



Research Article

## Renoprotective Effect of Agmatine Against Cyclosporin A- Induced Nephrotoxicity in Rats

Dalia H. El-Kashef \*, Asmaa E. El-Kenawi, Ghada M. Suddek and Hatem A. Salem

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

## ARTICLE INFO

Received 22/02/2018  
 Received in revised form  
 11/06/2018  
 Accepted 25/12/2018  
 Available online 01/01/2019

E-mail address:  
[elkashefdalia@yahoo.com](mailto:elkashefdalia@yahoo.com)

## ABSTRACT

In this study, the modulator effect of agmatine on the oxidative nephrotoxicity of cyclosporin (CsA) in the kidneys of rats was investigated by determining indices of lipid peroxidation and the activities of antioxidant enzymes, as well as by histological analyses. Furthermore, the effect of agmatine on CsA induced hypersensitivity of urinary bladder rings to ACh was estimated. Twenty-four male Sprague-Dawley rats were randomly divided into three groups, namely control, CsA and CsA plus agmatine. At the end of the study, all rats were sacrificed and then blood, urine samples and kidneys were taken. CsA administration caused a severe nephrotoxicity which was evidenced by an elevation of serum creatinine, blood urea nitrogen, serum lactate dehydrogenase and protein in urine with a concomitant reduction in serum albumin and creatinine clearance as compared with the controls. Moreover, a significant increase in renal contents of malondialdehyde, myeloperoxidase and tumor necrosis factor-alpha together with a significant decrease in renal reduced glutathione, superoxide dismutase activities and nitric oxide content was detected upon CsA administration with increasing the sensitivity of isolated urinary bladder rings to ACh. Agmatine protected kidney tissue against the oxidative damage and the nephrotoxic effect caused by CsA treatment. In addition, agmatine significantly reduced the responses of isolated bladder rings to ACh. The results from our study indicate that agmatine supplement attenuates CsA -induced renal injury via the amelioration of oxidative stress and inflammation of renal tubular cells. Exposure to CsA caused vacuolated tubular cells and thickened wall vessels, which was found to be prevented by agmatine concurrent treatment. Our study indicates that agmatine administration with CsA attenuates oxidative-stress associated renal injury by reducing oxygen free radicals and lipid peroxidation and inhibiting inflammatory mediators such as TNF- $\alpha$ .

**Keywords:** CsA; Agmatine; Nephrotoxicity; Urinary bladder; TNF- $\alpha$ ;

### 1. Introduction

Despite the widespread use of cyclosporin A (CsA) as an immunosuppressant in organ transplantation and in control of several autoimmune diseases (Kovarik and Burtin, 2003), its nephrotoxic impact continues to present serious challenges in

medicine. One key element in the initiation and progression of CsA nephrotoxicity is the, imbalance between renal vasodilator and vasoconstrictor capacities leading to renal ischemia and deterioration of renal function (El-Gowell et al., 2014). Apart from primates, the rat appears to be one of the few

laboratory species susceptible to cyclosporin-induced nephropathy and has been proposed as a model of the morphologic and functional changes that occur in the human (Moretti et al., 2003).

The exact mechanism of CsA-induced hypertension and nephrotoxicity remain obscure. Experimental studies revealed that several mechanisms may be involved. These include activation of the renin-angiotensin system (RAS) (Edwards et al., 1994) and increased synthesis of endothelins (Fogo et al., 1992). Several studies suggest that a defect in intracellular calcium handling (Lo Russo et al., 1996), magnesium deficiency (Mervaala et al., 1997), oxidative stress (De Nicola et al., 1993), and nitric oxide system (Navarro-Antolin et al., 1998) are involved. During the last decade, considerable attention has been focused on the involvement of reactive oxygen species (ROS) in various organs including the kidney. ROS have been proposed as mediators of different kidney diseases especially in toxic, ischemic or immunological conditions (Nath et al., 2000). The implications of ROS in CsA nephron toxicity was strengthened by the fact that many antioxidants and free radical scavengers provide marked functional and histopathological protection against CsA nephrotoxicity (Wongmekiat and Thamprasert, 2005).

Agmatine, (4-(aminobutyl) guanidine), is formed by decarboxylation of L-arginine (Arg) catalyzed by the enzyme arginine decarboxylase (ADC). Although agmatine and ADC have been recognized in plants, bacteria, and invertebrates, for many years they were not believed to be synthesized in mammals. Only in 1994, agmatine and ADC were discovered in the brain of rats and bovines (Li et al. 1994) and subsequently in many other mammalian organs and cell types (Raasch et al., 1995). Studies have shown the various biological activities of agmatine that include neuroprotective (Hong et al., 2009), cognitive, anxiolytic (Uzbay et al., 2012), anticonvulsant (Payandemehr et al., 2013), antinociceptive (Kotagale et al., 2013), and antidepressant properties (Neis et al., 2014).

Agmatine affects the synthesis of nitric oxide (NO) by activating the endothelial NOS (eNOS) while inhibiting inducible NOS (iNOS) (Auguet et al., 1995) and neuronal NOS (Demady et al., 2001). Previous studies have demonstrated that eNOS-derived NO inhibits airway inflammation by suppressing the activation of NF- $\kappa$ B, thereby inhibiting both the expression of iNOS and the production of inflammatory cytokines (Ten Broeke et al., 2006). On the other hand, iNOS derived NO may be involved in the infiltration of inflammatory cells (Schuiling et al., 1998), mucosal swelling, and epithelial damage (Flak and Goldman 1996; Schuiling et al., 1998).

Agmatine can activate  $\alpha$ -1 adrenoceptors and imidazolguanidine receptors (Li et al. 1994) and when

infused into the renal interstitial increases glomerular filtration and tubular reabsorption (Schwartz et al. 1997). Moreover, agmatine administration reduced collagen accumulation in kidneys of diabetic mice (Marx et al. 1995) as observed with low doses of arginine (Arg) administration (Lubec et al. 1997; Radner et al. 1994). ADC activity is high in the normal kidney (Morrissey et al. 1995) and therefore it is possible that agmatine may mediate some of the biological effects of Arg supplementation in renal disease. Another interesting feature is the ability of agmatine to induce eNOS activation and to reduce iNOS synthesis. Agmatine, in fact, is metabolized to agmatine aldehyde by diamine oxidase. It has been demonstrated that addition of this enzyme to cells in culture enhances the effects of agmatine as an inhibitor of iNOS, while inhibitors of aldehyde dehydrogenase further decrease NO generation (Blantz et al. 2000).

The present study aimed to evaluate the potential protective effect of agmatine in an experimental model of CsA-induced nephrotoxicity.

## 2. Materials and Methods

### 2.1. Chemicals

Agmatine sulfate was purchased as a pharmaceutical drug (Agmatine capsule 500 mg) obtained from USP labs (Dallas, Texas, USA). CsA was purchased as a pharmaceutical drug (Ciclosporin oral capsules 50 mg, Sandimmun neoral, Novartis, Egypt). All other chemicals and reagents used were of the highest analytical grade commercially available.

### 2.2. Animals

Twenty-four adult male Sprague-Dawley rats weighing 150-200 g purchased from Vacsera center, Helwan, Egypt were used for the experimental procedures. Rats were allowed 1 week to adapt to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments unless otherwise noted. Temperature was maintained at 25 °C with 12/12-h light/dark cycle. All animal experiments described in this study comply with the ethical principles and guidelines for the care and use of laboratory animals adopted by "Research Ethics Committee, Faculty of Pharmacy, Mansoura University" in agreement with the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985).

### 2.3. Experimental Design

The animals were randomly divided into three groups containing eight rats in each group. Group (1): Control group, rats did not receive any solvent or drug during the experiment and were on a usual diet. Group

(2): Cyclosporin (CsA) group, CsA was administered to rats (15 mg/kg, S.C.) diluted in olive oil with a ratio 1:1 for four weeks (Lei et al., 2014). Group (3): Cyclosporin /Agmatine (CsA/AG) group, rats were injected with CsA (15 mg/kg, S.C.) along with AG (50 mg/kg, orally) (Rushaidhi et al., 2012) from the 1<sup>st</sup> day till the end of experimental study.

After the last dose, all control and experimental animals were immediately kept in individual metabolic cages (without prior adaptation) for collection of 24 h urine samples. These samples were centrifuged for 15 min at 3000 rpm, and kept frozen until analyzed. Blood samples were obtained from overnight fasted animals through retro-orbital sinus, under diethyl ether anesthesia, into non-heparinized tubes. The collected blood samples were allowed to clot for 30 min. at 25 °C. After clotting, they were centrifuged at 1000 x g, 4 °C for 15 min. using cooling centrifuge (Damon /IEC Division, Model: CRU-5000, Needham, Mass., USA). Sera were collected and stored frozen for the determination of levels of Cr, BUN, albumin and LDH. The animals were sacrificed after anesthesia with diethyl ether by cervical dislocation, then the lower abdomen was opened and the contractile response of the isolated urinary bladder rings towards ACh was tested. Fatty adherents from the kidneys were removed and the kidneys were weighed using a digital balance to calculate the kidney body weight ratio. The left kidneys were excised immediately, rinsed in ice-cold normal saline (0.9% w/v), homogenized in 0.1 M phosphate buffer (pH 7.4) to yield 10% w/v tissue homogenates that were then used for biochemical assays. The right kidneys were harvested for histopathological examination.

#### 2.4. Determination of serum creatinine

Creatinine was measured in rat sera as described by Bartles et al. (1972). Creatinine in alkaline solution reacts with picric acid to form a coloured complex that was measured spectrophotometrically at 550 nm. The amount of the complex formed is directly proportional to the creatinine concentration. A kit from Biodiagnostic Co., Egypt was used.

#### 2.5. Determination of blood urea nitrogen (BUN)

Urea was measured enzymatically in rat sera according to (Fawcett and Scott, 1960). Urea in the sample was hydrolyzed by urease enzyme to yield ammonia and carbon dioxide. In a modified Berthelot reaction, the blue dye indophenol product reaction absorbs light proportional to initial urea concentration and was measured spectrophotometrically at 550 nm. A kit from Biodiagnostic Co. was used.

#### 2.6. Determination of creatinine clearance (Ccr)

Glomerular filtration was assessed by creatinine clearance based on serum and urine creatinine levels, with values expressed in ml/min, computed with the formula:

$$Ccr = \frac{\text{urine creatinine (mg/dl)} \times \text{urine flow (ml/min)}}{\text{Serum creatinine (mg/dl)}}$$

Urine flow was calculated dividing 24 hours of urine volume by 1,440, which corresponds to the number of minutes in 24 hours (60 min x 24h = 1,440): urine flow (ml/min) = value of urine volume (24h)/1,440.

#### 2.7. Determination of protein in urine:

Protein in urine was determined by Folin-Lowry colorimetric method; two reactions are involved: (a) an initial interaction of protein and  $\text{Cu}^{+2}$  in alkali (related to biuret reaction); (b) a reduction of the phosphotungstic and phosphomolybdic acids to molybdenum blue and tungsten blue both by the Cu-protein complex and by the tyrosine and tryptophan of the protein. The latter two give color in the absence of  $\text{Cu}^{+2}$  but the rest of the protein gives no color without  $\text{Cu}^{+2}$ . About 75% of the colour is dependent on the  $\text{Cu}^{+2}$ . The maximum absorption of the colored products is at 750 nm (Daughaday et al., 1952).

#### 2.8. Determination of serum albumin

Albumin, in the presence of bromocresol green at a slightly acid pH, produces a color change in the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample (Doumas et al., 1971).

#### 2.9. Determination of lactate dehydrogenase

Lactate dehydrogenase (LDH) activity was assessed according to the method described by Henry (1974). The method depends on the conversion of pyruvate to lactate by LDH consuming  $\text{NADH}^+$ , which absorbs at 340 nm. Its consumption is directly proportional to serum LDH concentration. LDH activity was calculated as units/liter (U/L).

#### 2.10. Determination of lipid peroxidation

Lipid peroxidation was determined by the method of (Ohkawa et al., 1979). The principle of this method being that malondialdehyde (MDA), an end product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a pink chromogen. For this assay, 0.2 ml of 8.1% w/v sodium dodecyl sulfate (SDS), 1.5 ml of 20% v/v acetic acid (pH 3.5) and 1.5 ml of 0.8% w/v thiobarbituric acid aqueous solution were added in succession in a reaction tube. To this reaction mixture, 0.2 ml of the kidney homogenate

was added, and the mixture was then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol: pyridine (15:1, v/v) solution was added. The mixture was then centrifuged at 2236 x g for 15 min following which the upper layer was separated, and the intensity of the resulting pink color was read at 532 nm. Tetramethoxypropane was used as an external standard and the level of lipid peroxides was expressed as nmol of MDA formed/g wet tissue.

#### **2.11. Estimation of superoxide dismutase (SOD) activity**

Superoxide dismutase activity in kidney homogenates, as an index of endogenous antioxidant activity, was measured spectrophotometrically by monitoring the SOD-inhabitable autooxidation of pyrogallol, as described by Marklund. In brief, the reaction mixture consisted of 24 mmol/L of pyrogallol in 10 mM HCl, Tris buffer (pH 7.8). The reaction was carried out at 25°C. The change in absorbance at 420 nm was recorded for 3 min at 1 min interval. One unit of enzyme activity is defined as 50 % inhibition of pyrogallol autooxidation under the assay conditions. The SOD activity was expressed as Unit/ g wet tissue (Marklund, 1985).

#### **2.12. Estimation of reduced glutathione (GSH)**

activity Reduced glutathione was determined according to the method described by Ellman (1959). The method based on the reduction of Ellman's reagent by (-SH) groups of GSH to produce a yellow colored 2-nitro-5-mercaptobenzoic acid. The produced chromogen is directly proportional to GSH concentration. The absorbance was measured spectrophotometrically at 412 nm and the concentrations were expressed as  $\mu\text{mol/g}$  wet tissue.

#### **2.13. Measurement of $\text{NO}_2^-/\text{NO}_3^-$ concentration**

Nitrite/nitrate ( $\text{NO}_2^-/\text{NO}_3^-$ ) production, an indicator of NO synthesis, was measured in the supernatant of the kidney homogenate using a commercially available NO assay kit (R&D Systems, Minneapolis, USA) following the manufacturer's instruction.

#### **2.14. Measurement of TNF- $\alpha$ level**

Tumor necrosis factor-alpha concentrations were measured in kidney tissue homogenates using an enzyme-linked immunosorbent assay kit (R&D Systems Inc., USA). Kidney tissue homogenate was added to a microtitre plate pre-coated with a monoclonal antibody specific for rat tumor necrosis factor-alpha. Incubation, plate washing and quenching of reactions were carried out according to the kit manufacturer's instruction and tumor necrosis factor-alpha levels were expressed as

pg/mg protein. (Bender Med Systems GmbH, Vienna, Austria).

#### **2.15. Determination of myeloperoxidase (MPO) activity**

The neutrophils accumulation in the kidney was measured by assaying MPO activity as described by Schierwagen et al (1990). Myeloperoxidase activity was assayed by measuring the  $\text{H}_2\text{O}_2$ -dependant oxidation of tetramethylbenzidine. Tetramethylbenzidine has a blue colour in its oxidized form, which can be monitored spectrophotometrically at 450 nm. MPO activity was calculated as milliunits/mg wet tissue (mU/mg).

#### **2.16. Isolation and preparation of urinary bladder rings**

Following the collection of blood samples, the rats were sacrificed by after anaesthesia with diethyl ether by cervical dislocation; the lower abdomen was opened and the urinary bladder was exposed, the connective tissue and accompanying blood vessels were cut away, and the bladder was cut into rings and placed in a warm physiological salt solution (PSS). The composition of the PSS (Mostafa et al., 2003) in g/L was as follows: NaCl, 6.9;  $\text{NaHCO}_3$ , 2.1; KCl, 0.35;  $\text{MgSO}_4$ , 0.15;  $\text{KH}_2\text{PO}_4$ , 0.16;  $\text{CaCl}_2$ , 0.28; and glucose, 2.0. The rings were mounted horizontally between a clamp and a force transducer for the measurement of the isometric tension in an organ bath that was filled with 10 mL of the PSS at a temperature of 37 °C and gassed with 95 %  $\text{O}_2$ -5 %  $\text{CO}_2$ . The rings were allowed to equilibrate for 30 min prior to the experiment under a resting load of 1 g (Nakamura et al., 1992). During this time, the bath solution was replaced every 5 min. Isometric tension generated by the smooth muscle was measured by means of an isometric transducer (serial no. 88576, Biegestab K30, Hugo Sachs Elektronik, Federal Republic of Germany) recorded with a Powerlab unit/400 linked to a PC running Chart v 4.2 software (ADInstruments Pty Ltd., Australia). Concentration-response curves to acetylcholine (ACh) were constructed. The rings were exposed to different concentrations of ACh in a cumulative manner. Exposure to each concentration of ACh was maintained until the maximal response to that concentration was reached. The responses of the bladder rings were calculated as g tension/g tissue.

#### **2.17. Histological examinations**

The kidney tissues of rats were fixed in buffered 10% neutral buffered formalin solution for 24 h and embedded in a paraffin wax. Tissues were then sectioned at 5- $\mu\text{m}$ , stained with hematoxylin eosin (H &

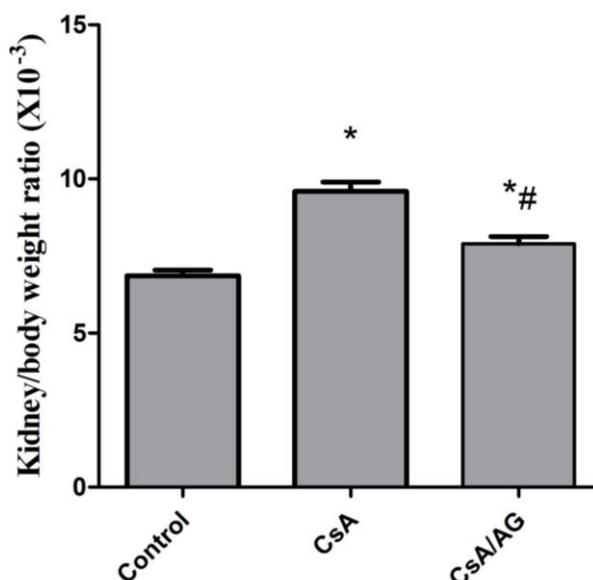


Figure 1. Effect of AG (40 mg/kg) on kidney/body weight ratio in CsA-treated rats. Values are expressed as means  $\pm$  standard error of mean (n = 8). Comparisons performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \* p < 0.05 vs. control; # p < 0.05 vs. CsA-treated group.

Table 1. Effect of AG (40 mg/kg) on kidney function parameters in CsA-treated rats

	Serum creatinine (mg/dl)	BUN (mg/dl)	CCr X10 <sup>-3</sup> (ml/min)	Proteinuria (mg/day)	Serum albumin (g/dl)
<b>Control</b>	0.48 $\pm$ 0.01	11.1 $\pm$ 0.86	0.36 $\pm$ 0.01	17.4 $\pm$ 0.64	4 $\pm$ 0.14
<b>CsA</b>	4.03 $\pm$ 0.1*	84.2 $\pm$ 4.5*	0.01 $\pm$ 0.0008*	95.1 $\pm$ 3.6*	2.4 $\pm$ 0.14*
<b>CsA/AG</b>	0.56 $\pm$ 0.02 <sup>#</sup>	17.8 $\pm$ 0.6 <sup>#</sup>	0.35 $\pm$ 0.005 <sup>#</sup>	69.5 $\pm$ 1.3 <sup>#</sup>	2.95 $\pm$ 0.2*

ANOVA, analysis of variance; CsA, cyclosporin A; AG, Agmatine; SEM, standard error of mean. Data expressed as means  $\pm$  SEM (n = 8). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.

\* p < 0.05 vs. control; # p < 0.05 vs. CsA.

E). A semiquantitative evaluation of renal tissues was accomplished by scoring the degree of severity according to the formerly published criteria (Teixeira et al., 1982).

### 2.18. Statistical analysis

The values are expressed as mean  $\pm$  standard error of mean (S.E.M), for 8 rats in each group. Bladder contraction was calculated as g tension/g tissue. The highest response obtained was considered as the maximum response ( $E_{max}$ ).  $pEC_{50}$  (negative log the concentration producing 50 % of maximal response) was determined from non-linear regression analysis (4-parameter curve fit). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by a Tukey-Kramer post-hoc test (Daniel, 1991). Differences were considered significant at P < 0.05. Statistical analyses were carried out using Graph

pad Prism software (GraphPad Software Inc. V4.03, San Diego, CA, USA).

## 3. Results

### 3.1. Kidney/Body weight ratio

The results in figure 1 show that CsA produced a significant increase in the kidney body weight ratio compared to control rats. In the CsA/AG-treated group, the kidney body weight ratio was significantly lower than that of the CsA-treated group.

### 3.2. Serum biochemical parameters

#### 3.2.1. Kidney functions

CsA caused a significant elevation in serum creatinine and BUN and protein in urine compared to the control group, with a significant decrease in serum albumin and creatinine clearance (CCr). AG administration significantly attenuated CsA-induced changes in serum creatinine, BUN, CCr and proteinuria

compared to CsA-treated rats. BUN and serum albumin still significantly different from control group (Table 1).

### 3.2.2. Serum LDH activity

Figure 2 shows that CsA produced a significant increase in LDH activity compared to control rats. In the CsA/AG-treated group, LDH activity significantly decreased compared to CsA-treated rats.

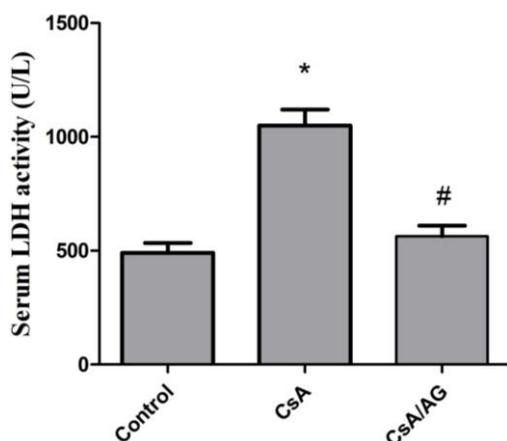


Figure 2. Effect of AG (40 mg/kg) on serum LDH activity in CsA-treated rats. Values are expressed as means  $\pm$  standard error of mean (n = 8). Comparisons performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \* p < 0.05 vs. control; # p < 0.05 vs. CsA-treated group.

### 3.2.3. Antioxidant status

Table 2 shows that after 4 weeks of treatment, CsA significantly increased the MDA levels in rat kidney tissue homogenate but decreased both GSH and SOD activities. AG significantly decreased the CsA-induced changes in MDA levels and significantly increased GSH and SOD activities.

Table 2. Effect of AG (40 mg/kg) on kidney anti-oxidant status in CsA-treated rats

	MDA (nmol/g)	GSH ( $\mu$ mol/g)	SOD (U/g)
Control	7.85 $\pm$ 0.5	0.06 $\pm$ 0.003	53.1 $\pm$ 0.5
CsA	23.2 $\pm$ 1.2*	0.02 $\pm$ 0.004*	45.7 $\pm$ 0.7*
CsA/AG	13.2 $\pm$ 1*#	0.06 $\pm$ 0.005#	52.8 $\pm$ 0.8#

ANOVA, analysis of variance; CsA, cyclosporin; AG, Agmatine; SEM, standard error of mean. Data expressed as means  $\pm$  SEM (n = 8). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \* p < 0.05 vs. control; # p < 0.05 vs. CsA

### 3.2.4. Renal nitric oxide (NOx) content

The results in figure 3 show that CsA produced a significant decrease in the renal NOx content compared to control rats. In CsA/AG-treated group, the renal NOx

content significantly increased when compared to CsA-treated group.

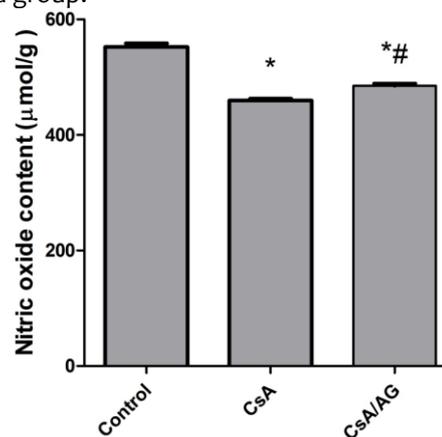


Figure 3. Effect of AG (40 mg/kg) on renal NOx content in CsA-treated rats. Values are expressed as means  $\pm$  standard error of mean (n = 8). Comparisons performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \* p < 0.05 vs. control; # p < 0.05 vs. CsA-treated group

### 3.2.5. Tumor Necrosis Factor- $\alpha$ (TNF- $\alpha$ ) level in kidney tissue homogenate

Figure 4 shows that CsA produced a significant increase in the renal TNF- $\alpha$  level compared to control rats. In the CsA/AG-treated group, the renal TNF- $\alpha$  level decreased significantly from the CsA-treated group.

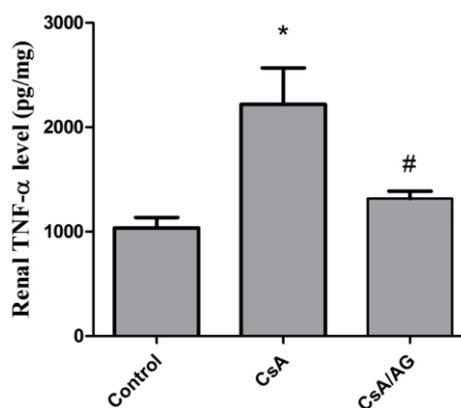


Figure 4. Effect of AG (40 mg/kg) on renal TNF- $\alpha$  level in CsA-treated rats. Values are expressed as means  $\pm$  standard error of mean (n = 8). Comparisons performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \* p < 0.05 vs. control; # p < 0.05 vs. CsA-treated group.

### 3.2.6. Myeloperoxidase (MPO) activity in kidney tissue homogenate

The results in figure 5 show that CsA produced a significant increase in the renal MPO activity compared

to control rats. In the CsA/AG-treated group, the renal MPO activity decreased significantly from the CsA treated group.

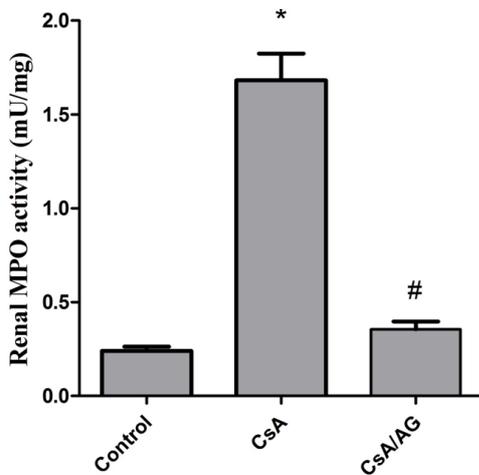
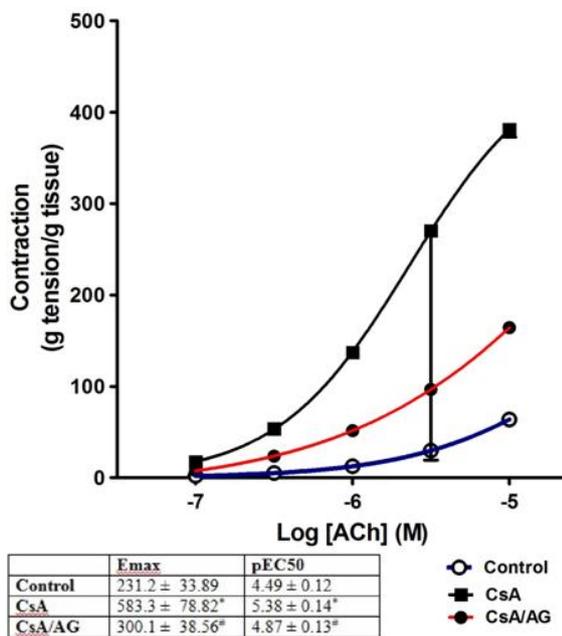


Figure 5. Effect of AG (50 mg/kg) on renal MPO activity in CsA-treated rats. Values are expressed as means  $\pm$  standard error of mean (n = 8). Comparisons performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \* p < 0.05 vs. control; # p < 0.05 vs. CsA-treated group.



	Emax	pEC50
Control	231.2 $\pm$ 33.89	4.49 $\pm$ 0.12
CsA	583.3 $\pm$ 78.82*	5.38 $\pm$ 0.14*
CsA/AG	300.1 $\pm$ 38.56*	4.87 $\pm$ 0.13*

Figure 6. Effect of CsA (15 mg/kg) and AG (40 mg/kg) on contractions to acetylcholine (ACh) in isolated rat urinary bladder rings. Values are expressed as means  $\pm$  standard error of mean (n = 5). Cumulative dose-response curve to ACh ( $10^{-7}$ – $10^{-5}$  M) was measured. P < 0.05 for Emax value compared with control group (\*) and compared with CsA group (#) using one way

analysis of variance (ANOVA) followed by Tukey-Kramer’s multiple comparison post hoc test.

**3.3 Vascular reactivity**

The effects of CsA and AL on the responses of bladder rings to ACh are shown in figure 6. The average increments in the control urinary bladder rings tension after ACh of 0.1, 0.3, 1, 3 and 10  $\mu$ M were 2  $\pm$  0.3, 5.2  $\pm$  0.5, 12.5  $\pm$  0.8, 29.8  $\pm$  0.7 and 63.8  $\pm$  1.9 g tension/g tissue, respectively. Treatment with CsA significantly enhanced the responsiveness of the rings toward ACh, as compared with the control group. Thus, the average increments in tension in response to ACh of 0.1, 0.3, 1, 3 and 10  $\mu$ M were 17.5  $\pm$  0.79, 53.6  $\pm$  2.5, 137.2  $\pm$  4.1, 250.8  $\pm$  5.2 and 380.9  $\pm$  8.1 g tension/g tissue, respectively. Urinary bladder rings, isolated from CsA/AL-treated rats showed a significant reduction in their responsiveness to ACh when compared to the CsA-treated ones. The average increments in their tensions in response to ACh of 0.1, 0.3, 1, 3 and 10  $\mu$ M were 6.2  $\pm$  0.27, 18.8  $\pm$  0.92, 43  $\pm$  1.9, 94.5  $\pm$  5.2 and 164.8  $\pm$  7.6 g tension/ g tissue, respectively.

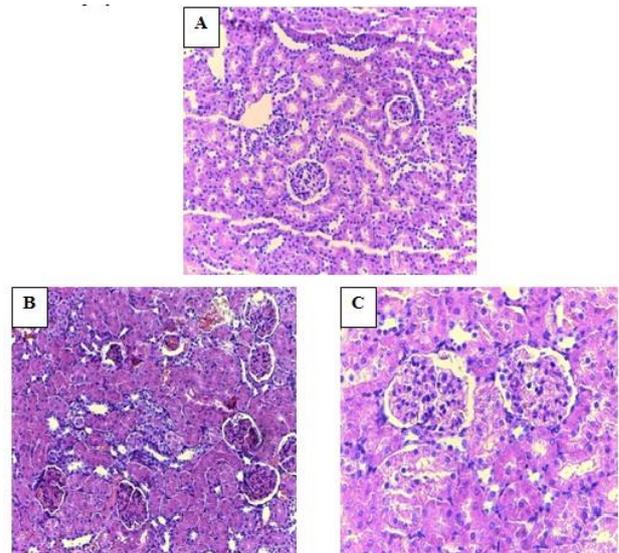


Figure 7. Light micrographs of sections from rat kidneys stained with hematoxylin and eosin. (A) control group (x100); (B) CsA group (x100); (C) CsA/AG group (X200).

**3.4. Histopathological results**

The kidney of control rats showed normal architecture of glomerulus and tubules (Fig. 7A). Kidney of ciclosporin-induced rats showed acute drug toxicity with clear vacuolated tubular cells and thickened wall vessels in group II (Fig. 7B) animals. Treatment of rats with 40 mg/kg/day agmatine along with CsA (group III: Fig. 7C), showed no abnormalities in glomeruli, minor tubular alteration in form of mild focal tubular injury.

**4. Discussion**

Cyclosporin continues to be the backbone of post-transplant immunosuppression. However, side effects associated with CsA treatment are numerous and kidney dysfunction is the main complication of CsA treatment (Chandramohan and Parameswari, 2013).

The present study demonstrated that CsA administration increased the kidney/body weight ratio of rats when compared to that of control group. This result agreed with our previous study El-Kashef et al. (2017). Administration of AG significantly decreased the kidney/body weight ratio of rats when compared to that of CsA group. This effect is mediated by its anti-inflammatory effect.

In agreement with reported studies (Herlitz and Lindelöw, 2000; El-Mas et al., 2003), the present findings that CsA caused significant rises in serum creatinine and BUN are consistent with impaired renal function. However, AG treatment decreased serum levels of creatinine, blood urea nitrogen when compared to CsA-treated group. This renoprotective effect may be due to its action on imidazoline receptor (Rouch and Kudo, 2002).

In the present study, CsA resulted in a significant increase in serum level of LDH. This result came in line with Ateyya (2015). On the other hand, AG administration significantly reduced LDH activity when compared to CsA-treated group. AG possesses anti-inflammatory effect after systemic administration (Santos et al., 2005). This anti-inflammatory action could account for its lowering effect on serum level of LDH.

CsA intoxication significantly reduced serum levels of albumin. This observation agreed with Goksu Erol et al. (2012) who detected that CsA caused lower serum albumin levels, probably as a result of its side effect of albuminuria. Administration of AG with CsA failed to retain the level of serum albumin, perhaps higher dose was required.

Proteinuria is an early useful clinical marker to detect cyclosporin (CsA) nephrotoxicity (Fernandez-Fresnedo et al., 2001). In this study, CsA administration resulted in increased levels of protein in urine. This result is in consistent with Shin et al. (2012) who stated that the 24-hour urine protein levels were significantly increased in the CsA compared with control group. However, AG administration resulted in decreased levels of protein in urine when compared to CsA-treated group.

In this study, treatment with CsA significantly reduced creatinine clearance when compared to the control group. This result is confirmed by an earlier study reported by Chander et al. (2005). AG efficiently reversed this CsA induced reduction in creatinine clearance. In the current study, CsA caused a significant increase in the MDA levels, while GSH and SOD levels

were significantly decreased in the kidney tissue homogenate when compared to control group. This result agreed with Ciarcia et al. (2012) who demonstrated an increase of MDA content in kidneys treated with CsA. In this study, it was shown that treatment with CsA significantly reduced SOD and GSH activities in kidney, which increased the susceptibility to oxidative stress of the cellular structures. Decreased SOD and GSH activities are probably due to the reduced synthesis or to an increase of the turn-over of such enzymes (Demian et al., 2001; Yilmaz et al., 2006). On contrast, AG administration significantly decreased renal MDA content and significantly increased both GSH and SOD activities. This observation is in agreement with Khanna et al. (1982).

The current study showed that CsA administration significantly decreased the levels of NOx in kidney tissue homogenate. This result agreed with Chander et al. (2005) who showed that CsA caused a significant decrease in tissue nitrite levels. Previous studies have shown that administration of CsA stimulates the production of vasoconstriction factors such as endothelin, thromboxane A<sub>2</sub> and angiotensin II (Perico et al., 1986a,b). Studies on endothelial cell cultures showed that exposure of cells to CsA results in structural damage (Zoja et al., 1996), and several in vitro studies reported that acetylcholine-induced vasodilation is impaired in vascular beds of CsA-treated animals, suggesting a deficient endothelial NO synthesis (Diederich et al., 1992; Takenaka et al., 1992), although these findings can also be explained by enhanced generation of free radicals that inactivates NO (Diederich et al., 1994). Mansour et al. (2002) have demonstrated that exogenous supplementation of L-arginine is effective in reducing renal damage induced by CsA, possibly through the NO pathway. In addition, De Nicola et al. (1993) have shown that oral supplementation of L-arginine prevents nephrotoxicity induced by chronic supplementation of CsA due to formation of more NO which may enhance vasodilation and consequently reduce the kidney function impairment. Administration of AG elevated the level of renal NO when compared to CsA group, but did not reach level of significance.

In this study, CsA-treated rats showed significant increase in TNF- $\alpha$  level in kidney tissue homogenate. This finding agreed with Abdel-latif et al. (2013) who stated that CsA-treated rats exhibited a significant increase in the renal expression of TNF- $\alpha$  which may be due to renal ischemia and vasoconstriction that are commonly associated with CsA administration. Many researchers reported that renal ischemia with or without reperfusion leads to increase in renal TNF- $\alpha$  levels (Donnahoo et al., 2000; Gabr et al., 2011). Furthermore, the oxidative stress and lipid peroxidation

products possibly serve as activators of transcription factors, leading to induction of gene expression of pro-inflammatory cytokines and release of many inflammatory cytokines including TNF- $\alpha$  (Mariappan et al., 2007). Concerning AG, it significantly decreased renal TNF- $\alpha$  level when compared to CsA group. This result is consistent with Gu et al. (2011).

In this study, treatment with CsA increased the levels of renal MPO activity. This finding agreed with Gökçe et al. (2009) who reported that the activity of MPO enzyme and MDA levels in the CsA group were significantly increased. Myeloperoxidase is a neutrophil and monocyte enzyme that amplifies the reactivity of hydrogen peroxide through the generation of hypochlorous acid, free radicals, and reactive nitrogen species (Heinecke et al., 1993). Myeloperoxidase and its oxidative products play a key role for the enzyme in promotion of lipid peroxidation, protein nitration, and other oxidative modifications (Nicholls and Hazen 2005). Malondialdehyde is a major lipid peroxidant end product, and our present observations are consistent with the previous findings indicating the increases of lipid peroxidation (Duru et al., 2008; Zal et al., 2007). The neutrophil accumulation and the activation of myeloperoxidase enzyme is probably indirectly responsible for lipid oxidation (Nicholls and Hazen 2005). Moreover, a correlation has been found between neutrophil and MDA levels (Belboul et al., 2000). The data obtained suggest that the accumulation of neutrophils leads to lipid peroxidation. Administration of AG significantly decreased renal MPO activity when compared to CsA group. This result agreed with Gu et al. (2011),

In the present study, CsA treatment produced significant increase in responsiveness of urinary bladder rings towards ACh. Afferent arteriole vasoconstriction is considered the main mechanism responsible for the pathogenesis of CsA-induced acute renal injury (Burdmann and Bennett, 2008). This event is mediated by the imbalance of vasoactive factors, i.e. an increase of vasoconstrictors (such as angiotensin II and endothelin) and a decrease in vasodilators (such as prostaglandins and nitric oxide) causing vasoconstriction (Myers, 1986; English et al., 1987; Burdmann and Bennett, 2008). On the other hand, AG administration retained normal response of urinary bladder rings towards ACh. The histological studies of kidney from CsA treated rats showed clear vacuolated tubular cells and thickened wall vessels. However, AG attenuated that change.

In summary, we have confirmed that AG has a protective role against nephrotoxicity induced by CsA exposure. According to our biochemical findings, which were supported by histopathological and sensitivity of urinary bladder rings to ACh, administration of AG

rescued the cells from the effects of CsA. These findings indicate that AG administration might reduce CsA-induced renal injury. Therefore, we propose that AG might be a potential candidate agent against CsA induced nephrotoxicity via its antioxidant and anti-inflammatory properties.

#### 5. Acknowledgment:

The author acknowledges Dr. Mona Abdel Rahim, Department of Pathology, Urology and Nephrology Center, Mansoura University for her help in the histopathological examination.

#### 6. References

- Abdel-latif, R.G., Morsy, M.A., El-Moselhy, M.A. and Khalifa, M.A. (2013): Sildenafil protects against nitric oxide deficiency-related nephrotoxicity in cyclosporin A treated rats. *Eur J Pharmacol*;705(1-3):126-134.
- Ateyya, H. (2015): Amelioration of cyclosporin induced nephrotoxicity by dipeptidyl peptidase inhibitor vildagliptin. *Int Immunopharmacol*, 28: 571-577.
- Auguet, M., Viosat, I., Marin, J.G., and Chabrier, P.E. (1995): Selective inhibition of inducible nitric oxide synthase by agmatine. *Jpn. J. Pharmacol.* 69: 285-287.
- Bartels, H., Böhmer, M. and Heierli, C. (1972): Serum creatinine determination without protein precipitation. *Clin Chim Acta* ;37: 193-197. [Article in German]
- Belboul, A., Lofgren, C., Storm, C. and Jungbeck, M. (2000): Heparincoated circuits reduce occult myocardial damage during CPB: A randomized, single blind clinical trial. *Eur J Cardiothorac Surg*;17: 580-586.
- Blantz, R.C., Satriano, J., Gabbai, F., and Kelly, C. (2000): Biological effects of arginine metabolites. *Acta Physiol. Scand.* 168:21-25.
- Burdmann, E. and Bennett, W. (2008): Nephrotoxicity of calcineurin and mTOR inhibitors. In: De Broe M, Porter G, Bennett W, editors. *Clinical Nephrotoxins*. New York: Springer. p. 403-471.
- Chander, V., Tirkey, N. and Chopra, K. (2005): Resveratrol, a polyphenolic phytoalexin protects against cyclosporin-induced nephrotoxicity through nitric oxide dependent mechanism. *Toxicology.* 15;210(1):55-64
- Chandramohan, Y. and Parameswari, C.S. (2013): Therapeutic efficacy of naringin on cyclosporin (A) induced nephrotoxicity in rats: involvement of hemeoxygenase-1. *Pharmacol Rep.*;65(5):1336-1344.
- Ciarcia, R., Damiano, S., Fiorito, F., Granato, G., Pagnini, F., Mastellone, V., Iovane, V., Alfano, L., Valenti, F., Florio, S., Giordano, A. (2012): Hydrocortisone

- attenuates cyclosporin A-induced nephrotoxicity in rats. *J Cell Biochem*; 113(3):997-1004.
- Daniel, W.W. (1991): Hypothesis testing, In: *Biostatistics: A Foundation for Analysis in the Health Sciences*. 5th ed., John Wiley & Sons. USA, Chichester, Brisbane, Toronto and Singapore. PP: 191-213.
- Daughaday, W.H., Lowry, O.H., Rosebrough, N.J. and Fields, W.S. (1952): Determination of cerebrospinal fluid protein with the Folin phenol reagent. *J Lab Clin Med*. ;39(4):663-665.
- De Nicola, L., Thomson, S.C., Wead, L.M., Brown, M.R. and Gabbai, F.B. (1993): Arginine feeding modifies cyclosporin nephrotoxicity in rats. *J. Clin. Invest.* 92, 1859–1865.
- Demady, D.R., Jianmongkol, S., Vuletich, J.L., Bender, A.T., and Osawa, Y. (2001): Agmatine enhances the NADPH oxidase activity of neuronal NO synthase and leads to oxidative inactivation of the enzyme. *Mol. Pharmacol.* 59: 24–29.
- Deman A, Ceysens B, Pauwels M, Zhang J, Houte KV, Verbeelen D, Van den Branden C. (2001): Altered antioxidant defense in a mouse adriamycin model of glomerulosclerosis. *Nephrol Dial Transplant* 16:147–150.
- Diederich, D., Skopec, J., Diederich, A., Dai, F.X. (1994): Cyclosporin produces endothelial dysfunction by increased production of superoxide. *Hypertension* 23; 957–961.
- Diederich, D., Yang, Z., Luscher, T.F. (1992): Chronic cyclosporin therapy impairs endothelium-dependent relaxation in the renal artery of the rat. *J. Am. Soc. Nephrol.* 2, 1291–1297.
- Donnahoo, K.K., Meldrum, D.R., Shenkar, R., Chung, C.S., Abraham, E., Harken, A.H. (2000): Early renal ischemia, with or without reperfusion, activates NF $\kappa$ B and increases TNF- $\alpha$  bioactivity in the kidney. *J. Urol.* 163, 1328–1332.
- Doumas, B.T., Watson, W.A. and Biggs, H.G. (1971): Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta*; 31(1):87-96.
- Duru, M., Nacar, A., Yonden, Z., Kuvandik, G., Helvacı, M.R., Koc, A., et al. (2008): Protective effects of N-acetylcysteine on cyclosporin A-induced nephrotoxicity. *Ren Fail.*; 30:453–459.
- Edwards, B.D., Chalmers, R.J., O'Driscoll, J.B., Mitchell, D.M., Smith, R.J., Lawson, R.S., Testa, H.J. and Ballardie, F.W. (1994): Angiotensin II as a risk factor for cyclosporin nephrotoxicity in patients with psoriasis. *Clin Nephrol* 41:350–356.
- El-Gowell, H.M., Helmy, M.W., Ali, R.M., El-Mas, M.M. (2014): Celecoxib offsets the negative renal influences of cyclosporin via modulation of the TGF- $\beta$ 1/IL-2/COX-2/endothelin ET(B) receptor cascade. *Toxicol Appl Pharmacol.* 1;275(2):88-95.
- El-Kashef, D.H., El-Kenawi, A.E., Suddek, G.M. and Salem, H.A. (2017): Allicin ameliorates kidney function and urinary bladder sensitivity in cyclosporin A-treated rats. *Hum Exp Toxicol.* 2;36(7):681-691.
- Ellman, G.L. (1959): Tissue sulfa hydryl groups. *Arch Biochem. Biophys.* 74: 214-226.
- El-Mas, M.M., Afify, E.A., Omar, A.G., Mohy El-Din, M.M., Sharabi, F.M., (2003): Testosterone depletion contributes to cyclosporin-induced chronic impairment of acetylcholine renovascular relaxations. *Eur. J. Pharmacol.* 468, 217–224.
- English, J., Evan, A., Houghton, D.C. and Bennet, W.M. (1987): Cyclosporin induced acute renal dysfunction in the rat. Evidence of arteriolar vasoconstriction with preservation of tubular function. *Transplantation* 44, 135–141.
- Fawcett, J.K. and Scott, J.E. (1960): A rapid and precise method for the determination of urea. *J Clin Pathol.* ;13:156-9.
- Fernández-Fresnedo, G., Escallada, R., Rodrigo, E. et al. (2001): Proteinuria as a useful clinical marker of cyclosporin nephrotoxicity in renal transplant patients. *Transplant Proc*, 33: 3373-3374.
- Flak, T.A., and Goldman, W.E. (1996): Autotoxicity of nitric oxide in airway disease. *Am. J. Respir. Crit. Care Med.* 154: S202–S206.
- Fogo, A., Hellings, S.E., Inagami, T. and Kon, V. (1992): Endothelin receptors antagonism in protective in vivo acute cyclosporin toxicity. *Kidney Int* 42:770–774.
- Gabr, M.M., Hussein, A.M., Sherif, I.O., Ali, S.I., Mohamed, H.E. (2011): Renal ischemia/reperfusion injury in type II DM: possible role of pro inflammatory cytokines, apoptosis and nitric oxide. *J. Physiol. Pathophysiol.* 2, 6–17.
- Gökçe, A., Oktar, S., Yönden, Z., Aydin, M., İlhan, S., Ozkan, O.V., Davarci, M. and Yalçınkaya, F.R. (2009): Protective effect of caffeic acid phenethyl ester on cyclosporin A-induced nephrotoxicity in rats. *Ren Fail*; 31(9):843-7
- Goksu Erol, A.Y., Avci, G., Sevimli, A., Ulutas, E. and Ozdemir, M. (2013): The protective effects of omega 3 fatty acids and sesame oil against cyclosporin A-induced nephrotoxicity. *Drug Chem Toxicol*; 36(2):241-248.
- Gu, Y., Fan, X., Zhang, C., Yang, X., Bao, Y. and Liang, H. (2011): The protective effects of agmatine in zymosan induced acute lung injury in mice. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue.* 23: 665-668 [abstract].

- Heinecke, J.W., Li, W., Francis, G.A. and Goldstein, J.A. (1993): Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative cross-linking of proteins. *J Clin Invest*; 91:2866–2872.
- Henry, J.B. Todd Sanford Davidsohn (1974): clinical diagnosis and management by laboratory methods, 16th edn, WB Saunders and Co., Philadelphia.
- Herlitz, H. and Lindelöw, B. (2000): Renal failure following cardiac transplantation. *Nephrol. Dial. Transplant.* 15, 311–314.
- Hong, S., Kim C.Y., Lee, J.E., and Seong G.J. 2009. Agmatine protects cultured retinal ganglion cells from tumor necrosis factor- $\alpha$ -induced apoptosis. *Life Sci.* 84: 28–32.
- Khanna, Y., Taneja, S., Raj, H. and Venkatasubramanian, T. (1982): Polyamines modify paraquat-induced changes in pulmonary superoxide dismutase and lipid peroxidation. *Res Commun Chem Pathol Pharmacol*, 35: 337–340.
- Kotagale, N.R., Shirbhate, S.H., Shukla, P., and Ugale, R.R. (2013): Agmatine attenuates neuropathic pain in sciatic nerve ligated rats: modulation by hippocampal sigma receptors. *Eur. J. Pharmacol.* 714:424–431.
- Kovarik, J.M. and Burtin, P. (2003): Immunosuppressants in advanced clinical development for organ transplantation and selected autoimmune diseases. *Expert Opin. Emerg. Drugs* 8, 47–62.
- Lei, D.M., Piao, S.G., Jin, Y.S., Jin, H., Cui, Z.H., Jin, H.F., Jin, J.Z., Zheng, H.L., Li, J.J., Jiang, Y.J., Yang, C.W. and Li, C. (2014): Expression of erythropoietin and its receptor in kidneys from normal and cyclosporin-treated rats. *Transplant Proc* ; 46(2):521–528.
- Li, G., Regunathan, S., Barrow, C.J., Eshraghi, J., Cooper, R., and Reis, D.J. (1994): Agmatine: an endogenous clonidine-displacing substance in the brain. *Science.* 263:966–969.
- Lo Russo, A., Passaquin, A.C., Andre, P., Skutella, M. and Ruegg, U.T. (1996): Effect of cyclosporinA and analogues on cytosolic calcium and vasoconstriction: Possible lack of relationship to immunosuppressive activity. *Br J Pharmacol* 118:885–892.
- Lubec, B., Hayn, M., Kitzmüller, E., Vierhapper, H., and Lubec, G. (1997): L-arginine reduces lipid peroxidation in patients with diabetes mellitus. *Free Radic. Biol. Med.* 22: 355–357.
- Mansour, M., Daba, M.H., Gado, A., Al-Rikabi, A., Al-Majed, A. (2002): Protective effect of L-arginine against nephrotoxicity induced by cyclosporin in normal rats. *Pharmacol. Res.* 45, 441–446.
- Mariappan, N., Soorappan, R.N., Haque, M., Sriramula, S. and Francis, J. (2007): TNF- $\alpha$ -induced mitochondrial oxidative stress and cardiac dysfunction: restoration by superoxide dismutase mimetic Tempol. *Am. J. Physiol. Heart Circ. Physiol.* 293, H2726–H2737.
- Marklund, S.L. (1985): Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. *Mutat. In mice Res.* 148: 129–134.
- Marx, M., Trittenwein, G., Aufricht, C., Hoeger, H., and Lubec, B. (1995): Agmatine and spermidine reduce collagen accumulation in kidneys of diabetic db/db mice. *Nephron.* 69: 155–158.
- Mervaala, E.M.A., Pere, A.K., Lindgren, L., Laasko, J., Tera“va“inen, T.L., Karjala, K., Vapaatalo, J., Ahonen, J. and Karppanen H. (1997): Effects of dietary sodium and magnesium on cyclosporineA-induced hypertension and nephrotoxicity in spontaneously hypertensive rats. *Hypertension* 29:822–827.
- Moretti, M.E., Sgro, M., Johnson, D.W., Sauve, R.S., Woolgar, M.J., Taddio, A., et al. (2003): Cyclosporin excretion into breast milk. *Transplantation*; 75: 2144–2146.
- Morrissey, J., McCracken, R., Ishidoya, S., and Klahr, S. (1995): Partial cloning and characterization of an arginine decarboxylase in the kidney. *Kidney Int.* 47: 1458–1461.
- Mostafa, A.M., Nagi, M.N., Al-Shabanha, O.A. and El-Kashef, H.A. (2000): Effect of aminoguanidine and melatonin on the response of isolated urinary bladder to acetylcholine in normal and diabetic rats. *Med. Sci. Res.* 28: 33–37.
- Myers, B. (1986): Cyclosporin nephrotoxicity. *Kidney Int.*;30: 964–974.
- Nakamura, I., Takahashi, C. and Miyagawa, I. (1992): The alterations of norepinephrine and acetylcholine concentrations in immature rat urinary bladder caused by streptozotocin induced diabetes *J. Urol.* 148: 423–426.
- Nath, K.A. and Norby, S.M. (2000): Reactive oxygen species and acute renal failure. *Am J Med*, 109, 665–678.
- Navarro-Antolin, J., Hernandez-Pereira, O., Lopez-Ongil, S., Rodriguez- Puyol, M., Lamas, S. (1998): CsA and FK-506 up-regulate eNOS expression: Role of reactive oxygen species and AP-1. *Kidney Int* 68:S20–S24.
- Neis, V.B., Manosso, L.M., Moretti, M., Freitas, A.E., Daufenbach, J., and Rodrigues, A.L. (2014): Depressive-like behavior induced by tumor necrosis factor- $\alpha$  is abolished by agmatine administration. *Behav. Brain Res.* 261:336–344.

- Nicholls, S.J. and Hazen, S.L. (2005): Myeloperoxidase and cardiovascular disease. *Arterioscler Thromb Vasc Biol*; 25: 1102–1111.
- Okhawa, H., Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351-358.
- Perico, N., Benigni, A., Zoja, C., Delaini, F., Remuzzi, G., 1986b. Functional significance of exaggerated renal thromboxane A<sub>2</sub> synthesis induced by cyclosporin A. *Am. J. Physiol.* 251, F581–F587.
- Perico, N., Zoja, C., Benigni, A., Bosco, E., Rossini, M., Morelli, C., Cattaneo, G.M., Remuzzi, G., 1986a. Renin-angiotensin system and glomerular prostaglandins in early nephrotoxicity of cyclosporin. *Contrib. Nephrol.* 51, 120–125.
- Payandemehr, B., Rahimian, R., Bahremand, A., Ebrahimi, A., Saadat, S., Moghaddas, P., et al. (2013): Role of nitric oxide in additive anticonvulsant effects of agmatine and morphine. *Physiol. Behav.* 118:52-57.
- Raasch, W., Regunathan, S., Li, G., and Reis, D.J. 1995. Agmatine, the bacterial amine, is widely distributed in mammalian tissues. *Life Sci.* 56: 2319–2330.
- Radner, W., Höger, H., Lubec, B., Salzer, H., and Lubec, G. (1994): L-arginine reduces kidney collagen accumulation and N-epsilon- (carboxymethyl) lysine in the aging NMRI-mouse. *J. Gerontol.* 49:M44–M46.
- Rouch, A. and Kudo, L. (2002): Agmatine inhibits arginine vasopressin-stimulated urea transport in the rat inner medullary collecting duct. *Kidney Int*; 62: 2101-2108.
- Rushaidhi, M., Collie, N.D., Zhang, H., and Liu, P. (2012): Agmatine selectively improves behavioural function in aged male Sprague-Dawley rats. *Neuroscience.* 218: 206-215.
- Santos, A., Gadotti, V., Oliveira, G. et al. (2005): Mechanisms involved in the antinociception caused by agmatine in mice. *Neuropharmacology*, 48: 1021-1034.
- Schierwagen, C., Bylund-Fellenius, A.C. and Lundberg, C. (1990): Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity *J Pharmacol Methods.* 23(3):179-186.
- Schuling, M., Meurs, H., Zuidhof, A.B., Venema, N., and Zaagsma, J. (1998): Dual action of iNOS-derived nitric oxide in allergen-induced airway hyperreactivity in conscious, unrestrained guinea pigs. *Am. J. Respir. Crit. Care Med.* 158: 1442–1449.
- Shin, B.C., Kwon, Y.E., Chung, J.H. and Kim, H.L. (2012): The antiproteinuric effects of green tea extract on acute cyclosporin-induced nephrotoxicity in rats. *Transplant Proc* ;44(4):1080-2.
- Schwartz, D., Peterson, O.W., Mendonca, M., Satriano, J., Lortie, M., and Blantz, R.C. (1997): Agmatine affects glomerular filtration via a nitric oxide synthase-dependent mechanism. *Am. J. Physiol.* 272: F597–F601.
- Takenaka, T., Hashimoto, Y., Epstein, M. (1992): Diminished acetylcholine-induced vasodilation in renal microvessels of cyclosporin-treated rats. *J. Am. Soc. Nephrol.* 3, 42–50.
- Ten Broeke, R., De, C.R., Van Haperen, R., Verweij, V., Leusink-Muis, T., Van Ark, I., et al. (2006): Overexpression of endothelial nitric oxide synthase suppresses features of allergic asthma in mice. *Respir. Res.* 7: 58.
- Teixeira, R.B., Kelly, J., Alpert, H., Pardo, V. and Vaamonde, C.A. (1982): Complete protection from gentamicin-induced acute renal failure in the diabetes mellitus rat. *Kidney Int.* 21: 600-612.
- Uzbay, T.I. (2012): The pharmacological importance of agmatine in the brain. *Neurosci. Biobehav. Rev.* 36: 502–519.
- Wongmekiat, O. and Thamprasert, K. (2005): Investigating the protective effects of aged garlic extract on cyclosporin-induced nephrotoxicity in rats. *Clin Pharmacol*, 19, 555–562.
- Yilmaz, S., Atessahin, A., Sahna, E., Karahan, I., Ozer, S. (2006): Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. *Toxicology* 218:164–171.
- Zal, F., Mostafavi-Pour, Z. and Vessal, M. (2007): Comparison of the effects of vitamin E and/or quercetin in attenuating chronic cyclosporin A-induced nephrotoxicity in male rats. *Clin Exp Pharmacol Physiol*;34:720–724.
- Zoja, C., Furci, L., Ghilardi, F., Zilio, P., Benigni, A., Remuzzi, G. (1996): Cyclosporin-induced endothelial cell injury. *Lab. Invest.* 55, 455–462.