

Original Article**Renoprotective Effects of Naringenin and Olive Oil against Cyclosporine-Induced Nephrotoxicity in Rats**Said Said Elshama*¹, Hosam-Eldin Hussein Osman², Ayman El-Meghawry El-Kenawy³

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ABSTRACT

Background: Prolonged use of cyclosporine A for prevention of allograft rejection is associated with nephrotoxicity development. Naringenin and olive oil are beneficial dietary antioxidants with potential renoprotective properties. This study aimed to evaluate the therapeutic effect of naringenin and olive oil in alleviating cyclosporine induced nephrotoxicity in rats by the assessment of renal function and lesions, and redox status parameters.

Methods: Eighty adult male rats were divided into four groups during a 45 days treatment period; control group received saline; the second group treated with 25 mg/kg/d of cyclosporine while the third and fourth groups received 100 mg/kg/d of naringenin and 1.25 ml/kg/d of virgin olive oil respectively, together with the same cyclosporine dose.

Results: Cyclosporine-treated rats presented renal dysfunction and damage, as viewed by the elevated serum markers of renal function and kidney histopathological lesions, when compared to the control animals with an increase in the blood cyclosporine level and impaired redox status parameters.

Conclusion: Co-administration of naringenin or virgin olive oil with cyclosporine alleviated nephrotoxicity by serum urea and creatinine levels reduction, renal lesions amelioration, as well as the improved antioxidant parameters. Naringenin and virgin olive oil have potential to act as natural renoprotective agents against cyclosporine -induced nephrotoxicity.

Keywords: Cyclosporine, Naringenin, Nephrotoxicity, Olive Oil.

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INTRODUCTION

Organ transplantation becomes the best modern trend for some chronic diseases treatment such as end-stage hepatic and renal failure. Prevention of allograft incompatibility is a major challenge because of the high risk of transplanted organ rejection. This problem has been clinically managed by using immunosuppressive agents [1]. Cyclosporine A (CsA), a calcineurin inhibitor, is considered one of the most potent immunosuppressive drugs; however, its clinical use has been associated with undesirable side-effects, including nephrotoxicity, which leads to shorten the long- term allograft and the patients survival rates [2].

Although acute and chronic CsA-induced nephrotoxicity are well established, the underlying mechanism remains debatable [3], with several distinct possibilities, including a direct effect due to calcineurin inhibition, overexpression of

fibrotic mediators, such as the transforming growth factor- β 1 (TGF- β 1), the impairment of vasoactive substances, including angiotensin II, nitric oxide (NO), thromboxane A (TXA), serotonin, catecholamine and leukotriene, as well as, increased generation of reactive oxygen species "ROS" and consequent oxidative stress [4, 5].

Folk medicine, based on plants and herbs, has been used as an old trend by the grandfathers for many diseases treatment, and are now viewed as putative beneficial modulators of some drugs toxicity as a new trend in the modern medicine [6, 7]. Naringenin (NGN) is bioflavonoids highly distributed in citrus fruits, tomatoes, cherries, grapefruit and coca, and has many pharmacological actions such as anti-inflammatory, antitumor, antiviral, anti-diabetic and antioxidant [8, 9] associated with renoprotective properties against CsA-induced

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nephrotoxicity, despite the precise mechanisms remain to be fully elucidated [10].

Virgin olive oil, containing monounsaturated fatty acids (MUFAs) and phytochemicals, such as polyphenolic compounds, is an important element in the human diet because of its health benefits [11, 12]. MUFAs reduce the circulating lipoprotein's sensitivity to peroxidation because it decreases endothelial activation and the susceptibility of low density lipoprotein (LDL) to oxidation, while phenolic compounds show potent antioxidant properties against lipids, DNA and LDL oxidation; in addition, it has also anti-cancer and anti-inflammatory effects, and ability for immune response modification [13, 14]. However, the putative renoprotective effects of virgin olive oil remain to be clarified, namely against CsA-induced nephrotoxicity.

Therefore, the present study aimed to compare the renoprotective effects of naringenin and olive oil against cyclosporine-induced nephrotoxicity in rats, focusing on renal dysfunction and damage, as well as antioxidant properties.

MATERIAL AND METHODS

Eighty healthy adult male Sprague-Dawley rats weighing 200 - 250 g were obtained from the animal house of King Abdel Aziz University-Jeddah, Saudi Arabia. Rats were maintained at a day-night regimen of 12h/12h and free access to water and rat chow during the experimental period of 45 days. Rats were divided into four groups (n=20 each one). The control group received physiological saline, the second group treated with 25 mg/kg/d of cyclosporine dissolved in physiological saline [15], while the third and fourth groups received 100 mg/kg/d of naringenin [16], and 1.25 ml /kg /d of virgin olive oil [17], respectively, together with the same cyclosporine dose. Cyclosporine, naringenin and virgin olive oil doses were given daily by gastric gavage. Cyclosporine was available in a soft gelatin capsule (50 mg) that was manufactured by R.P. Scherer GmbH & Co. KG, Eberbach / Baden - Germany- for Novartis Pharma AG, Basle, Switzerland while naringenin was available in a powder form > 95% that was produced by Sigma-Aldrich Co., (USA).The virgin olive oil was obtained from Wadi Food Industries Co., Egypt whereas it is cold press and cholesterol free while its acidity is below 0.8%.

Blood Sample Collection

In the last day of experiment, rats were anesthetized by diethyl ether whereas the blood samples were collected from the orbital sinus under anesthesia using the covered test tubes. The samples were centrifuged 2000 rounds at 4 °C for 10 min to separate the serum for renal function tests assay (urea & creatinine) that were determined by the routine colorimetric methods using commercial kits and then it quantifies on clinical biochemistry autoanalyser [18]. Cyclosporine blood level assay was measured in the whole blood sample that was taken after 12 h from the last administration of drug dose by a monoclonal antibody-based fluorescence polarization immunoassay system on TDx analyzer (Abbott lab, USA) [19].

Histopathological Studies

Rats were weighed and then sacrificed under an excess anesthesia after 24 h from the last administration of cyclosporine, naringenin and olive oil. An abdominal incision was carried out for renal excision from four groups; kidneys were weighed and then fixed in 10% neutral buffered formalin whereas the fixed specimens were trimmed, washed and dehydrated in the ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 μm thickness and stained by haematoxylen and eosin, periodic acid Schiff and Mallory stain for the light microscope examination [20].

Renal tissue specimens were prepared for ultrastructure studies via fixation in 2.7% glutaraldehyde solution and 0.1 M phosphate buffer for 1.5 h at 4 °C and then washed in 0.15 M phosphate buffer (pH 7.2), and post-fixed in 2% osmic acid solution in 0.15 M phosphate buffer for one hour at 4 °C. Dehydration was carried out in acetone while the inclusion was in the epoxy embedding resin Epon 812. The blocks were cut with an ultramicrotome type LKB at 70 nm thickness. The sections were doubly contrasted with the solutions of uranyl acetate and lead citrate for analysis by using a transmission electron microscope [21].

Immunohistochemical Study

The paraffin-embedded kidney sections were immunohistochemically stained by standard avidin-biotin peroxidase method for caspase-3 expression identification whereas it was

deparaffinized and hydrated then an endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for five minutes. The sections were incubated overnight with mouse monoclonal primary antibody to caspase-3 with dilution 1-150 (31A1067 - NOVUS biological), it was rinsed in a phosphate buffered saline, and few drops of biotinylated goat - anti-mouse polyvalent secondary antibody were applied for ten minutes. It was incubated with streptavidin conjugated peroxidase for thirty minutes whereas a brown colored reaction was developed by exposing sections to 3, 3-diaminobenzidine tetrahydrochloride solution (DAB) for five minutes and then it was washed in a distilled water. The sections were counter stained with haematoxylen while the control slides were stained without primary antibody [22].

Tissue Preparation

Renal tissues (500 mg) were homogenized in 4 ml of the buffer solution of phosphate buffered saline at pH 7.4 whereas the homogenates were centrifuged at 10.000 xg for 15 min at 4 °C. The resultant supernatant was used for peroxidative stress assay such as malondialdehyde "MDA" and antioxidant enzymes assay such as catalase "CAT", superoxide dismutase "SOD", glutathione peroxidase "GPX" and glutathione "GSH" [23].

Statistical Analysis

SPSS version 17 (Chicago, IL, USA) was used for statistical analysis. Results were analyzed using one-way ANOVA, post-hoc multiple comparisons test (TUKEY) to investigate the difference among groups. The results variability

was expressed as mean + SD while *P* value of 0.05 was considered statistically significant.

Ethical Considerations

The most appropriate animal species was chosen for this research. Promotion the high standard of care and animal well-being at all times was done. An appropriate sample size was calculated by using the fewest number of animals to obtain statistically the valid results. Painful procedures were carried out under anesthesia to avoid distress and pain. Our standards of the animal care and administration met those required by an applicable international laws and regulations.

RESULTS

Body and Kidney Weight Changes in the Different Experimental Groups

There was a statistical significant decrease in the rats' body weight of second group (cyclosporine) in comparison with the control group. Furthermore, the rats' body weight of third group (cyclosporine and naringenin) and fourth group (cyclosporine and an olive oil) had a statistical significant increase in comparison with the second group (cyclosporine) Figure 1. The rats' kidney weight of second group (cyclosporine) had a statistical significant decrease in comparison with the control group while the rats' kidney weight of third group (cyclosporine and naringenin) and fourth group (cyclosporine and an olive oil) had a statistical significant increase in comparison with the second group (cyclosporine) Figure 2. *P* < 0.001 indicates to a statistical significant difference between the groups.

Weight (gm)

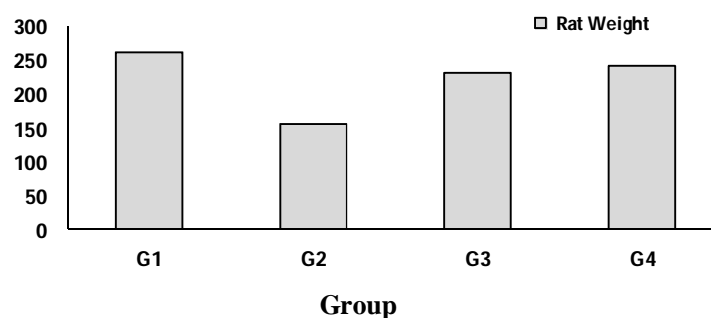


Figure 1. Comparison between the rats' body weight in the different groups

G1: Physiological saline (Control).

G2: Cyclosporine.

G3: Cyclosporine and Naringenin.

G4: Cyclosporine and olive oil.

Number per group: 20

Weight (gm)

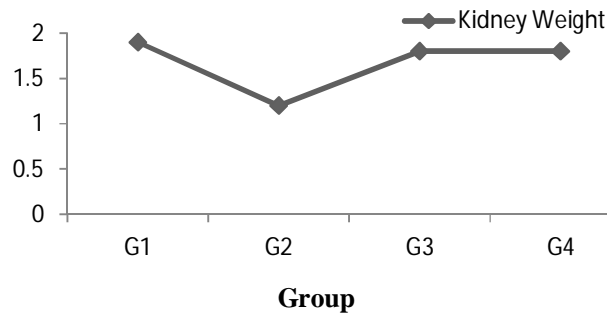


Figure 2. Comparison between the rats' kidney weight in the different groups

G1: Physiological saline (Control).

G2: Cyclosporine.

G3: Cyclosporine and Naringenin.

G4: Cyclosporine and olive oil.

Number per group: 20

Cyclosporine level (ng/ml)

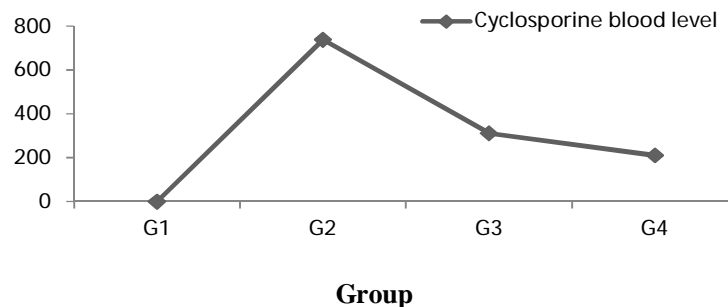


Figure 3. Comparison between the rats' cyclosporine blood level in the different groups

G1: Physiological saline (Control).

G2: Cyclosporine.

G3: Cyclosporine and Naringenin.

G4: Cyclosporine and olive oil.

Number per group: 20

The Cyclosporine Blood Level Assay

The rats' cyclosporine blood level of second group (cyclosporine) was increased statistically significantly in comparison with the control group while the rats' cyclosporine blood level of third group (cyclosporine and naringenin) and fourth group (cyclosporine and an olive oil) were decreased statistically significantly in comparison with the second group (cyclosporine) Figure 3. $P < 0.001$ indicates to a statistical significant difference between the groups.

Biochemical Findings

A-Renal Biochemical Markers

The serum urea and creatinine levels of second group (cyclosporine) were increased statistically significantly in comparison with the control group while the serum urea and creatinine levels of third group (cyclosporine and naringenin) and fourth group (cyclosporine and an olive oil) were decreased statistically significantly

in comparison with the second group (cyclosporine) Table 1.

B- Redox Status Parameters

There was a statistical significant decrease in the values of catalase, peroxidase, glutathione and superoxide dismutase in the second group (cyclosporine) in comparison with the control group while these values were increased statistically significantly in the third group (cyclosporine and naringenin) and fourth group (cyclosporine and an olive oil) in comparison with the second group (cyclosporine). Conversely, the values of malondialdehyde (MDA) and nitric oxide were increased statistically significantly in the second group (cyclosporine) in comparison with the control group and then it decreased statistically significantly in the third group (cyclosporine and naringenin) and fourth group (cyclosporine and an olive oil) in comparison with the second group (cyclosporine) Table 2.

Table 1. Comparison between Mean + SD of renal function test in the rats' different groups.

Group	First Control	Second CsA	Third CsA+ NGN	Fourth CsA+ Olive oil	F
Parameter	M±SD	M±SD	M±SD	M±SD	
Urea	27.75±4.39	81.2±6.06*	33.3±8.93**	33.65±1.98**	356.406
Creatinine	0.86±0.213	5.78±0.27*	0.72±0.12**	0.83±0.078**	3.597

First group: Physiological saline.

Second group: Cyclosporine (CsA).

Third group: Cyclosporine and Naringenin (CsA+ NGN).

Fourth group: Cyclosporine and olive oil (CsA+ Olive oil).

Number per group: 20

SD: Standard deviation

* **P < 0.001:** Significant difference in comparison with the control group

** **P < 0.001:** Significant difference in comparison with the second group

Table 2. Comparison between Mean +SD of redox status parameters in the rats' different groups.

Group Parameter	First Control	Second CsA	Third CsA+ NGN	Fourth CsA+ Olive oil	F
	M±SD	M±SD	M±SD	M±SD	
Catalase	36.84±1.66	14.52±2.58*	37.28±1.82**	36.58±2.31**	555.513
Peroxidase	15.51±1.22	8.79±1.05*	15.89±1.22**	14.44±1.09**	164.854
GSH	93.32±1.51	62.52±4.85*	95.02±3.33**	94.52±2.86**	447.757
MDA	23.78±0.59	54.3±2.57*	24.30±2.57**	24.85±1.95**	1.031
Nitric oxide	48.66±4.35	59.92±6.41*	43.47±2.04**	44.12±1.91**	68.208
Superoxide Dismutase	25.41±1.78	14.21±0.92*	25.58±2.67**	26.56±1.65**	196.237

First group: Physiological saline (Control).

Second group: Cyclosporine (CsA).

Third group: Cyclosporine and Naringenin (CsA+ NGN).

Fourth group: Cyclosporine and olive oil (CsA+ Olive oil).

Number per group: 20.

SD: Standard deviation.

GSH: Glutathione.

MDA: Malondialdehyde.

* **P < 0.001:** Significant difference in comparison with the control group

** **P < 0.001:** Significant difference in comparison with the second group

Histopathological Findings

A- Renal Histopathological Findings by the Light Microscope

Examination of renal tissues in the rats of first group (control) showed normal renal structure (Figs.4a & 5a), positive periodic acid Schiff (PAS) stain in the renal glomeruli and the luminal brush border of renal tubules (Fig. 6a), with weak caspase -3 expression in glomeruli and cytoplasm of renal tubular cells (Fig.7a). But, the renal tissues in the rats of second group which received cyclosporine, showed atrophy of tubular cells with a cystic dilatation, destructed epithelium of Bowman's capsule with enlarged glomeruli (Fig.4b) and interstitial fibrosis (Fig.5b). Intense positive periodic acid Schiff reaction (PAS) stain was appeared in the media of renal blood vessels and glomerular capillaries (Fig. 6b) with marked

caspase -3 expression in the cytoplasm of renal tubular cells and the lining cells of glomerular capillaries (Fig.7b). Transverse section of renal tissues in the rats of third group, which received cyclosporine and naringenin, showed nearly normal renal structure, highly acidophilic cytoplasm and vesicular nuclei (Fig.4c), a minimal renal interstitial connective tissue (Fig.5c) with nearly positive periodic acid Schiff (PAS) stain (Fig.6c) and weak caspase -3 expression (Fig.7c). The renal tissues of fourth group rats that received cyclosporine and virgin olive oil showed nearly normal renal structure, a slight congestion and a minimal inflammatory cell reaction (Fig.4d), a minimal renal interstitial connective tissue (Fig.5d) with positive periodic acid Schiff (PAS) stain (Fig.6d) and weak caspase -3 expression (Fig.7d).

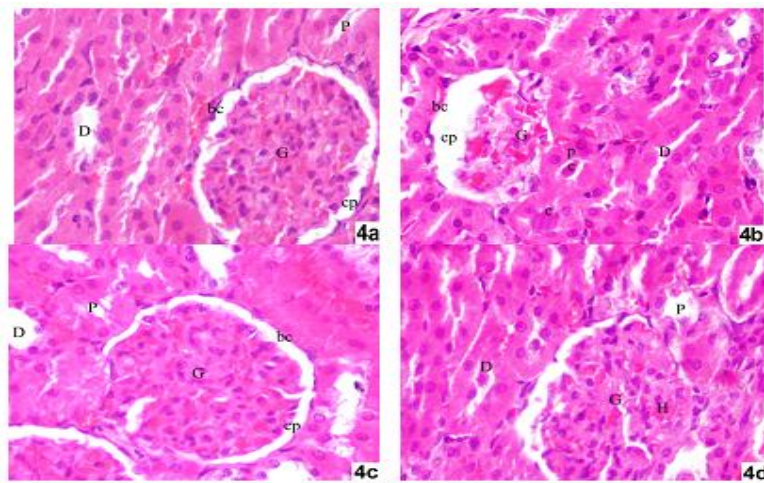


Figure 4. A: a photomicrograph of section in the control rat kidney shows normal glomeruli (G) and glomerular capsular space (CP), a flat epithelium lining the glomerular capsule (bc) and distinct capsular space (cp), a typical thick cubic epithelium of proximal convoluted tubules (P) and a low simple cubic epithelium of distal convoluted tubules (D). (H&E X400)

B: a photomicrograph of section in the second group rat kidney shows enlarged vascular glomeruli (G), widening the glomerular capsular space (cp), destructed flat epithelium lining of Bowman's capsule (bc), tubular cell atrophy of proximal (P) and distal tubules (D), a cystic dilation of renal tubules with appearance of tubular casts (c). (H&E X400)

C: a photomicrograph of section in the third group rat kidney shows nearly normal glomeruli (G), a flat epithelium lining the glomerular capsule (bc), distinct capsular space (cp), with normal proximal (P) and distal (D) convoluted tubules. (H&E X400)

D: a photomicrograph of section in the fourth group rat kidney shows nearly normal glomeruli (G), a flat epithelium lining the glomerular capsule (bc), distinct capsular space (cp), normal proximal (P) and distal (D) convoluted tubules with congestion and minimal inflammatory cell reaction. (H&E X400)

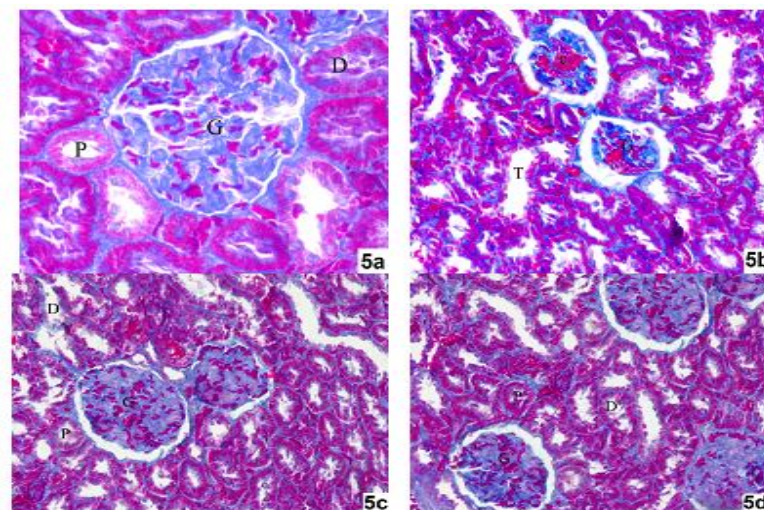


Figure 5. A: a photomicrograph of section in the control rat kidney shows normal glomeruli (G), normal proximal (P) and distal (D) tubules with a minimal connective tissue in the renal interstitial tissues. (Mallory X400)

B: a photomicrograph of section in the second group rat kidney shows vascular congestion (c) of glomeruli (G) with multiple foci of tubular interstitial fibrosis. (Mallory X400)

C: a photomicrograph of section in the third group rat kidney shows nearly normal glomeruli (G), proximal (P) and distal (D) renal tubules with minimal renal interstitial connective tissue. (Mallory X400)

D: a photomicrograph of section in the fourth group rat kidney shows nearly normal glomeruli (G), proximal (P) and distal (D) renal tubules with mild renal interstitial connective tissue. (Mallory X400)

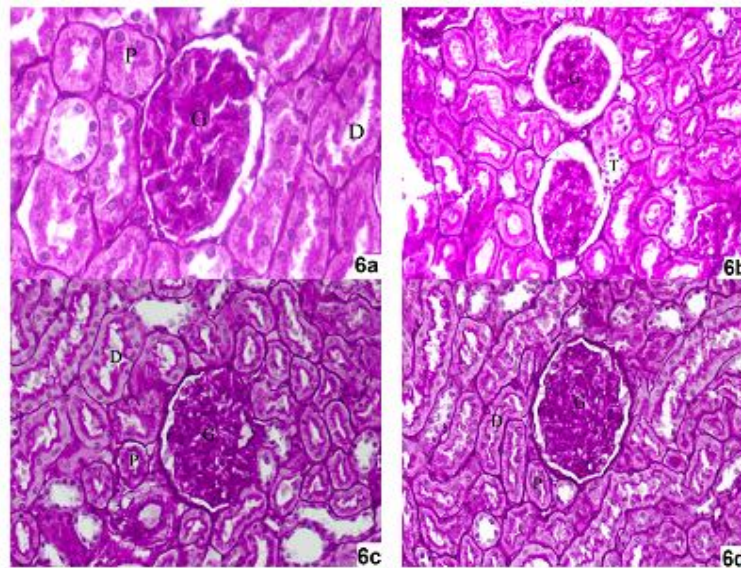


Figure 6. A: a photomicrograph of section in the control rat kidney shows positive reaction in renal glomeruli (G), luminal brush border of proximal (P) and distal (D) renal tubules. (PAS X400)
 B: a photomicrograph of section in the second group rat kidney shows strong positive reaction in renal glomeruli (G) and moderate reaction in the renal tubules (T). (PAS X400)
 C: a photomicrograph of section in the third group rat kidney shows positive reaction in renal glomeruli (G) and the luminal brush border of proximal (P) and distal (D) renal tubules. (PAS X400)
 D: a photomicrograph of section in the fourth group rat kidney shows positive reaction in renal glomeruli (G) and the luminal brush border of proximal (P) and distal (D) renal tubules. (PAS X400)

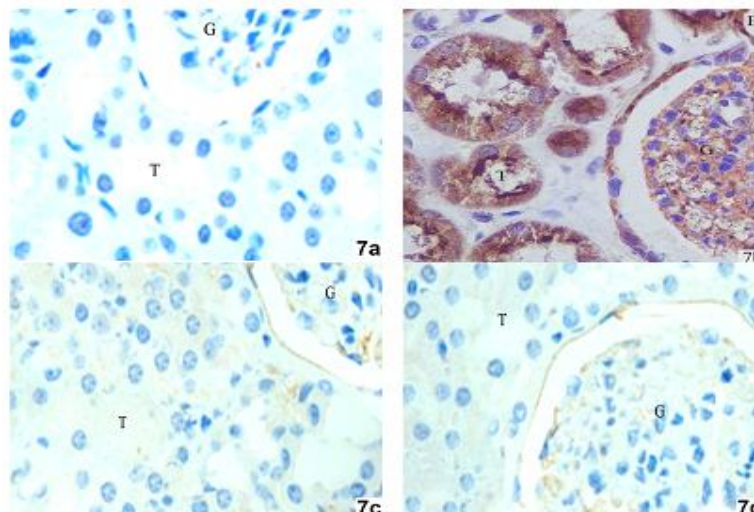


Figure7. A: a photomicrograph of section in the control rat kidney shows weak caspase -3 expression in renal glomeruli (G) and cytoplasm (T) of renal tubular cell. (X1000)
 B: a photomicrograph of section in the second group rat kidney shows strong caspase -3 expression in glomerular capillaries (G), cytoplasm (T) of renal tubular cell and endothelial cells of blood vessels (E). (X1000)
 C: a photomicrograph of section in the third group rat kidney shows weak caspase -3 expression in the glomerular capillaries (G) and cytoplasm (T) of renal tubular cells. (X1000)
 D: a photomicrograph of section in the fourth group rat kidney shows weak caspase -3 expression in the glomerular capillaries (G) and cytoplasm (T) of renal tubular cells. (X1000)

B- Renal Histopathological Findings by the Transmission Electron Microscope

The rats of first group (control) showed normal ultrastructure of renal cells (Fig. 8a). However, the renal cells in the rats of second group which received cyclosporine, showed shrinkage nuclei in the cells of proximal convoluted tubules, destructed cytoplasmic organelles with many vacuoles, remarkable swollen mitochondria with a decrease in the number, thickened tubular basement membrane and missed microvilli in the apical brush border

(Fig. 8b). The ultrastructure of renal cells in the rats of third group, which received cyclosporine and naringenin showed an increase in scattered normal mitochondria number in cytoplasm of proximal convoluted tubules cells and well developed microvilli with a large spherical nucleus (Fig. 8c) while the renal cells of fourth group rats which received cyclosporine and virgin olive oil showed nearly the same ultrastructure of third group whereas nucleus and mitochondria were nearly normal with well-developed microvilli (Fig. 8d).

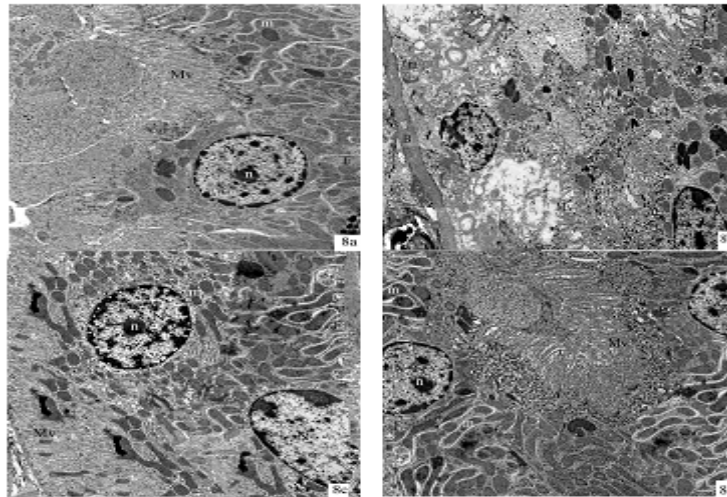


Figure 8.A: an electron micrograph of proximal convoluted tubules cells of the control rat kidney shows a large spherical nucleus (N) with a small nucleolus (n), a large number of mitochondria (m) with basal infolding (F), and microvilli (Mv). (X8000)

B: an electron micrograph of proximal convoluted tubules cells of the second group rat kidney shows destruction of cytoplasmic organelles with presence of vacuoles (v), swollen mitochondria (m), destructed microvilli (Mv) and shrunken nucleus (N) with thickened basement membrane (B). (X8000)

C: an electron micrograph of proximal convoluted tubules cells of the third group rat kidney shows nearly a normal nucleus (N) with a small nucleolus (n), a large number of mitochondria (m) and basal infolding (F) with nearly normal microvilli (Mv). (X8000)

D: an electron micrograph of proximal convoluted tubules cells of the fourth group rat kidney shows nearly a normal nucleus (N) with a small nucleolus (n), an increase in the number of mitochondria (m) and basal infolding (F) with nearly normal microvilli (Mv). (X8000)

DISCUSSION

Cyclosporine is still the first choice treatment for transplanted organ rejection because of its immunosuppressive action. However, some adverse effects of its long use may be a challenge leading to a limitation for its use in the future. Nephrotoxicity is considered one of the most common side effects of cyclosporine use whereas many researches tried to find a solution for it by using some natural agents. Therefore, the current study aimed to investigate the role of alternative

medicine in overcoming cyclosporine nephrotoxicity by evaluating the therapeutic efficacy of some dietary natural agents such as naringenin and olive oil in amelioration cyclosporine induced nephrotoxicity.

The current study showed that there was a significant decrease in the rats' body and kidney weight of second group which received cyclosporine only in comparison with the control group because cyclosporine toxicity causes anorexia and catabolic effect leading to the body

weight loss in agreement with a previous study [24]. Our immunohistochemical study showed that an increase in apoptotic cells number due to cyclosporine toxicity is consistent with a previous study [25] who referred to renal apoptosis as a cause for kidney weight reduction.

There was a significant gain in the rats' body and kidney weight of third group which received naringenin with cyclosporine because of antioxidant effect of naringenin that counteracts effects of cyclosporine induced oxidative stress such as the weight loss in consistent with a previous study [26], but this is in contrast with a study [27] who reported that naringenin reduces the body weight gain based on its insulin-like properties that correct many metabolic disturbances linked to the insulin resistance without any effect on the appetite. There was also a significant gain in the rats' body weight of fourth group, which received an olive oil with cyclosporine in comparison with the second group, which received cyclosporine only, and the third group, which received naringenin with cyclosporine in consistent with [28] who reported that long-term ingestion of virgin olive oil produces weight gain and insulin resistance.

Our results revealed that cyclosporine induced renal histopathological and ultrastructural changes, significant increase of renal biomarkers blood level (urea & creatinine) in consistent with previous studies [29, 30], and renal cells apoptosis in consistent with Buffoli et al. [31]. Renal changes and dysfunction are based on cyclosporine toxicity induced oxidative stress that generates reactive oxygen species (ROS) leading to lipid peroxidation [32], in consistent with a study [33], and our results that showed a significant increase in oxidative parameters (malondialdehyde "MDA" and nitric oxide) with a significant decrease in glutathione and antioxidant enzymes (catalase, peroxidase and superoxide dismutase) as a reflection to high blood level of cyclosporine that causes oxidative damage such as enzyme inactivation, mitochondrial dysfunction and cell death [34]. The present study indicated that concomitant administration of naringenin with cyclosporine in the third group leads to amelioration of cyclosporine toxicity manifestations in comparison with the second group which received cyclosporine only in agreement with a previous study [35] who showed that naringenin was a primary antioxidant because

it prevents chain reactions via an electron donation to the peroxy radical of fatty acids inducing regulation of antioxidative capacity based on the increase of glutathione, superoxide dismutase, peroxidase and catalase activities by upregulation its gene expression. Lin et al. [36], add that naringenin upregulates hemeoxygenase enzyme expression in renal tissues maintaining its level and then it reduces renal oxidative stress damage and its oxidative parameters such as malondialdehyde "MDA" and nitric oxide. Chandramohan and Parameswari [26], are also in consistent with our results for an improvement in renal histopathological and ultra-structural changes, cyclosporine blood level, renal functions and redox status parameters due to concurrent use of naringenin and cyclosporine.

The current study referred that co-administration of virgin olive oil with cyclosporine modulates its toxicity manifestations for renal morphological and ultra-structural changes, caspase-3 stain reaction, oxidative and antioxidant parameters at the same degree of improvement that is exerted by naringenin but it has a different manner for serum creatinine level and cyclosporine blood level which are more decreased significantly in comparison with naringenin effect in the third group in consistent with [37] who showed that phenolic compounds of olive oil are potent lipid peroxidation inhibitors whereas it is a free radical scavenging and chelators for metal ions that catalyze free radical generation reactions and then it is considered potent antioxidants [38].

CONCLUSION

Long use of cyclosporine may lead to renal oxidative stress damage that is manifested by histopathological and ultra-structural changes with renal dysfunction. Concurrent use of naringenin or virgin olive oil with cyclosporine induces an improvement in nephrotoxicity manifestations by restoration the efficacy of oxidant - antioxidant pathways. The concurrent use of naringenin or virgin olive oil as dietary neutral agents with prolonged use of cyclosporine is recommended in the organs transplanted patients to modulate its nephrotoxicity.

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