ejpmr, 2017,4(10), 105-112

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

WHEAT GERM OIL ATTENUATES CYCLOSPORIN A-INDUCED RENAL INJURY VIA INHIBITION OF ROS, INOS, AND NF-κB EXPRESSION

Amany Balah*

Pharmacology and Toxicology Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.

***Corresponding Author: Dr. Amany Balah**

Pharmacology and Toxicology Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.

Article Received on 07/08/2017 **Article Revised on 28/08/2017** **Article Accepted on 18/09/2017**

ABSTRACT

Cyclosporine A (CsA) is one of the most efficient immunosuppressive drugs that are widely used in organ transplantation. However, its clinical use is strongly limited by acute and chronic nephrotoxicity. One of the possible mechanisms of CsA-induced nephrotoxicity is thought to be reactive oxygen species (ROS) formation. The present work was designed to investigate the potential protective effect of wheat germ oil (WGO) as antioxidant on CsA-induced nephrotoxicity in rats. It was found that treatment of rats with CsA alone significantly increased the levels of blood urea nitrogen (BUN) and serum creatinine. In addition, CsA has also reduced the renal content of reduced glutathione (GSH), as well as the renal enzymatic activities of superoxide dismutase (SOD) and catalase (CAT). Furthermore, these effects were associated with an increase in lipid peroxidation, inducible NO-synthase (iNOS) and nuclear factor kappa-B (NF-κB) expression. Moreover, histopathological examination showed severe damage of the renal tissue in animals treated with CsA alone. Most interestingly, concomitant administration of WGO along with CsA ameliorated all these negative effects. Furthermore, the immunosuppressive effect of CsA was not affected by WGO. Collectively, this study demonstrates that WGO markedly attenuated CsA-induced renal injury and improved renal function via inhibition of ROS, iNOS and NF-κB expression.

KEYWORDS: Cyclosporin A, Wheat germ oil, Nephrotoxicity, ROS, NF-κB, iNOS.

INTRODUCTION

Organ transplantation is one of the most miraculous achievements in modern medicine due to its contribution in decreasing the mortality rate of patients with organs failure. However, the risk of transplant rejection threatens the success of the transplantation process. Transplant rejection can be prevented by the use of an immunosuppressive agent to suppress the reaction of the immune system to the transplanted tissue. Cyclosporine A (CsA) is one of the calcineurin inhibitors that suppress T-cell activation by inhibiting the cellular phosphatase calcineurin.[1] Calcineurin inhibitors (CNI) are among the most efficient immunosuppressive drugs and therefore are widely used in transplantation and for the treatment of many inflammatory diseases including psoriasis and rheumatoid arthritis. However, the clinical use of the calcineurin inhibitor CsA is limited by acute and chronic nephrotoxicity which remain a major clinical problem.[2,3] The mechanisms of CsA-induced renal injury are not fully elucidated. However, one of the possible mechanisms of CsA-induced nephrotoxicity is thought to be over production of reactive oxygen species (ROS) and a consequent imbalance between oxidants and endogenously produced antioxidants.[4-6] The harmful effects of ROS induced by CsA can be antagonized by using a powerful antioxidant agent.[7,8] Wheat germ oil (WGO) is the richest known natural source of vitamin E.[9] In addition to the most powerful natural antioxidant vitamin E,[10,11] WGO is also rich in unsaturated fatty acids, mainly oleic and α-linoleic acids that may exert inhibiton of oxidative stress.[12,13] Furthermore, WGO is rich in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione.[14] Furthermore, it has been reported that WGO intake results in a rapid increase in the content of vitamin E in different rat tissues and gives high protection for these tissues against oxidative damage.[15,16] Moreover, it has been shown that WGO has the ability to inhibit hepatotoxicity induced by CsA via inhibition of reactive oxygen species (ROS) in rats.[17] Thus it was interesting to investigate the potential protective effect of WGO against CsA-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats weighing 200-250 g were used in this study. The experimental protocol used in this study was approved by the Institutional Animal Ethics Committee. The rats were housed in a 12-hour dark/light cycle animal facility with controlled humidity and constant temperature. The animals were fed a standard diet and water was supplied ad libitum. The animals

were kept under observation for one week before the treatments for adaptation.

Drugs and Chemicals

Wheat germ oil (WGO) was purchased from SEDICO Pharmaceutical Co., 6 October City, Egypt. Cyclosporin A (CsA) was purchased from Sandoz Ltd, Basel, Switzerland. Inducible NO-synthase (iNOS), nuclear factor kappa-B (NF-κB) and catalase (CAT) ELISA kits were purchased from EIAab Science Co., Ltd., China. Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and Superoxide dismutase (SOD) assay kits were purchased from Cell Biolabs (USA), Oxford biomedical research (Oxford MI, USA) and Trevigen (USA) respectively. Creatinine and blood urea nitrogen (BUN) assay kits were purchased from Bioassay system (USA). Interleukin-2 (IL-2) ELISA kit was purchased from Uscn Life Science Inc, Wuhan, China.

Experimental Design

Twenty-four male Wistar albino rats were used in this study. The animals were randomly divided into four groups, 6 animals in each. The first group (Control) was administered the vehicle of CsA intraperitoneal (i.p.) daily for 21 days. The second group received CsA (25 mg/kg/day i.p.) daily for 21 days.[18] The third group was administered WGO (900mg/kg/day by oral gavage)[19] 5 days before and concurrently during CsA administration daily for 21 days. The fourth group received WGO alone (as previously mentioned in group 3). Twenty-four hours after the last treatment, blood samples were collected for the determination of serum levels of creatinine, BUN as well as IL-2. After terminal bleeding, animals were sacrificed by cervical dislocation. The left kidney was dissected immediately after death, washed with ice cold phosphate buffered saline (PBS) and kept at -20ºC till used. The right kidney was fixed in 10% neutral-buffered formal saline for histopathological investigation.

Assessment of BUN and Serum creatinine

BUN and serum creatinine levels were determined by commercial kits according to the manufacturer's instructions (Bioassay system, USA).

Assessment of Serum IL-2

The level of IL-2 in serum was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Uscn Life Science Inc, Wuhan, China).

Determination of oxidative damage markers

Determination of Lipid Peroxides

The level of malondialdehyde (MDA) [the by-product of lipid peroxidation] in renal tissues was measured using a TBARS assay kit according to the manufacturer's instructions (Cell Biolabs, Inc., USA). Briefly, the unknown MDA containing samples or MDA standards are first reacted with thiobarbituric acid (TBA) at 95°C. After a brief incubation, the samples and standards can be read spectrophotometrically.

Determination of iNOS expression

The protein level of iNOS in renal tissues was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EIAab Science Co., Ltd., China).

Determination of NF-κB expression

The level of Total NF-κB in renal tissues was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EIAab Science Co., Ltd., China).

Determination of endogenous antioxidants

Determination of GSH

GSH in renal tissues was determined by colorimetric assay according to the manufacturer's instructions (Oxford biomedical research, Oxford MI, USA). Briefly, the method is based on the reaction of GSH with Ellman's reagent (5, 5´-dithiobis-2-nitrobenzoic acid (DTNB) which gives rise to a product that can be quantified spectrophotometrically at 412 nm.

Determination of SOD

SOD activity in renal tissues was determined by assay kit according to the manufacturer's instructions (Trevigen, USA). In Trevigen's superoxide dismutase assay, superoxide radicals generated from the conversion of xanthine to uric acid and hydrogen peroxide by xanthine oxidase (XOD), converts nitroblue tetrazolium (NBT) to NBT-diformazan. NBT-diformazan absorbs light at 550 nm. SODs reduce superoxide radical concentrations and thereby lower the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity.

Determination of CAT activity

CAT activity in renal tissues was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EIAab Science Co., Ltd., China).

Histopathological Examination

Kidney specimens from all animals were dissected immediately after death and fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6µm thick were cut and stained with Haematoxylin and eosin for histopathological investigation.[20]

Statistical Analysis

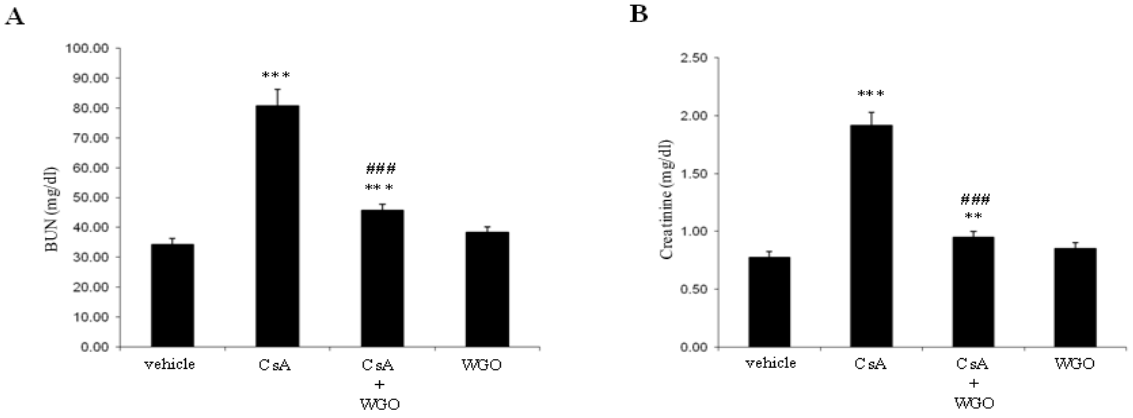
Results are expressed as means ± SD. Statistical analysis was performed using Student's *t* test and for multiple comparisons the ANOVA test for significance. *P*-values below 0.05 were considered as indication for statistically significant differences between conditions compared.

RESULTS AND DISCUSSION

CsA is one of the most important immunosuppressive agents that are commonly used in organ transplantation and in the treatment of autoimmune diseases. However, the clinical use of CsA is strongly limited by acute and chronic nephrotoxicity which remain a major clinical problem. The mechanisms of CsA-induced nephrotoxicity are still not fully elucidated. It has been demonstrated that over production of ROS and consequently oxidative stress plays a major role in CsA-induced renal damage.[4-6] Previously, it has been reported that antioxidants have the ability to attenuate nephrotoxicity induced by CsA.[21-24] WGO was found to contain the highest content of the most powerful natural antioxidant vitamin E than any other natural source.[9-11] Recently, it has been reported that WGO intake results in a rapid increase in the content of vitamin E in different rat tissues and gives high protection for these tissues against oxidative damage.[15,16] In order to investigate the potential protective effect of WGO against CsA-induced nephrotoxicity, male Wistar albino rats were received either CsA, WGO or WGO 5 days before and concurrently during CsA administration daily for 21 days. In agreement with previous studies,[25,26] treatment of rats with CsA resulted in a decline in renal function as indicated by significant increase in BUN (Fig. 1A) and serum creatinine level (Fig. 1B). However, concomitant administration of WGO along with CsA improved the renal function as indicated by significant decrease in BUN (Fig. 1A) and serum creatinine level (Fig. 1B) compared with CsA alone-treated rats. Alterations in renal function and structural damage have been shown to be associated with lipid peroxidation induced by CsA.[23,27] In the present study, treatment of rats with CsA significantly induced lipid peroxidation as indicated by an increase in the by-product of lipid peroxidation malondialdehyde (Fig. 2A). On the other hand, lipid peroxidation is significantly attenuated in animals treated with CsA in combination with WGO as compared to CsA alone treated animals (Fig. 2A). Previously, superoxide radical has been shown to be induced by CsA in renal cells.[28-30] Furthermore, the highly reactive peroxynitrite ($ONOO^-$) that is usually produced by the reaction between nitric oxide (NO) and superoxide (O_2^-) has been demonstrated to be induced by CsA in the kidney[31-34] and involved in renal cellular damage.[35] Moreover, it has been reported that CsA-induced renal injury is associated with induction of the pro-oxidant enzyme iNOS[36] that catalyze the production of NO. Therefore, the involvement of iNOS in the renal toxicity induced by CsA was investigated. As shown in Fig 2B, treatment of rats with CsA significantly induced iNOS expression. On the other hand, administration of WGO along with CsA significantly attenuated iNOS expression induced by CsA (Fig. 2B). The transcription factor NF-κB has been reported to be involved in the transcription of the inflammatory enzyme iNOS and other inflammatory genes in response to oxidative stress.[37,38] Thus, the involvement of NF-κB in CsA-induced renal damage has also been investigated. As shown in Fig. 2C, CsA significantly induced NF-κB expression. However, NF-κB expression in animals treated with CsA in combination with WGO was highly reduced compared to CsA alone treated animals. (Fig. 2C). An efficient endogenous antioxidant defense system operates to scavenge the reactive oxygen species. The most important endogenous antioxidants are GSH, SOD and CAT. GSH plays a major role in cells protection against oxidative damage and detoxification of xenobiotics including CsA.[39,40] Therefore, the renal content of GSH was investigated. In agreement with many studies,[27,41,42] treatment of rats with CsA produced a significant decrease in renal GSH level (Fig. 3A). On the other hand, concomitant administration of WGO along with CsA significantly increased the level of renal GSH as compared to CsA alone-treated animals (Fig. 3A). Also, SOD and CAT play a major role in cells protection against oxidative damage by scavenging ROS from the cell where SOD metabolizes superoxide radical into hydrogen peroxide (less toxic) that can be detoxified by catalase, which converts H_2O_2 into H_2O and O_2.[43,44] Therefore, the renal activities of SOD and CAT were investigated. In agreement with previous findings,[9,41,42] treatment of rats with CsA produced a significant decrease in the levels of the antioxidant enzymes SOD (Fig. 3B) and CAT (Fig. 3C). However concomitant administration of WGO along with CsA significantly increased the renal activities of the antioxidant enzymes SOD (Fig.3B) and CAT (Fig. 3C) as compared to CsA alone-treated group. Moreover, histopathological examination showed sever damage of the renal tissue in CsA alone-treated rats as indicated by interstitial hemorrhage, disrupted glomerular basement membrane and hyaline deposition of the blood vessels (Fig. 4B). However, concomitant administration of WGO along with CsA improved these histopathological changes to a great extent as indicated by improvement in the glomerular damage (the glomeruli don't have any noticed change) and decrease in the interstitial hemorrhage (Fig. 4C) indicating that WGO has the ability to protect against oxidative damage induced by CsA. Finally, to test whether the immunosuppressive efficiency of CsA would also be affected in the presence of WGO, the serum level of IL-2 was measured. As shown in fig 5, treatment of rats with CsA produced a significant reduction in IL-2 level as expected but most interestingly this inhibition of IL-2 by CsA was not altered in the presence of WGO indicating that the immunosuppressive efficiency of CsA was not affected in the presence of WGO.

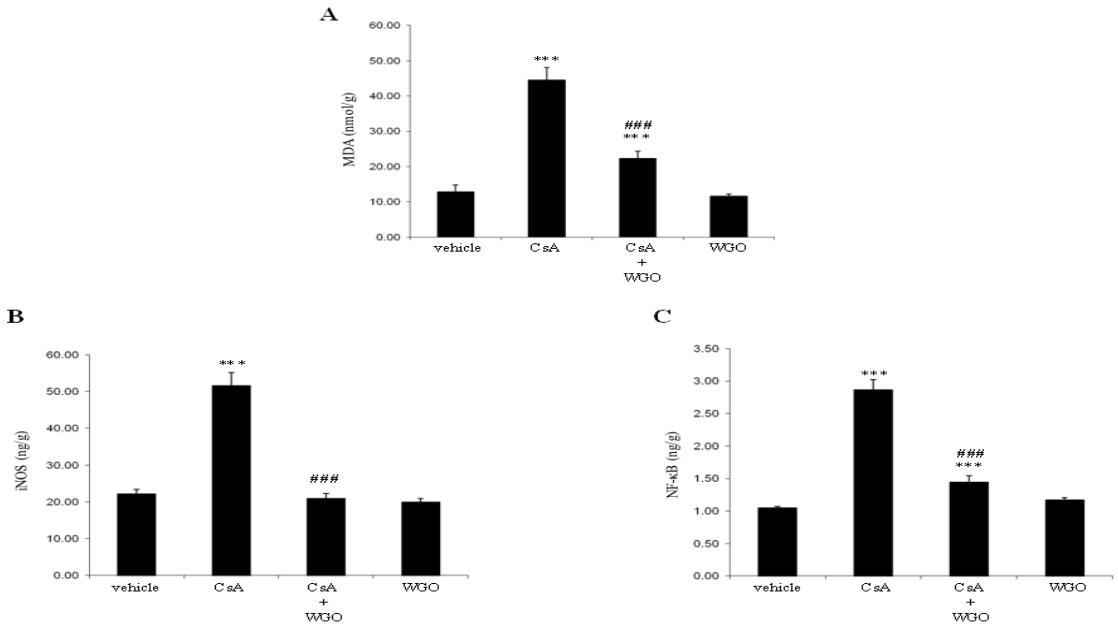
Figures:

Figure 1



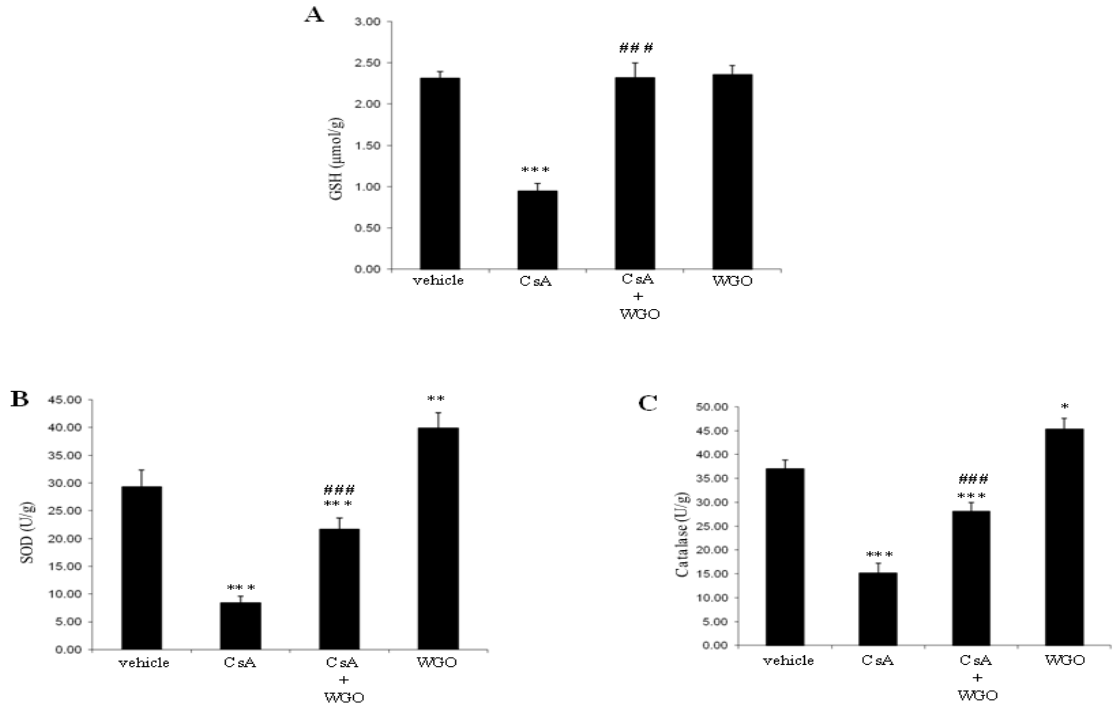
Effects of CsA and/or WGO on BUN (A) and serum creatinine (B) in male Wistar albino rats.
Data represent means ± S.D. (n=6), ** $p < 0.01$, *** $p < 0.001$ versus control, ### $p < 0.001$ versus CsA alone-treated animals.

Figure 2



Effects of CsA and/or WGO on renal content of MDA (A) as well as iNOS (B) and NF-□B expression (C) in male Wistar albino rats.
Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control, ### $p < 0.001$ versus CsA alone-treated animals.

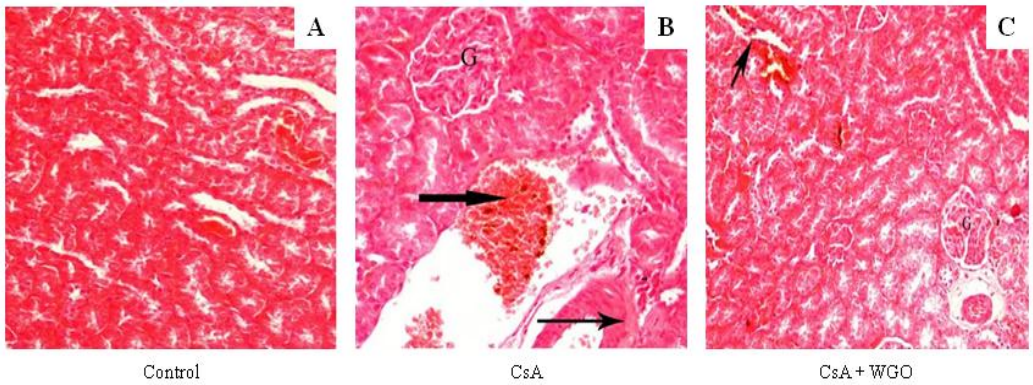
Figure 3



Effects of CsA and/or WGO on renal content of GSH (A) as well as renal enzymatic activities of SOD (B) and CAT (C) in male Wistar albino rats.

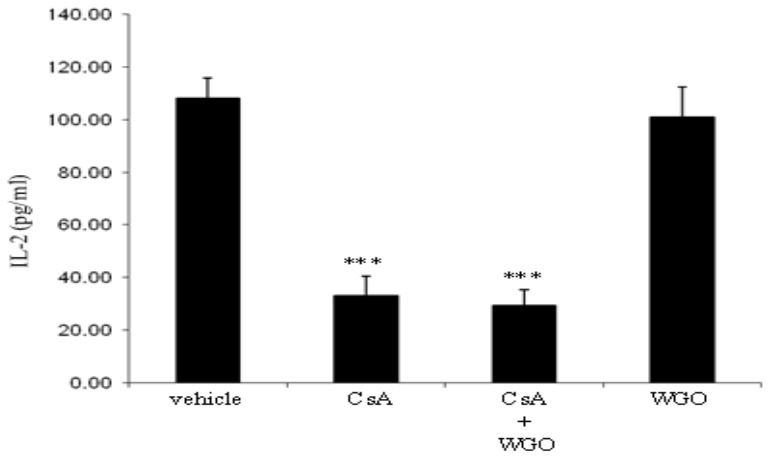
Data represent means ± S.D. (n=6), * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus control, ### $p < 0.001$ versus CsA alone-treated animals.

Figure 4



A. A photomicrograph of a section of the kidney of a control rat showing the normal structure of the tissue. (H&E X100). **B**. A photomicrograph of a section of the kidney of CsA alone-treated rats showing sever damage of the renal tissue at which there are interstitial hemorrhage (thick arrow) with disrupted glomerular basement membrane (G), as well as mild to moderate thickened blood vessels due to hyaline deposition (thin arrow). (H&E X200). **C**. A photomicrograph of a section of the kidney of rats received CsA + WGO showing mild interstitial hemorrhage (arrow) while the glomeruli don`t have any noticed change (G). (H&E X100).

Figure 5



Effects of CsA and/or WGO on serum level of IL-2 in male Wistar albino rats. Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control.

CONCLUSION

The findings of the present study demonstrate that WGO has the ability to improve not only the impairment of renal function but also the histopathological changes induced by CsA via inhibition of the oxidative damage markers lipid peroxidation, iNOS and NF-κB expression. This inhibition of the oxidative damage markers was associated with induction of the most important endogenous antioxidants GSH, SOD and CAT which play a major role in cells protection against oxidative damage. In addition, the present study demonstrates that the immunosuppressive efficiency of CsA was not affected in the presence of WGO. Finally, these findings suggest that concomitant use of WGO might be useful in reducing renal toxicity induced by CsA via inhibition of ROS, iNOS and NF-κB expression.

REFERENCES

1. Schreiber SL and Crabtree GR. The mechanism of action of cyclosporine A and FK506, Immunol Today, 1992; 13(4): 136-42.
2. Mihatsch MJ, Kyo M, Morozumi K, Yamaguchi Y, Nickeleit V, Ryffel B. The side-effects of ciclosporine-A and tacrolimus. Clin Nephrol, 1998; 49(6): 356-63.
3. Fellstrom B. Cyclosporine nephrotoxicity. Transplant Proc, 2004; 36(2): 220-23.
4. Akool El-S, Doller A, Babelova A, Tsalastra W, Moret K, Schaefer L, Pfeilschifter J, Eberhardt W. Molecular mechanisms of TGF beta receptor-triggered signaling cascades rapidly induced by the calcineurin inhibitors cyclosporin A and FK506. J Immunol, 2008; 181(4): 2831-45.
5. Ichikawa I, Kiyama S, Yoshioka T. Renal antioxidant enzymes: their regulation and function. Kidney Int, 1994; 45(1): 1-9.
6. Serino F, Grevel J, Napoli KL, Kahan BD, Strobel HW. Oxygen radical formation by the cytochrome P450 system as a cellular mechanism for cyclosporine toxicity. Transplant Proc, 1994; 26(5): 2916-7.
7. Tariq M, Morais C, Sobki S, Alsulaiman M, Alkhader A. N-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. Nephrol Dial Transplant, 1999; 14(4): 923-29.
8. Uz E, Uz B, Kaya A, Akdeniz D, Ruzgaresen NB, Uz E, Turgut FH, Bayrak R, Akcay A. Protective Effect of Erdosteine on Cyclosporine Induced Chronic Nephrotoxicity in Rats. Nephro-Urol Mon, 2011; 4: 280-84.
9. Shurpalekar SR, Rao PH. Wheat germ. Adv Food Res, 1977; 23: 187-304.
10. Wolf R, Wolf D, Ruocco V. Vitamin E: the radical protector. J Eur Acad Dermatol Venereol, 1998; 10(2): 103-17.
11. Ohkatsu Y, Kajiyama T, Arai Y. Antioxidant activities of tocopherols. Polymer degradation and stability, 2001; 72(2): 303-11.
12. Sjovall O, Virtalaine T, Lapvetelainen A, Kallio H. Development of rancidity in wheat germ analyzed by headspace gas chromatography and sensory analysis. J Agr Food Chem, 2000; 48(8): 3522-27.
13. Alessandri C, Pignatelli P, Loffredo L, Lenti L, Del Ben Roberto CM, Perrone A, Ferro D, Angelico F, Violi F. Alpha-Linolenic Acid-Rich Wheat Germ Oil Decreases Oxidative Stress and CD40 Ligandin Patients With Mild Hypercholesterolemia.

Arterioscler Thromb Vasc Biol, 2006; 26(11): 2577-8.
14. Zhu K, Zhou HM, Qian HF. Proteins extracted from defatted wheat germ, nutritional and structural properties. Cereal Chem, 2006; 83(1): 69-75.
15. Mehranjani MS, Abnosi MH, Mahmodi M. Preventing effects of wheat germ oil on sex hormones, liver enzymes, lipids and proteins in rat serum following treatment with p-nonylphenol. J Biol Sci, 2007; 7(8): 1406-11.
16. Field R, Verghese M, Walker LT, Boateng J. Feeding wheat germ meal and wheat germ oil reduced azoxymethane-induced aberrant crypt foci in fisher 344 male rats. Int J Cancer Res, 2008; 4(4): 127-36.
17. Akool El-S. Molecular mechanisms of the protective role of wheat germ oil against cyclosporin A-induced hepatotoxicity in rats. Pharm Biol, 2015; 53: 1311-17.
18. Pari L, Sivasankari R. Effect of ellagic acid on cyclosporine A-induced oxidative damage in the liver of rats. Fundam Clin Pharmacol, 2008; 22: 395-401.
19. Abd El-Hameed AM, Soliman HA, Abd El-Reheem ES. Protective role of wheat germ oil in clozapine-induced oxidative stress and biochemical alterations in liver of male albino rats. J Am Sci, 2013; 9: 268-74.
20. Drury RVA, Walligton EA. Carltons Histological techniques. 5th ed., New York; Oxford University Press, Pronto: 1980.
21. Burdmann EA, Andoh TF, Yu L, Bennett WM, Cyclosporine nephrotoxicity. Semin Nephrol, 2003; 23(5): 465-76.
22. Capasso G, Di Gennaro CI, Della RF, Manna C, Ciarcia R, Florio S, Perna A, Pollastro RM, Damiano S, Mazzoni O, Galletti P, Zappia V. In vivo effect of the natural antioxidant hydroxytyrosol on cyclosporine nephrotoxicity in rats. Nephrol Dial Transplant, 2008; 23(4): 1186-95.
23. Parra Cid T, Conejo García JR, Carballo Alvarez AF, de Arriba G. Antioxidant nutrients protect against cyclosporine A nephrotoxicity. Toxicology, 2003; 189(1-2): 99-111.
24. Rezzani R, Rodella L, Buffoli B, Goodman AA, Abraham NG, Lianos EA, Bianchi R. Change in renal heme oxygenase expression in cyclosporine A-induced injury. J Histochem Cytochem, 2005; 53(1): 105-12.
25. Abdelsattar MH. Garlic oil as an effective protective agent against CsA-induced nephrotoxicity in rats. Int J Med Arom Plants, 2013; 3(1): 85-92.
26. Mason J. The pathophysiology of Sandimmun (cyclosporin) in man and animals. Pediatr nephrol, 1990; 4(5): 554-74.
27. Wongmekiat O, Leelarugrayub N, Thamprasert K. Beneficial effect of shallot (Allium ascalonicum L.) extract on cyclosporine nephrotoxicity in rats. Food Chem Toxicol, 2008; 46(5): 1844-50.
28. Raymond MA, Mollica L, Vigneault N, Désormeaux A, Chan JS, Filep JG, Hébert MJ. Blockade of the apoptotic machinery by cyclosporin A redirects cell death toward necrosis in arterial endothelial cells: regulation by reactive oxygen species and cathepsin D. FASEB J, 2003; 17(3): 515-17.
29. Vetter M, Chen ZJ, Chang GD, Che D, Liu S, Chang CH. Cyclosporin A disrupts bradykinin signaling through superoxide. Hypertension, 2003; 41(5): 1136-42.
30. Parra T, de Arriba G, Arribas I, Perez de Lema G, Rodriguez-Puyol D, Rodriguez-Puyol M. Cyclosporine A nephrotoxicity: role of thromboxane and reactive oxygen species J Lab Clin Med, 1998; 131(1): 63-70.
31. Amudha G, Josephine A, Varalakshmi P. Role of lipoic acid in reducing the oxidative stress induced by cyclosporine A. Clin Chim Acta, 2006; 372(1-2): 134-9.
32. Ramzy D, Rao V, Tumiati LC, Xu N, Miriuka S, Delgado D, Ross HJ. Role of endothelin-1 and nitric oxide bioavailability in transplant-related vascular injury: comparative effects of rapamycin and cyclosporine. Circulation, 2006; 114(1): I214-19.
33. Jennings P, Koppelstaetter C, Aydin S, Abberger T, Wolf AM, Mayer G, Pfaller W. Cyclosporine A induces senescence in renal tubular epithelial cells. Am. J Physiol Renal Physiol, 2007; 293(3): 831-38.
34. Uz E, Bayrak O, Uz E, Kaya A, Bayrak R, Uz B, Turgut FH, Bavbek N, Kanbay M, Akcay A. Nigella sativa oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. Am J Nephrol, 2008; 28(3): 517-22.
35. Josephine A, Amudha G, Veena CK, Preetha SP, Varalakshmi P. Oxidative and nitrosative stress mediated renal cellular damage induced by cyclosporine A: role of sulphated polysaccharides. Biol Pharm Bull, 2007; 30(7): 1254-59.
36. Navarro-Antolín J, Redondo-Horcajo M, Zaragoza C, Alvarez-Barrientos A, Fernández AP, León-Gómez E, Rodrigo J, Lamas S. Role of peroxynitrite in endothelial damage mediated by Cyclosporine A. Free Radic Biol Med, 2007; 42(3): 394-403.
37. Aktan F. iNOS-mediated nitric oxide production and its regulation. Life Sci, 2004; 75(6): 639-53.
38. Tak PP, Firestein GS. NF-κB: a key role in inflammatory diseases. J Clin Invest, 2001; 107(1): 7-11.
39. Inselmann G, Lawerenz HU, Nellessen U, Heidemann HT. Enhancement of cyclosporin A induced hepato- and nephrotoxicity by glutathione depletion. Eur J Clin Invest, 1994; 24(5): 355-59.
40. Meister A, Anderson ME. Glutathione. Ann Rev Biochem, 1983; 52(1): 711-60.
41. Anjaneyulu M, Tirkey N, Chopra K. Attenuation of cyclosporine-induced renal dysfunction by catechin: possible antioxidant mechanism. Ren Fail, 2003; 25: 691-707.
42. Hagar HH, Etter E, Arafa M. Taurine attenuates hypertension and renal dysfunction induced by

cyclosporine A in rats. Clin Exp Pharmacol Physiol, 2006; 33(3): 189-96.
43. McCord JM, Keele BB Jr, Fridovich I. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc Natl Acad Sci U S A, 1971; 68(5): 1024-27.
44. Deisseroth A, Dounce AL. Catalase: Physical and chemical properties, mechanism of catalysis and physiological role. Physiol Rev, 1970; 50(3): 319-75.