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Evaluation of reno-protective effect of grape seed proanthocyanidin extract on Cyclosporine A- induced Nephrotoxicity by mitigating inflammatory response, oxidative stress and apoptosis in rats

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ABSTRACT

Cyclosporine A (CsA) is a drug used as immunosuppressive agent in organ transplant and non-transplant medicine. The main secondary effect results from CsA treatment is nephrotoxicity. A protective effect of Grape seed proanthocyanidin extract (GSPE) against CsA-induced nephrotoxicity in rats was assessed. Thirty male rats were divided into three equal groups. Group I: (Normal control), received no drugs, Group II: (CsA treated), rats received oral dose of CsA (25 mg/kg b.wt/day) for 21 successive days. Group III: (GSPE protected+ CsA), rats received GSPE (200 mg/kg b.wt/day) orally 7 days before and during 21 days of CsA treatment. The obtained results showed a significant increase in serum urea and creatinine concentration in addition to L-MDA levels in kidney tissue while a marked decrease in renal catalase activity and GSH concentration in CsA treated rats. Moreover, a significant down-regulation in Bcl-2 and up-regulation of NF-κB, PAI-1, Caspase-3 and p53 gene expressions were observed in kidney tissues of CsA treated rats. Meanwhile, GSPE potentially improved renal function and oxidative alterations related to CsA near its normal ranges. Also, various histopathological alterations were detected in kidneys of CsA treated rats. Interestingly, histopathological findings supported that where GSPE markedly attenuated the harmful effects induced by CsA and protected kidney tissues. Our research could conclude that, GSPE has an ameliorating role as potent antioxidant, anti-inflammatory and anti-apoptotic agent via inhibition of inflammatory (NF-κB, PAI-1) and apoptotic (Caspase-3, p53) signaling pathway in modulation of CsA-induced nephrotoxicity.

1. INTRODUCTION

Cyclosporins are immunosuppressant drugs isolated from *Tolypocladium inflatum* (Cragg and Newman, 2013). Cycloeporine A nephrotoxicity is related to its chronic use in numerous autoimmune diseases and in organ transplantation especially kidney transplantation (Raeisi et al., 2016). Oxidative stress has important role in Cyclosporine A nephrotoxicity. Cyclosporine A-induced nephrotoxicity results from the endoplasmic reticulum stress activation, the increased production of the mitochondrial reactive oxygen species (ROS) which modifies the redox balance enhancing lipid peroxidation ends with nephrotoxicity (Wu et al., 2018).

Procyanidins are the most common flavonoids present in grapes. Grape seed proanthocyanidin extract (GSPE) is rich in polyphenols which play an important role as a metabolic regulator and reactive oxidative species (ROS) scavenger (Gil-Cardoso et al., 2017). GSPE significantly protects against oxidative stress damage more effective than vitamins C, E and beta-carotene (Hassan et al, 2013).

This study aims to evaluate the possible protective effect of grape seed proanthocyanidin extract (GSPE) against the nephrotoxic effect of Cycosporine A long term administration in adult male rats through investigation of renal functions, oxidative stress and antioxidant biomarkers, pro-inflammatory mediators, apoptotic and anti-apoptotic gene expression markers in kidney tissues in addition to histopathological alterations of kidneys.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Thirty-white male albino rats, 4-5 weeks old with average body weight 140-160 g were used in this study. Rats were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. All rats were acclimatized for two weeks prior to the beginning of study. The experimental protocols were approved by the Animal Care and Use committee at Benha University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Chemicals and antioxidant agent:

2.2.1. Cyclosporine (CsA): CsA presents in the form of soft gelatin capsules containing 50 mg cyclosporine under traditional name (Sandimmune®, Neoral®) obtained from (Novartis Pharma AG, Basilea, Suiza). CsA was freshly dissolved in propylene glycol. Nephrotoxicity induced in

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rats after oral treatment with CsA (25mg/kg b.wt/day) for 21 successive days according to Chia et al., (2012).

2.2.2. Grape Seed proanthocyanidin Extract (GSPE): GSPE was purchased from (Al Debeiky Pharma Company for Pharmaceutical industries, Al Obour, Cairo, Egypt). GSPE was dissolved in Dimethyl sulfoxide (DMSO) (100%) and diluted to the appropriate concentration by sterilized saline solution similar to previous study (El-Gawish et al., 2006).

2.3. Experimental design:

Rats were randomly divided into three groups (10 rats each) placed in individual cages and classified as follow:

2.3.1. Group I: (Normal control group): Rats fed only with ordinary diet without any treatment during the experimental period.

2.3.2. Group II: (Cyclosporine A nephrotoxic group): Rats orally received CsA (25 mg/kg b.wt/day) for 21 successive days.

2.3.3. Group III: (GSPE protected group): Rats orally received GSPE at a dose of (200 mg/kg b.wt/day) 7 days prior to CsA administration and concurrently during CsA treatment for 21 days later.

2.4. Sampling:

2.4.1. Blood samples

Blood sample was collected from retro-orbital plexus of eyes puncture. Blood was allowed to clot then centrifuged for 15 minutes at 3,000 rpm. Sera were separated in dry sterile tubes by automatic pipette, and then stored at -20 °C in a deep freezer until determination of urea and creatinine.

2.4.2. Tissue samples:

After blood collection rats were sacrificed by decapitation according to Animal Ethics Committees and abdomen was opened, then kidneys were collected.

2.4.3. For biochemical analysis:

Briefly, one gram of kidney tissues were cut and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates, then centrifuged for 15 minutes at 6000 r.p.m at 4°C. The supernatant used directly for determination of reduced glutathione (GSH), catalase (CAT), and L-malondialdehyde (L-MDA).

2.4.4. For molecular analysis (kidney):

About 0.5 g of kidney tissue was put in Eppendorf tubes, kept immediately in liquid nitrogen and stored at -80°C till RNA extraction for determination of caspase-3, tumor suppressor protein p53, B cell lymphoma-2 (Bcl-2), nuclear factor kappa B (NF-κB) and plasminogen activator inhibitor-1 (PAI-1) gene expressions.

2.4.5. For histopathological examination:

Small tissue specimens were collected from the kidneys of rats in all groups and then immediately fixed in 10% neutral buffered formalin solution then subjected to histopathological examination according to the technique described by Bancroft and Gamble, (2008).

2.5. Analysis:

2.5.1. Biochemical analysis

Serum urea and creatinine were measured according to described methods by Kaplan and Kohn (1992) and Jaffe, (1986), respectively. Also, kidney tissue catalase activity, GSH and L-MDA concentrations were measured according to described methods by Weydert and Cullen (2010), Moron et al. (1979) and Lahouel et al. (2004), respectively.

2.5.2. Molecular analysis:

The mRNA expression contents of *Caspase-3, p53, Bcl-2, NF-kB* and *PAI-1*were determined using real time quantitative polymerase chain reaction analysis (real-time qPCR) in rat kidney. Forward and reverse primers sequence for real time PCR is shown in table (1). β-actin was used as load control. Total RNA was isolated from the kidney using High Kit for isolation of pure RNA (Thermo Scientific, Fermentas, #K0731) RNA Extraction kit according to manufacturer's instructions. With each cDNA sample was reverse transcribed using RevertAid TM First Strand CDNA synthesis kit (#EP0451, Thermo Scientific, Fermentas, USA). Then, real-time quantitative PCR amplification was performed on Faststart Universal SYBR Green Master (Roche, GER). The target gene was normalized with β-actin by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analysis:

Results were expressed as mean ± SE using SPSS (13.0 software, 2009). Data were analyzed using one-way ANOVA followed by Duncan's test. Values were statistically significant at $p < 0.05$.

Table 1 Forward and reverse primers sequence for real time PCR.

Gene	Forward primer (′5 ------ ′3)	Reverse primer (′5 ------ ′3)
Caspase-3	GGTATTGAGACAGACAGTGG	CATGGGATCTGTTTCTTTGC
p53	ATGGCTTCCACCTGGGCTTC	TGACCCACAACTGCACAGGGC
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
NF-κB	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
PAI-1	TTCCTCCACAGCCATTCTAGTCT	GAAAGGATCGGTCTAAAACCATCTC
B-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

3. RESULTS

3.1. Biochemical findings

Data were presented in table (2) showed that, serum urea and creatinine were significantly elevated in CsA rats when compared with normal control group. However, GSPE significantly reduced these concentrations comparing with CsA nephrotoxic group.

The obtained results in table (3) showed a significant increase in L-MDA concentration and marked decrease in GSH concentration and CAT activity in kidney tissue of CsA treated rats as compared with normal control group. However, GSPE protected group showed a significant decrease in L-MDA level with marked increase in GSH concentration and CAT activity when compared to CsA nephrotoxic group.

The presented data in table (4) showed a significant up-regulation in NF-κB and PAI-1 gene expressions of kidney in CsA treated rats as compared with normal control group. In contrast to GSPE administration before and during CsA treatment reveals significant down-regulate of these gene expressions as compared with CsA nephrotoxic group.

The results presented in table (5) exhibited a significant up-regulation of caspase-3, p53 and down-regulation in Bcl-2

kidney gene expressions in CsA treated rats when compared to normal control group. Meanwhile, GSPE administration before and during CsA treatment resulted in significant down-regulation in Caspase-3, p53 and up-regulation in Bcl-2 kidney gene expressions when compared with CsA nephrotoxic group.

Table 2 The Protective effect of GSPE on serum urea and creatinine concentrations in Cyclosporine A-induced nephrotoxicity in rats.

Animal groups	Urea (mg/dl)	Creatinine (mg/dl)
Group I: Normal control	$35.44^{e} \pm 0.13$	$0.92^{d} \pm 0.01$
Group II: CsA group	$59.07^{a} \pm 0.23$	$1.58^{a,b} \pm 0.06$
Group III: GSPE + CsA	$44.40^{c} \pm 0.31$	$1.36^{b} \pm 0.02$

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 3 The Protective effect of GSPE on Kidney tissue L-MDA, GSH concentrations and CAT activity in Cyclosporine A-induced nephrotoxicity in rats.

Animal groups	L-MDA (mmol/g. tissue)	GSH (ng/g. tissue)	CAT (U/g. tissue)
Group I: Normal control	$3.34^{f} \pm 0.09$	$34.73^{a} \pm 0.32$	$9.75^{a} \pm 0.15$
Group II: CsA group	$17.03^{a} \pm 0.35$	$10.77^{d} \pm 0.28$	$2.41^{e} \pm 0.30$
Group III: GSPE + CsA	$10.45^{c} \pm 0.22$	$23.08^{c} \pm 0.57$	$7.47^{b} \pm 0.22$

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 4 The Protective effect of GSPE on kidney tissue NF-kB and PAI-1 gene expression levels in Cyclosporine A-induced nephrotoxicity in rats.

Animal groups	Nuclear factor kappa B(NF-kB) Fold change		Plasminogen activator inhibitor-1 (PAI-1) Fold change	
	Mean	SEM	Mean	SEM
Group I: Normal control	1.00^{e}	0.07	1.00^{f}	0.09
Group II: CsA group	4.35^{b}	0.21	7.21^{b}	0.24
Group III: GSPE + CsA	2.04^{d}	0.11	2.14^{e}	0.1

Data are presented as (Mean ± SEM). SEM = Standard error mean. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 5 The Protective effect of GSPE on kidney tissue apoptotic (Caspase-3, p53) and anti-apoptotic (Bcl-2) gene expression levels in Cyclosporine A-induced nephrotoxicity in rats.

Animal groups	Caspase-3 Fold change		Tumor suppressor protein P53 Fold change		BCl-2 Fold change	
	Mean	SEM	Mean	SEM	Mean	SEM
Group I: Normal control	1.00^{f}	0.07	1.00^{e}	0.06	1.00^{a}	0.05
Group II: CsA group	6.28^{b}	0.27	3.92^{b}	0.15	0.14^{f}	0.01
Group III: GSPE + CsA	3.29^{d}	0.14	3.07^{c}	0.15	0.49^{d}	0.02

Data are presented as (Mean ± SEM). SEM = Standard error mean. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

3.2. Histopathological examination:

Microscopical examination of kidney tissues obtained from normal control rats (Group I) revealed normal histological structure with normal glomeruli and proximal and renal tubules. The examined renal tissues obtained from CsA treated rats in (Group II) revealed various histopathological alterations including congestion of inter-tubular blood capillaries and renal blood vessels (Figure 1a) with perivascular mononuclear leukocytic cellular infiltrations mainly lymphocytes and macrophages in renal cortex with vacuolar degeneration in the wall of renal blood vessels (Figure 1b). Vacuolation of the lining endothelial cells of the glomerular tuft as well as Hyper-cellularity of the glomerular tuft with adhesions between Bowman's capsule and glomerular tufts were seen. (Figure1c). Accidently, necrosis, shrinkage and disintegration of the glomerular tuft were also observed (Figure 1c). The lining epithelium of some proximal and distal convoluted tubules in renal cortex exhibited extensive degenerative changes such as vacuolar and hydropic degeneration with necrosis of the lining epithelium of other renal tubules (Figure 1d&e). Meanwhile, microscopical examination of the kidney tissues obtained from the protected group with GSPE (Group III), revealed moderate improvement in the renal tissue histology as mild congestion of the inter-tubular blood capillaries and renal blood vessels with proliferation and hyper-cellularity of the endothelial cell lining of the glomerular tuft with adhesions between Bowman's capsule and glomerular tufts and absence of Bowman's spaces were detected (Figure 1e). Multifocally, the lining epithelium of some proximal and distal convoluted tubules in renal cortex exhibited mild degenerative changes such as cloudy swelling (Figure 1f)

4. DISCUSSION

Nephrotoxicity could be the major problem in transplant medicine as a side effect caused by Cyclosporine A (CsA) when used as an immunosuppressive drug**.** Oxidative stress has a crucial role in nephrotoxicity induced by Cyclosporine A (Raeisi et al., 2016). Apoptosis increased oxidative stress, and mitochondrial dysfunction were reported in CsA nephrotoxicity (Hausenloy et al., 2012). Renal failure was the end stage resulted from nephrotoxicity associated with long term administration of Cyclosporine A (Caires et al., 2018).

Cyclosporine A treated rats revealed a significant increase in serum concentration of urea and creatinine. Abdel-Wahab, (2015) demonstrated that CsA administration for 21 days impaired the proper kidney function and induced the renal oxidant/antioxidant homeostasis disturbance. Urea and creatinine levels were increased, while glomerular filtration rate (GFR) was decreased on chronic CsA administration. This was related to reactive oxygen species (ROS) formation in kidney revealed by increased lipid peroxidation (LPO), decreased GSH in addition to increased inducible nitric oxide synthase (iNOS) level establishing the oxidative stress role in pathogenesis of renal damage, vascular injury, and endothelium dysfunction induced by Cyclosporine A (Hewedy and Mostafa, 2016).

Administration of GSPE to CsA treated rats revealed significant decreases in serum levels of urea and creatinine, similar data were recorded by Yun et al., (2020) who recorded that administration of GSPE exhibited better recovery against renal oxidative damage induced in rats. The improvement in kidney function is related to the antioxidant properties of proanthocyanidins with flavonoid structure that give the ability to decrease blood levels of creatinine and urea by increasing glomerular filtration rate (Nazima et al., 2015).

A significant increase in kidney tissue L-MDA concentration with marked decreases in GSH level and CAT activity were observed in CsA treated rats. The increased lipid peroxidation increases peroxy and hydroxyl radicals resulting in oxidative damage (Mustafa et al., 2018). Our results agreed with Hussein et al., (2014) who demonstrated that a significant increase in renal L-MDA level resulted by CsA treatment to normal rats, suggesting the role of oxygen free radicals involved in renal injury. ROS production by CsA may be due to the drug action as an uncoupler and inhibitor of the mitochondrial electron transport system and metabolism of CsA by cytochrome P450 3A (Ateyya, 2015).

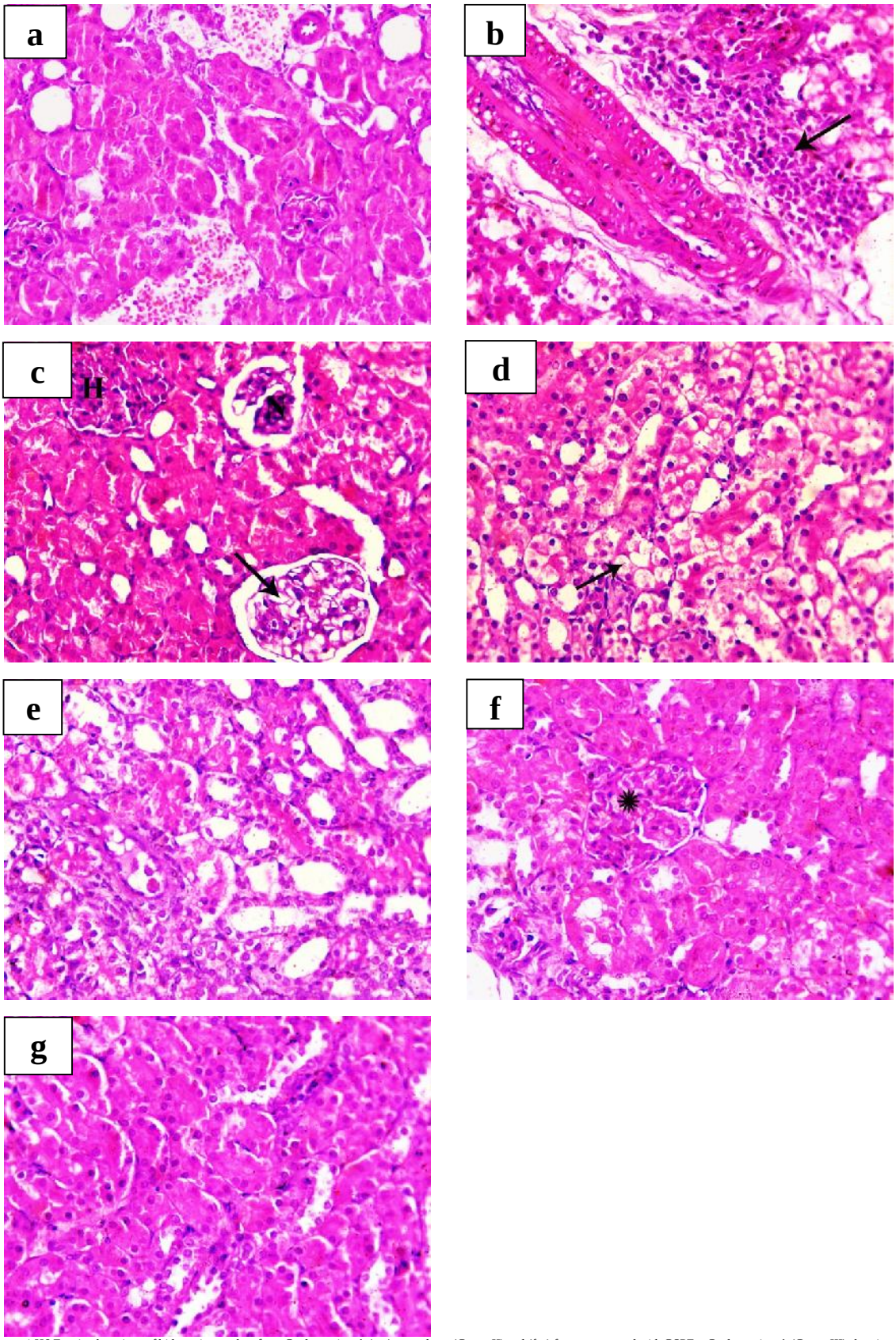

Figure 1 H&E stained sections of kidney tissue taken from Cyclosporine-A (a-e) treated rats (Group II) and (f-g) from rats treated with GSPE + Cyclosporine-A (Group III) showing (a) congestion of the renal blood vessel (x200), (b) perivascular leukocytic cellular infiltration (arrow) with vacuolar degeneration in the wall of renal blood vessels (x400), (c) vacuolation of endothelial cells lining the glomerular tuft (arrow) with coagulative necrosis and shrinkage of glomerular tuft (N), notice also hypercellularity of the endothelial cell lining the glomerular tuft (H, x200), (d) extensive vacuolation of the lining epithelium of some proximal and distal convoluted tubules (arrow, x200), (e) extensive degenerative changes and entire necrosis of the lining epithelium of renal tubules (x400), (f-g) hypercellularity of the endothelial cell lining the glomerular tuft (asterisk) with mild degenerative changes in the lining epithelium of renal tubules (x200).

Endogenous antioxidant defense system decreased due to results from ROS production. Excessive ROS decrease the renal antioxidant capacity and make the kidney more susceptible to oxidative damage. Significant decreases in

kidney GSH concentration and CAT activity was reported in CsA treated rats (Abdel-Wahab, 2015). It is possible that depletion of the production of NADPH which is required for activation of catalase from its inactive form during CsA-Treatment could decrease the catalase activity (Hussein et al., 2014).

On the other hand, GSPE administration resulted in a significant decrease in kidney tissue L-MDA and significantly increased renal tissue GSH concentration and CAT activity. These results agreed with Yun et al., (2020) who reported that L-MDA level was significantly reduced after GSPE administration. GSPE, as an antioxidant is thought to suppress ROS over-generation, therefore thought to eliminate the intracellular ROS level of kidney tissue in experimental rats, so GSPE offers the potential for enhancement of antioxidants and protects against tissue lipid peroxidation (Amin et al., 2018). GSPE treatment increased CAT activity in rats by increasing activity of glucose-6-phosphate dehydrogenase (G6PD) which produces NADH that is necessary for CAT activation and increased GSH content by protection of sulfhydryl groups in glutathione from oxidative damage by the quenching action of free radicals of the di-OH (catechol) structure in proanthocyanidins B-ring (Nazima et al., 2015) .

Our study showed that CsA treatment induced significant up-regulation of kidney tissue NF-κB and PAI-1 gene expression levels. CsA administration reduced the renal content of GSH, CAT and SOD activities resulted in oxidative damage expressed by increase in lipid peroxidation, iNOS and NF-κB expression (Balah, 2014). The NF-kB transcription factor has a role involved in the transcription of inflammatory iNOS and other inflammatory genes in response to oxidative stress (Aktan, 2004). Plasmogen activator inhibitor-1 (PAI-1) is a pro-fibrotic cytokine considered as the major inhibitor of fibrinolysis (Ghosh and Vaughan, 2012). PAI-1 increased expression *in vivo* inhibits fibrinolysis, and leading to the pathological fibrin deposition and tissue damage (Aso, 2007). CsA nephrotoxicity mechanism involved endothelial function impairment, vasoconstrictors and vasodilators mediators unbalance, activation of renin–angiotensin–aldosterone system and increased cell proliferation. These mediators increased transforming growth factor-β1 (TGF-β1) expression and plasminogen activator inhibitor-1 (PAI-1) which led to disruption of tissue architecture by increasing extracellular matrix synthesis and decreasing its degradation, resulting in fibrosis of tubule-interstitial and arteriolopathy (Young et al., 1995).

Meanwhile, GSPE administration significantly down-regulated NF-κB and PAI-1 gene expressions in kidney tissues compared with CsA treated rats. Similarly, Hussein et al., (2018) showed that GSPE significantly decreased kidney iNOS, TNF-α, NFκB and caspase-3. GSPE administration declined renal injury markers and oxidative damage, and inactivated the inflammatory pathway by NF-κB inhibition (Wei et al., 2012). Proanthocyanidins have many protective effects including anti-oxidation and anti-inflammation (Limtrakul et al., 2016). Li et al., (2016) indicated that GSPE significantly reduced mRNA expressions of inflammatory cytokine genes including PAI-1 in small intestine. Moreover, Sandra et al., (2010) showed that GSPE down-regulated urokinase-type plasminogen activator and plasminogen activator inhibitor-1.

Cyclosporine A treated rats showed significant up-regulation in kidney tissue gene expressions of apoptotic (caspase-3, p53) and down-regulation of antiapoptotic (Bcl-2). Renal cell apoptosis and kidney dysfunction were important features of chronic CsA nephrotoxicity (Xiao et al., 2013). The significant up-regulation in kidney tissue p53 gene expression in CsA treated rats came in accordance with (Moon and Kim, 2019**)**. Activation of p53 induced a series of programs, including cell-arrest, cellular senescence and apoptosis (Hsin et al., 2006). Cell apoptosis signaling pathway is activated by the mitochondrial and endoplasmic reticulum (ER) stress. Besides, mitochondrial injury induces mitochondria release of cytochrome C, resulting in mitochondrial transmembrane potential loss (Muthuraman et al., 2011) and activation of caspase family of proteases; caspase-3 and caspase-9 (Liu et al., 2019). The anti-apoptotic Bcl-2 protein is able to inhibition of Bax-induced apoptosis (Rossé et al., 1998). The observed down-regulation in Bcl-2 protein expression came in accordance with Ateyya, (2015). CsA down-regulated Bcl-2 and Bcl-XL resulted in translocation of Bax to the mitochondria and impaired Bcl-2 and Bax balance in renal tubular cells (Xiao et al., 2013).

Conversely, GSPE administration exhibited down-regulation in kidney tissues Caspase-3 and p53 and up-regulation of Bcl-2 gene expression levels. The anti-apoptotic GSPE protective effect by p53 tumor suppressor protein modulation was confirmed by Yousef et al., (2018). GSPE treatment resulted in a gradual decreased in caspase-3 level, this decline in caspaes-3 restored the enzyme pathway. The increase in Bcl-2 protein expression was related to presence of both hydrophobic and hydrophilic residues within the flavan-3-ol molecules, giving these molecules the ability to interact with the general structure of BCl-2 family (Nazima et al., 2015).

5. CONCULOSIONS

It could be concluded that, GSPE has a reno-protective effect by enhancing antioxidant defense system, and attenuates CsA induced nephrotoxicity and oxidative stress. Moreover, GSPE has a strong anti-inflammatory effect through modulation of NF-κB, PAI-1 gene expressions, and anti-apoptotic effect by inhibiting caspase-3, p53 and activating Bcl-2 signaling pathways.

6. REFERENCES

1. Abdel-Wahab, W. M. 2015. Therapeutic Efficacy of Thymoquinone and Selenium Against Cyclosporine A Nephrotoxicity in Rats. Journal of Pharmacology and Toxicology. 10 (2): 60-70.
2. Aktan, F. 2004. iNOS-mediated nitric oxide production and its regulation. Life Sciences. 75: 639-653.
3. Amin, K. A., Ahmed, R. R., Hozayen, W. G., Mahmoud, S. S., Alshehri, F. S. and Al-Muzafar, H. M. 2018. Renoprotective effect of grape seed extract in diabetic nephropathy by attenuating hyperglycemia-facilitated oxidative stress. Int J Clin Exp Med. 11 (11): 12285-12293.
4. Aso, Y. 2007. Plasminogen activator inhibitor (PAI)-1 in vascular inflammation and thrombosis. Front Biosci. 12: 2957–2966.
5. Ateyya, H. 2015. Amelioration of cyclosporine induced nephrotoxicity by dipeptidyl peptidase inhibitor vildagliptin. International immunopharmacology. 28 (1): 571-577.
6. Balah, A. 2014. Wheat germ oil attenuates cyclosporin A-induced renal injury in rats via inhibition of ROS, iNOS, and NF-κ B expression. Al-Azhar Journal of Pharmaceutical Sciences. 50 (2): 55-66.
7. Bancroft, J.D. and Gamble, M. 2008. Theory and Practice of Histological Techniques. 6th Edition, *Churchill Livingstone*, Elsevier, China.
8. Caires, A., Fernandes, G.S., Leme, A.M., Castino, B., Pessoa, E.A., Fernandes, S.M., Fonseca, C.D., Vattimo, M.F., Schor, N. and Borges, F.T. 2018. Endothelin-1 receptor antagonists protect

the kidney against the nephrotoxicity induced by cyclosporine-A in normotensive and hypertensive rats. Braz. J. Med. Biol. Res. 51 (2): e6373.

9. Chia, T.Y.. Sattar, M.A., Abdullah, M.H., Ahmad, F.D., Ibraheem, Z.O., Lia, K.J; Pei, Y. P., Rathore, H. A., Singh, G. K. C., Abdullah, N. A. and Johns, E. J. 2012. Cyclosporine A-induced nephrotoxicity sprague –dawley rats are more susceptable to altered vascular function and haemodynamics. Int. J. Pharmacy and Pharma. Scie. 4: 431-439.
10. Cragg, G. M., and Newman, D. J. 2013. Natural products: a continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA)-General Subjects. 1830 (6): 3670-3695.
11. El-Gawish, M. A., Ebrahim, A. T. and Eid, F. A. 2006. Protective Role Of Proanthocyanidin Against Carbon Tetrachloride Induced Toxicity In Irradiated Rats. *The Egyptian Journal of Hospital Medicine*. *23*(1): 231-244.
12. Ghosh, A.K. and Vaughan, D.E. 2012. PAI-1 in tissue fibrosis. J Cell Physiol. 227: 493-507.
13. Gil-Cardoso, K., Ginés, I., Pinent, M., Ardévol, A., Arola, L., Blay, M. and Terra, X. 2017. Chronic supplementation with dietary proanthocyanidins protects from diet induced intestinal alterations in obese rats. Mol. Nutr. Food Res. 61(8): oi: 10.1002/mnfr.201601039.
14. Hassan, H.A., Isa, A.M., El-Kholy, W.M. and Nour, S.E. 2013. Testicular disorders induced by plant growth regulators: cellular protection with proanthocyanidins grape seeds extract. Cytotechnology. 65(5): 851-862.
15. Hausenloy, D. J., Boston-Griffiths, E. A., and Yellon, D. M. 2012. Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent. British journal of pharmacology. 165(5): 1235-1245.
16. Hewedy, W. A. and Mostafa, D. K. 2016. Nebivolol suppresses asymmetric dimethylarginine and attenuates cyclosporine-induced nephrotoxicity and endothelial dysfunction in rats. Pharmacological Reports. 68(6): 1319-1325.
17. Hsin, Y.H., Cheng, C.H., Tzen, J.T.C., Wu, M.J., Shu, K.H. and Chen, H.C. 2006. Effect of aristolochic acid on intracellular calcium concentration and its links with apoptosis in renal tubular cells. Apoptosis. 11: 2167–2177.
18. Hussein, S. A., Hassanein, R., Mohamed, R. and Awadalla, M. A. 2018. The Ameliorative Effect of Proanthocyanidinsagainst Streptozotocin Induced Diabetic Nephropathy in Rats. Benha Veterinary Medical Journal. 34(3): 42-56.
19. Hussein, S. A., Ragab, O. A. and El-Eshmawy, M. A. 2014. Protective effect of green tea extract on cyclosporine A: Induced nephrotoxicity in rats. J of Biol. Sc. 14: 248-57.
20. Jaffe, A. 1986. Technological opportunity and spillovers of R&D: Evidence from firms' patents, profits, and market value. Am. Econ. Rev. 76: 984-1001.
21. Kaplan, A.A. and Kohn, O.F. 1992. Fractional excretion of urea as a guide to renal dysfunction. American journal of nephrology. 12: 49-54.
22. Lahouel, M., Boulkour, S., Segueni, N. and Fillastre, J.P. 2004. The flavonoids effect against vinblastine, cyclophosphamide and paracetamol toxicity by inhibition of lipid-peroxydation and increasing liver glutathione concentration. Pathologie-biologie. 52: 314-322.
23. Li, H., Parry, J., Weeda, S., Ren, S., Castonguay, T. W. and Guo, T. L. 2016. Grape pomace aqueous extract (GPE) prevents high fat diet-induced diabetes and attenuates systemic inflammation. Food and Nutrition Sciences. 7(07): 647. DOI:10.3390/antiox6020030
24. Limtrakul, P., Yodkeeree, S., Pitchakarn, P. and Punfa, W. 2016. Anti-inflammatory effects of proanthocyanidin-rich red rice extract via suppression of MAPK, AP-1 and NF-kappaB pathways in Raw 264.7 macrophages. Nutr Res Pract.10:251–258.
25. Liu, C., Zhu, P., Fujino, M., Isaka, Y., Ito, H., Takahashi, K., Nakajima, M.,Tanaka, T., Zhuang, J. and Li, X. K. 2019. 5-aminolaevulinic acid (ALA), enhances heme oxygenase (HO)-1 expression and attenuates tubulointerstitial fibrosis and renal apoptosis in chronic cyclosporine nephropathy. Biochemical and Biophysical Research Communications. 508(2): 583-589.
26. Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 25(4): 402-408.
27. Moron, M.S., Depierre, J.W. and Mannervik, B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et biophysica acta. 582: 67-78.
28. Moon, D. and Kim, J. 2019. Cyclosporin A aggravates hydrogen peroxide-induced cell death in kidney proximal tubule epithelial cells. Anatomy & cell biology. 52(3): 312-323.
29. Mustafa, A. G., Alfaqih, M. A. and Al-Shboul, O. 2018. The 4-hydroxynonenal mediated oxidative damage of blood proteins and lipids involves secondary lipid peroxidation reactions. Experimental and Therapeutic Medicine. 16(3): 2132-2137.
30. Muthuraman, A., Singla, S. K., Rana, A., Singh, A. and Sood, S. 2011. Reno-protective role of flunarizine (mitochondrial permeability transition pore inactivator) against gentamicin induced nephrotoxicity in rats. *Yakugaku Zasshi*. 131(3): 437-443.
31. Nazima, B., Manoharan, V. and Miltonprabu, S. 2015. Grape seed proanthocyanidins ameliorates cadmium-induced renal injury and oxidative stress in experimental rats through the up-regulation of nuclear related factor 2 and antioxidant responsive elements. Biochemistry and Cell Biology. 93(3): 210-226.
32. Raeisi, S., Ghorbanihaghjo, A., Argani, H., Dastmalchi, S., Ghasemi, B., Ghazizadeh, T., Rashtchizadeh, N. Mesgari Abbasi, M., Bargahi, N., Nemati, M., Mota, A., Mota, A. and Vatankhah, A.M. 2016. The effects of valsartan on renal glutathione peroxidase expression in alleviation of cyclosporine nephrotoxicity in rats. BioImpacts: BI. 6: (3), 119-124.
33. Rossé, T., Olivier, R., Monney, L., Rager, M., Conus, S., Fellay, I. and Jansen, B., Borner, C. 1998. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. Nature. 391: 496–499.
34. Sandra, D., Radha, M., Harishkumar, M., Yuichi, N., Sayuri, O. and Masugi, M. 2010. Downregulation of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 by grape seed proanthocyanidin extract. Phytomedicine. 17(1): 42-46.
35. Weydert, C.J. and Cullen, J.J. 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 5: 51-66.
36. Wei, R., Ding, R., Tang, L. and Wang, Y. 2012. Grape seed proanthocyanidin extract reduces renal ischemia/reperfusion injuries in rats. The American journal of the medical sciences. 343(6): 452-457.
37. Wu, Q., Wang, X., Nepovimova, E., Wang, Y., Yang, H. and Kuca, K. 2018. Mechanism of cyclosporine A nephrotoxicity: Oxidative stress, autophagy, and signalings. Food Chem. Toxicol. 118: 889-907.
38. Xiao, Z., Shan, J., Li, C., Luo, L., Lu, J., Li, S., Long, D. and Li, Y. 2013. Mechanisms of cyclosporine-induced renal cell apoptosis: a systematic review. American journal of nephrology. 37(1): 30-40.
39. Young, B.A., Burdmann, E.A., Johnson, R.J., Alpers, C.E., Giachelli, C.M., Eng, E., Andoh, T., Bennett, W.M. and Couser, W.G. 1995. Cellular proliferation and macrophage influx precede interstitial fibrosis in cyclosporine nephrotoxicity. Kidney Int. 48:439–48.
40. Yousef, M. I., Khalil, D. K. and Abdou, H. M. 2018. Neuro-and nephroprotective effect of grape seed proanthocyanidin extract against carboplatin and thalidomide through modulation of inflammation, tumor suppressor protein p53, neurotransmitters, oxidative stress and histology. Toxicology reports. 5: 568-578.
41. Yun, S., Chu, D., He, X., Zhang, W. and Feng, C. 2020. Protective effects of grape seed proanthocyanidins against iron overload-induced renal oxidative damage in rats. Journal of Trace Elements in Medicine and Biology. 57: 126407. Jan;57:126407. doi: 10.1016/j.jtemb.2019.126407.