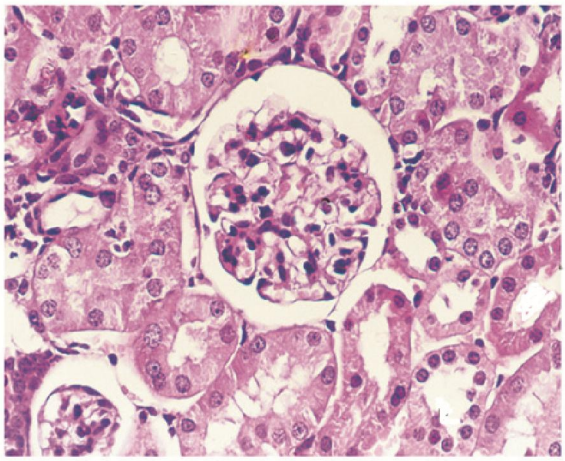


Fig. H 1: A photomicrograph of renal cortex of a control rat showing normal glomeruli , proximal convoluted tubules and distal convoluted tubules . (H&E...x400)

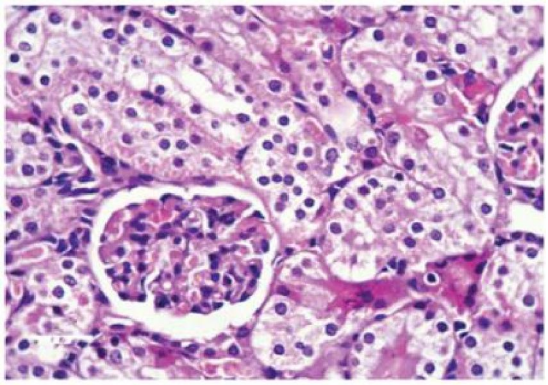


Fig. H2: A photomicrograph of renal cortex of a CsA-treated rat showing necrotic changes of the renal tubular cells in addition to cytoplasmic vacuolation (H&E...x400)

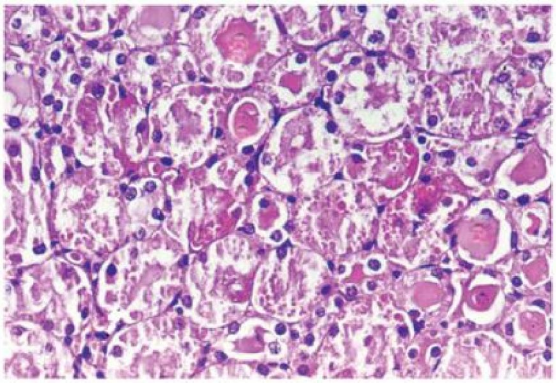


Fig. H3: A photomicrograph of renal cortex of a CsA - treated rat showing desquamated epithelial cells in the lumina of the tubules and intratubular casts (H&E...x400).

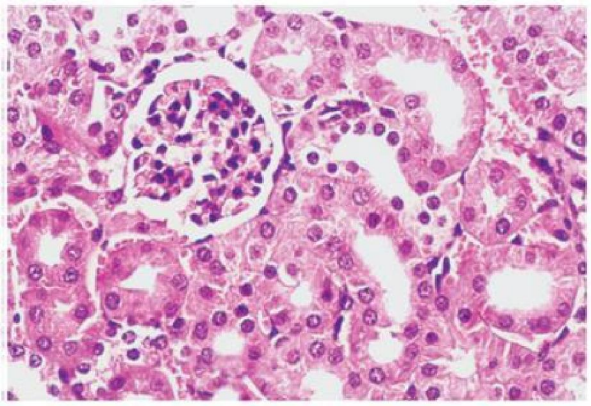


Fig. H4: A photomicrograph of renal cortex of a Lyc and CsA treated rat showing nearly similar appearance to that of the control group. (H&E...x400)

4.Discussion

The present investigation revealed that administration of CsA (15 mg kg^{-1} day^{-1} i.p. for 10

days) resulted in an overt nephrotoxicity as evidenced in the serum by the marked elevations of urea and creatinine concentrations. In addition, nephrotoxicity was also reflected in the kidney as the depletion of reduced glutathione content which is associated with marked reduction in the activities of glutathione peroxidase, glutathione superoxide dismutase enzymes and significant elevation of lipid peroxide. The nephrotoxic potential of CsA, however, limits its clinical use.[24] Consequently, there is much great interest in developing new methods to abrogate renal damage by using a combination of various agents with CSA.[25]

In the present study, treatments of rats with Lyc (40 mg kg^{-1} day^{-1}, p.o) produced significant changes in the measured biochemical parameters. However, pretreatment of rats with Lyc (40 mg kg^{-1} day^{-1} p.o) five days before and concomitantly with CsA administration renders rats less susceptible to kidney damage induced by treatment with CsA. This protection was evidenced in the serum as the elevated level in both urea and creatinine were markedly lowered below those elicited by the nephrotoxicant. In addition, Lyc greatly ameliorated reduced level in GSH-Px activity, reduced GSH content in kidney tissue and prevented the rise in lipid peroxides in kidney tissues. These findings may indicate a possible protective effect of Lyc against neprhotoxicity induced by CsA treatment.

CsA is one of the most common nephrotoxic drug.[4] It could lead to oxidative stress. So we have been trying to find out the protective role o Lyc against oxidative stress. Results of previous investigators showed that kidney damage caused by CsA decrease the level of GSH.[17] The mechanism of this CsA -induced change in renal GSH level is not completely understood. However, GSH may modulate chemical reduction, and the thiol portion is very reactive with several compounds. In response, GSH is increased to resist the injury derived from oxidative stress. Pre-treatment with natural antioxidants such Lyc may reduce the burden of GSH. We can therefore find the depletion of GSH in renal cortex. Apart from protective antioxidants, for example GSH, the antioxidant enzymes reducing free radical scavenging, such as SOD and GSH-px, also protect kidney from CsA induced nephrotoxicity.[26]

A key issue that we have been trying to address in our ongoing research is to identify primary molecular mechanisms that are involved in the stages of CsA induced renal damage. Taken together, the results of the present studies strongly indicate that the accumulation of CsA in kidney can damage renal mitochondria and generate ROS.This is in accordance with other studies.[3] Excessive ROS and the resulting oxidative stress play a pivotal role in apoptosis and the kidney is therefore injured. Renal damages caused by CsA are inferred from the morphological changes in renal sections.[27]

The antioxidant enzymes represent a first line of defense against toxic reactants by metabolizing them to innocuous byproducts.[28,29] When cells are exposed to oxidative stress they increase the activity and expression of antioxidant enzymes as a compensatory mechanism to better protect them from the damage. Moderate levels of toxic reactants induce rises in antioxidant enzymes while very high levels of reactants reduce enzyme activities as a result of damage of the molecular machinery that is required to induce these enzymes.[30]

In normal condition, per-oxidation injuries will promote anti-oxidation adaptation within human body. SOD and GPx are two different anti-oxidation enzymes major in the cytosol of living cells. We measured these two enzymes from renal tissue following the protocols of Randox, respectively. Function of intracellular GPx is degradation of H_2O_2 and hydroperoxides of free fatty acids, whereas in plasma GPx catalyses degradation of H_2O_2 and hydroperoxides of phospholipids. In addition GPx exerts a protective effect on membrane phospholipids by inhibiting their peroxidation processes.[31] Changes in activity of antioxidant enzymes are accompanied by intensification of lipid peroxidation processes, which is confirmed by elevated MDA levels that we observed in rats receiving CsA. In quantitative terms, MDA is the most important component among reactive aldehydes originating from lipid peroxidation. For this reason, it is commonly considered as an index of oxidative stress severity.[32]

As an effective free radical scavenger, Lyc is the most efficient biological carotenoid, being 10 folds, 47 folds and 100 folds more effective in quenching singlet oxygen than alpha tocopherol, beta carotene and vitamin E respectively.[33,34,35] Lyc removes free radicals produced by Hg in physical and chemical ways, while improving the body's antioxidant enzymes such as SOD, GSHpx to prevent the oxidative damage caused by CsA, enhancing the body antioxidant capacity, reducing the levels of lipid peroxidation and maintaining cell membrane permeability.

This findings is in harmony with the previous finding of Mansour *et al.,*2002 demonstrating that exogenous supplementation of antioxidant is effective in reducing renal damage induced by CsA, possibly through NO pathway.[17] In addition the previous reports showed that oral supplementation of L-arg prevents nephrotoxicity induced by chronic administration of CsA due to formation of more NO which may enhance vasodilatation and consequently reduce the impairment kidney function .[36,37]

The rational for Lyc dose schedule in this study was to maintain sufficient plasma concentration before, during and after the critical periods of CsA induced renal damage. Since the biochemical changes that occure in the kidney after CsA administration are of crucial importance in determining the extent of nephrotoxicity.[17]

In conclusion, CsA induced renal damage involves oxidative stress triggered by ROS generation. In our study the Lyc pretreatment was performed with the aim of raising the levels of both SOD and GPx thus strengthening the antioxidant defenses. Lyc is considered as a highly promising agent in protecting renal tissues against CsA induced renal damage.

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References

1. Andoh TF; Gardner MP; Bennett WM. Protective effects of dietary L-arginine supplementation on chronic cyclosporine nephrotoxicity. *Transplantation*. 1997; **64**: 1236-40.
2. Naidu MU; Kumar JV; Shifow AA, Prayag A, Ratnakar KS. Lacidipine protects against cyclosporine induced nephrotoxicity in rats. *Nephron* 1999; **81**: 60-6.
3. O'Connell S, Tuite N, Slattery C, Ryan MP, McMorrow T. Cyclosporine A—induced oxidative stress in human renal mesangial cells: a role for ERK 1/2 MAPK signaling. Toxicol Sci. 2012;126(1):101-13.
4. Bloom IT; Bentley FR; Spain DA, Garrison RN. An experimental study of altered nitric oxide metabolism as a mechanism of cyclosporine induced renal vasconstriction. *Br. J. Surg*. 1995; **82**: 195-8.
5. Bobadilla NA; Tapia E; Franco M, Lopez P, Mendoza S, Garcia-Torres R, Alvarado JA, Herrera-Acosta J. Role of nitric oxide in renal hemodynamic abnormalities of cyclosporine nephrotoxicity. *Kidney Int*. 1994; **46**: 773-9.
6. Keown PA; Stiller CR: Cyclosporine-allograft rejection: In Massry S6; Glassack RJ (eds): Textbook of nephrology, 2nd ed. Baltimore, Williams & Wilkins. 1995; **2**: 1648-1655.
7. Amore A; Gianglio B; Ghigo D, Peruzzi L, Porcellini MG, Bussolino F, *et al.* A possible role for nitric oxide in modulating the functional cyclosporine toxicity by arginine. *Kidney Int*. 1995; **47**: 1507-14.
8. Serino F; Grevel J; Napoli KL, Kahan BD, Strobel HW. Oxygen radical formation by the cytochrome P450 system as a cellular mechanism for cyclosporin toxicity. *Transplant Proc*. 1994; **26**: 2916-7.
9. Wolf A; Cleman N; Frieauff W, Ryffel B, Cordier A. Role of reactive oxygen formation in the cyclosporine mediated impairment of renal functions. *Transplant Proc*. 1994;**16**: 2902-7.
10- Agarwal A, Shen H, Agarwal S, Rao AV. Lycopene content of tomato products: its stability, bioavailability and *in vivo* antioxidant properties. J Med Food 2001;**4**:9–15.
11- Augusti PR, Conterato GM, Somacal S, Einsfeld L, Ramos AT, Hosomi FY, Graça DL, Emanuelli T. Effect of lycopene on nephrotoxicity induced by mercuric chloride in rats. Basic Clin Pharmacol Toxicol. 2007;100(6):398-402.
12- Mortensen A, Skibsted LH, Truscott TG. The interaction of dietary carotenoids with radical species. Arch Biochem Biophys 2001;**385**:13–9.
13- Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspects Med 2003;**24**:345–51.
14- Atessahin A, Yilmaz S, Karahan I, Ceribarsi AO, Karaoglu A. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. Toxicology 2005;**212**:116–23.
15- Reifen R, Nissenkom A, Matas Z, Bujanover Y. 5-ASA and lycopene decrease the oxidative stress and inflammation induced by iron in rats with colitis. J Gastroenterol 2004;**39**:514–9.
16- Matos HR, Marques SA, Gomes OF *et al.* Lycopene and betacarotene protect *in vivo* iron-induced oxidative stress damage in rat prostate. Braz J Med Biol Res 2006;**39**:203–10.
17- Mansour M, Daba MH, Gado A, Al-Rikabi A, Al-Majed A. Protective effect of L-arginine against nephrotoxicity induced by cyclosporine in normal rats. Pharmacol Res. 2002 , Jun;45(6):441-6.
18- Bartles H, Bohmer M, Heieri C. Serum kreatininbestimmung ohne Enteiweissen. *Clin. Chim. Acta* 1972; **37**: 193-7.
19- Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem*. 1977; **49**: 464-9.
20- Ellman GL. Tissue Sulfahydryl groups. Arch. Biochem. Biophys. 1959; **82**: 70-7
21- Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in normal tissues by thiobarbituric

acid reaction.*Anal. Biochem.*1979; **95**:351-8.
22- Kraus RJ, Ganther HE. Reaction of cyanide with glutathione peroxidase. *Biochem Biophys Res Commun*. 1980; **16**: **96(3)**:1116-22.
23-Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxidedismutase. J Biol Chem 1972;**247**:3170–5.
24-Fujinaga S, Hirano D, Murakami H, Ohtomo Y, Shimizu T, Kaneko K. Nephrotoxicity of once-daily cyclosporine A in minimal change nephrotic syndrome. Pediatr Nephrol. 2012 Apr;27(4):671-4. doi: 10.1007/s00467-011-2076-6.
25- Zhu S, Wang Y, Chen M, Jin J, Qiu Y, Huang M, Huang Z. Protective effect of schisandrin B against cyclosporine A-induced nephrotoxicity in vitro and in vivo. Am J Chin Med. 2012;40(3):551-66.
26- Duru M, Nacar A, Yönden Z, Kuvandik G, Helvaci MR, Koç A, Akaydin Y, Oksüz H, Söğüt S. Protective effects of N-acetylcysteine on cyclosporine-A-induced nephrotoxicity. Ren Fail. 2008;30(4):453-9.
27-de Arriba G, Calvino M, Benito S, Parra T. Cyclosporine A-induced apoptosis in renal tubular cells is related to oxidative damage and mitochondrial fission. Toxicol Lett. 2013 Mar 27;218(1):30-8.
28- Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res. 2004 Jan;36(1):1-9.
29- Eşrefoglu M, Gül M, Dogru MI, Dogru A, Yürekli M. Adrenomedullin fails to reduce cadmium-induced oxidative damage in rat liver. Exp Toxicol Pathol. 2007 Apr;58(5):367-74.
30- Pillai A, Gupta S. Antioxidant enzyme activity and lipid peroxidation in liver of female rats coexposed to lead and cadmium: effect of vitamin E and Mn2+. Free Radic Res 2005;39:707–712.
31-Rybus-Kalinowska B, Zwirska-Korczala K, Kalinowski M, Kukla M, Birkner E, Jochem J. Activity of antioxidative enzymes and concentration of malondialdehyde as oxidative status markers in women with newly diagnosed Graves-Basedow disease and after thiamazole therapy leading to euthyroidism. Pol Arch Med Wewn. 2008 Jul-Aug;118(7-8):420-5.
32- Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among vitamin C, vitamin E, and beta-carotene. Am J Clin Nutr. 1995 Dec;62(6 Suppl):1322S-1326S.
33-.Sandhir R, Mehrotra A, Kamboj SS. Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system. Neurochem Int, 2010; 57(5), 579-87.
34.Stahl W, Sies H. Carotenoids and protection against solar UV radiation. Skin Pharmacol Appl Skin Physiol, 2002; 15(5), 291-6.
35. Breinholt V, Lauridsen ST, Daneshvar B, Jakobsen J. Dose-response effects of lycopene on selected drug-metabolizing and antioxidant enzymes in the rat. Cancer Lett. 2000 Jun 30;154(2):201-10.
36- De Nicola L, Thomson SC, Wead LM, Brown MR, Gabbai FB. Arginine feeding modifies cyclosporine nephrotoxicity in rats. J. Clin. Invest. 1993; 92: 1859-65.
37- Assis SM, Monteiro JL; Seguro AC. L-arginine and allopurinol protect against cyclosporine-nephrotoxicity. *Transplantation* 1997; **63**: 1070-3.

5/15/2013