Potential Antioxidant Effect of Date Pits Extract on Nephrotoxicity induced by Cyclosporine-A in Male Rats

Eman A. Abduljawad

Food and Nutrition Department, Faculty of Home Economics, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT

Cyclosporine A (CsA) is a potent immunosuppressive drug, it is commonly used to treat rheumatoid arthritis and polymyositis; however, treatment with CsA develops nephrotoxicity. Natural plants are perfect remedies as they cost less, and they are easier to obtain without any troubles. Date fruit is utilized as a main food in the Gulf region. Date pits are the spin-off product of dates through industrial processing. They have antioxidative activity. This research aimed at assessing the impact of date pits' aqueous extract (DPE) on CsA- induced nephrotoxicity. Male rats (=40) were classified to 4 groups. (I): control negative; (II) CsA: rats injected subcutaneously (SC) with CsA at a dose of (15 mg/kg) for 28 d: (III &IV): DPE + CsA: rats which received DPE orally at a dose of 4 and 6 ml/kg/d, respectively and recieved SC CsA through injection. After 28 d, the rats were sacrificed, and the blood samples were collected. Serum creatinine, urea, uric acid, sodium were measured. The concentration of both lipid peroxidation product (malondialdehyde) and reduced glutathione as well as catalase enzyme activity were determined in kidney tissues. Also, the renal tissues from rats in different groups were examined using light microscope. The results showed that CsA injection induced significant increase in the kidney functions, total protein and ionic potassium, with a significant decrease in albumin, ionic sodium, as well, it induced oxidative stress compared with the control group ($P<0.05$). The administrated DPE either at low or high dose induced a significant amelioration ($p<0.05$) in tested parameters compared to CsA values. Furthermore, it protected against CsA-induced histopathological changes. The high dose was the most effective dose compared with the low dose. The results of this study revealed that DPE attenuated CsA-induced nephrotoxicity via an antioxidant mechanism.

Key Words: *Cyclosporine A, nephrotoxicity, date pits extract, rats, antioxidant, histopathological changes*

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INTRODUCTION

Cyclosporine A (CsA), a member of calcineurin inhibitors, remains as one of the major immunosuppressive drugs preventing allograft rejection following solid organ transplantation and being used to treat rheumatoid arthritis, other rheumatic conditions and certain autoimmune diseases [1]. Treatment with CsA has been limited due to the concomitant development of nephrotoxicity [2]. Furthermore, CsA induces many harmful impacts on many other organs. Kahan et al. [3] notified that nearly 50% of the observed CsA treated patients have had some sort of liver illness. In addition, CsA has been known to influence the pancreas, both in animals and human being [4, 5].

CsA nephrotoxicity has been characterized by renal functional derangements and morphological damages. CsA enhances the generation of oxidative stress measures, which plays a vital function in creating the structural and functional deterioration of the kidney. Given long term, CsA can lead to an irreversible renal failure due to renal vasoconstriction, tubular epithelial cell changes, tubulointerstitial fibrosis, and glomerular changes [1, 6]. Trials have been undertaken to understand the pathogenic mechanisms underlying CsA nephrotoxicity with a view to finding a possible protective agent against this adverse consequence. Antioxidants are molecules that react and disrupt the reactive oxygen species (ROS), and subsequently, prohibit their damage. The hindering of ROS activity is an essential procedure in the control of several illnesses [1, 7].

Corresponding author: Eman A. Abduljawad
Address: Food and Nutrition Department, Faculty of Home Economics, King Abdulaziz University, Jeddah, Saudi Arabia.
E-mail: ✉ abduljawad.a@ gamail.com
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The fruits of the date palm *(Phoenix dactylifera*) are popular plants in many countries. Date fruits have been used as a staple food in the Middle East for thousands of years. Date pits, by products, have been found to contain extremely high levels of phenolic [8]. Date pits extract have shown an important role in diseases prevention through antioxidant, anti-inflammatory and antibacterial activities [9-11]. Studies have demonstrated that date pits have in vitro chemo-protective effects versus hydrogen peroxide-induced damage in skin cell lines [12, 13]. Furthermore, Habib and Ibrahim have demonstrated that diet containing date seeds reduces the level of lipid peroxidation in liver of rats while does not affect the antioxidant enzyme capacity of normal tissues [14]. Habib and coworkers showed that the date pits constitute one of the highest sources of total polyphenols, exceeding tea, grapes, flaxseed, nut seeds and even date flesh [8].

Based on the involvement of oxidative stress and formation of oxygen free radicals in the mechanism of CsA-induced nephrotoxicity and the antioxidant action of DPE, the following study was prepared to examine the impact of DPE on CsA-produced kidney toxicity.

MATERIALS AND METHOD:

Preparation of date pits Extract

The date pits were manually separated from date flesh. The pits were dried at 40°C, then the dried powder was soaked in cold distilled water (1:10 ratio, g/mL) under agitation, and kept for 48 hours in a refrigerator (4°C) with continuous stirring. After that, the extract was filtered and the aqueous supernatant was then used [15]. Aqueous extract was selected because most of the antioxidants and active components in dates are extracted in water [16]. The date pits extract was freshly prepared during the experiment and administrated to rats by oral gavage at a dose of 4 and 6 ml/kg/d; the dose of 4 ml has been proved as an effective dose [15].

Experimental design

Male Wister albino rats (n=40 rats) weighing about (200 - 230 g) were used in this research. The rats were permitted to be adapted with animal house circumstances one week before the experiment. The rats were fed basic usual nourishment according to Reeves [17], and were freely exposed to drinking water ad libitum. After the adaptation period, the animals were assorted to 4 categories. First, the control negative (Cont) rats (n=10) were fed based on diet and given subcutaneously (SC) 0.5 ml of NaCl/d for 28 d. Second (CsA); the rats were given subcutaneously CsA at a dose of (15 mg/kg/d) for 28 d [18]. Third and fourth; the rats orally received DPE at a dose of 4 and 6 ml/ kg/ d and CsA at a dose of (15 mg/kg/d) for 28 d. Twenty-eight days after CsA injection, the food was restricted for 12 h, then the rats were anesthetized with diethyl ether. The blood samples were obtained from the retro-orbital plexus in heparinized capillary tubes, for serum separation. The kidney of each animal was dissected out, and then fixed in 10% formalin for histopathological examination.

Biochemical analysis

Serum creatinine, blood urea nitogen, uric acid, sodium, potassium, total protein and albumin levels were measured according to the manufacture instructions.

Determination of renal antioxidant status

Kidney tissue contents of malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) activity were quantified using ELISA kits obtained from MyBiosource, San Diego, California, USA according to the manufacture's instructions.

Histological examination of kidney tissues

A histological examination was done to assess the histopathological alternations in kidney tissues.

Statistical analysis

The obtained results were analyzed statistically by the analysis of variance, for statistical significance ($p \leq 0.05$) using L.S.D. test, one way ANOVA, post hoc multiple comparisons. An IBM computer with a software system SPSS version 22 was used for these calculations [19].

RESULTS

Impact of DPE on serum kidney functions

In CsA group, there was a significant increase in the creatinine, blood urea nitrogen and uric acid compared with Cont group ($P<0.05$). The administration of DPE either at low or high dose significantly reduced ($p< 0.05$) the tested kidney functions parameters compared to CsA values. There was a significant difference ($P<0.05$) between DPE (4 ml/kg) and DPE (6 ml/kg) results (Table 1).

Table 1: Impact of DPE on serum creatinine, urea and uric acid against CsA- produced kidney toxicity in rats

Groups	Creatinine (µmol/L)	BUN (mmol/L)	Uric acid (umol/L)
Cont	24.10 ± 1.09	7.66 ± 0.34	64.18 ± 1.97
CsA	54.97 ±2.68 [a]	20.04 ± 0.92 [a]	121.60 ± 3.71 [a]
DPE (4 ml/ kg) **+ CsA**	32.46 ±1.28 [b]	9.79 ± 0.52 [b]	78.75 ± 4.03[b]
DPE (6 ml/ kg) **+ CsA**	27.46 ± 1.25 [b, c]	7.90 ± 0.29 [b, c]	68.20 ± 2.53 [b, c]

Results have been represented as mean ± SE (n = 10). [a] Significant versus Cont values, [b] significant versus CsA values. [C] significant between DPE (4 ml/kg) and DPE (6 ml/kg) values. $p \leq 0.05$

Impact of DPE on some serum protein metabolism parameters

In CsA group, there was a significant decrease in the total protein with significant increase in albumin levels compared with Cont group (P<0.05). The administration of DPE either at low or high doses induced a significant improvement (p< 0.05) in the tested protein metabolism parameters compared to CsA values. There was a significant difference (P<0.05) between DPE (4 ml/kg) and DPE (6 ml/kg) results (Table 2).

Impact of DPE on serum ionic sodium and potassium

In CsA group, there was a significant decrease in the sodium (Na^+) with a significant increase in potassium (K^+) levels compared with Cont group (P<0.05). The administration of DPE either at low or high doses induced a significant improvement (p< 0.05) in the tested ionic Na^+ and K^+ compared to CsA values. There was a significant difference (P<0.05) between DPE (4 ml/kg) and DPE (6 ml/kg) results (Table 3).

Table 2: Impact of DPE on serum total protein and albumin against CsA- produced kidney toxicity in rats

Groups	Total protein (g/dl)	Albumin (g/dl)
Cont	7.17 ± 0.26	4.28 ± 0.15
CsA	6.11 ± 0.15 [a]	5.47 ± 0.19 [a]
DPE (4 ml/ kg) + **CsA**	6.88 ± 0.12 [b]	4.55 ±0.14 [b]
DPE (6 ml/ kg) + **CsA**	7.09 ± 0.29 [b]	4.27 ± 0.25 [b]

Results have been represented as mean ± SE (n = 10). [a] Significant versus Cont values, [b] significant versus CsA values. [C] significant between DPE (4 ml/kg) and DPE (6 ml/kg) values. p ≤ 0.05

Table 3: Impact of DPE on serum sodium (Na^+) and potassium (K^+) against CsA- produced kidney in rats

Groups	Na^+(nmol/L)	K^+ (nmol/L)
Cont	144.7 ± 1.04	5.17 ± 0.14
CsA	98.82 ± 2.02 [a]	6.16 ± 0.11 [a]
DPE (4 ml/ kg) + **CsA**	140.36 ± 1.09 [b]	5.03 ±0.13 [b]
DPE (6 ml/ kg) + **CsA**	142.69 ± 0.85 [b]	4.84 ± 0.10 [b]

Results have been represented as mean ± SE (n = 10). [a] Significant versus Cont values, [b] significant versus CsA values. [C] significant between DPE (4 ml/kg) and DPE (6 ml/kg) values. p ≤ 0.05

Impact of DPE on renal antioxidant status

In CsA group, there was a significant decrease in the MDA with a significant increase in GSH and CAT levels compared with Cont group (P<0.05). The administration of DPE either at low or high doses induced a significant improvement (p< 0.05) in the renal antioxidant status compared to CsA values. There was a significant difference (P<0.05) between DPE (4 ml/kg) and DPE (6 ml/kg) results (Figures 1-3).

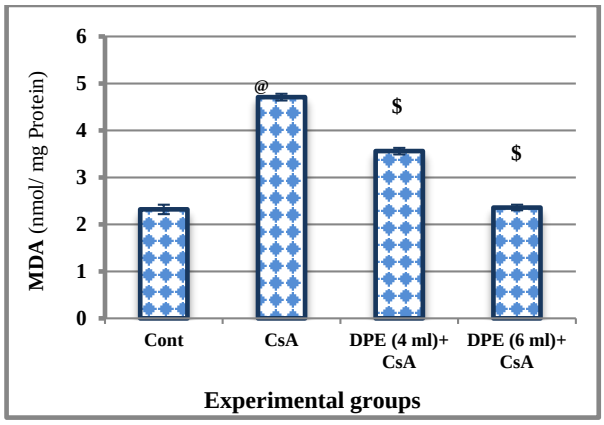

Figure 1: Impact of DPE on renal malondialdehyde (MDA) levels against CsA- Induced nephrotoxicity in rats

Results have been represented as mean ± SE (n = 10). [@] Significant versus Cont values, [$] significant versus CsA values. [#] significant between DPE (4 ml/kg) and DPE (6 ml/kg) values. p ≤ 0.05

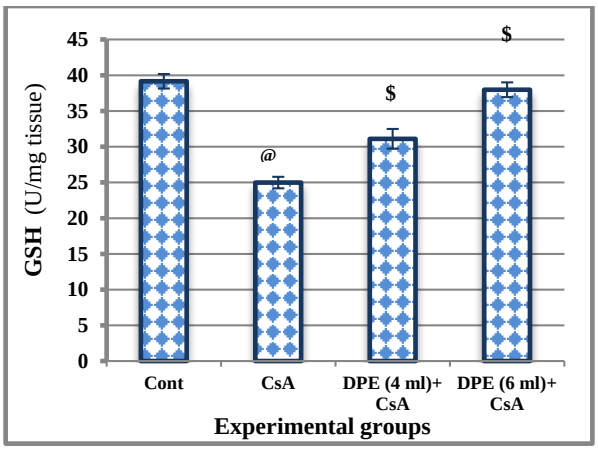

Figure 2: Impact of DPE on renal glutathione (GSH) levels against CsA- Induced nephrotoxicity in rats

Results have been represented as mean ± SE (n = 10). [@] Significant versus Cont values, [$] significant versus CsA values. [#] significant between DPE (4 ml/kg) and DPE (6 ml/kg) values. p ≤ 0.05

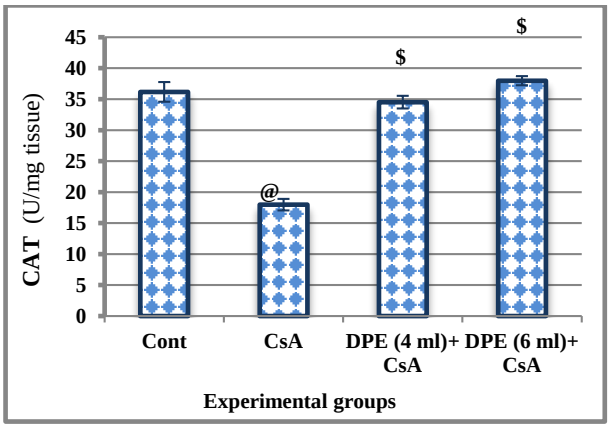

Figure 3: Impact of DPE on renal CAT activity against CsA- Induced nephrotoxicity in rats

Results have been represented as mean ± SE (n = 10). [@] Significant versus Cont values, [$] significant versus CsA values. [#] significant between DPE (4 ml/kg) and DPE (6 ml/kg) values. $p \leq 0.05$

Histological results

Figures 4 and 5 show the effects of DPE on the renal tissue histopathological changes in CsA-induced nephrotoxicity in rats. Figures 4 A-D represent Cont group with normal histology. The injection of CsA induced coagulative necrosis of epithelial lining renal tubules and cystic dilatation of renal tubules with thickening of basement membrane of renal tubules, as well as focal interstitial nephritis. The renal tissues of the rats treated with DPE (4ml)+CsA group, showed the congestion of glomerular tufts and few periglomerular inflammatory cells infiltration (Figures 5 A-B). On the other hand, the administration of CsA injected on rats with DPE (6ml) resulted in apparent normal renal parenchyme, except some sections in which there was a slight congestion of glomerular tufts (Figures 5 C-D).

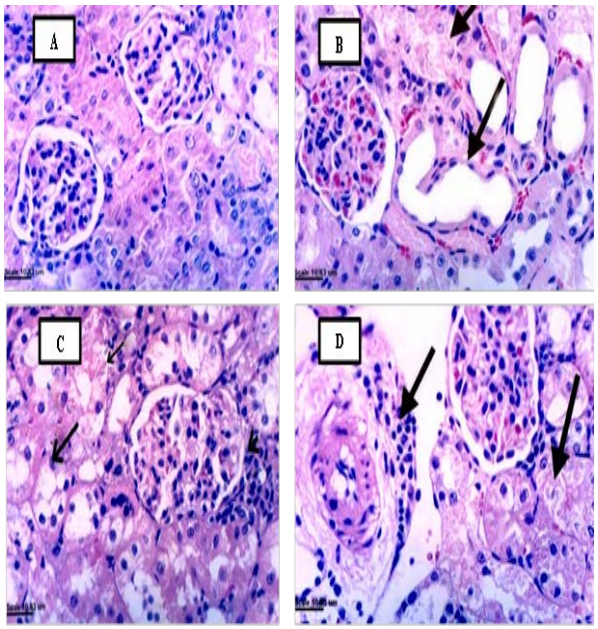

Figure 4: Impact of DPE on the renal tissue histopathological changes detected by H & E staining in CsA-induced nephrotoxicity in rats. Photo A represents Cont group, with no histopathological changes. Photo B represents CsA group, showing coagulative necrosis of epithelial lining renal tubules and cystic dilatation of renal tubules with thickening of basement membrane of renal tubules (arrows). Photo C represents necrosis of renal tubules, vacuolation of epithelial lining renal tubules, and focal interstitial nephritis. Photo D represents coagulation necrosis of renal tubules and perivascular inflammatory cells infiltration (arrows).

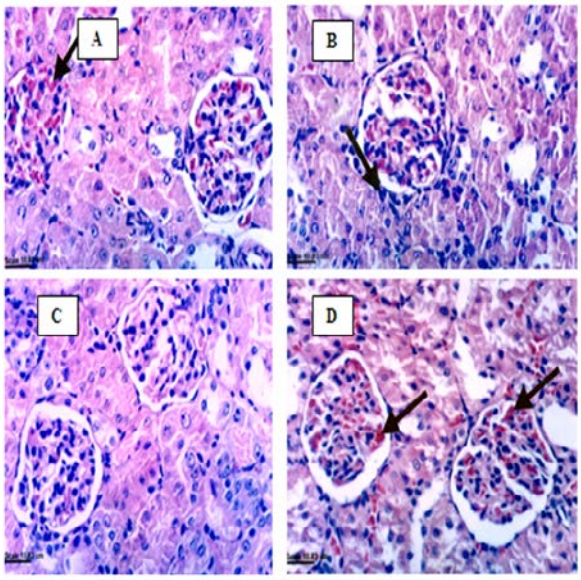

Figure 5: Impact of DPE on the renal tissue histopathological changes detected by H & E staining in CsA-induced nephrotoxicity in rats. Photo A represents DPE (4 ml)+ CsA group, showing the congestion of glomerular tufts (arrows), as well as few periglomerular inflammatory cells' infiltration (Photo B). Photo C represents DPE (6 ml)+ CsA group, showing the apparent normal renal parenchyme, except some sections of slight congestion of glomerular tufts (arrows) (Photo D).

DISCUSSION

This study was carried out to assess the protective effect and antioxidant activity of DPE against CsA induced nephrotoxicity. The results obtained revealed that SC injection of CsA in a dose of 15 mg/kg/d for 28 d resulted in the deterioration of renal function and the development of histopathological changes in the renal tissues. This was evidenced by a significant increase in serum levels of creatinine, blood urea nitrogen and uric acid compared with the Cont group. The obtained results were consistent with the most reported experimental procedures [2, 20, 21]. In addition, there was an elevation of serum albumin and ionic potassium with the reduction of serum total protein and ionic sodium levels as compared with the control rats. This effect confirmed the CsA nephrotoxicity effect. The obtained results were in agreement with the previously reported lesions of the CsA-induced nephrotoxicity [22].

The oxidative stress can encourage the production of multiple vasoactive intermediates which influence the kidney function immediately by inducing kidney vasoconstriction or lowering the glomerular filtration rate. The high levels of kidney functions would indicate several disturbances in kidney [23]. Sodium depletion has been associated with CsA treatment [24]. CsA-induced nephrotoxicity has been characterized by 20–30%

reduction in glomerular filtiration rate and up to 40% reduction in renal blood [25]. Previously, it was observed that CsA treatment produced a significant reduction in serum total protein which was measured 2 and 4 weeks after CsA [26].

The mechanism of the CsA-produced kidney toxicity has not been completely established. The development of CsA nephrotoxicity in the present study was associated with a significant decrease in renal GSH and CAT activity, and significant increase in renal MDA indicating that oxidative stress played a crucial role in the pathogenesis of CsA-induced nephrotoxicity. The obtained results may be due to the compensatory mechanisms for the overproduction of free radicals and oxidative stress [27, 28]. The increase in MDA level suggested the enhanced peroxidation leading to tissue damage and failure of the antioxidant mechanisms to prevent the production of excessive free radicals.

Different pathways have been suggested to be involved in CsA-produced kidney toxicity, specifically, the enhancement of the renin-angiotensin system and the increased sympathetic output [29], the increased synthesis of endothelin [30], and the inductions of cytochrome P450 enzymes in renal microsomes [31], kidney vasoconstriction revealed to the elevated formation of vasoactive compounds and lowered production of vasodilator compounds especially nitric oxide [32], and the oxidative stress, which resulted in structural and functional ailments of the kidney [33].

Antioxidants have healing effects on renal functions and histological damages related to CsA. The date palm (*Phoenix dactylifera*) have been popular plants in many countries and have been a vital component of arid and semiarid regions of the world. The obtained results suggested that DPE has been effective in preventing the renal functional impairment in CsA-induced nephrotoxicity in the rats model. There were significant factors inhibiting the elevated renal functions, disturbance in protein metabolism and ionic sodium and potassium levels compared with CsA group. This effect may be related to the antioxidant properties of date seeds since it has been found that ROS may be involved in the impairment of glomerular filtration rate [34, 35]. In addition, Habib *et al*., [8] revealed that date seeds possess high antioxidant activity due to the abundance of phenolic compounds and flavenouids.

In the present study, cotreatment with DPE significantly attenuated the toxic effects of CsA on the depletion in antioxidant status probably due to its rich antioxidant phenolics and flavenoids compounds, which have had a strong free-radical scavenging capacity, thereby diminished the oxidative injuries [12, 36].

CONCLUSION

The administration of DPE was experimentally demonstrated to be remarkably combat the oxidative stress and nephrotoxicity induced by CsA. This effect might be attributed to high antioxidant contents in DPE. The data obtained in this research proposed that DPE is a commonly present foodstuff. Besides, it is considered an important source of natural antioxidants. The proved prophylactic action attained in this research gave powerful confirmation which would support future clinical research of standardized DPE in the management of oxidative stress-linked kidney ailments.

REFERENCES

1. Capasso, G., Di Gennaro, C., Ragione, F., Manna, K., Ciarcia, R., Florio, S., Perna, A., Pollastro, R., Damiano, S., Mazzoni, O., Galletti, P. and Zappia, V. (2008). In vivo effect of the natural antioxidant hydroxytyrosol on cyclosporine nephrotoxicity in rats. Nephrol Dial Transplant, 23 (4), 1186-95.
2. Agnieszka, K., Mariusz, M., Grażyna, C., Krzysztof, C. and Elżbieta, K. (2010). Experimental models of cyclosporine a nephrotoxicity, Bull Vet Inst Pulawy 54: 59-62.
3. Kahan,B.D., Flechner, S.M., Lorber, M.I., Golden, D., Conley,S. and Van Buren,C.T. (1987). Complications of cyclosporine-prednisone immunosuppression in 402 renal allograft recipients exclusively followed at a single center for from one to five years. Transplantation. 43(2):197-204.
4. Lorber, M.I., Van Buren,C.T., Flechner, S.M., Williams, C. and Kahan, B.D. (1987). Hepatobiliary and pancreatic complications of cyclosporine therapy in 466 renal transplant recipients. Transplantation, 43(1):35-40.
5. Remuzzi, G. and Perico, N. (1995). Cyclosporine-induced renal dysfunction in experimental animals and humans. Kidney Int Suppl. , 52:S70-4.
6. Burdmann, E., Andoh ,T., Rosen, S., Lindsley, J., Munar ,M., Elxinga, W. and Bennet,W. (1994). Experimental nephrotoxicity, hepatotoxicity and pharmacokinetics of cyclosporin G versus cyclosporin A. Kidney Int , 45: 684-91.
7. Bobadilla, N. and Gamba, G. (2007). New insights into the pathophysiology of cyclosporine nephrotoxicity: a role of aldosterone. Am J Renal Physiol, 293 (1), 2-9.
8. Habib, H.M., Platat, C., Meudec, E., Cheynier, V. and Ibrahim,W.H. (2014). Polyphenolic compounds in date fruit seed (Phoenix dactylifera): Characterisation and quantification by

using UPLC-DAD-EDI-MS. J Sci Food Agric, 94 (6):1084-9.
9. Al-Farsi, M.A. and Lee, C.Y. (2008). Nutritional and functional properties of dates: a review, Critical Reviews in Food Science and Nutrition, 48 (10): 877–87.
10. Juhaimi,F.A., Ghafoor, K. and Özcan, M.M. (2012). Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (Phoenix dactylifera L.) varieties, Int Jof Food Sci and Nutr, 63 (1): 84–9.
11. Besbes, S., Blecker, C., Deroanne, C., Lognay, G., Drira, N.E. and Attia, H. (2004). Quality characteristics and oxidative stability of date seed oil during storage. Food Sci and Technol Int, 10: 333–8.
12. Dammak, I., Boudaya, S., Abdallah, F., Ben, H.T. and Attia, H. (2009). Date seed oil inhibits hydrogen peroxide-induced oxidative stress in normal human epidermal melanocytes. Connective Tissue Res, 50 (5): 330–5.
13. Cabr´e, M., Camps, J., Paternáin, J.L., Ferré, N. and Joven, J. (2000). Time course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride induced cirrhosis. Clin and Experiment Pharmacol and Physiol, 27 (9): 694-9.
14. Habib, H.M. and Ibrahim,W.H. (2011). Effect of date seeds on oxidative damage and antioxidant status in vivo. J of the Sci of Food and Agri, 91(9): 1674-9.
15. Al-Qarawi, A. A., Mousa, H. M., Ali, B. E. H., Abdel- Rahman, H. and El-Mougy, A.A. (2004). Protective effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats. Int J of Applied Res in Veterinary Med, 2 (3):176–80.
16. Al-Farsi, M., Alasalvar, C. , Morris, A., Baron, M. and Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. J of Agriand Food Chem, 53(19):7592–9.
17. Reeves, P.G.(1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. J. of Nutr., 127:838-41.
18. Mihatch, M..J., Ryffel, B. and Hermle, M. (1986). Morphology of cyclosporine nephrotoxicity in the rat. Clin Nephrol, 25 (1): 2-8.
19. Snedecor, G.W. and Cochron,W.G. (1989) Statistical methods & Application. 8th ed., Lowa State Univ. Press, Ames, Lowa., USA.
20. Ciarcia, R., Damiano S, Fiorito F, Granato,G., Pagnini,F., Mastellone, V., Iovane,V., Alfano,L., Valenti,F., Florio,S. and Giordano,A. (2012). Hydrocortisone attenuates cyclosporin A-induced nephrotoxicity in rats. J Cell Biochem. 103:997-1004.
21. Wongmekiat, O., Gomonchareonsiri, S. and Thamprasert, K. (2011). Caffeic acid phenethyl ester protects against oxidative stress-related renal dysfunction in rats treated with cyclosporin A. Fundam Clin Pharmacol., 25 (5):619-26.
22. Naesens, M., Kuypers, D.R. and Sarwal, M. (2009). Calcineurin inhibitor nephrotoxicity. Clin J Am Soc Nephrol. 4 (2):481-508.
23. Elshater, A.A., Salman, M.A. and Moussa, M.A. (2008). Effect of green tea consumption on level of glucose, lipid profile and kidney functions in alloxan induced-diabetic rats Egypt. Acad. J. biolog. Sci. 1(2): 125–34.
24. 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 susceptible to altered vascular function and hemodynamics. Inter. J. of Pharmacy and Pharma. Sci. 4 :0975- 1491.
25. Mohamadin, A.M., El-Beshbishy, H.A. and El-Mahdy, M.A. (2005). Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. Pharmacological Research. 51(1): 51–7.
26. Elsayed,A.S.I., Bayomy,M.F.F. and Azab, A.E. (2016). Effect of acute and chronic treatment of Cyclosporin A on liver and kidney functions in rats. J. of Applied Pharmaceutical Sci., 6 (03): 116-119.
27. Somani, R., Singhai, A.K., Shivgunde, P. and Jain, D. (2012). Aspragus racemosus Willd (Liliaceae) ameliorates early diabetic nephropathy in STZ induced diabetic rats, Indian J of Experimental Biology, 50 (7): 469-475.
28. Hazem, H., Kataya, H. and AlaaEldin, A. (2008). Red cabbage (Brassica oleracea) ameliorates diabetic nephropathy in rats, eCAM, 5(3): 281–7.
29. Dell, K., Bohler, T., Gaedeke, J., Budde, K., Neumayer, H.H. and Waiser, J. (2003). Impact of PGE1 on cyclosporine A induced up-regulation of TGF-beta1, its receptors, and related matrix production in cultured mesangial cells. Cytokine., 22:189-93.
30. Ramirez, C., Olmo, A. and O'Valle, F. (2000). Role of intrarenal endothelin 1, endothelin 3, and angiotensin II expression in chronic cyclosporin A nephrotoxicity in rats. Exp Nephrol., 8 (3):161-72.

31. Serino, F., Grevel, J., Napoli, K.L., Kahan, B.D., Strobel, H.W. (1994). Oxygen radical formation by the cytochrome P450 system as a cellular mechanism for cyclosporine toxicity. Transplant Proc., 26 (5):2916-7.
32. Yoon, H.E. and Yang, C.W. (2009). Established and newly proposed mechanisms of chronic cyclosporine nephropathy. Korean J Intern Med., 2009; 24 (2):81-92.
33. Burdmann, E.A., Andoh, T.F., Yu, L. and Bennett, W.M. (2003). Cyclosporine nephrotoxicity. Semin Nephrol, 23 (5):465-76.
34. Hughes, A.K., Stricklett, K., Padilla, E. and Kohan, D.E. (1996). Effect of reactive oxygen species on endothelin-1 production by human mesangial cells, Kidney Int 49:181-189.
35. Farombi, E.O. and Ekor, M. (2006). Curcumin attenuates gentamicin induced renal oxidative damage in rats, Food Chem Toxicol, 44 (9), 1443-8.
36. Dammak, I., Abdallah, F. Ben Boudaya, S., Besbes, S., Keskes, L., El Gaied, A., Turki, H., Attia, H. and Hentati, B. (2007). Date seed oil limit oxidative injuries induced by hydrogen peroxide in human skin organ culture, BioFactors (Oxford, England), 29 (2-3): 137–45.