New insights on the anti-inflammatory effect of some Egyptian plants against renal dysfunction induced by cyclosporine

A.B. SHALBY[1], A.H. HAMZA[2], H.H. AHMED[1]

[1]Hormones Department, National Research Center, Dokki (Egypt)
[2]Biochemistry Department, Faculty of Science (girls), King Abdulaziz University, Jeddah (Kingdom of Saudi Arabia)

Abstract. – ***Objective:*** **This study was undertaken to investigate the anti-inflammatory role of fennel, carob, doum and mixture of them in improving the renal dysfunction induced in rats by the cyclosporine.**

Design: **Sixty female albino rats were divided into six groups: healthy control rats, cyclosporine injected rats (positive control), other groups were injected by cyclosporine for 7 days and then diet was supplemented with either fennel, doum, carob or mixture of them. After 45 days of supplementation, rats were scarified. Serum and urinary samples were obtained for different biochemical analysis.**

Methods: **The analysis included the determination of creatinine levels in serum and urinary samples, serum ammonia, transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α), urinary N-acetyl-beta D-glucosaminidase (NAG), β2 microglobulin as well as calculating the creatinine clearance. Also, an histopathological examination for the kidney tissue was performed.**

Results: **The present study showed that cyclosporine induced the nephrotoxicity as appeared by elevation of serum and urinary levels of creatinine, urinary level of β2 microglobulin, serum levels of ammonia, TGF-β1 and TNF-α and the NAG level while decreased the creatinine clearance. Addition of fennel, carob, doum or mixture of them significantly improved the kidney functions. Moreover, anti-inflammatory status of animals injected with cyclosporine and supplemented with fennel, carob, doum and mixture of them, showed a significant amelioration in the kidney functions as compared to animals injected with the cyclosporine only. Histopathological investigation of kidney tissue of rat treated with the cyclosporine showed a moderate degree of renal damages. While, groups fed on fennel, carob, doum or mixture of them showed a great improvement in the kidney morphological structure.**

Conclusions: **Diet supplementation with fennel, carob, doum have a promising anti-inflammatory influence on attenuating the complications associated with the renal dysfunction.**

Key Words:

Fennel, Carob, Doum, Cyclosporine, Renal dysfunction, Anti-inflammatory effect.

Introduction

Cyclosporine (CsA) is an immunosuppressive agent frequently used for prevention of allograft rejection and for the treatment of autoimmune diseases. However, its clinical use is often limited by severe side effects. CsA caused gingival hypertrophy, tremor, increased blood pressure, and its hepato- and nephrotoxic capacity[1]. It has been demonstrated that these negative effects determine histopathological changes in several organs, i.e. thymus, kidney, liver, heart, pancreas and nervous system[2]. Kidney dysfunction is the main complication of CsA treatment[2].

The toxic impact of CsA can be divided into acute and chronic effects[3]. Acutely, the drug can cause afferent glomeruloarteriolar constriction with a hemodynamically mediated decrease in the glomerular filtration rate (GFR), solute transport alterations such as thrombotic microangiopathy related to the direct endothelial injury[4].

Chronic administration of CsA inhibits the renin-angiotensin-aldosterone axis; activates profibrotic genes such as that for transforming growth factor-β; and maintains relative intrarenal ischemia, which leads to cycles of injury and repair in the regions of the kidney that are most vulnerable to hypoxia. This process culminates in a distinct form of chronic organ dysfunction that is easily apparent in recipients of extrarenal transplants in which other mechanisms of renal injury are not present to cloud histologic mechanisms[2].

Corresponding Author: Aziza Shalby, MD; e-mail: drazizanrc@yahoo.com

Fennel [*Foeniculum (F.) vulgare*] is a plant belonging to the Umbelliferae (*Apiaceae*) family, known and used by humans since antiquity. It was cultivated in every country surrounding the Mediterranean Sea because of its flavour[5]. The renewed interest in natural product rather than synthetic agents has again focused attention on plants as a source of flavouring compounds[6]. Fennel is an aromatic edible plant whose seed are used for savoury formulations, sauces, liqueurs, confectionery, etc.[7]. Due to unique and preferred flavour and aroma, the swollen bases of fennel are freshly consumed in salad or cooked as kitchen vegetable[8]. The fennel (*F. vulgare*) seeds, leaves and fruits is a potential source of natural antioxidant[9].

Carob [*Ceratonia (C.) siliqua L.*] is the bean-like fruit of *C. siliqua*, which grows widely in the Mediterranean region and belongs to the genus leguminosae. Historically, due to its high content of sugars, the brown pod was consumed as food, especially in ancient times as a candy for children or in emergency situations such as war. The carob pulp contains 40–60% of low-molecular-weight carbohydrates, mainly sucrose, depending on carob species, origin and climate[10]. Apart from carbohydrates, high amounts of dietary fibre and polyphenols are characteristic of this Mediterranean food. Owen et al.[10] study show that carob fibre has a high content of phenolic antioxidants. Carob fibre also contains a rich variety of individual components from several classes: simple phenolic acids, cinnamic acid and its derivatives, flavonoids, isoflavones, lignans, anthocyans and tannins. Therefore, when included in the daily diet, these compounds have antioxidative, antimutagenic, anticarcinogenic, antiproliferative, or antioestrogenic activity[10].

Doum [*Hyphaene (H.) thebaica]* is a desert palm native to Egypt, sub-Saharan Africa and West India. It is known in Egypt as the Doum or gingerbread palm which grows to 6 or 9 m and usually has forked stems with fanshaped leaves, 65-75 cm long. It is listed as one of the useful plants of the world[11]. Research on the fruit pulp showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid[12]. Identification of compounds, by thin-layer chromatography, showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids, and the fruit also lowers blood pressure in animal models[13]. Also, Hsu et al[13] reported that the hot water extract from the fruit of the Doum palm, *H. thebaica* has an antioxidant activity.

Materials and Methods

Drugs and Plant Materials

Cyclosporine (Sandimmun) was purchased from Novartis Co. Egypt. Fennel seeds, carob pods and doum fruit were purchased from local markets, finely powdered in electric miller for use in animal diet.

Cyclosporine was dissolved in corn oil and injected to rats with a dose of 50 mg/kg daily for 7 days successively according to Seki et al[14]. 17 g fennel/kg diet was used[15]. Carob was added in 18 g/kg diet to the animal diet according to Zunf et al[16], while doum in dose of 21 g/kg diet[17] was added to the experimental animal's diet. Mixture of fennel, carob and doum was prepared in the same doses.

Experimental Animals

The current study was conducted on sixty, 3 months old female Sprague Dawley rats (120-130 g). The animals were obtained from the Animal House Colony of the National Research Centre, Dokki, Cairo, Egypt and maintained in standard laboratory conditions. The animals were housed in plastic cages at room temperature (25±2˚C) and humidity (55%). Rats were controlled constantly with a 12 h light dark cycle at National Research Centre, Animal Facility Breeding Colony. They were provided with tap water and standard laboratory diet *ad libitum*. The standard laboratory diet consists of casein 10%, salts mixture 4%, vitamins mixture 1%, corn oil 10% and cellulose 5% completed to 100 g with corn starch[18]. Animals were allowed to acclimate for two weeks to the housing conditions and received human care in compliance with the guidelines of the Ethical Committee of Medical Research of the National Research Centre, Cairo, Egypt. After 2 weeks of adaptation, they were randomly assigned into six groups: 10 rats/group as follows: Gp (1) rats were fed on standard control diet and injected with corn oil daily for 7 days. This group set as healthy control group. Gp (2) fed standard control diet and injected with 50 mg/kg cyclosporine daily for 7 days (positive control). Gp (3-6) was injected by cyclosporine also for 7 days then fennel, carob, doum and mixture of them was added to the diet of these groups respectively. The experiment lasts for 45 days.

Samples Collection

At the end of the experimental period, rats were fasted over-night, kept individually in metabolic cages where urine samples were collected without faecal contamination under paraffin oil to avoid evaporation. The urine samples were stored at –80˚C until analysis. Then the rats were anesthetized and the blood samples were collected in EDTA-free tubes, left to cold, and then they were centrifuged at 3000 rpm for 10 minutes at 4˚C where the clear sera were obtained and stored at –80˚C for further analytical assays.

Histological Investigation

The other kidney for each animal was fixed in 10% neutral buffered formalin and embedded into paraffin blocks. Histopathological examination was carried out on 5 μm-thick, hematoxylin–eosin (H&E) stained sections.

Blood Chemistry

Creatinine levels were determined colorimetrically according to the methods of Larsen[19]. Quantitative estimation of urine beta$_2$-microglobulin (b2MG) was carried out by enzyme linked immunosorbant assay (ELISA) procedure as described by Crisp et al[20]. Urinary N-acetyl-β-glucosaminidase (NAG) activity was evaluated by liberating P-nitrophenol in alkaline medium which was proportional to the enzymatic activity according to the method described by Gressner and Roebruk[21]. Serum transforming growth factor-β (TGF-β) and tumor necrosis factor (TNF-α) were detected by using ELISA procedure as described by Kim et al[22] and Corti et al[23], respectively.

Statistical Analysis

In the present study, all results were expressed as mean ± S.E of the mean. Statistical Package for the Social Sciences (SPSS) program, version 11.0 (SPSS Inc., Chicago, IL, USA) was used to compare significance between each two groups. Difference was considered significant when $p \leq 0.05$.

Results

Table I shows the effect of diet supplemented with fennel, carob, doum or their mixture after cyclosporine (CsA) administration on different biomarkers of kidney function. CsA administration significantly increased each of serum and urinary creatinine, and serum ammonia while decreased creatinine clearance as compared to untreated control group. Fennel, carob, doum or their mixture administration after injection with CsA showed significant reduction in serum creatinine, urinary creatinine and serum ammonia levels while they improved the creatinine clearance as compared to CsA-treated group.

Table II represents the results of urinary beta2-microglobulin (b2MG) and N-acetyl-β-glucosaminidase (NAG) activity in CsA-treated group and groups fed on fennel, carob, doum or their mixture. The levels of these biomarkers were significantly elevated in CsA-treated group as compared to control group. The influence of diet supplement with fennel, carob, doum or their

Table I. Effect of diet supplemented fennel, carob, doum or their mixture on various renal function biomarkers (values are mean ± S.E.).

Groups	Parameters			
	Serum creatinine (mg/dl)	Urinary creatinine (mg/dl)	Creatinine clearance (ml/min)	Serum ammonia (μmol/L)
Control -ve	0.9870 ± 0.05	7.30 ± 0.4	1.794 ± 0.01	367.3 ± 4.6
CsA	3.0575 ± 0.1[a]	9.10 ± 0.4[a]	0.968 ± 0.02[a]	526.1 ± 8.8[a]
Fennel	1.1612 ± 0.04[b]	7.57 ± 0.4[b]	1.531 ± 0.02[b]	380.6 ± 4.0[b]
Carob	1.2578 ± 0.04[b]	7.60 ± 0.4[b]	1.426 ± 0.02[b]	392.0 ± 4.7[b]
Doum	1.8797 ± 0.06[b]	7.90 ± 0. 4[b]	1.161 ± 0.02[b]	426.4 ± 8.0[b]
Mix	1.6499 ± 0.1[b]	8.83 ± 0. 3	1.343 ± 0.02[b]	398.0 ± 5.8[b]

[a]Significant change at $p < 0.05$ in comparison with control group. [b]Significant change at $p < 0.05$ in comparison with CsA-treated group.

Table II. Effect of diet supplemented with fennel, carob, doum and their mixture on urinary beta2-microglobulin (b2MG) and N-acetyl-β-glucosaminidase (NAG) activity (values are mean ± S.E.).

Groups	Parameters	
	Urine b2MG (μg/ml)	Urine NAG (U/L)
Control -ve	0.023 ± 0.002	51.64 ± 2.5
CsA	0.042 ± 0.002[a]	213.22±9.3[a]
Fennel	0.027 ± 0.001[b]	73.21 ±3.1 b
Carob	0.028 ± 0.001[b]	88.76 ± 3.6[b]
Doum	0.034 ± 0.001[b]	154.23 ± 3.3[b]
Mix	0.030 ± 0.003[b]	98.84 ± 2.7[b]

[a]Significant change at $p < 0.05$ in comparison with control group. [b]Significant change at $p < 0.05$ in comparison with CsA-treated group.

mixture on kidney biomarkers revealed a significant decrease in their levels when compared to the CsA-treated group only.

The data in Table III showed the effect of diet supplemented with fennel, carob, doum or their mixture on serum inflammatory indices. The levels of serum TNF-α and TGF-β were significantly elevated in CsA-treated group as compared to control group. The influence of diet supplement with fennel, carob, doum or their mixture on serum inflammatory biomarkers revealed a significant decrease in their levels when compared to the CsA-treated group only.

Microscopic examination of the kidney tissues of control rats showed normal structure; normal glomerular and tubular architecture was observed in Figure 1. Figure 2: Section of kidney of cyclosporine (CsA)-treated rats shows moderate degrees of tubular damages. The lumina of the tubules filled with degenerate and desquamated epithelial cells. Intercellular hemorrhage and edema are noticed. Figure 3: Section of kidney of rat treated with CsA and fennel show the renal tubules that appear more or less as control one. Figure 4: Section of kidney of rat treated with CsA and carob showed the tubules that appear more or less like normal. Notice the hemorrhage area in the interstitium. Figure 5: Section of kidney of rat treated with CsA and doum show some healthy tubules while the other shows degenerate and desquamated materials in its lumen. Figure 6: Section of kidney of rat treated with CsA and mixture of them shows desquamation of the epithelial cells of the tubules and presence of cell debris in the lumen of the tubules cloudy degeneration.

Table III. Effect of diet supplemented with fennel, carob, doum and their mixture on serum TNF-α and TGF-β levels (values are mean ± S.E.).

Groups	Parameters	
	Serum TNF-α (pg/ml)	Serum TGF-β (pg/dl)
Control -ve	7.92 ± 0.3	193.91 ± 13.4
CsA	55.81 ± 2.9[a]	1191.77 ± 26.5[a]
Fennel	18.14 ± 0.9[b]	292.50 ± 5.4[b]
Carob	26.20 ± 1.4[b]	356.20 ± 8.4[b]
Doum	38.10 ± 1.6[b]	656.74 ± 14.2[b]
Mix	33.10 ± 1.6[b]	416.64 ± 9.1[b]

[a]Significant change at $p < 0.05$ in comparison with control group. [b]Significant change at $p < 0.05$ in comparison with CsA-treated group.

Discussion

Cyclosporine (CsA), widely used in the clinic as part of the protocol of immunosuppression, may present serious adverse effects[1]. Kidney dysfunction is the main complication of CsA treatment[1]. The results of the present study revealed that CsA induced nephrotoxicity in rats which was indicated by elevated serum creatinine, urinary creatinine and serum ammonia while decreased creatinine clearance as compared to untreated control group. These data agreed with those of Hosogai et al[24].

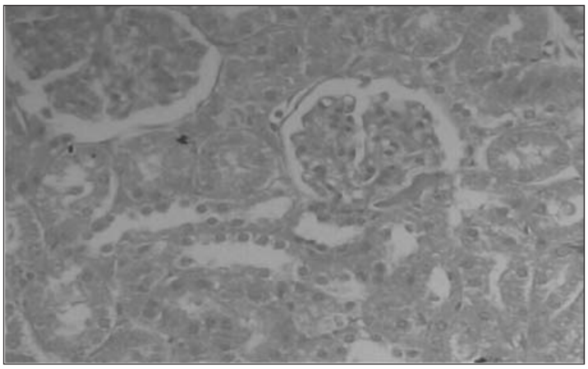

Figure 1. Microscopic examination of the kidney tissues of control rats shows normal structure; normal glomerular and tubular architecture (H&E ×40).

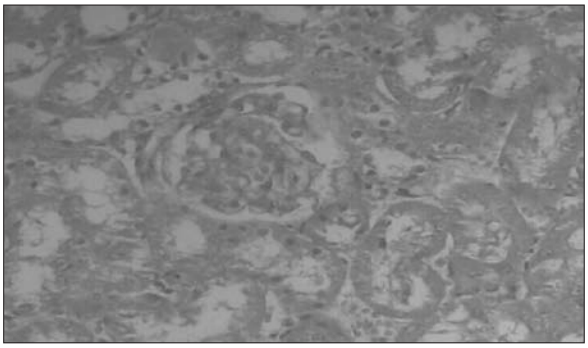

Figure 2. Section of kidney of cyclosporine (CsA)-treated rats shows moderate degrees of tubular damages. The lumina of the tubules filled with degenerate and desquamated epithelial cells. Intercellular hemorrhage and edema are noticed (H&E ×40).

Our findings of the increased urinary NAG and of the b2MG excretion in CsA-treated animals highlighted a tubular dysfunction, which was supported by the results of Burdmann et al[25] and Pereira et al[26]. Where, N-acetyl-b-D-glucosaminidase (NAG) and b2MG are biomarkers of proximal tubular injury because they are freely filtered at the glomerulus and then reabsorbed and metabolized in the proximal tubule[27,28].

Additionally, treatment with CsA caused increasing serum TNF-α which may be attributed to the increased expression[29]. Treatment with CsA increased serum TGF-β level. This finding may be due to the increased TGF-β expression, which promotes renal fibrosis by increasing the production and decreasing the degradation of extracellular matrix proteins[30] and apoptotic cell death in the renal tissue of rats[31,32].

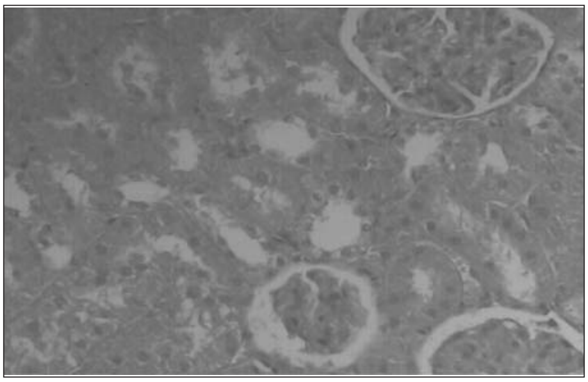

Figure 3. Section of kidney of rat treated with CsA and fennal show the renal tubules that appear more or less as control one (H&E ×40).

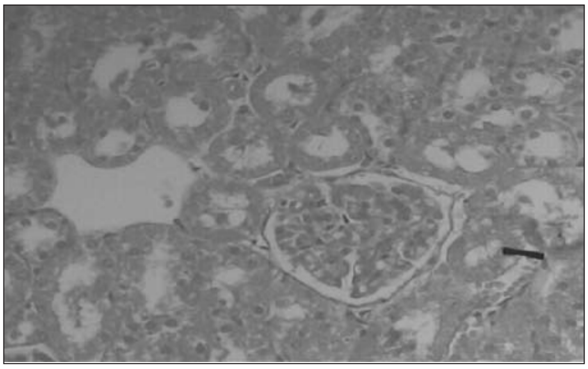

Figure 4. Section of kidney of rat treated with CsA and carob showed the tubules that appear more or less like normal. Notice the hemorrhage area in the interstitium (H&E ×40).

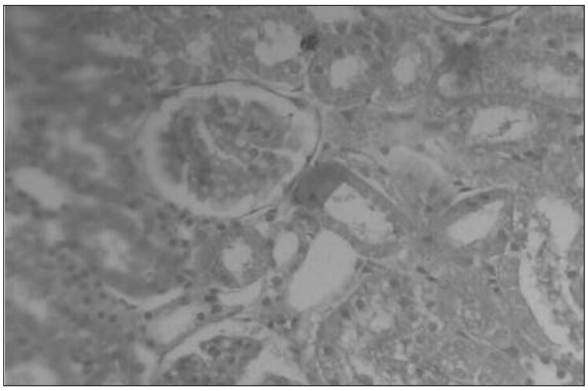

Figure 5. Section of kidney of rat treated with CsA and doum show some healthy tubules while the other shows degenerate and desquamated materials in its lumen (H&E ×40).

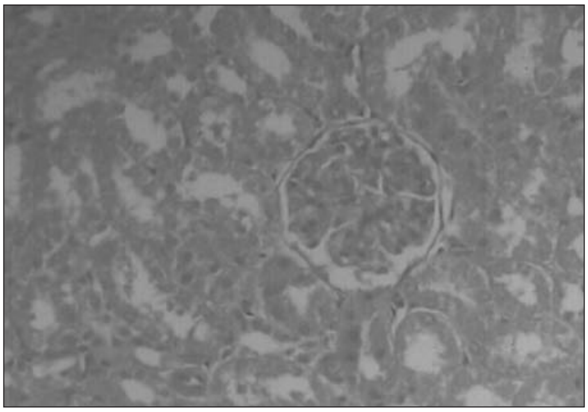

Figure 6. Section of kidney of rat treated with CsA and mixture of them shows desquamation of the epithelial cells of the tubules and presence of cell debris in the lumen of the tubules cloudy degeneration (H&E ×40).

It is clear from our results that addition of fennel, carob, doum or mixture of them to the diet of the experimental animals produced a significant improvement in kidney functions and inflammatory status.

The nephroprotective effect of fennel may be attributed to its anethole, a chief constituent of fennel, which has been shown to block the inflammation. Anethole is a potent inhibitor of TNF-α induced NF-B activation, IkappaBalpha (IB) phosphorylation and degradation, and NF-B reporter gene expression[33]. Chainya et al[34] stated that anethole and its structural analogues act at an early step in the cascade of TNF-dependent signal transduction.

Carob also showed moderate nephroprotective effect against CsA. Carob contains phytochemicals including several classes of polyphenolic compounds: simple phenolic acids, cinnamic acid and its derivatives, flavonoids, isoflavones, lignans, anthocyans and tannins. The simple phenols and polyphenols detected in carob fiber are potent scavengers of reactive oxygen species (ROS) while the flavonoids, in addition, inhibit enzymes such as xanthine oxidase[10], cyclooxygenase[35] and the production of cytokines[36]. Also, Jung et al[37] reported that polyphenolic compounds significantly inhibited the expression of NF-kappaB regulated proinflammatory genes (TNF-alpha, IL-1beta, CXCL9, CXCL10), inflammatory relevant enzymes (COX-2, CYP3A4), and transcription factors (STAT1, IRF1). Therefore, the observed nephroprotective influence of carob could be attributed to its polyphenolic compounds which have antioxidant and anti-inflammatory activities.

The effect of doum in ameliorating nephrotoxicity was minimal as compared to the effect of fennel and carob. The anti-inflammatory activity of doum was possibly due to its saponin content which acts against the oxidative damage and suppresses the TGF-beta1 expression[38]. So, doum administration declines the oxidative damage and the renal interstitial fibrosis in rats. The slight nephroprotective effect of doum may explain the less nephroprotective effect of the mixture (fennel, carob, and doum).

The Authors suggested that the improvement in kidney functions as inducted by decreased levels of serum and urinary creatinine and the reduced level of serum ammonia accompanied with elevated creatinine clearance value could be attributed to the anti-inflammatory action of these dietary supplements.

Our findings have been confirmed by the histological examinations of kidney tissues in which kidney histology of rats treated by CsA showed moderate degrees of tubular damages; the lumina of the tubules filled with degenerate and desquamated epithelial cells, and the intercellular hemorrhage as well as edema were observed. These data agreed with Shu et al[4]. On the other hand, groups fed on fennel, carob, doum or mixture of them showed a great improvement in the kidney morphological structure.

In conclusion, the present study shed light on the anti-inflammatory activity of some Egyptian plants (fennel, carob and doum) against CsA-induced renal dysfunction in *vivo*. Noteworthy, fennel showed the best nephroprotective effect as compared to other groups while doum was less effective.

References

1) Böhmer AE, Souza DG, Hansel G, Brum LM, Portela LV, Souza DO. Long-term cyclosporine treatment in non-transplanted rats and metabolic risk factors of vascular diseases. Chem Biol Interac 2010; 185: 53-58.
2) Rezzani R. Cyclosporine A and adverse effects on organs: histochemical studies. Prog Histochem Cytochem 2004; 39: 85-128.
3) Calne RY. Immunological tolerance: the liver effect. J Gastroenterol Hepatol Rev 2002; 17: 488-490.
4) Shu Z, Pu X, Xiong X, Li Q, Wang Y, Zhai S. Differential expression of plasma protein in cyclosporine A- induced rat acute nephrotoxicity. Biosci Biotech Biochem 2009; 73: 592-598.
5) Muckensturm B, Foechterlen D, Reduron JP, Danton P, Hildenbrand M. Pythochemical and chemotaxonomic studies of Foeniculum vulgare. Biochem Syst Ecol 1997; 25: 353-358.
6) Yaylayan VA. Flavour technology: Recent trends and future perspectives. Can I Food Sci Tech J 1991; 24: 2-5.
7) Guilled MD, Manzanons MJ. A study of several parts the plant Foeniculum vulgare as a source of compounds with industrial interests. Food Res Int 1996; 29: 85-88.
8) Atta-Aly MA. Fennel swollen base yield and quality as affected by variety and source nitrogen fertilizer. Sci Hortic 2001; 88: 191-202.
9) Barros A, Heleno SA, Carvalho AM, Ferreira ICFR. Systematic evaluation of the antioxidant potential of different parts of Foeniculum vulgare Mill. from Portugal. Food Chem Toxicol 2009; 47: 2458-2464.

10) Owen RW, Haubner R, Hull WE, Erben G, Spiegelhalder B, Bartsch H, Haber B. Isolation and structure elucidation of the major individual polyphenols in carob fibre. Food Chem Toxicol 2003; 41: 1727-1738.

11) Fletcher R. Listing of useful plants of the world. Australian New Cropshttp: htm .//www.newcrops.uq.edu.au/listing/hyphaenetheb aica.1997.

12) Cook JA, VanderJagt DJ, Pastuszyn A, Mounkaila G, Glew RS, Millson M, Glew RH. Nutritional and chemical composition of 13 wild plant foods of Niger. J Food Composition Anal 2000; 13: 83-92.

13) Hsu B, Coupar IM, Ng K. Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. Food Chem 2006; 98: 317-328.

14) Seki T, Ishimoto T, Sakurai T, Yasuda Y, Taniguchi K, Doi M, Sato M, Roman RJ, Miyata N. Increased excretion of urinary 20-HETE in rats with cyclosporine-induced nephrotoxicity. J Pharmacol Sci 2005; 97: 132-137.

15) Zhu M, Wong PY, Li RC. Effect of oral administration of fennel (Foeniculum vulgare) on ciprofloxacin absorption and disposition in the rat. J Pharm Pharmacol 1999; 51: 1391-1396.

16) Zunf HJ, Luder W, Harde A, Haber B, Graubaum HJ, Gruenwald J. Carob pulp preparation for treatment of hypercholesterolemia. Adv Ther 2001;18: 230-236.

17) Kamis AB, Modu S, Zanna H, Oniyangi TA. Preliminary biochemical and haematological effects of aqueous suspension of pulp of hyphaene thebaica [1] mart in rats. Biokemistri 2003; 13: 1-7.

18) A. O. A. c. Official Methods of Analysis, 16th ed., Association of Official Analysis, Washington B., DC., 1995.

19) Larsen K. Creatinine assay by a reaction kinetic principle. Clin Chim Acta 1972; 41: 209-217.

20) Crisp AJ, Coughlan RJ, Mackintosh D, Clark B, Panayi GS. β2-microglobulin plasma level reflect disease activity in rheumatoid arthritis. J Rheumatol 1983; 10: 954-956.

21) Gressner AM, Roebruk P. Predictive values of serum N-acetyl-beta-D glucosaminidase for fibroid liver disorders, correlation with monoamine oxidase activity. Clin Chim Acta 1982; 124: 315-326.

22) Kim SJ, Romeo D, Yoo YD, Park K. Transforming growth factor-beta; Expression in normal and pathological conditions. Horm Res 1994; 42: 5-8.

23) Corti A, Fassino J, Marcucci F, Barbanti E, Cassani G. Oligometric tumor necrosis factor-? slowly converts into the reactive forms at bioactive levels. Biochem J 1992; 284: 905-910.

24) Hosogai N, Tomita M, Hamada K, Ogawa T, Hirosumi J, Manda T, Mutoh S. Phosphodiesterase type 5 inhibition ameliorates nephrotoxicity induced by cyclosporin A in spontaneous hypertensive rats. Eur J Pharmacol 2003; 477: 171-178.

25) Burdmann EA, Andoh TF, Nast CC, Evan A, Connors BA, Coffman TM, Lindsley J, Bennett WM. Prevention of experimental cyclosporin-induced interstitial fibrosis by losartan and enalapril. Am J Physiol 1995; F491-F499.

26) Pereira BJ, Castro I, Burdmann EA, Malheiros DM, Yu L. Effects of sirolimus alone or in combination with cyclosporine A on renal ischemia/reperfusion injury. Braz J Med Biol Res 2010; 43: 737-744.

27) Ekor M, Emerole GO, Farombi EO. Phenolic extract of soybean (Glycine max) attenuates cisplatin-induced nephrotoxicity in rats. Food Chem Toxicol 2010; 48: 1005-1012.

28) Dajak M, Ignjatović S, Stojimirović B, Gajić S, Majkić-Singh N. Urinary beta-trace protein as a tubular marker of renal dysfunction in patients with chronic kidney disease. Clin Chim Acta 2010; 411: 1154-1155.

29) Park JW, Bae EH, Kim IJ, Ma SK, Choi C, Lee J, Kim SW. Paricalcitol attenuates cyclosporine-induced kidney injury in rats. Kidney Int 2010; 77: 1076-1085.

30) Bobadilla NA, Gamba G. New insights into the pathophysiology of cyclosporine nephrotoxicity: a role of aldosterone. Am J Physiol 2007; 293: F2-F9.

31) Disel U, Paydas S, Dogan A, Gulfiliz G, Yavuz S. Effect of colchicine on cyclosporine nephrotoxicity, reduction of TGF-beta overexpression, apoptosis, and oxidative damage: an experimental animal study. Transplant Proc 2004; 36: 1372-1376.

32) Vítko S, Viklický O. Cyclosporine renal dysfunction. Transplant Proc 2004; 36: S243-S247.

33) Budavari S ed. The Merck Index. Merck & Co, Inc, Rahway NJ. 1996; pp. 108-109.

34) Chainya GB, Mannaa SK, Chaturvedi MM, Aggarwal BB. Anethole blocks both early and late cellular responses transduced by tumor necrosis factor: effect on NF-κB, AP-1, JNK, MAPKK and apoptosis. Oncogene 2000; 19: 2943-2950.

35) Soriani M, Rice-Evans C, Tyrrell RM. Modulation of the UVA activation of haem oxygenase, collagenase and cyclooxygenase gene expression by epigallocatechin in human skin cells. FEBS Lett 1998; 439: 253-257.

36) Wadsworth TL, Koop DR. Effects of the wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages. Biochem Pharmacol 1999; 57: 941-949.

37) Jung M, Triebel S, Anke T, Richling E, Erkel G. Influence of apple polyphenols on inflammatory gene expression. Mol Nutr Food Res 2009; 53: 1263-1280.

38) Xie XS, Liu HC, Yang M, Zuo C, Deng Y, Fan JM. Ginsenoside Rb1, a panoxadiol saponin against oxidative damage and renal interstitial fibrosis in rats with unilateral ureteral obstruction. Chin J Integr Med 2009; 15: 133-140.