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The Role of Senna and Fennel In Ameliorating Cardiovascular Disease In Diabetic Rats

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ABSTRACT

Diabetes alters the biochemical pathways of body and increases reactive oxygen species (ROS) leading to cardiovascular diseases. Compounds showing antioxidant activity could have a protective role against cardiovascular disease in diabetes. The current study aimed to investigate the cardioprotective effects of senna (*Cassia angustifolia*) and/or fennel (*Foeniculum vulgare*) against streptozotocin (STZ) induced diabetes in rats. Rats were divided into five groups: control, diabetic (60 mg·kg^{-1}of STZ), diabetic rats treated with senna (150mg/kg/day), diabetic rats administered with fennel (120mg/kg/day) and diabetic rats administered with a combination of senna and fennel. Diabetic rats demonstrated a substantial increment in the levels of blood glucose, lipid profile, creatine kinase, lactate dehydrogenase, thiobarbituric acid reactive substances, nitric oxide and xanthine oxidase accompanied with a noteworthy decline in reduced glutathione content, vitamin C level and catalase activity in heart tissues with comparison to the control group. Daily oral treatment of senna or fennel aqueous extract for 4 consecutive weeks showed a marked attenuation of oxidative stress in heart tissues. Combination of both senna and fennel extracts exhibited more amelioration than these extracts alone and reversed the adverse effect of diabetes in rats by bringing blood glucose levels and lipid profile near to that of control. The present investigation has demonstrated that treatment with a combination of senna and fennel extracts in STZ induced diabetes in rats show significant antidiabetic activity and pronounced cardioprotective effects.

Keywords: Antioxidant, diabetes, senna, fennel, cardioprotection

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INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing rapidly in developed as well as developing countries (1). According to the latest report of International Diabetes Federation (IDF) (2015), it is estimated that 415 million people are affected by DM worldwide, and that the total number of individuals diagnosed with DM will increase to 642 million by the year 2040 (2). With respect to the diabetic cases, Saudi Arabia is known to have second highest rank in Middle East and seventh in the world as reported by World Health Organization (WHO) (3). Diabetes is a metabolic syndrome that leads to an increase in oxidative stress which seems to be the basic cause of all the diabetic complications such as neuropathy, nephropathy, myocardial injury and retinopathy (4,5). One of the major diabetic complications is the manifestation of cardiovascular disease (CVD) which mostly shows as atherosclerosis and cardiomyopathy. Oxidative stress generated by diabetes leads to endothelial dysfunction of vascular tissues which seems to be the underlying cause of CVD in diabetes (6). Thus regulation of anti-oxidant potential of a system could avert the major complicacies caused by diabetes.

To put a check on this disorder, many anti-diabetic pharmaceutical drugs are available in the market, but a safer alternative to these medicines are herbal drugs which are in high demand nowadays for their safer course of action. A variety of plant derived compounds such as phenols are known to possess strong antioxidant properties and exhibiting cardioprotective effects (7). Senna (*Cassia angustifolia*) and Fennel (*Foeniculum vulgare*) are two such plants belonging to family Leguminosae and Umbelliferae, respectively, whose extracts have shown to possess strong hepatoprotective, anti-inflammatory, antioxidant, hypoglycemic, antimutagenic and anticancer properties (8,9). Due to its antioxidant properties, the phenolic compounds in these plants are associated with the prevention of diseases such as cancer, cardiovascular diseases, and many inflammatory disorders (10). A thorough investigation on the ameliorative potential of these plant extracts in cardiovascular system affected by diabetes could lead to the development of a safer and more effective cardioprotective drug.

Based on the already reported properties of senna and fennel extracts, this study was designed to explore the ameliorative and cardioprotective potential of the aqueous extracts of these plants in streptozotocin induced diabetes in male albino rats.

MATERIALS AND METHOD

Plant material

Senna leaves and seeds of fennel were purchased from the local traditional market in Jeddah,

Saudi Arabia.

Preparation of the herbal extracts

The senna leaves and fennel seeds were ground with the help of a grinder and 100 g of this powder was dissolved by bringing to a boil for 30 min with 400 ml of distilled water. The mixture was filtered and centrifuged at 3500 rpm for 20 min. The solution thus obtained was stored at 4ºC prior to its drying, till usage.

Experimental animals

Male albino rats (n=50) weighing about (150 to 200 g) were obtained from the animal experimental unit of King Fahd Center for Medical Research, King Abdul-Aziz University. The animals were kept in plastic cages and were fed with tap water and rat chow *ad libitum* in a stable environment (temperature 28 ± 2ºC, humidity 60 ± 5%) with 12 hour light and dark cycle. Animal procedures were carried out according to the instruction of the Ethics Committee, King Fahad Medical Research Center which were in compliance with the international guidelines for proper use and care of laboratory animals.

Induction of diabetes

Diabetes was induced by an injection of STZ (60 mg/kg body weight) given intraperitoneally and the animals were considered diabetic after 72 hours of injection. Diabetes was confirmed by blood glucose and serum insulin levels tests.

Experimental Design

A total of 50 animals were divided into five groups, each one having 10 rats. Group 1 was control (receiving 0.1 M citrate buffer (pH 4.5), group 2 was diabetic, group 3 included rats having diabetes treated with senna aqueous extract (150 mg/kg body weight) (11), group 4 comprised of diabetic rats treated with fennel aqueous extract (150 mg/kg body weight) (12) and group 5 had diabetic rats treated with mixture of aqueous extracts from senna (150 mg/kg) and fennel (150 mg/kg). Each aqueous extract was given by oral gavage for a period of 30 days. Blood was collected from the retro-orbital plexus of rats under anesthesia with diethyl ether by heparinized capillary tube at the end of experimental time of 4 weeks. Animals were sacrificed after overnight fasting and the heart of each animal was removed. Each organ was homogenized with 0.1M chilled phosphate buffer (pH 7.4) and centrifugation was done at 10,000g for 15 min. Supernatant thus obtained was used for biochemical estimations.

Biochemical estimations

Blood glucose levels were measured by enzymatic kits according to the protocol of Kunst et al., (13). Serum insulin levels were determined by solid phase enzyme-linked immune-sorbent assay

using Immunospec Insulin Quantitative Test Kit (model E29-88). Total cholesterol, triglycerides, LDL-C and HDL-C concentrations were assessed by using enzymatic colorimetric kits. The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured as described by Bergmeyer et al., (1978); Bergmeyer et al., (1976) respectively (14, 15). Total activity of creatine kinase (CK) and serum lactate dehydrogenase (LDH) was measured according to the methods of Gay et al. and Rosalki, respectively (16,17).

Thiobarbituric acid reactive substances (TBARS) was used to measure lipid peroxidation. Xanthine oxidase (XO) activity was determined using the method of Bergmeyer et al. (18). Nitrite assay was measured as an end product for knowing nitric oxide (NO) concentration according to the method of Miranda *et al*. (19). The activity of CAT was determined using the method of Aebi, (20). The content of reduced glutathione (GSH) was measured by following the protocol of Beutler et al. (21). Ascorbic acid in heart tissue was determined by using commercial kits (CAT NO AS2516).

Statistical analysis:

Analysis of data was done by Statistics Package for Social Sciences (SPSS) version 20. The data was expressed as arithmetic mean and standard deviation of the mean (SD). One-way analysis of variance (ANOVA), least significant difference (LSD) equation for parametric parameters, was used to analyze the differences between groups. A *p*-value less than or equal 0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 represents blood glucose and plasma insulin levels of both control and experimental rats. A significant elevation in blood glucose levels accompanied by lower plasma insulin levels in diabetic rats compared with those of the control rats was observed. Treatment with senna and fennel extracts individually tends to bring these parameters significantly towards the normal value. However, treatment of combination of both senna and fennel extracts showed more pronounced effects than the individual treatments.

Table 1: Anti-diabetic effect of aqueous extract of senna or/ and fennel on glucose and insulin in all groups.

Dependent variable	Control	Diabetes	Dia + senna	Dia + fennel	Dia + Mix (senna+fennel)
Insulin (µU/ml)	9.07±.540	3.47±.559***	5.56±0437*** ###	6.12±.475*** ###	8.025±.463*** ###
Glucose (mg/dl)	4.20±.309	25.1±1.42***	8.04±.537*** ###	7.97±.505*** ###	5.48±.795*** ###

Each value represents the mean of 10 rats ± SD. Significantly different from control value at P<0.05[*], 0.01[**], 0.001[***] Significantly different from the untreated diabetic group at P<0.05[#], 0.01[##], 0.001[###]

The animals injected with STZ showed a significant elevation of serum TC, TG, LDL-C levels which is associated with a significant reduction of serum HDL-C compared to those of control animals. In comparison with diabetic group, the rats that received an aqueous extract of senna and fennel individually showed a significant decrease in the lipid profile, except HDL-C which was significantly higher. The group treated with the mixture of both the plants extract showed a significant amelioration of the enzyme levels (Table 2).

Table 2: Effect of aqueous extract of senna or/ and fennel on serum lipid profile in STZ-diabetic rats.

Dependent variables	Control	Diabetes	Dia +Senna	Dia+ Fennel	Dia+Mix Senna +fennel
TC (mg/dl)	2.57±.760	7.53±1.03***	4.39±.626*** ###	4.54±.562*** ###	3.27±.444* ###
TG (mg/dl)	1.30±.312	4.85±.847***	2.88±.480*** ###	2.87±.374*** ###	1.80±.503* ###
LDL-C (mg/dl)	.287±.095	6.01±.523***	2.22±.493*** ###	2.18±.518*** ###	.817±.168*** ###
HDL-C (mg/dl)	1.31±.097	.418±.078***	.854±.057*** ###	.808±.096*** ###	.975±.175*** ###

Each value represents the mean of 10 rats ± SD. Significantly different from control value at P<0.05*, 0.01**, 0.001*** Significantly different from the untreated diabetic group at P<0.05[#], 0.01[##], 0.001[###].

The activities of serum ALT, AST, CK and LDH was found significantly raised in diabetic group as compared to control as shown in Table 3. On the other hand, 30 days treatment of diabetic rats with aqueous extracts of senna and/or fennel daily exhibited a significant decrease in the all enzyme activities when compared with untreated diabetic animals.

Table 3: Effect of aqueous extract of senna or/ and *fennel* on serum alaninetransaminase (ALT), aspartate transaminase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) activities in diabetic rats.

Dependent variables	Control	Diabetes	Dia +Senna	Dia +Fennel	Dia+Mix senna+fennel
AST (U/ml)	50.60±6.36	109.40±21.42 ***	76.60±2.36*** ###	71.00±4.61 *** ###	63.00±8.69 *** ###
ALT (U/ml)	19.20±2.61	87.10±11.72***	32.60±4.94*** ###	32.00±3.33 ***	26.40±5.79 ***

				###	###
CK (U/l)	172.70±60.66	487.10±60.56 ***	259.70±23.87 *** ###	253.20±20.37 *** ###	229.90±12.83 *** ###
LDH (U/l)	150.30±20.99	301.10±44.19 ***	201.70±8.76 *** ###	208.40±9.00 *** ###	170.00±22.63 ###

Each value represents the mean of 10 rats ± SD. Significantly different from control value at P<0.05*, 0.01**, 0.001*** Significantly different from the untreated diabetic group at P<0.05#, 0.01##, 0.001###

There was a significant elevation of TBARS, NO and XO levels in heart tissues of diabetic rats in comparison to the control group. Treatment with either senna or fennel showed individually a significant protective effect in diabetic rats, which was more pronounced when the mixture of both the plant extracts was administered (Table 4).

Table 4: The Effect of aqueous extract of senna or/ and fennel on cardiac oxidative stress Biomarkers in STZ-induced diabetic rats.

Dependent variable	Control	Diabetes	Dia + senna	Dia + fennel	Dia + Mix Senna + fennel
Nitric Oxide (Heart) (µmol/L)	7.13±.479	15.83±2.56***	9.4140±.937*** ###	9.10±.397*** ###	8.25±1.282* ###
TBRAS (Heart) (nmol/ g wet tissue)	41.10±6.40	97.97±6.55***	57.05±7.55*** ###	56.11±4.83*** ###	47.45±5.38* ###
Xanthine oxidase (Heart) (U/mg)	.0221±.0044	.0404±.0053***	.0254±.0031* ###	.0268±.0050* ###	.0209±.00264###

Each value represents the mean of 10 rats ± SD. Significantly different from control value at P<0.05*, 0.01**, 0.001*** Significantly different from the untreated diabetic group at P<0.05#, 0.01##, 0.001###

Table (5) illustrates the effect of senna and fennel on the antioxidant enzymes. A marked reduction was noted in the level of non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin C and the activity of enzymatic antioxidant (CAT) in the heart of STZ-induced diabetic rats when compared with control rats. Administration of senna and fennel extract individually or in mixture for 30 days to STZ-induced diabetic rats significantly increased vitamin C, GSH levels and the activity of CAT.

Table 5: The Effect of aqueous extract of *senna* or/ and *fennel* on cardiac antioxidant enzymes in STZ-induced diabetic rats.

Dependent variable	Control	Diabetes	Dia + senna	Dia + fennel	Dia + Mix Senna + fennel
VitC(heart) (mg/L)	554.11±83.07	236.14±51.05***	369.85±47.12*** ###	378.80±41.38*** ###	496.51±59.99*** ###
CAT(heart) (U/g)	130.85±15.70	29.87±4.48***	79.79±6.53*** ###	83.38±5.77*** ###	101.09±5.23*** ###
GSH(heart) (U/g)	113.50±9.50	71.66±6.40***	82.59±4.11*** ###	83.78±4.85*** ###	94.15±4.49*** ###

Each value represents the mean of 10 rats ± SD. Significantly different from control value at P<0.05*, 0.01**, 0.001*** Significantly different from the untreated diabetic group at P<0.05#, 0.01##, 0.001###

DISCUSSION

Despite antidiabetics being highly prevalent in market globally, non-toxic drugs having lesser side effects are much preferred than the synthetic pharmaceutics. This has led to a surge in research towards development of herbal medicines that are potentially safer being close to natural products. Therefore, an attempt was made to evaluate the hypoglycemic activity of aqueous extract of senna and fennel in STZ-induced diabetic rats and also study their cardio protective effects.

Diabetes was confirmed by presence of higher levels of glucose and lower insulin in this study. The diabetic group of rats also showed a significant increase in serum TC, TG and LDL-C levels, which might be due to abnormal lipid metabolism, as reported by other studies (22). Diabetic rats receiving extracts of senna and fennel showed a significant decline in blood glucose and a rise in plasma insulin which has been confirmed in other studies as well (23,24). However, a combination of both the extracts proved to be more efficient in reversing these antagonistic effects caused by diabetes. Hypoglycemic effect of senna is reported to be either due to enhancement of insulin secretion or blood glucose transport (25).

In this study, significant elevations in the levels of AST, ALT, CK and LDH activities were noticed in the diabetic group which did not receive any treatment. Similar has been reported in other study as well which might be the cause of myocardial damage induced by diabetes (26,27). From the results obtained, it could also be observed that treatment with senna and/or fennel caused an amelioration in these marker enzymes suggesting a protective effect of these plant extracts against tissue damage which might be due to strong antioxidant properties of senna and

fennel. This finding is reported in other study as well (24). However, a combination of extracts from both the plants showed more ameliorative potential than each extract alone.

Increased lipid peroxidation (TBARS), serum NO levels and the activity of XO in heart tissue indicated presence of oxidative stress in hearts of diabetic rats. Hyperglycemia in particular has been found to promote lipid peroxidation of LDL, which results in the generation of free radicals (28). Increase in the levels of NO leads to further manifestation of oxidative stress (29). Increase in the activity of XO in the heart tissues in diabetic rats in the present study falls in line with some previous studies as well (30).

This study depicted senna and fennel to be efficient cardioprotective agents since both these compounds alone, as well as in combination, showed a strong lipid lowering effect on all components of lipid profile in diabetic rats and also caused an amelioration in the peroxide enzymes of diabetic heart; the ameliorative effects being stronger when both extracts were present in combination. The cardioprotective effect of senna and fennel maybe due to the presence of flavonoids, tannins and sennosides in these plants (31,32). These findings are in agreement with many other studies as well (33,34).

In this study, STZ-induced diabetes showed a decrease in GSH, vitamin C levels and the activity of CAT in heart tissues which is in agreement with other finding (35). The decreased levels of GSH and vitamin C in heart tissues of diabetic rats might be due to their utilization in scavenging the oxygen radicals produced by the increased level of glucose. Furthermore, the decreased level of vitamin C might also be due to the decrease in the levels of GSH, which is necessary for its recycling (36). The result also revealed that oral administration of aqueous extract of senna and fennel or their mixture to STZ-induced diabetic rats reversed the negative changes in these antioxidant parameters and brought it to near normal values in comparison to the diabetic untreated group. The potent free radical scavenging activity of senna has been attributed to the presence of flavanoides and phenolic active components like alaternin and nor-rubrofusarin glucoside in its extract as already described (31).

CONCLUSION

To conclude, it is suggested that the extracts of leaves of senna and the seeds of fennel are effective attenuators of diabetes-induced alterations in enzymes. A combination of these extracts showing stronger amelioration could be developed as potential cardio protective agent for a system exposed to diabetes.

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REFERENCES

1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Rao M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2· 7 million participants. The Lancet 2011; 378: 31-40.
2. International Diabetes Federation (IDF), Diabetes Atlas: 7th edition 2015.
3. Al Dawish MA, Robert AA, Braham R, Al Hayek AA, Al Saeed A, Ahmed RA, Al Sabaan FS. Diabetes mellitus in Saudi Arabia: a review of the recent literature. Curr Diabetes Rev 2016; 12(4):359-368.
4. Ansley DM, Wang B. Oxidative stress and myocardial injury in the diabetic heart. J Pathol 2013; 229(2): 232-241.
5. Mukherjee A, Diksha R, Supriya S, Shaik J. Diagnosis of diabetic retinopathy . Int J Eng Res Appl 2015; 5(2): 21-24.
6. Deedwania PC. Diabetes is a vascular disease: the role of endothelial dysfunction in pathophysiology of cardiovascular disease in diabetes. Cardiol clin 2004; 22(4): 505-509.
7. Shabir H, Kundu S, Basir SF, Khan LA. Modulation of Pb(II) caused aortal constriction by Eugenol and Carvacrol. Biol Trace Elem Res 2014; 161:116-122.
8. El Baz FK, Salama ZA, Baky HHA, Gaafar AA. Hepatoprotective effect of sweet fennel (*Foenculum vulgare l.*) methanol extract against carbon tetrachloride induced liver injury in rats. Int J Pharm Sci Rev Res 2014; 25(2): 194-201.
9. Rezq A. Benificial health effects of fennel seeds on male rats feeding high fat diet. Med J Cairo Univ 2012; 80(2).
10. Mohamad RH, El-Bastawesy AM, Abdel-Monem MG, Noor AM, Al-Mehdar HAR, Sharawy SM, El-Merzabani MM. Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (*Foeniculum vulgare*). J Med Food 2011; 14(9), 986-1001.
11. Shanmugasundaram R., Devi KV, Soris TP, Maruthupandian A, Mohan VR. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata (L.) Roxb.* leaves in alloxan induced diabetic rats. Int J Pharm Tech Res, 2011; *3*(2), 747-56.

12. Sadrefozalayi S, Farokhi F. Effect of the aqueous extract of *Foeniculum vulgare* (fennel) on the kidney in experimental PCOS female rats. Avicenna J phytomed 2014; 4(2): 110.
13. Kunst A, Drager B, Ziegenhorn J. UV methods with hexokinase and glucose-6-phosphate dehrydrogenase. In: Bergmeyer H, editor. Methods of Enzymatic analysis. 1983. p. 163-72.
14. Bergmeyer HU, Bowers GN, Horder M, Moss DW. IFCC method for aspartate aminotransferase. Appendix A. Description of pertinent factors in obtaining optimal conditions for measurement. Clinica chimica acta; Int J Clin Chem 1973; 70(2), F31-40.
15. Bergmeyer HU, Scheibe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem 1978; 24(1): 58-73.
16. Gay R J, Mc Comb RB, Bowers GN. Optimum reaction conditions for human lactate dehydrogenase isoenzymes as they affect total lactate dehydrogenase activity. Clin Chem 1968; 14(8): 740-753.
17. Rosalki SB. An improved procedure for serum creatine phosphokinase determination. J Lab Clin Med 1967; 69(4): 696-705.
18. Bergmeyer HU, Gawehn K, Grassl M. Enzymes as biochemical reagents. Methods of enzymatic analysis 1974; 1 425-522.
19. Miranda KM, Espey MG, Wink DA. A rapid simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5: 67-71.
20. Aebi H. Catalase in vitro: Methods in enzymology 1984; 105:121-126.
21. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-888.
22. Suckling KE, Jackson B. Animal models of human lipid metabolism. Prog Lipid Res 1993; 32(1): 1-24.
23. Al-Adhal A. The effect of *Cassia angustifolia* (senna) leaves on the fasting blood sugar in a sample of mild diabetic Yemeni patients. Yemeni journal for medical sciences 2009; 3: 9.
24. Anitha T, Balakumar C, Ilango KB, Jose CB, Vetrivel D. Antidiabetic activity of the aqueous extracts of *Foeniculum vulgare* on streptozotocin-induced diabetic rats. Int J Adv Pharm Biol Chem 2014; 3(2): 487-494.
25. Pari L., & Latha, M. (2002). Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. *Singapore Med J*, *43*(12), 617-621.

26. Pari L, Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J 2002; 43(12): 617-621.
27. Anwer T, Sharma M, Khan G, Alam MF, Alam N, Ali MS, Alam MS. Preventive role of *Withania somnifera* on hyperlipidemia and cardiac oxidative stress in streptozotocin induced type 2 diabetic rats. Trop J Pharm Res 2017; 16(1): 119-125.
28. Maritim AC, Sanders A, Watkins J. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17(1), 24-38.
29. Asahi M, Fujii J, Suzuki K, Seo HG, Kuzuya T, Hori M, Taniguchi N. Inactivation of glutathione peroxidase by nitric oxide Implication for cytotoxicity. J Biol Chem 1995; 270(36): 21035-21039.
30. Ghaffari T, Nouri M, Saei AA, Rashidi MR. Aldehyde and xanthine oxidase activities in tissues of streptozotocin-induced diabetic rats: effects of vitamin E and selenium supplementation. Biol Trace Elem Res 2012; 147(1-3), 217-225.
31. Neharkar V, Gautam G, Pandhare R. Cardioprotective potential of *Hygrophila auriculata* (l) leaves methanolic extract and it's sub-fraction against doxorubicin induced cardiotoxicity in rats. World Journal of Pharmacy and Pharmaceutical Sciences 2016; 5: 1574-1590.
32. Laghari AQ, Memon S, Nelofar A, Laghari AH. Extraction, identification and antioxidative properties of the flavonoidric fractions from leaves and flowers of *Cassia angustifolia*. Am J Analyt Chem 2011; 2(08): 871.
33. Oulmouden F, Ghalim N, El Morhit M, Benomar H, Daoudi EM, Amrani S. Hypolipidemic and anti-atherogenic effect of methanol extract of fennel (Dia+Fennel) in hypercholesterolemic mice. IJSK 2014; 3: 42-52.
34. Parsaeyan N. The effect *of Foenicumlum vulgarae* (fennel) extract on lipid profile, lipid peroxidation and liver enzymes of diabetic rat. Iranian Journal of Diabetes & Obesity 2016; 8(1).
35. Subash-Babu P, Alshatwi AA, Ignacimuthu S. Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin-induced pancreatic β-cells damage in Wistar rats. Biomolecules & therapeutics 2014; 22(1): 47.
36. Moser U, Bendich A. Handbook of vitamins: 2nd Ed. Marcel Dekker. New York 1991; p. 195–232.