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Research article

Nephroprotective potential of whey protein concentrate and fennel seed extract against tienilic acid -induced renal dysfunctions in albino rats

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Abstract

The objective of this study was to evaluate the nephroprotective potential of whey protein concentrate (WPC) and methanolic fennel seed extract (FSE) against tienilic acid (a hypotensive drug) -induced renal dysfunctions in albino rats. Sixty rats were divided into six equal groups and treated orally for six weeks as follows: control group; FSE alone (200 mg/ kg/day); WPC alone (0.5 g/kg/day); TA alone (1g/kg/twice a week); TA (1g/kg/twice a week) plus FSE (200 mg/kg/day). TA (1g/kg/twice a week) plus WPC (0.5g/kg/day). TA administration significantly increased serum levels of creatinine, urea and potassium while decreased significantly sodium and corticosterone levels. On the other hand, Na+/ K+-ATPase activity and total antioxidant capacity content in kidney tissue were diminished concomitant with a significant rise in the levels of kidney lipid peroxidation and nitric oxide. Furthermore, serum tumor necrosis factor-α and interleukin-6 levels were significantly elevated while serum calcium was unchanged. The treatment with either WPC or FSE to TA–treated animals significantly protected the kidney tissue against the injurious effects of tienilic acid. They effectively ameliorated most of the changes induced by TA towards the normal values of the controls. It could be concluded that the renoprotective effect of these agents might be through the prevention or scavenging of free radicals. This may be attributed to the high content of antioxidant compounds in WPC and fennel extract.

Introduction

The kidney represents the major control system maintaining the body homeostasis [1]; it is considered as the major organ for filtration, re-absorption and also excretion of drugs or drug metabolites [2]. As a consequence, the kidney is vulnerable to toxic revilement, by various drugs or xenobiotics, and thus nephrotoxicity is one of the major concerns in preclinical safety evaluation. Despite the morphological complexity of the kidney, the renal tubular epithelial cells emerge as one of the most sensitive components in the kidney and are thus highly susceptible to damage [2].

Tienilic acid is a diuretic with hypotensive and marked hypouricaemic properties [3]. Therefore, it has been proposed as the drug of first choice in patients with hypertension who already have gout [4]. The clinical use of tienilic acid is, however, associated with particular side effects such as hepatotoxicity [5-7], and autoimmune diseases [8,9].

Fennel (*Foeniculum vulgare* M, *Apiaceae*) is a well-known mediterranean aromatic small, hardy perennial herb widely used as food and with an established role as herbal remedy [10]. Both infusions and essential oils obtained from the fruits and the aerial parts of the plant are included in the herbalist armamentarium for their dysmenorrhea [11], relaxant [12], analgesic and anti-inflammatory properties [13]. The essential oil showed also antioxidant [13], antimicrobial [14] and immuno-protective activity [15]. The dried, aromatic fruits are widely employed in culinary preparations for flavouring bread and pastry, in candies and in alcoholic liqueurs of French type, as well as in cosmetic and medicinal preparations [16].

Whey proteins, a byproduct of cheese manufacture [17]. It recognized as a valuable food ingredient with important nutritional and functional properties is gaining acceptance

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as functional food ingredient. Whey proteins have a high content of sulfur-containing amino acids, which support antioxidant functions [18] owing to the abundance of cysteine or the presence of glutamyl cysteine which facilitate glutathione (GSH) synthesis [19-21]. Feeding whey proteins would prevent aflatoxins-induced liver damage [22]. It would protect also against CCl_4-induced erythrocyte damage [23] and against tienilic acid -induced liver toxicity in rats [24].

Therefore, the objective of this study was to evaluate the renoprotective batteries of two antioxidant and safe agents derived from different natural sources, WPC as animal product and FSE as plant product, against kidney damage induced by tienilic acid in rats as an experimental model for nephrotoxicity.

Experimental

Materials

Fennel (*Foeniculum vulgare*) seeds were obtained from Abd El-Rahman Harraz (Bab El-Khalk zone, Cairo, Egypt). The herb was identified in the Botany Department, National Research Centre. Tienilic acid: (purity 99.7%) was purchased from DAIICHI SANKYO Co., Ltd., Tokyo, Japan. It was suspended in 1% methylcellulose (from Wako Pure Chemical Industries, Osaka, Japan). Whey proteins concentrate (WPC) powder contains 80% proteins was obtained from Davisco Foods International, Inc., Eden Prairie, Minnesota, USA. The WPC was prepared according the method described by Kennedy *et al.,* [25] by mixing with water and stirring with a magnetic stirrer and left in refrigerator overnight to fully hydrate. All other chemicals were of the highest analytical grade available.

Herb extraction

Alcoholic extract was prepared from fennel seeds according to the procedure described by Anwar *et al.*, [26]. Ground dry sample (100 g) was extracted with 1000 ml 80% methanol (80:20, methanol: water, v/v) using an orbital shaker for 8 hours at room temperature. The extract was separated from solids by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 45°C, using a rotary vacuum evaporator and stored at –4°C until used for further analyses. This procedure resulted in an average yield of 11.8 % (w/w) of methanolic extract based on the dry weight.

Experimental design

Adult male Sprague–Dawley (SD) rats weighting 130-150g were obtained from animal house colony, Giza, Egypt. One week of acclamation before the administration, the animals were housed in wire-mesh cages in an air-conditioned room and allowed free access to tap water and commercial rodent chow purchased from Meladco Feed Co (Aubor City, Cairo, Egypt) at the Animal House Lab, National Research Centre, Dokki, Cairo, Egypt.

After one week of acclimation, animals were randomly arranged in six groups (10 rats each) and treated for six weeks as follows: group (1) animals given physiological saline and served as control group, group (2) animals treated orally with WPC (0.5 g/kg/day), group (3) animals treated orally with fennel seed extract (FSE) (0.2g/kg/day) [13], group (4) animals treated orally with tienilic acid (1g/kg twice a week) [6] dissolved in 1% methylcellulose solution, group (5) animals treated orally with tienilic acid (1g/kg twice a week) combined with WPC (0.5 g/kg/day) and group (6) animals treated orally with tienilic acid (1g/kg twice a week) combined with FSE (0.2 g/kg/day).

Blood and tissue sampling

At the end of the treatment period, blood samples were collected from the retro-orbital venous plexus from each animal under diethyl ether anesthesia. Blood samples were left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated at once by micro pipette, divided into aliquots and stored at -70°C, until analysis.

After blood collection, all animals were rapidly sacrificed and the left kidney of each animal was dissected out, washed with saline, dried, rolled in a piece of aluminum foil and stored at -70°C until homogenization for tissue biochemical determinations. Samples of the kidneys were weighed (approximately 0.05–0.1 g) and homogenized in phosphate buffer (pH 7.4) to give 20 % (w/v) homogenate and centrifuged at 5000 rpm for 20 minutes. The clear supernatants were drown out, divided into aliquots and stored at -70°C till the determination of the requested biochemical parameter.

Biochemical determinations

An enzymatic procedure was used to determine serum urea level using a kit obtained from Biodiagnostic Co. (Egypt). Serum creatinine concentration was kinetically evaluated using a kit obtained from Biodiagnostic Co. (Egypt). Serum sodium and potassium levels were evaluated using kits purchased from DiaSys GmbH, Germany using MEDICA Easylyte Na/K ANALYZER (USA). Total calcium in serum was estimated by colorimetric method using a kit purchased from Randox Laboratories LTD Co., UK. Serum corticosterone level was determined using ELISA reagent kit purchased from Immunospect, Canoga Park, USA. Renal total antioxidant capacity (TAC) and nitric oxide (NO) levels of the homogenates were determined using the reagent kits produced by Biodiagnostic, Dokki, Giza, Egypt. While kidney level of lipid peroxidation end product,

malondialdehyde (MDA) and Na^+/K^+-ATPase activity were determined according to the modified chemical methods of Ruiz-Larrea [27] and Tsakiris [28] respectively. Serum tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels were determined using enzyme-linked immuno-sorbent assay (ELISA) using enzyme immunoassay kits for the quantitative determination of TNF-α and IL6 purchased from Ray Biotech Co., USA.

Statistical Analysis

The obtained data were subjected to one way analysis of variance (ANOVA). The analysis was performed using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. Tukey test was used to evaluate the significance between the individual groups at $p \leq 0.05$ [29].

Results and Discussion

Results

Figure (1) shows the effect of tienilic acid administration individually or in combination with fennel seed methanolic extract (FSE) or whey protein concentrate (WPC) on the specific biomarkers of kidney function. Administration of tienilic acid significantly increased serum urea and creatinine concentrations when compared with those of control. The treatment with WPC or FSE alone resulted in no significant effects on these parameters as compared with the normal rats indicating safety of both agents. Administration of WPC or FSE in combination with tienilic acid showed significant reduction in serum urea and creatinine levels compared with tienilic acid-treated group, although their levels still higher than the control values.

Serum potassium, sodium, calcium and corticosterone levels in the different studied groups are depicted in figure (2). Tienilic acid administration significantly decreased sodium and corticosterone concentrations while potassium concentration was significantly increased as compared to those of the control group. Oral administration of WPC in combination with tienilic acid produced significant increase in corticosterone level while it caused insignificant changes in sodium and potassium concentrations as compared to those in the tienilic acid -treated group. On the other hand, administration of FSE in combination with tienilic acid resulted in significant increase in sodium and corticosterone levels but not affect potassium level when compared to those in the tienilic acid -treated group. Figure (2) shows also non significant differences in serum calcium among the different studied groups.

Data in figure (3) show the effects of different treatments on oxidative stress markers and Na^+/K^+-ATPase activity of the animals. It is clearly indicated that tienilic acid increased significantly the levels of renal MDA and NO while it decreased significantly the values of renal TAC level and Na^+/K^+-ATPase activity compared to control group. Treatment with WPC or FSE alone had no significant effect on renal MDA, NO, TAC or Na^+/K^+-ATPase as compared to those in the normal rats. The administration of WPC or FSE succeeded in ameliorating the tienilic acid-induced significant changes in the mentioned parameters and reversed their values toward the values of the controls.

Figure (4) illustrates the effect of different treatments on serum inflammatory markers. Serum TNF- α and IL-6 were significantly increased in the group received tienilic acid alone compared to the controls. However, the levels of both cytokines in animals that treated with WPC or FSE alone were comparable to the control group. On the other hand, animals received tienilic acid and treated with WPC or FSE showed a significant improvement in TNFα level but insignificantly affect IL-6 level.

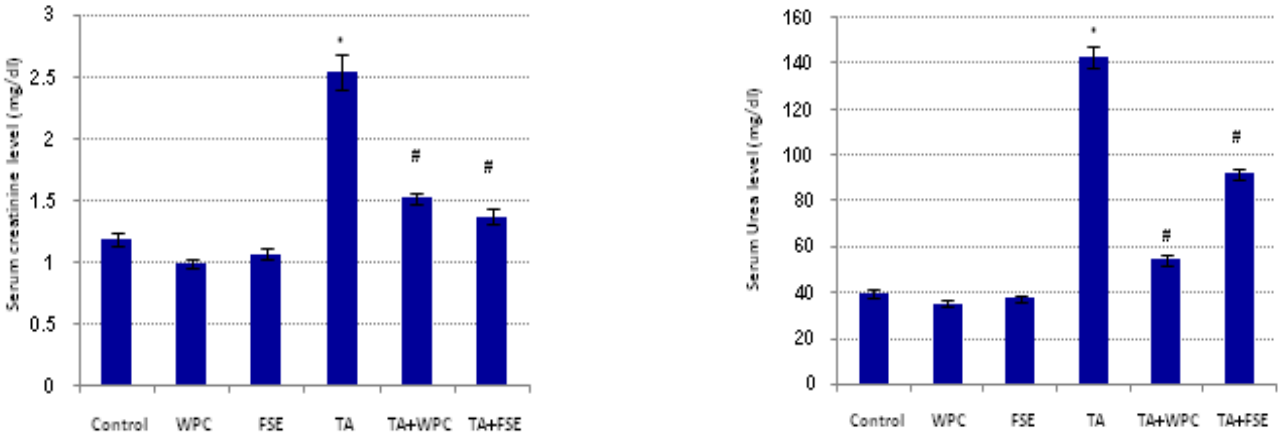


Figure 1. Serum creatinine and urea levels in rats treated with whey protein concentrate (WPC), fennel seeds methanolic extract (FSE), tienilic acid (TA), TA+WPC and TA+FSE. Data represent the mean ± standard error (n = 10). *p ≤ 0.05 compared to normal control rats; # p ≤ 0.05 compared to TA-treated rats.

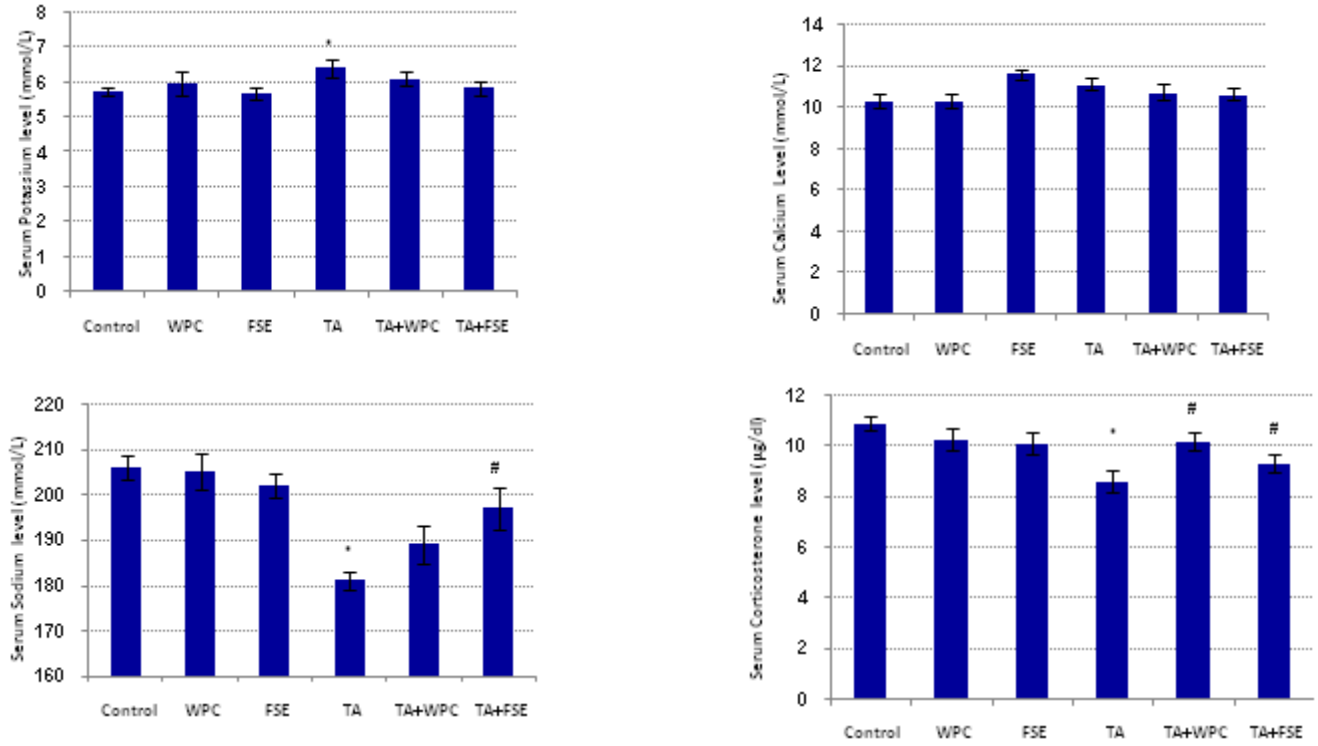


Figure 2. Serum potassium; sodium; calcium and corticosterone levels in rats treated with whey protein concentrate (WPC), fennel seeds methanolic extract (FSE), tienilic acid (TA), TA+WPC, and TA+FSE. Data represent the mean ± standard error (n = 10). * $p \leq 0.05$ compared to normal control rats; # $p \leq 0.05$ compared to TA -treated rats.

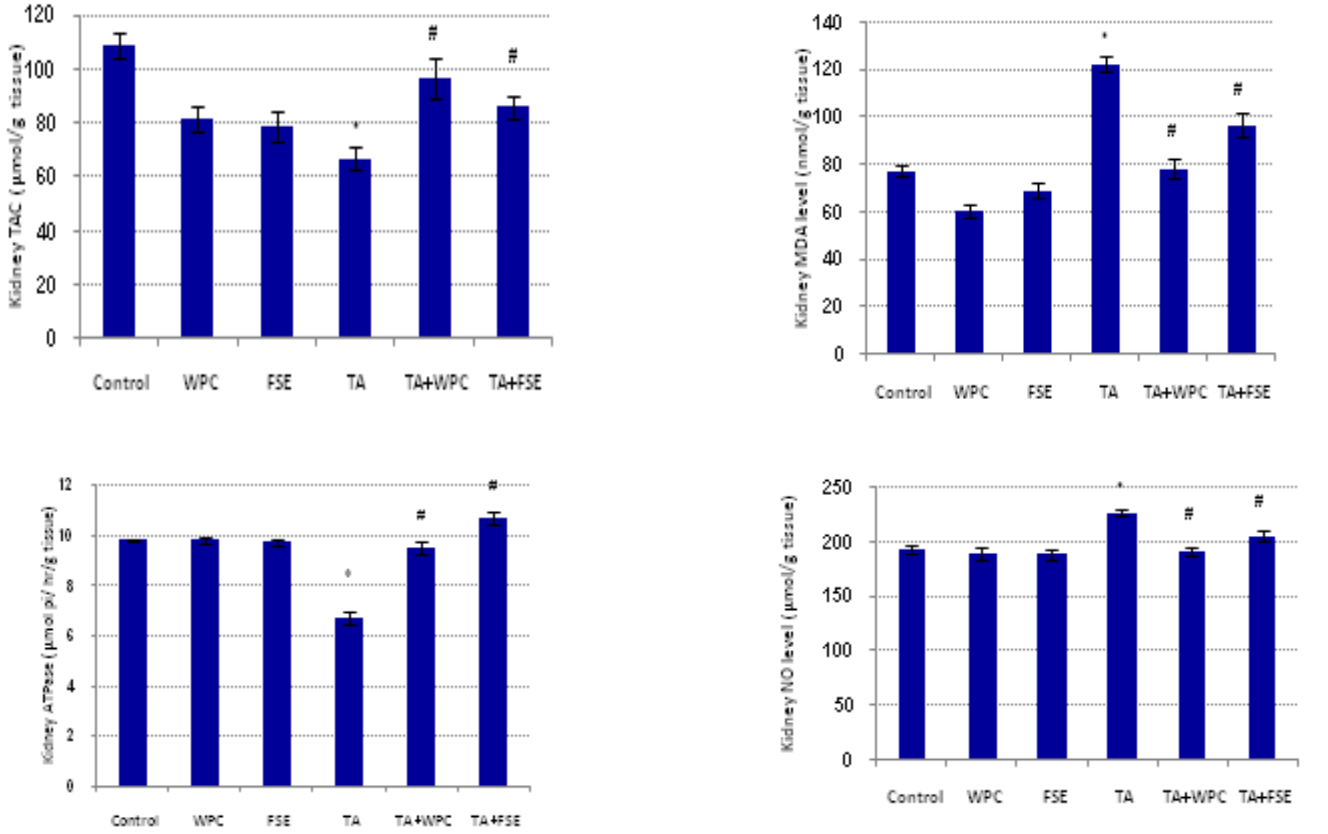


Figure 3. Kidney total antioxidant capacity (TAC); kidney Na^+/ K^+-ATPase activity (ATPase); kidney malondialdehyde (MDA) content; and serum nitric oxide level (NO) in rats treated with whey protein concentrate (WPC), fennel seeds methanolic standard error (n = 10). * $p \leq 0.05$ compared to normal control rats; # $p \leq 0.05$ compared to TA -treated rats.

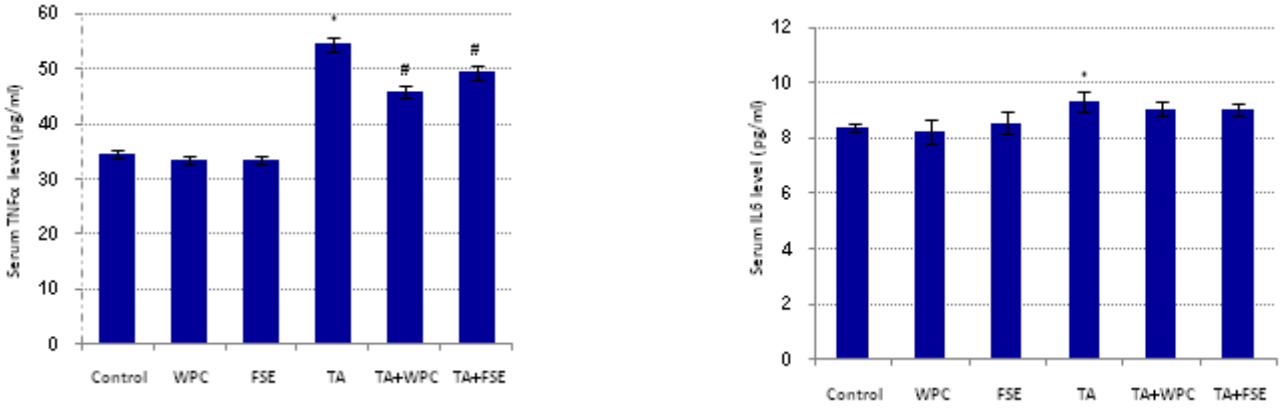


Figure 4. Serum tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels in rats treated with whey protein concentrate (WPC), fennel seeds methanolic extract (FSE), tienilic acid (TA), TA+WPC and TA+FSE. Data represent the mean ± standard error (*n* = 10). * $p \leq 0.05$ compared to normal control rats; # $p \leq 0.05$ compared to TA -treated rats.

Discussion

In the present study, we evaluated the protective effects of fennel methanolic extract (FSE) and whey protein concentrate (WPC) against the nephrotoxicity induced by tienilic acid administration in rats. In our study, treatment with tienilic acid resulted in a significant increase in urea and creatinine levels. These results may indicate impairment in kidney function. The measurements of serum urea [30] and creatinine [31] levels are considered to be good indicators for glomerular filtration rate. It was suggested that the reduction in glomerular filtration rate with an increase in serum blood urea nitrogen and creatinine levels indicates induction of acute renal failure [32]. A significant increase in plasma creatinine level was observed in patients with secondary hyperuricemia received tienilic acid [33]. Others found that tienilic acid treatments does not alter creatinine level [34]. This discrepancy is returned to the variation in the duration and dose of tienilic acid. Several reports linked renal failure in cases on tienilic acid treatments with urate deposition in the kidney [35-37]. Inadequate fluid intake and high starting doses of tienilic acid appear to be common to these cases [38].

The present data demonstrate significant decrease in plasma sodium and corticosterone levels concomitant with an increase in potassium level by tienilic acid treatment as compared to untreated control. Sodium (Na+) and potassium (K+) transport through the distal tubules is regulated by the action of adrenocortical hormones and aldosterone which promotes the increase in Na+ reabsorption in exchange for K+ [39]. Corticosterone, a mineralocorticoid, is one of the adrenocortical steroid hormones that regulate salt homeostasis (sodium conservation and potassium loss) and extracellular fluid volume [40, 41]. Mineralocorticoid deficiency leads to dehydration with hypotension, hyponatremia, and hypercalemia [42].

Tienilic acid may be suppress production of renin by the kidney and consequently decreases the plasma corticosterone level which in turn decreases the reabsorption of Na+ with concomitant decrease of K+ and H+ excretion. Therefore, plasma Na+ level was decreased while that of K+ was increased. In healthy kidney, receptors located in the juxtaglomerular cells when affected by decreased plasma Na+, they stimulate the production of renin enzyme. Renin is released into the blood, where it hydrolyzes its substrate angiotensinogen that is synthesized in the liver, to produce angiotensin I which is rapidly converted to angiotensin II by angiotensin- converting enzyme. Angiotensin II stimulate adrenal gland to produce mineralocorticoid hormones to maintain electrical neutrality [43].

Alternatively, It was reported by McLeod [44] that the antihypertensive nonselective β-adrenergic-receptor antagonists (β-blockers) are more effective than β-blockers in suppressing plasma renin activity when given in low doses and that they may thus be more appropriate for the treatment of hypertension in patients with elevated plasma renin activity.

The nephrotoxicity induced by tienilic acid may be a consequence of the increase in oxidative stresses. Oxidative stress *in vivo* can result from a reduction in endogenous antioxidant, burst formation of reactive oxygen species (ROS), or other imbalances between antioxidants and ROS. [45].

Tienilic acid is metabolized in the hepatic cells, where the thiophene ring of tienilic acid is oxidized by cytochrome P450 (CYP) 2C9 in humans, and similarly by CYP2C11 in rats [46,5,47] to form the labile electrophilic reactive intermediates, S-oxide and arene oxide, which are converted non-enzymatically to the major metabolite, 5-hydroxy tienilic acid (5-OHTA) [48]. 5-OHTA scavenged by intracellular reduced glutathione (GSH), or covalently

bound to macromolecules including metabolizing enzyme [49,50].

The covalently bound complex causes immune-mediated mechanisms that leads to toxicity, this asserted by the presence of the anti-liver and -kidney microsomal type 2 autoantibodies that specifically recognize CYP450 2C9 in humans [49] and the corresponding isoform 2C11 in rats [8].

In the present study, rats received tienilic acid showed a significant increase in the levels of renal malondialdehyde (MDA) and nitric oxide (NO) accompanied by a significant decrease in total antioxidant capacity (TAC) value. These results indicate the evolution of a state of oxidative stresses.

Nitric oxide serves beneficial roles as a messenger and host defense molecule but excessive NO production can be cytotoxic. The overproduction of NO leads to its reaction with reactive oxygen and form the most potent injurious peroxynitrite molecule [51]. Excessive NO production contribute to the pathogenesis of a number of renal diseases characterized by inflammation and injury such as tubulointestinal renal failure [52], glomerulo-nehritis [53] and postscemic renal failure [54].

The oxidative stress mainly results from endogenous antioxidant depletion due to the conjugation of GSH with tienilic acid metabolites. It was reported that GSH depletion leads to alteration of redox state in kidney and consequently leads to an increase in generation of superoxide and other oxygen radicals [55] that attach the lipids of cell membrane causing lipid peroxidation and increase MDA production.

TAC includes enzymes such as SOD, catalase and GPX and non-enzymatic antioxidants such as GSH and trace elements. TAC may provide more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids [56]. The decrease in TAC and the increase in MDA reported herein in tienilic acid -treated rats leading to an indirect increase in oxidative DNA damage and in turn cell damage.

The current study revealed also a significant reduction in Na^+/ K^+-ATPase activity in tienilic acid - treated rats. Na^+/ K^+-ATPase is present in the plasma membranes of the cells and organelles. ATP degradation is an essential process for survival, and ATP is catalyzed by Na^+/ K^+-ATPase [57]. It concerned with the active transport of sodium-potassium across the cell membrane and responsible for a large part of the energy consumption constituting the cellular metabolic rate [58]. Moreover, it is highly dependent on lipids, therefore membrane lipid peroxidation affects the activity of this enzyme [59]; hence the oxidative stress induced by tienilic acid leads to lipid peroxidation that disturbs the lipid structure of membranes causing loss of Na+/ K+-ATPase activity.

Our study showed that tienilic acid increased the blood levels of IL-6 and TNF-α. IL-6 and TNF-α are proinflammatory cytokines involved in a variety of inflammatory responses, including differentiation, maturation and activation of inflammatory cells such as macrophages, neutrophils, T cells and natural killer cells [60]. The covalent binding of electrophilic metabolites of tienilic acid with proteins induces their structural modification, which may be recognized as a foreign antigen, leading to immune cells stimulation [61] and autoantibodies production. It is reported by Ramseyer and Garvin [62] that TNF-α alters renal hemodynamics and nephron transport, affecting both activity and expression of transporters. It also mediates organ damage by stimulating immune cell infiltration and cell death.

There is good evidence indicating that uraemia in general is associated with enhanced oxidative stress [63]. Accordingly, the impairment in kidney function reported in the present work in response to tienilic acid intake could be mediated by oxidative stress. Therefore, natural antioxidants have been proposed as therapeutic agents to protect against kidney damage.

Our data demonstrated that WPC and FSE are considered as potent antioxidant agents since they could produce marked decrease in MDA and NO formation that increased by the tienilic acid towards the normal values. We observed also that the lowered values of TAC and ATPase caused by tienilic acid tratment were effectively countered by WPC and FSE. Additionally, WPC and FSE could also ameliorate serum corticosterone and sodium but not affect calcium or potassium. These positive alterations were more pronounced in the animals received WPC.

WPC and FSE may increase the endogenous antioxidant capacity of the kidney to overcome oxidative stress induced by tienilic acid; this in turn improves kidney integrity and function and consequently enhance renal excretory function of urea and creatinine as well as enhances the kidney to maintain electrical neutrality and improve body homeostasis. In previous studies, it was found that WPC [22] and FSE [24] have free radical scavenging activity and in turn prevents the attack and exhaustion of the endogenous antioxidants. WPC and FSE contain a number of bioactive compounds which are generally believed to be the active constituents responsible for their antioxidant activity. In previous studies, we determined the major components responsible for the antioxidant activity of the tested materials that included thiol (SH) groups which was expressed as cysteine equivalents in WPC [22] and total phenolic compounds which was expressed as catechin equivalents in FSE [24].

The antioxidant activity of WPC may attributed to its higher content of cysteine and glutamate residues which are suggested that their intake contribute to increase the level of free cysteine, and consequent production of GSH

[64,65,21]. GSH, a tripeptide product synthesized from cysteine, glutamate, and glycine, is a low-molecular-weight thiol reductant present in most cells [66]. GSH was reported to be an antioxidant and anticarcinogenic, and thereby improving protection against oxidant-induced cell damage [67].

Whey protein contains also alpha-lactalbumin, lactoferrin, glycomacropeptide, beta-lactoglobulin and immuno-globulins which demonstrate immune-regulating properties [68]. The present data revealed that WPC administration counteracted the levels of blood IL6 and TNF-α that increased by tienilic acid. The study of Badr *et al.* [69] revealed that WPC could regulate levels of TNF, IL-1α, IL-1β and IL-10, thus decreasing inflammation. There is evidence suggesting that the development of diseases is associated with spontaneous increases in proinflammatory cytokines [70]; thus, the ability of WPC in decreasing these cytokines improves immune response and decreases the risk of the associated diseases.

The possible protective effects reported in this study are generally associated with the antioxidant activity of the polyphenolics. Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens [71]. Epidemiological studies strongly suggest that long term consumption of diets rich in plant polyphenolic compounds may help to prevent oxidative damage such as lipid peroxidation which is associated with cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [72,73].

The methanolic extract of fennel seed was reported to increase the plasma superoxide dismutase and catalase activities and decreases lipid peroxidation in mice [74] and rats [13] models. The extract was reported to have also remarkable anti-inflammatory [75,13] and also anticancer properties [74].

Conclusion

In conclusion, the increase in the endogenous antioxidants and the reduction of proinflammatory cytokines and lipid peroxidation by WPC and FSE may result in reducing the deleterious effects due to the accumulation of free radicals in kidney tissue. The renoprotective effect of these agents might be through the prevention or scavenging of free radicals. This may be attributed to the high content of antioxidant compounds in WPC and fennel extract.

References

1. Ajith TA, Usha S and Nivitha V. Ascorbic acid and a-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice a comparative study. Clin. Chim. Acta 2007; 375: 82–86.
2. Jiang Y, Gerhold DL, Holder DJ, Figueroa DJ, Bailey WJ, Guan P, Skopek TR, Sistare FD and Sina JF. Diagnosis of drug-induced renal tubular toxicity using global gene expression profiles. J. Transl. Med 2007; 5:47. doi:10.1186/1479-5876-5-47
3. Lemieux G, Beauchemin M, Gougoux A and Vinay P. Treatment of arterial hypertension with tienilic acid, a new diuretic with uricosuric properties. Can. Med. Assoc. J 1978; 118: 1074-80.
4. Roberts CJC, Marshall AJ, Heaton S and Barritt DW. Comparison of natriuretic uricosuric and antihypertensive properties of tienilic acid, bedrofluazide and spironolactone. Br. Med. J 1979; 1(6158):224-6.
5. Dansette PM, Amar C,Smith C, Pons C and Mansuy D. Oxidative activation of the thiophene ring by hepatic enzymes. Hydroxylation and formation of electrophilic metabolites during metabolism of tienilic acid and its isomer by rat liver microsomes. Biochem. Pharmacol 1990; 39: 911–918.
6. Nishiya T, Mori K, Hattori C, Kai K, Kataoka, H, Masubuchi N, Jindo T and Manabe S. The crucial protective role of glutathione against tienilic acid hepatotoxicity in rats. Toxicology and Applied Pharmacology 2008; 232: 280-291.
7. Nishiya T, Kato M, Suzuki T, Maru C, Kataoka H, Hattori C, Mori K, Jindo T, Tanaka Y and Manabe S. Involvement of cytochrome P450-mediated metabolism in tienilic acid hepatotoxicity in rats. Toxicology Letters 2008; 183: 81-89.
8. Bonierbale E, Valadon P, Pons C, Desfosses B, Dansette P and Mansuy D. Opposite behaviors of reactive metabolites of tienilic acid and its isomer toward liver proteins: use of specific anti-tienilic acid protein adducts antibodies and the possible relationship with different hepatotoxic effects of the two compounds. Chemical Research in Toxicology 1999; 12: 286-296.
9. Methogo RM, Parick M and Dansette KK. Identification of liver protein targets modified by tienilic acid metabolites using a two-dimensional Western blot-mass spectrometry approach. International Journal of Mass Spectrometry 2007; 268: 284–295.
10. Badgujar SB, Patel VV and Bandivdekar AH: Foeniculum vulgare Mill: A Review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Research International 2014 http://dx.doi.org/10.1155/2014/842674
11. Modaress MV and Asadipour M. Comparison of the effectiveness of fennel and mefenamic acid on pain intensity in dysmenorrhoea. East Mediterranean Health J 2006; 12: 423–427.
12. Boskabady MH, Khatami A and Nazari A. Possible mechanism(s) for relaxant effects of Foeniculum vulgare on guinea pig tracheal chains. Pharmazie 2004; 59:561–564.
13. Choi E and Hwang J. Antiinflammatory, analgesic and antioxidant activities of the fruit of Foeniculum vulgare. Fitoterapia 2004; 75: 557-565.
14. Ruberto G, Baratta MT, Deans SG and Dorman HJD. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta Med 2000; 66: 687–693.
15. Ibrahim NA, Mannaa FA, Ibrahim SS, Abdel-Wahhab KG and Mohamed SG.Protective potential of chamomile flowers and fennel seeds extracts against Hemo-.and immune-toxicity of azathioprine in male Sprague-Dawley rats. Journal of Environmental Immunology and Toxicology 2015; DOI: 10.7178/jeit.29.
16. Ozbek H, Ugras S, Dulgerc H, Bayram I, Tuncer I, Ozturke G and Ozturk A. Hepatoprotective effect of Foeniculum vulgare essential oil. Fitoterapia 2003; 74: 317–319.
17. Morr CV and Ha EYW. Whey protein concentrates and isolates: processing and functional properties. Critical Reviews in Food Science and Nutrition 1993; 33(6):431–476.
18. Lahl WJ and Windstaff DA. Spices and seasonings: hydrolysed proteins. In Proceedings of the 6th SIFST symposium on food ingredients – applications, status and safety. Singapore Institute of Food Science and Technology 1989; 51–65.
19. Bounous G and Gold P. The biological activity of undenatured dietary whey proteins: role of glutathione. Clin. Invest. Med 1991; 14: 296-309.
20. Lands LC, Grey VL and Smountas AA. Effect of supplementation with a cysteine donor on muscular performance. J. Appl. Physiol 1999; 87: 1381-1385.
21. Micke P, Beeh KM, Schlaak JF and Buhl R. Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients. Eur. J. Clin. Invest 2001; 31: 171–178.
22. El-Kady A, Sharaf HA, Gad AS, Mannaa FA, Hassan NS and Abdel-Wahhab MA. Whey Protein Concentrate and Ginseng Extract Exhibit Antioxidant Properties in vitro and Reduce Hepatotoxicity and Oxidative Stress of Aflatoxin in vivo. New York Science Journal 2010; 3(11): 37-51.

23. Abdel-Wahhab KG, Mannaa FA and Abdel-Wahhab MA. The protective effects of whey protein and spirulina against CCl4-induced erythrocyte damage in rats. J. of Appl. Sci. Res 2013; 9: 2063-2071.
24. Abdel-Wahhab KG, Fawzi H and Mannaa FA. Paraoxonase-1 (PON1) inhibition by tienilic acid produces hepatic injury: Antioxidant protection by fennel extract and whey protein concentrate. Pathophysiology 2016; 23:19-25.
25. Kennedy RS, Konok GP, Bounous G, Baruchel S and Lee TD. The use of whey protein concentrates in the treatment of patients with metastatic carcinoma: a phase I-II clinical trial study. Anticancer Res.1995; 15: 2643-2649.
26. Anwar F, Ali M, Hussain A and Shahid M. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare Mill.) seeds from Pakistan. Flavour and Fragrance Journal 2009; 24:170-176.
27. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M and De Groot H. Antioxidant effects of estradiol and 2- hydroxyestradiol on iron induced lipid peroxidation of rat liver microsome. Steriod 1994; 59: 383- 388.
28. Tsakiris S, Schulpis KH, Marinous K and Behrakis P. Protective effect of L-cysteine and glutathione on the modulated sukling rat brain Na+/K+-ATPase and Mg2+-ATPase activities induced by the in vitro gulactosaemia. Pharmacological Research 2004; 49:475-479.
29. Steel R and Torrie G. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, 1980; New York, USA.
30. Kassirer JP. Clinical evaluation of kidney function: Glomerular function. N. Engl. J. Med 1971; 285: 385-389.
31. Perrone RD, Madias NE and Levey AS. Serum creatinine as an index of renal function. New insights into old concepts. Clin. Chem 1992; 38: 1933-1953.
32. Atessahin A, Ceribasi AO, Yuce A, Bulmus O and Cikim G. Role of ellagic acid against cisplatin-induced nephrotoxicity and oxidative stress in rats. Basic Clin. Pharmacol. Toxicol 2007; 100:121–126.
33. Torrenté A: Tienilic acid, hyperuricemia and kidney function. Schweiz Med Wochenschr 1981; 111: 381-385.
34. Eisalo A, Tötterman KJ and Haapanen E. Tienilic acid in patients with impaired renal function. Acta Med Scand Suppl 1982; 668:1103.
35. Bennett WM, Vanzee BE and Hutchings R. Acute renal failure from ticrynafen. New Engl. J. Med 1979; 301: 1179-1180.
36. Mclain DA, Garriga FJ and Kantor OS. Adverse reactions associated with Ticrynafen use. J. Am.med. Ass 1980; 243 (8): 763-764.
37. Gary L, Paddack MD, Richard C, Wahl MD, Richard E, Holman MD, Wagner J, Schorr MD, John W and Lacher MD. Acute Renal Failure Associated With Ticrynafen. JAMA 1980; 243(8):764-765.
38. Andrews DI, Bailey RR, Campbell DG, Dickson DSP, Kiddle GB, Lewis GRJ, Maling TJB, Simpson FO, Turner AS and Waal-Manning HJ. 1983. Multicentre controlled trial of tienilic acid in hyperuricaemic, hypertensive subjects, with intensive monitoring of liver function. Br. J. clin. Pharmac 1983; 15: 513-518.
39. Latner AL. Renal function. In: Clinical biochemistry, seventh edition, W.B. Saunders Company, Philadelphia, 1975:454-546.
40. Biglieri EG and Kater CA. Mineralocorticoids. In: Basic and Clinical Endocrinology.3rd ed. Greenspan, F.S. and Forsham, P.H. Eds. East Norwalk, CT, Appleton and Lange, 1992; 310-325.
41. Ehrlic EN, Friedman AL and Shenker Y. Hormonal regulation of electrolyte and water metabolism. In: Endocrinology. 3rd ed. M. Besser, HG Durger & JL Jameson et al., Eds. Philadelphia, WB Saunders Co. 1995:1685-1710.
42. Loriaux DL and McDoland WJ. Adrenal insufficiency. In: Endocrinology. 3rd ed. M. Besser, HG Durger & JL Jameson et al., Eds. Philadelphia, WB Saunders Co. 1995: 1731-1740.
43. Demers LM and Whitley RJ. Function of the adrenal cortex. In: Burtis C.A., Ashwood, E.R. (eds). Tietz Textbook of Clinical Chemistry. Sawnders W.B. Co, Philadelphia, London. 1999; 1530- 1569.
44. McLeod SM. Evolution of antihypertensive therapy. CMA. J 1979; 120: 517-518.
45. Sun J, Charles S and Elizabeth M. S-Nitrosylation: NO-Related Redox Signaling to Protect Against Oxidative Stress. Antioxid Redox Signal 2006; 8(9-10): 1693–1705.
46. Mansuy D, Dansette PM, Foures C, Jaouen M, Moinet G and Bayer N. Metabolic hydroxylation of the thiophene ring: isolation of 5-hydroxy-tienilic acid as the major urinary metabolite of tienilic acid in man and rat. Biochem. Pharmacol 1984; 33: 1429–1435.
47. Dansette PM, Amar C, Valadon P, Pons C, Beaune PH and Mansuy D. Hydroxylation and formation of electrophilic metabolites of tienilic acid and its isomer by human liver microsomes. Catalysis by a cytochrome P450 IIC different from that responsible for mephenytoin hydroxylation. Biochem. Pharmacol 1991; 41: 553–560.
48. Belghazi M, Jean P, Poli S, Schmitter J, Mansuy D and Dansette P. Use of isotopes and LC–MS-ESITOF for mechanistic studies of tienilic acid metabolic activation. Advances in Experimental Medicine and Biology 2001; 500: 139-144.
49. Lopez-Garcia MP, Dansette PM and Mansuy D. Thiophene derivatives as new mechanism-based inhibitors of cytochromes P-450: inactivation of yeast-expressed human liver cytochrome P-450 2C9 by tienilic acid. Biochemistry 1994; 33: 166-175.
50. Rademacher PM, Woods CM, Huang Q, Szklarz GD and Nelson SD. Differential oxidation of two thiophene-containing regionisomers to reactive metabolites by cytochrome P450 2C9. Chem Res Toxicol 2012; 25: 895–903.
51. Levonen AL, Patel RP, Brookes P, Go YM, Jo H, Parthasarathy S, Anderson PG and Darley-Usmar VM. Mechanisms of cell signaling by nitric oxide and peroxynitrite: from mitochondria to MAP kinases. Antioxid Redox Signal 2001;3(2): 215-29.
52. Gabbai FB, Hammond TC, Thomson SC, Khang S and Kelly CJ. Effect of acute iNOS inhibition on glomerular function in tubuinterstitial nephritis. Kidney Int 2002; 61: 851-854.
53. Narita I, Border WA, Ketteler M and Noble NA. Nitric oxide mediates immunologic injury to kidney mesangium in experimental glomerulonephritis. Laboratory Investigation 1995; 72(1): 17-24.
54. Noiri E, Nakao A, Uchida K, Tsukahara H, Ohno M, Fujita T, Brodsky S and Goligorsky MS. Oxidative and nitrosative stress in acute renal ischemia. Am J Physiol Renal Physiol. 2001; 281(5): 48-57.
55. Santos NA, Catao CS, Martins NM, Curti C, Bianchi ML and Santos AC. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch. Toxicol 2007; 81: 495–504.
56. Gad AS, Khadrawy YA, El-Nekeety AA, Mohamed SR, Hassan NS and Abdel-Wahhab MA. Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. Nutr.2011; 5: 582-589.
57. Wang X, Haiyuan Z, Yilan S, Pei W, Yanru W, Weiqian Z, Jing J, Yan C and Zhigang Z. Nephroprotective effect of astaxanthin against trivalent inorganic arsenic-induced renal injury in wistar rats. Nutrition Research and Practice. Nutr. Res. Pract.2014; 8(1): 46-53.
58. Dixon M, Nimmo J and Prescott L. Experimental paracetamol-induced hepatic necrosis: A histopathological study. Pathology 1971; 103: 225-229.
59. Masuda Y, Yano I, Sumida S and Murano T. Studies on the function of cell membrane, 10th 2Report: Effects of CCl4 on the marker enzyme activities and fine structures of rat liver plasma membranes and microsomes in vitro. Journal of Pharmacology 1975; 25: 151-160.
60. Yousef MI and Hussien HM. Cisplatin-induced renal toxicity via tumor necrosis factor-α, interleukin 6, tumor suppressor P53, DNA damage, xanthine oxidase, histological changes, oxidative stress and nitric oxide in rats: Protective effect of ginseng. Food and Chemical Toxicology 2015; 78: 17–25.
61. Obermayer-Straub P, Strassburg CP and Manns MP. Autoimmune hepatitis. Journal of Hepatology 2000; 32: 181-197.
62. Ramseyer VD and Garvin JL. Tumor necrosis factor-α: regulation of renal function and blood pressure. Am. J. Physiol. Renal Physiol 2013; 304: F1231–F1242.
63. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P and Descamps-Latscha B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int.1996; 49(5): 1304-1313.
64. Wong CW, Seow HF, Husband AJ, Regester GO and Watson DL. Effects of purified bovine whey factors on cellular immune functions in ruminants. Veterinary Immunology and Immunopathology 1997; 56: 85–96.
65. Gill HS, Doull F, Rutherfurd KJ and Cross ML. Immunomodulatory properties of bovine milk. The British Journal of Nutrition 2000; 84: S111–S1117.
66. Tseng YM, Shu-Kai L, Jen-Kuei H, Ing-Jun C, Jang-Hwa L, Szu-Hsien W and Li-Yu T. Whey protein concentrate promotes the production of glutathione (GSH) by GSH reductase in the PC12 cell line after acute ethanol exposure. Food and Chemical Toxicology 2006; 44: 574–578.
67. Bounous G, Gervais F, Amer V, Batist G and Gold P. The influence of dietary whey protein on tissue glutathione and disease of aging. Clinical and Investigative Medicine 1989; 12: 343–349.
68. Horton B. The whey processing industry, Into the 21st Century, In: Proceedings of the Second International whey conference, Chicago, USA, International Dairy Federation. 1997; 12-25.
69. Badr G, Ebaid H, Mohany M and Abuelsaad A. Modulation of immune cell proliferation and chemotaxis towards CC chemokine ligand (CCL)-21

and CXC chemokine ligand (CXCL)-12 in undenatured whey protein-treated mice. J. of Nutrit. Biochem 2012; 23: 1640-1646.
70. Holmes CC, Cunningham C, Zotova E, Culliford D and Perry VH. Proinflammatory cytokines, sickness behavior, and Alzheimer disease. Neurology 2011; 77: 212-218.
71. Pandey KB and Rizvi SI. Plant polyphenols as dietary ntioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2009; 2(5): 270-278.
72. Seeram NP, Cichewicz RH, Chandra A and Nair MG. Cyclooxygenase inhibitory and antioxidant compounds from crabapple fruits. J. Agric. Food. Chem 2003; 51: 1948-1951.
73. Joseph JA, Shukitt-Hale B and Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. Am. J. Clin. Nutr 2005; 81: 313-316.
74. Mohamad RH, El-Bastawesy AM, Abdel-Monem MG, Noor AM, Al-Mehdar HA, Sharawy SM and El- Merzabani MM. Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (Foeniculum vulgare). Journal of Medicinal Food 2011; 14: 986-1001.
75. Jahromi B, Tartifizadeh A and Khabnadideh S. Comparison of fennel and mefenamic acid for the treatment of primary dysmenorrheal. International Journal of Gynecology & Obstetrics 2003; 80: 153-157.