

Experimental study of renal toxicity of cyclosporine and the ameliorative effect of N-acetylcysteine in albino rat

Mai M. Abdelkader¹, Maha A. Hilal¹, Abdel Rahman Torky², Hoda M. Elsayed³ and Walaa A. Allam¹

¹ Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Sohag University, Sohag, Egypt.

² Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Helwan University, Cairo, Egypt.

³ Department of Histology, Faculty of Medicine, Sohag University, Sohag, Egypt.

Abstract

Introduction: Cyclosporine A (CsA) is considered powerful immunosuppressive drug which has improved the quality of life and survival rate of transplant patients and also used in autoimmune diseases. However, its use is limited by many side effects mainly nephrotoxicity. NAC is an antioxidant found to reduce CsA toxicity. **Aim of the work:** The study aims to determine the effect of exposure to cyclosporine on the kidney and to investigate the protective role of NAC. **Methods:** the study conducted on 50 adult male albino rats for 4 weeks, divided into 5 groups, group A the negative control group, group B the olive oil group (0.5 ml/d orally), group C the cyclosporine group (25mg/kg/d orally), group D the NAC group (600mg/kg/d orally) and group E the cyclosporine+NAC group. At the end of the study the evaluation was done by biochemical analysis and histopathology. **Results:** cyclosporine significantly affects the kidney by morphological changes in the form of dilatation of urinary space with congestion and lobulation of glomerular capillaries in the renal corpuscle. Proximal convoluted tubules showed degeneration of their cells with irregularity and destruction of brush border. Degeneration of distal convoluted tubules with exfoliation of some cells inside the lumen and the peritubular capillaries were congested and extravasated. Also cyclosporine affects the kidney by increasing serum urea and creatinine levels, while co-administration of NAC with cyclosporine attenuate its effects. **Conclusion:** cyclosporine causes renal injury through oxidative stress and NAC as an antioxidant attenuates but not fully protect against cyclosporine induced injuries.

Key words cyclosporine, N-acetylcysteine, nephrotoxicity, histopathology

Introduction

Organ transplantation is an important progress in the medical field that has extended human life. Also, immunosuppressive drugs are used to decrease organ rejection (Lu et al., 2020).

There are many immunosuppressive agents used in transplant medicine one of them is cyclosporine A (CsA) which is a neutral agent that is derived from *Cylindrocarpum lucidum* Booth and *Tolypocladium inflatum* Gams fungi. Cyclosporine is used as an antibiotic, antifungal and antiparasitic agent (Fahr, 1993 & Alazouny et al., 2014).

Additionally, cyclosporine is used in the treatment of various autoimmune disorders such as rheumatoid arthritis idiopathic nephritic syndrome, psoriasis, uveitis and inflammatory bowel disease (Najafi et al., 2016).

T lymphocytes are responsible for the immune response to a transplanted organ. Cyclosporine A suppress the synthesis of many cytokines as interleukin IL-2, IL-4, interferon- γ and granulocyte-macrophage colony stimulating factor which are responsible of T cells activation (Damaino et al., 2015).

The immune and inflammatory reactions are also regulated by many cytokines such as interleukins 1a and 1b, interleukin6, gamma-interferon and other

lymphokines that can be suppressed by cyclosporine to decrease graft rejection (Lee, 2010).

However, the use of cyclosporine is accompanied by several adverse effects such as nephrotoxicity, hepatotoxicity, malignancies, increased risk of cardiovascular events, hypertension, dyslipidemia, gingival hyperplasia and hypertrichosis (Rezzani, 2006).

The exact mechanism of chronic CsA toxicity has not been fully defined. By Experimental studies it was found that CsA enhances ROS formation. Furthermore, cyclosporine causes cell membrane and mitochondrial degeneration by increasing calcium influx into the cell which leads to oxidative stress and apoptosis. Also, CsA stimulates the release of some vasoactive mediators as angiotensin II, thromboxane and endothelin, that increase renal vascular resistance leading to renal hypoperfusion and injury (Farag et al., 2015).

Cyclosporine A increases ROS formation which can affect many intracellular molecules as unsaturated fatty acids and transmembrane proteins. The oxidation of these molecules leads to increased cellular membrane permeability with disruption of many membrane functions and metabolic processes. Kidney dysfunction is the most common complication of CsA usage as about 30% of patients treated with CsA have moderate to severe kidney affection (cid et al., 2003).

Cyclosporine A affects the kidney by decrease in creatinine clearance and increase creatinine and blood urea and potassium levels. Also, it causes renal tubular acidosis (*Patel & Kobashigawa, 2004*).

Cyclosporine A nephrotoxicity can be acute or chronic. In the acute form there is renal vasoconstriction due to increased vasoactive mediator release. While, the chronic nephrotoxicity is characterized in addition by the development of structural damage, as arteriopathy and tubulointerstitial fibrosis, these changes are irreversible that can lead to end-stage renal disease (*Bobadilla & Gamba, 2007*).

There are many mechanisms responsible for cyclosporine nephrotoxicity including the activation of the renin-angiotensin system, enhancement of sympathetic tone, increased formation of endothelin, activation of cytochrome P450 enzymes in renal microsomes, and renal vasoconstriction. In addition, oxidative stress plays an important role in producing structural and functional renal affection (*Mostafa-Hedeab et al., 2015*).

The vascular dysfunction caused by CsA is due to the imbalance between the vasoactive factors such as thromboxane and endothelin, and the vasodilator factors such as NO, prostacyclins and prostaglandin E2 (*Shahbazi et al., 2020*).

Additionally, chronic cyclosporine nephrotoxicity is mediated by many other mediators such as transforming growth factor beta (TGF beta), platelet derived growth factor (PDGF) and tumour necrosis factor alpha (TNF-alpha) (*Fellstrom, 2004*).

As ROS plays an important role in CsA induced toxicity, antioxidants have been used to ameliorate the damages induced by CsA (*Damiano et al., 2015*).

In experimental studies on acute CSA nephrotoxicity, antioxidants such as vitamin E, melatonin and carvedilol were found to improve renal function (*Burdmann et al., 2003*).

N-acetyl-L-cysteine (NAC) is a powerful antioxidant as its usage increase glutathione formation to detoxify oxygen derived free radicals (ODFR) and other foreign substances. Also NAC improve tissue perfusion through decreasing vascular resistance (*Tariq et al., 1999 & Kaya et al., 2008*).

So this study was done to determine the effect of exposure to cyclosporine on the kidney and to investigate the possible protective role of NAC.

Materials and Methods:

Animals

The present work was conducted on 50 sexually mature male albino rats purchased from animal house, faculty of medicine Assiut University. Their weight ranged from (180:220) gm at the beginning of the experiment. The animals were housed in stainless metal cages in a ventilated animal room under ambient temperature, 21 ± 3 °C. Animals were fed with standard pellet feed and water. They were

acclimatized to the laboratory condition for one week before starting the treatment protocol. The protocol of ethics and husbandry conditions of animal research were considered and the study was approved by the ethical committee of Faculty of Medicine, Sohag University.

Chemicals

- 1) Cyclosporine A, cyclosporine in the form of soft gelatin capsules with a commercial name (Sandimmune, Neoral)® purchased from Novartis pharma and freshly dissolved in olive oil.
- 2) N-acetyl cystein, NAC is water soluble powder, purchased from Pharco Co. Egypt, 25g /container.
- 3) Olive oil, from commercial market.
- 4) Kits of urea& creatinine, were purchased from Beckman Coulter Company for measurement of serum urea and creatinine.
- 5) Hematoxyline and Eosin stains, from ALPHACHEMIKA.

Animal groups and methods

Rats were divided into 5 groups 10 rats each

Group A: Negative control group, where animals received no treatment.

Group B: Positive control group where animals received olive oil with a dose of 0.5 ml / day orally by gavage for 4 weeks.

Group C: CsA-treated group, where animals were treated by CsA dissolved in olive oil with a dose of 25 mg/kg/day [double the therapeutic dose (*Feagan et al., 1994*)] orally by gavage for 4 weeks (*Zal et al., 2007*).

Group D: NAC-treated group, where animals were treated by NAC dissolved in water at a dose of 600 mg/kg/day orally by gavage for 4 weeks (*Saleh, 2014*).

Group E: Combined CsA and NAC by the same doses as mentioned in groups C&D orally by gavage for 4 weeks.

Collection of blood samples

Blood samples were collected before scarification from retro-orbital plexus into clean dry tubes. Blood samples then centrifuged [4000 Revolutions per minute (Rpm) for 5 minutes]. The serum was separated and transferred to sterile screw capped vials for measurement of serum urea and creatinine levels.

Histopathological examination

At the end of the study rats were sacrificed by cervical decapitation under anesthesia by ether. The kidneys for all animals were fixed in formalin 10% and paraffin embedding. Then specimens were embedded in blocks for sectioning at 5 micro thicknesses. After this sections were processed for staining with hematoxylin and eosin (H&E) stain. Sections of the kidney were examined then photographed.

Statistical Analysis

The data are demonstrated as mean \pm SD. Differences between groups were determined by one way analysis of variance (ANOVA) and a post-hoc test of least significant difference (LSD) was assessed by Statistical package for social sciences (SPSS) software, version 24 for windows. Probability value for significance (P values) less than 0.05 were considered to be statistically significant.

Results:

Biochemical results:

For urea and creatinine there was no significant changes in mean value of serum urea & creatinine between negative control group (group A) on one side and positive control (group B) & NAC treated group (group D) respectively on the other side as shown in table (1).

The mean value of serum urea and creatinine levels in cyclosporine treated group (group C) showed significant increase ($P < 0.05$) as compared to negative control group (group A), positive control group (group B) & NAC treated group (group D) as shown in table (2).

Combination of NAC with cyclosporine (group E) showed no significant increase in the mean value of serum urea compared to negative control group, positive control group and NAC treated group. While there was significant increase in serum creatinine ($P < 0.05$) compared to negative control group, positive control group and NAC treated group as shown in table (3).

As regard comparison of cyclosporine + NAC treated group (group E) with cyclosporine group (group C), there was significant decrease in the in the mean value of serum urea and creatinine levels ($P < 0.05$) as shown in table (4).

Table (1): Statistical analysis of serum Urea & Creatinine in the negative control group (A) compared to positive control group (B) and NAC treated group (D) using ANOVA

Parameters	Mean \pm SD			P-Value	
	Group A (Negative control)	Group B (Positive control)	Group D (NAC)	Negative control vs positive control	Negative control vs (NAC)
Urea (mg/dl)	38.75 \pm 0.975	38.00 \pm 2.88	36.20 \pm 2.58	0.547 NS	0.094 NS
Creatinine (mg/dl)	0.495 \pm 0.020	0.494 \pm 0.016	0.48 \pm 0.04	0.955 NS	0.531 NS

* $P < 0.05$ (significant), NS: Non significant, SD: Standard deviation

Table (2): Statistical analysis of serum Urea & Creatinine in the cyclosporine group (C) compared to negative control group (A), positive control group (B) and NAC treated group (D) using ANOVA:

Parameters	Mean \pm SD				P-value		
	Group C (Cyclosporine)	Group A (Negative control)	Group B (Positive control)	Group D (NAC)	Cyclosporine Vs Negative control	Cyclosporine Vs Positive control	Cyclosporine Vs NAC
Urea(mg/dl)	59.44 \pm 8.23	38.75 \pm 0.975	38.00 \pm 2.88	36.20 \pm 2.58	0.000*	0.003*	0.004*
Creatinine (mg/dl)	0.755 \pm 0.036	0.495 \pm 0.020	0.494 \pm 0.016	0.48 \pm 0.04	0.000*	0.000*	0.000*

* $P < 0.05$ (significant), SD: Standard deviation

Histopathological findings:

Light microscopic examination of the negative control group (group A), positive control group (group B) and NAC treated group (group D) showed normal histological appearance of the kidney in the form of renal corpuscles, proximal convoluted tubules, loop of henel, distal convoluted tubules and collecting tubules with minimal interstitial tissue in between. The renal corpuscles were composed of glomerular capillaries surrounded by Bowman's space and capsule. The proximal convoluted tubules (P.C.T) were lined with simple cuboidal epithelium with acidophilic cytoplasm and had a brush border. The distal convoluted tubule (D.C.T) lined with cuboidal cells but had a wider lumen than the P.C.T with no signs of congestion or inflammation as shown in figure (1, 2& 4).

Serial H&E stained sections from kidney of animals tested with cyclosporine (group C) showed dilatation of urinary space with congestion and lobulation of glomerular capillaries in the renal corpuscle. Proximal convoluted tubules showed degeneration of their cells with irregularity and destruction of brush border. Degeneration of distal convoluted tubules with exfoliation of some cells inside the lumen and the peritubular capillaries were congested and extravasated as shown in figure (3).

Examination of the cyclosporine+NAC treated group revealed milder histopathological changes compared to group C (treated with cyclosporin) expressed as narrower urinary space and less congested glomerular capillaries. Some of P.C.T & D.C.T were more or less similar to the control group, while other tubules were degenerated (fig 5).

Table (3): Statistical analysis of serum Urea & Creatinine in the cyclosporine + NAC treated group (E) compared to negative control group (A), positive control group (B) and NAC treated group (D) using ANOVA:

Parameters	Mean \pm SD				P-value		
	Group E (NAC + Cyclosporine)	Group A (Negative control)	Group B (Positive control)	Group D (NAC)	NAC+ Cyclosporine Vs Negative control	NAC+ Cyclosporine Vs Positive control	NAC+ Cyclosporine Vs NAC
Urea (mg/dl)	43.33 \pm 3.87	38.75 \pm 0.975	38.00 \pm 2.88	36.20 \pm 2.58	0.4 NS	0.14 NS	0.06 NS
Creatinine (mg/dl)	0.600 \pm 0.026	0.495 \pm 0.020	0.494 \pm 0.016	0.48 \pm 0.04	0.000*	0.000*	0.000*

*P: < 0.05 (significant), NS: Non significant, SD: Standard deviation

Table (4): Statistical analysis of serum Urea & Creatinine in the cyclosporine + NAC treated group (E) compared to cyclosporine group (C) using ANOVA:

Parameters	Groups	Mean \pm SD		P-Value
		Group E (NAC+Cyclosporine)	Group C (cyclosporine)	
Urea (mg/dl)		43.33 \pm 3.87	59.44 \pm 8.23	0.006*
Creatinine (mg/dl)		0.600 \pm 0.026	0.755 \pm 0.036	0.000*

*P: < 0.05 (significant), SD: Standard deviation

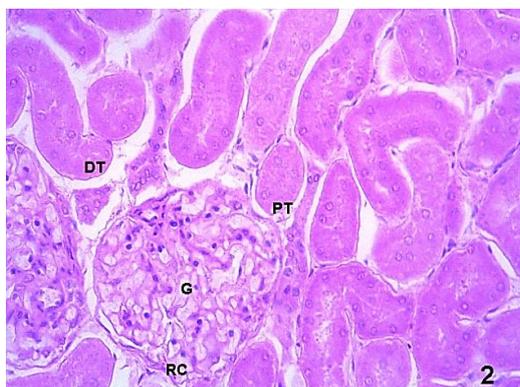


Fig. 1: A photomicrograph of a magnified section in the kidney of the negative control group showing normal renal corpuscle (RC) with narrow urinary space, glomerular capillaries (G), proximal tubules (PT) with narrow lumen and brush border and distal tubules (DT) with wide lumen. H&E stain X 400

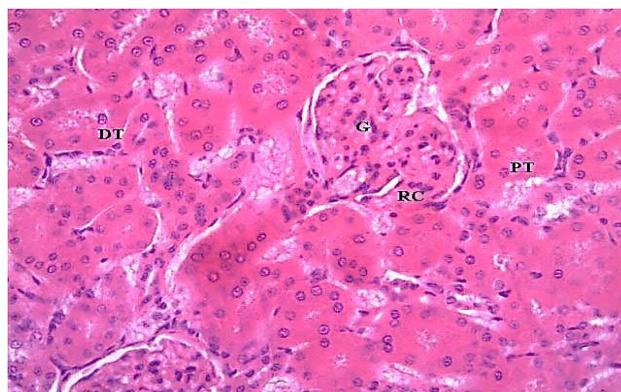


Fig. 2: A photomicrograph of a magnified section in the kidney of the positive control group showing renal corpuscle (RC) with narrow urinary space, glomerular capillaries (G), proximal tubules (PT) with narrow lumen and brush border and distal tubules (DT) with wide lumen similar to the negative control group. H&E stain X 400

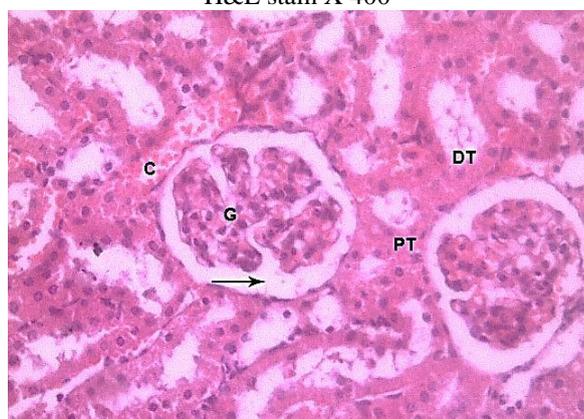


Fig. 3: A photomicrograph of a magnified section in the kidney of the cyclosporine group showing renal corpuscle with wide urinary space (arrow), congested glomerular capillaries (G), proximal tubules (PT) and distal tubules (DT) showing degeneration with congestion of peritubular capillaries (C). H&E stain X 400

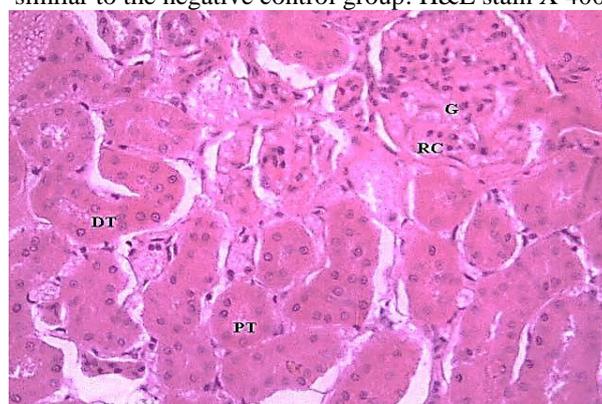


Fig. 4: A photomicrograph of a magnified section in the kidney of the NAC treated group showing renal corpuscle (RC) with narrow urinary space, glomerular capillaries (G), proximal tubules (PT) with narrow lumen and brush border and distal tubules (DT) with wide lumen similar to the negative control group. H&E stain X 400

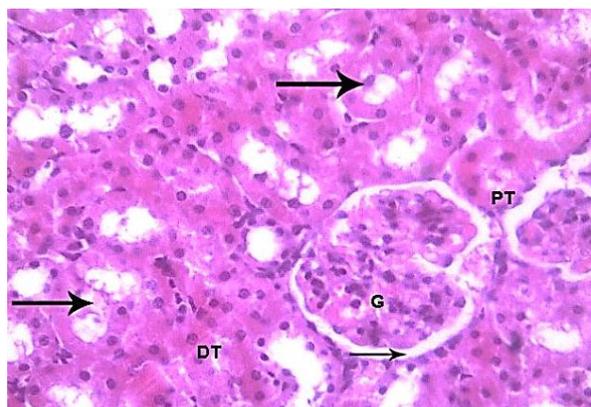


Fig. 5: A photomicrograph of a magnified section in the kidney of the cyclosporine+NAC treated group showing renal corpuscle with narrow urinary space (thin arrow), decrease glomerular capillaries congestion (G) compared to the cyclosporine group, proximal tubules (PT) and distal tubules (DT) show milder affection and others are still degenerated (thick arrow). H&E stain X 400

Discussion

The kidney is one of the organs related to the detoxification process. Several drugs, chemicals, and heavy metals affect the kidney by causing structural and functional changes (*Parameshappa et al., 2012*).

One of the main complications of CsA treatment is kidney dysfunction. CsA nephrotoxicity can be divided into two major subgroups: (a) functional (basic) toxicity characterized by slight increase of serum creatinine and decreased glomerular filtration rate without significant morphologic lesions; (b) morphological forms of toxicity with tubular and/or vascular-interstitial lesions (*Mihatsch et al., 1988*).

In the present study, it was found that the cyclosporine treated animals had significantly higher urea and creatinine levels compared to the control groups.

Liu and Tan (2020) stated that cyclosporin A caused an increase in serum urea and creatinine levels, and decrease in their clearance levels as compared to the control group

Kanchana and Parameswari (2013) reported that oral administration of cyclosporine by a dose of 25 mg/kg for 21 days was associated with significant elevation in the circulating levels of blood urea nitrogen and serum creatinine. These results were in agreement with the results recorded by Farag et al. (2015) and Haleagrahra et al. (2009) who stated that in CsA-treated rats, serum creatinine and urea levels were significantly increased compared to control rats.

The increased levels of urea and creatinine observed in the present work were in accordance with the results of (Mostafa-Hadeab et al., 2015; Hussein et al., 2014; Xiang et al., 2013; Goksu Erol et al., 2013 and Duru et al., 2008).

In the present study, treatment with NAC alone did not cause significant change in urea and

creatinine levels. Combination of NAC and cyclosporine caused significant decrease of urea and creatinine levels compared to cyclosporine treated group but these levels still higher than those of control groups.

These results were consistent with Duru et al. (2008) who found that cyclosporine caused significant increases in serum urea and creatinine levels, while the treatment with NAC alone showed no significant change in serum urea and creatinine levels.

Also Haleagrahra et al. (2009) stated that when NAC given with cyclosporine the renal function was significantly improved compared to CsA alone treatment group. But this improvement is partial compared to the control and NAC treated groups as the renal function still higher than normal levels.

In the present study there was pathological changes found in the rat kidney of cyclosporine group. These changes were in the form of dilatation of urinary space with congestion and lobulation of glomerular capillaries in the renal corpuscle. P.C.T showed degeneration of their cells with irregularity and destruction of brush border and dilatation of the lumen. There was degeneration with exfoliation of some cells inside the lumen in the D.C.T and the peritubular capillaries were congested and extravasated.

Liu and Tan (2020) reported that in cyclosporin A treated group there was marked vacuolar degeneration, atrophy, necrosis and exfoliation of kidney tubules, interstitial fibrosis, glomerular atrophy, and vascular congestion. Also, Al-Sa'aidi et al. (2019) found that cyclosporine caused obvious atrophy of glomeruli, necrosis of Bowman capsule lining, cystic dilation of renal convoluted tubules and necrosis of their lining.

These results were in accordance with Mostafa-Hadeab et al. (2015) who found that the

nephrotoxic effects of cyclosporine cause prominent histopathological changes in the form of marked vacuolization of the cortical distal convoluted tubular epithelium, hyaline arteriosclerosis, and cystic dilatation of the Bowman capsule. Moreover, interstitial nephritis and shrunken glomerular tufts were observed. Also in agreement with Kanchana and Parameswari (2013) reported that there were marked necrosis and dilatation of proximal tubules with tubular cell desquamation and massive infiltration with inflammatory cells after cyclosporine administration. Cid et al. (2003) also reported loss of proximal tubular cells brush border with dilatation, necrosis, and infiltration of white blood cells in the tubules of renal cortex.

Haleagrahra et al. (2009) reported that there was tubular necrosis and hyalinization with thickening of blood vessels and congestion of interstitium of the kidney. There was also marked glomerular atrophy.

There are several mechanisms responsible for chronic CsA induced nephrotoxicity including 1) activation of the renin-angiotensin-aldosterone system, 2) renal vasoconstriction that lead to renal hypoxia and generation of reactive oxygen species that cause cellular injury and apoptosis and 3) increased transforming growth factor (TGF) production which increases extracellular matrix proteins production and decrease its degradation leading to renal fibrosis (Bobadilla & Gamba, 2007).

Cyclosporine A increases renal ROS formation which increases cellular membrane permeability, changes ionic gradients, disrupts membrane functions and metabolic processes through its interaction with many intra-cellular molecules (Cid et al., 2003).

In conditions of oxidative stress the cell induce several enzymatic and non-enzymatic systems to counteract the ROS and other free radicals but they are insufficient (Kanchana & Parameswari, 2013).

In conditions of stress, swelling of the cell is the first response. This occurs due to impairment of pump systems as a result of energy metabolism imbalance leading to the accumulation of excessive fluid which appear as vacuoles in the cytoplasm (Goksu Erol et al., 2013).

Cyclosporine A induced nephrotoxicity due to oxidative stress can be attenuated by inhibition of ROS formation or by increasing antioxidant activity (Lu et al., 2020).

Therefore, in the last several years attention was forwarded to use antioxidants as new drug that could ameliorate the damages induced by CsA (Damiano et al., 2015).

Some of these antioxidants are vitamin E, N-acetylcysteine, melatonin and carvedilol which were found to improve renal function in experimental models of acute CSA induced nephrotoxicity (Burdmann et al., 2003).

From previous studies, antioxidants cause an increase in the antioxidants levels and decrease in the peroxidation and ROS levels which can lead to improvement of the morphological and cyto-histological structure of the tissues (Al Khatib et al., 2019).

In the present study when NAC were administered with cyclosporine the morphological changes were less compared to cyclosporine treated group in the form of decrease of urinary space of renal corpuscles and decrease congestion of glomerular capillaries. Some of P.C.T & D.C.T were more or less similar to the control group, while other tubules were degenerated. This indicates NAC role in attenuating cyclosporine effect on the kidney.

These results were in agreement with Duru et al. (2008) who stated that administration of NAC with CsA caused milder tubular atrophy and epithelial vacuolizations in the proximal tubules and the glomeruli maintained a better morphology when compared to the CsA-treated group alone. Also, Tariq et al. (1999) stated that combination of NAC with CsA significantly decrease histopathological changes caused by CsA.

Haleagrahra et al. (2009) reported that combination of NAC with CsA attenuate pathological changes as most of the glomeruli appeared normal with minimal blood vessel thickening and decreased areas of congestion. Also, tubular atrophy was milder.

N-acetylcysteine (NAC) is one of the powerful antioxidants as its combination with CsA increase the total antioxidant level both in the serum and kidneys. Also, N-acetylcysteine directly scavenges superoxide radicals. Additionally, the NAC intake increase GSH levels to detoxify ROS and other reactive substances (Duru et al., 2008).

N-acetylcysteine can decrease vascular resistance and improve tissue perfusion resulting in decrease in oxygen free radicals formation and attenuation of cellular damage induced by CsA in the kidney (Haleagrahra et al., 2009).

N-acetylcysteine has a direct relaxing action on vascular smooth muscle resulting in reduction of vascular resistance and improvement of tissue perfusion. Also, NAC can inhibit rennin angiotensin system, stimulate NO production and modulate endothelin action on microvessels (Tariq et al., 1999).

Additionally CsA cause nephrotoxicity by increasing intracellular calcium. This can be attenuated by NAC through the blockage of calcium channels and maintenance of calcium homeostasis (Haleagrahra et al., 2009).

Concomitant administration of NAC with cyclosporine produced a mild decrease in blood cyclosporine level. Thus the alteration in bioavailability of cyclosporine by NAC may to some extent contribute to its protective effect (Tariq et al., 1999).

Conclusion:

It has been found that cyclosporine has toxic effects on kidney functionally and pathologically. These effects attributed to oxidative stress. N-acetylcystein is an antioxidant and has the ability to attenuate renal toxicity induced by cyclosporine but not provide full protection.

References:

- Alazouny, Z. M.; Mohamed, E. M., and Ahmed, G. A. (2014): Effect of cyclosporine A on the structure of adult albino rat testis and the role of lycopene supplementation: a histological and immunohistochemical study. *Egyptian Journal of Histology*, 37(2):+ 292-303.
- Al-Khatib, B. Y.; Al-Hamdani, N. M., and Gumaih, H. S. (2019): Ameliorated and antioxidant effects of Fucoïdan against cyclosporine A-induced kidney injury in rats. *The Journal of Basic and Applied Zoology*, 80(1): 1-11.
- Al-Sa'aidi, J. A.; Al-Kalby, J. M., and Alozy, M. A. (2019): Protective effect of Silymarin against cyclosporine nephrotoxicity in male rats. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 18(1): 39-46.
- Bobadilla, N. A., and Gamba, G. (2007): New insights into the pathophysiology of cyclosporine nephrotoxicity: a role of aldosterone. *American Journal of Physiology-Renal Physiology*, 293(1): F2-F9.
- Burdmann, E. A.; Andoh, T. F.; Yu, L., and Bennett, W. M. (2003): Cyclosporine nephrotoxicity. In *Seminars in nephrology*, 23(5): 465-476.
- Cid, T. P.; Garcia, J. C.; Alvarez, F. C., and De Arriba, G. (2003): Antioxidant nutrients protect against cyclosporine A nephrotoxicity. *Toxicology*, 189(1-2): 99-111.
- Damiano, S.; Ciarcia, R.; Montagnaro, S.; Pagnini, U.; Garofano, T.; Capasso, G. and Giordano, A. (2015): Prevention of nephrotoxicity induced by cyclosporine A: role of antioxidants. *Journal of cellular biochemistry*, 116(3): 364-369.
- Duru, M.; Nacar, A.; Yönden, Z.; Kuvandık, G.; Helvacı, M. R.; Koç, A., and Söğüt, S. (2008): Protective effects of N-acetylcysteine on cyclosporine-A-induced nephrotoxicity. *Renal failure*, 30(4): 453-459.
- Fahr, A. (1993): Cyclosporin clinical pharmacokinetics. *Clinical pharmacokinetics*, 24(6): 472-495.
- Farag, M. M.; Ahmed, G. O.; Shehata, R. R., and Kazem, A. H. (2015): Thymoquinone improves the kidney and liver changes induced by chronic cyclosporine A treatment and acute renal ischaemia/reperfusion in rats. *Journal of Pharmacy and Pharmacology*, 67(5): 731-739.
- Feagan, B. G., McDonald, J., Rochon, J., Laupacis, A., Fedorak, R. N., Kinnear, D., and Valberg, B. (1994): Low-dose cyclosporine for the treatment of Crohn's disease. *New England Journal of Medicine*, 330(26): 1846-1851.
- Fellström, B. (2004): Cyclosporine nephrotoxicity. In *Transplantation proceedings*, 36(2): 220-223.
- Goksu Erol, A. Y.; Avcı, G.; Sevimli, A.; Ulutas, E., and Ozdemir, M. (2013): The protective effects of omega 3 fatty acids and sesame oil against cyclosporine A-induced nephrotoxicity. *Drug and chemical toxicology*, 36(2): 241-248.
- Haleagrahara, N.; Tan, M. Y.; Chakravarthi, S., and Nagarajah, L. (2009): Protective effect of N-acetylcysteine on cyclosporine A-induced changes in lipid hydroperoxide levels and renal dysfunction in rats. *Archives of Medical Science*, 5(1): 16-22.
- Hussein, S. A.; Ragab, O. A., and El-Eshrawy, M. A. (2014): Renoprotective effect of dietary fish oil on cyclosporine A: Induced nephrotoxicity in rats. *Asian Journal of Biochemistry*, 9(2): 71-85.
- Kanchana, K., and Parameswari, C. S. (2013): Protective effect of *Phyllanthus niruri* against cyclosporine A-induced nephrotoxicity in rats. *Comparative Clinical Pathology*, 22(5): 885-893.
- Kaya, H.; Koc, A.; Sogut, S.; Duru, M.; Yilmaz, H. R.; Uz, E., and Durgut, R. (2008): The protective effect of N-acetylcysteine against cyclosporine A induced hepatotoxicity in rats. *Journal of Applied Toxicology*, 28(1): 15-20.
- Lee, J. (2010): Use of antioxidants to prevent cyclosporine a toxicity. *Toxicological research*, 26(3): 163-170.
- Liu, B., and Tan, P. (2020): PPAR γ /TLR4/TGF- β 1 axis mediates the protection effect of erythropoietin on cyclosporin A-induced chronic nephropathy in rat. *Renal Failure*, 42(1): 216-224.
- Lu, Y.; Li, C. F.; Ping, N. N.; Sun, Y. Y.; Wang, Z.; Zhao, G. X., and Liu, J. J. (2020): Hydrogen-rich water alleviates cyclosporine A-induced nephrotoxicity via the Keap1/Nrf2 signaling pathway. *Journal of Biochemical and Molecular Toxicology*, 34(5): e22467.
- Mihatsch, M. J.; Thiel, G., and Ryffel, B. (1988): Hazards of cyclosporine A therapy and recommendations for its use. *Journal of Autoimmunity*, 1(6): 533-543.
- Mostafa-Hedeab, G.; Sati, L. M.; Elnaggar, H. M.; Elgatlawey, Z. O.; Eltwab, A. A.; Elsaghayer, W. A., and Ali, H. (2015): Ameliorating Effect of Olive Leaf Extract on Cyclosporineinduced Nephrotoxicity in Rats. *Iranian journal of kidney diseases*, 9(5): 361-368.
- Najafi, G.; Farokhi, F.; Jalali, A. S., and Akbarizadeh, Z. (2016): Protection against cyclosporine-induced reprotoxicity by *Satureja khuzestanica* essential oil in male rats. *International journal of fertility & sterility*, 9(4): 548.
- Parameshappa, B.; Ali Basha, M. S.; Sen, S.; Chakraborty, R.; Kumar, G. V.; Sagar, G. V., and Lakshmi, A. V. (2012): Acetaminophen-induced nephrotoxicity in rats: protective role of *Cardiospermum halicacabum*. *Pharmaceutical biology*, 50(2): 247-253.

- Patel, J. K., and Kobashigawa, J. A. (2004): Cardiac transplant experience with cyclosporine. In Transplantation proceedings, 36(2): 323-330.
- Rezzani, R. (2006): Exploring cyclosporine A-side effects and the protective role-played by antioxidants: the morphological and immunohistochemical studies. Histology and histopathology, 21: 301-316.
- Saleh, A. A. S. (2014): Synergistic effect of N-acetyl cysteine and folic acid against aspartame induced nephrotoxicity in rats. Int. J. Adv. Res, 5: 363-373.
- Shahbazi, F.; Farvadi, F.; Dashti-Khavidaki, S.; Ataei, S., and Shojaei, L. (2020): Potential nephroprotective effects of resveratrol in drug induced nephrotoxicity: a narrative review of safety and efficacy data. Advances in Traditional Medicine, 1-16.
- Tariq, M.; Morais, C.; Sobki, S.; Al Sulaiman, M., and Al Khader, A. (1999): N-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association, 14(4): 923-929.
- Xiang, Y.; Piao, S. G.; Zou, H. B.; Jin, J.; Fang, M. R.; Lei, D. M., and Li, C. (2013): L-carnitine protects against cyclosporine-induced pancreatic and renal injury in rats. In Transplantation proceedings, 45(8): 3127-3134.
- Zal, F.; Mostafavi-Pour, Z., and Vessal, M. (2007): Comparison of the effects of vitamin e and/or quercetin in attenuating chronic cyclosporine a induced nephrotoxicity in male rats. Clinical and experimental pharmacology and physiology, 34(8): 720-724.

دراسة تجريبية عن السمية الكلوية للسيكلوسبورين والدور المحسن لأن اسيتيل سيستين في الفئران البيضاء

مى مصطفى عبد القادر^١، مها عبد الحميد هلال^١، عبد الرحمن وجيه تركي^٢، هدى محمد السيد^٣، ولاء احمد علام^١

الملخص العربي

المقدمة: يعد عقارالسيكلوسبورين مثبط قوى للمناعة وقد ادى استخدامه الى ارتفاع معدل البقاء على قيد الحياة لمرضى زراعة الأعضاء كما يستخدم ايضا لعلاج امراض المناعة الذاتية. ولكن استخدام السيكلوسبورين يحمل العديد من الآثار الجانبية منها التسمم الكلوى. ويعد الاسيتيلسيستين احد مضادات الاكسدة التى تستخدم للحد من سمية السيكلوسبورين.

الهدف من الدراسة: تحديد تأثير التعرض للسيكلوسبورين على الكلى وفحص الدور الوقائي للاسيتيلسيستين.

طريقة البحث: اجريت الدراسة على ٥٠ من ذكور الفئران البيضاء البالغة لمدة ٤ اسابيع مقسمة إلى ٥ مجموعات ، المجموعة الضابطة السلبية ، مجموعة زيت الزيتون (٠.٥ مل / يوم عن طريق الفم) ، مجموعة السيكلوسبورين (٢٥ مجم / كجم / يوم عن طريق الفم) ، مجموعة الاسيتيلسيستين (٦٠٠ مجم / كجم / يوم عن طريق الفم) ومجموعة السيكلوسبورين + الاسيتيلسيستين. تم إجراء التحليل الكيميائي الحيوي والفحص النسيجي في نهاية البحث.

نتائج البحث: يؤثرالسيكلوسبورين بشكل كبير على الكلى ويسبب تغيرات مورفولوجية مثل اتساع المساحة البولية واحتقان الشعيرات الدموية. كما يسبب تكسر في خلايا الاناييب الكلوية وظهور بعض منها في تجويف الاناييب واحتقان الشعيرات الدموية المحيطة. ويؤدى ايضا الى زيادة مستويات اليوريا والكرياتينين. ولكن تناول الاسيتيلسيستين يحد من سمية السيكلوسبورين.

الخلاصة: يتسبب السيكلوسبورين في إصابة الكلى عن طريق الإجهاد التأكسدي ولكن الاسيتيلسيستين كاحد مضادات الاكسدة يقلل ولكن لا يوفر الحماية الكاملة ضد الإصابات الناجمة عن السيكلوسبورين.

١. قسم الطب الشرعي والسموم الإكلينيكية ، كلية الطب ، جامعة سوهاج ، سوهاج ، مصر.

٢. قسم الطب الشرعي والسموم الإكلينيكية ، كلية الطب ، جامعة حلوان ، القاهرة ، مصر.

٣. قسم الأنسجة - كلية الطب - جامعة سوهاج - سوهاج - مصر.