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Effect of Walnut Oil Fortified With β-Sitosterol on Hematological and Histological Parameters in the Liver of Diabetic Rats

Monireh Ghorbani[1], Abdolhossein Shiravi[1*], Gholamhasan Vaezi[1], Hamid Sepehri[2], Vida Hojati[1]

[1]Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran

[2]Golestan Neuroscience Research Center, Golestan University of Medical Sciences, Gorgan, Iran

ABSTRACT

Diabetes mellitus is a metabolic disorder in body in which body fails to produce insulin or represent resistance against insulin and therefore, insulin cannot have its normal application in body. One of the plants to cure this disease is walnut (Juglans regia).

Walnut oil is rich in polyunsaturated fatty acids and is fundamentally important from nutritional and medicinal viewpoints. The present study was thus designed to determine the effect of β-sitosterol-fortified walnut oil on blood factors and hepatic enzymes in diabetic rats. For this aim, diabetes was induced to male rats (200 g) using STZ injection.

Overall, five treatments were considered: normal saline gavage, not-enriched walnut oil gavage, gavage of walnut oil enriched with low, average, and high concentrations of β-sitosterol. Glucose, cholesterol, triglyceride, high-density cholesterol (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin and albumin were measured. In addition, hepatic tissue was analysed by H&E (haematoxylin and eosin) staining, trichrome staining, and TUNEL assay.

The results obtained from the study revealed that gavage of β-sitosterol-enriched walnut oil improved blood parameters and hepatic tissue in diabetic rats.

Key words: Walnut oil, β-sitosterol, Hematological parameters, Liver tissue, Diabetic rats

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Corresponding author: Abdolhossein Shiravi
e-mail ✉: Shiravi738@yahoo.com
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INTRODUCTION

Diabetes mellitus is a common endocrine disorder and is characterized by increase in blood sugar and malfunction in metabolism of carbohydrates, lipids, and proteins [1]. It is thus a metabolic disorder, which adversely influences insulin production or insulin resistance in body and consequently, insulin cannot have its normal function. In this disorder, various tissues such as pancreas, kidney, liver, brain, and heart are influenced. Liver is very dependent on insulin and therefore, diabetes mellitus might increase the risk of hepatic diseases [2]. Although insulin injection is the most common medication method for diabetes mellitus, nutritional considerations have also gained attention in treating this disorder [3]. Before the discovery of insulin and common antidiabetics, diabetic patients were cured using medicinal plants and traditional medications. Positive effects of many of these plants have been shown on reduction of blood sugar and amelioration of consequences caused by diabetes mellitus. For instance, there were reports showing the positive effects of the extracts of *Citrus maxima* leaves [4], *Heracleum persicum* extract [5], *Ilex paraguariensis* extract [3], polysaccharides from *Suillellus luridus* [6], walnut powder extract [7], walnut leaves powder [8], *Pistachia lentiscus* leaves extract [9], *Callicarpa arborea* extract [2] among others, on diabetes mellitus.

Studies showed that walnut has positive effects on nervous system [10], motor and cognitive functions [11], learning and memory [12,13], lipid metabolism as well as it is antioxidant properties [14]. Furthermore, walnut contains phytosterols, especially β-sitosterol, which has been shown to render anti-cancer properties. Walnut oil is rich in polyunsaturated fatty acids, which are very important from nutritional and medicinal perspectives. Consumption of walnut and its products may improve endothelial performance in type II diabetic patients and therefore, it can reduce the risk of cardiovascular disease [15]. However, few studies have been performed on protective effect of walnut oil in diabetes mellitus and hepatic enzymes; also, no study, to the best of our knowledge, has been carried out on the effect of

fortification of walnut oil with β-sitosterol on diabetes mellitus and hepatic enzymes in diabetic rats. The present study is an attempt to determine the influence of β-sitosterol on diabetes mellitus and hepatic enzymes.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (Steinheim, Germany) and β-sitosterol was bought from Merck (Darmstadt, Germany). Also, normal saline, chloroform, and formalin were purchased from Merck. All other materials were of analytical grade obtained from Merck (Darmstadt, Germany).

Extraction and analyses of walnut oil

Walnut oil is extracted through cold press method by the staff of Golkaran Co. (Kashan, Iran). Analyses of the extracted oil were performed by gas chromatography (GC) and high-performance liquid chromatography (HPLC).

The HPLC (Younglinm South Korea) with SP930D pump equipped with UV730D detector, manual injection system with ul20 loop and AUTOchrom software was used. Separation was performed using a 250 × 4.6 mm C18 column *via* a gradient of acetonitrile in water and 0.1% phosphoric acid at a flow rate of 1 mL/min at 30°C.

Fatty acid composition was measured by an Agilent 6890 (Santa Clara, CA, USA) equipped with a TR-CN100 Teknokroma column (100 m × 0.25 mm, 0.2 μm) at the flow rate of 5 ml/min. The injection and detection temperatures were 280°C and 320°C, respectively. The initial oven temperature was 165°C and was raised to 210°C at 40 min. After comparing the retention times of authentic standards and peak integrations, quantification of fatty acids was performed by comparing integrated areas. Sterol profile was measured by a Young Lin Instrument Co., Ltd (Anyang, South Korea) equipped with a Restek, RSS column (30 m × 0.25 mm, 0.25 μm) at the flow rate of 2 ml/min. The injection and detection temperatures were 280°C and 300°C, respectively. The oven temperature was isothermal at 260°C. After comparing the retention times of authentic standards and peak integrations, sterol profile was obtained by comparing integrated areas.

Laboratory animals

Male rats (200 g) were purchased from Pasteur Institute (Amol, Iran) and kept at lab condition (temperature of 22°C ± 1°C and moisture of 60%) at 12 h light and 12 h darkness. The rats were given free access to standard food and water.

Experimental

Diabetes mellitus was induced to rats by intraperitoneal injection of STZ at 65 mg/kg. After 72 h, blood sugar was measured in blood obtained from the tail area of the rats. To ensure the successful induction of diabetes mellitus, blood sampling was repeated a week later and the rats with >300 mg/dL sugar in their blood serum (16.6 mM) were considered diabetic. Overall, five treatments were taken into account as follows:

1. Gavage of normal saline (control group)
2. Gavage of pure walnut oil without fortification with β-sitosterol;
3. Gavage of walnut oil with low dose of β-sitosterol;
4. Gavage of walnut oil with medium dose of β-sitosterol;
5. Gavage of walnut oil with high dose of β-sitosterol.

Oil gavage was performed for 4 weeks at 0.5 ml/kg on a daily basis. For performing measurements, the rats were anesthetized using ether and blood samples were taken from their heart using a syringe.

After centrifuge of the blood samples, appropriate amount of serum was obtained and kept at -20°C until experiments. Blood factors including insulin, cholesterol, triglycerides, high density cholesterol (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, and albumin, were measured. After collection of blood samples, the rats were killed and their livers were separated for histological tests. After separation, the livers were washed with physiologic serum and were then placed in 10% formalin for hydration and preparation for subsequent steps. After dehydration, tissue cuts were prepared and staining was carried out *via* three protocols, i.e. hematoxylin and eosin (H&E stain), trichrome, and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).

Statistical analysis

Data analysis was performed by Analysis of Variance (ANOVA) and differences between means were determined by Tukey test. All the statistical operations were carried out in the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., USA). Differences were considered significant at $p<0.05$.

RESULTS

Characterization of the extracted oil

Fatty acid profile: Fatty acid profile of walnut oil is shown in Table 1. As seen in this table, linoleic acid (C18:2c) is the most abundant fatty acid in walnut oil (around 60% of total fatty acids) followed by linolenic

acid (C18:3), both of which are known as very important fatty acids.

Also, the table shows that PUFAs are the most abundant fatty acids in the oil, indicating the high nutritional and medicinal value of this oil.

Table 1: Fatty acid profile of extracted oil from walnut

Fatty acid	Percentage			ppm		
	1st	2nd	Mean ± SD	1st	2nd	Mean ± SD
C16:0	6.2	6.3	6.26 ± 0.05	49159.8	52046.26	50603.03 ± 1443023
C16:1	0.07	0.09	0.08 ± 0.01	696.456	756.228	726.342 ± 29.886
C17:0	0.04	0.04	0.04 ± 0	379.84	450.18	415.01 ± 35.17
C17:1	0.01	0.01	0.01 ± 0	117.686	177.93	147.808 ± 30.122
C18:0	3.76	3.71	3.735 ± 0.025	29699.78	30334.52	30017.15 ± 317.3695
C18:1t	0.01	0.02	0.015 ± 0.005	156.4	177.9	167.15 ± 10.75
C18:1c	2.22	2.22	2.22 ± 0	174120.9	187277.6	180699.3 ± 6578.324
C18:2t	0.04	0.04	0.04 ± 0	313.611	266.904	290.2575 ± 23.3535
C18:2c	59.15	59.11	59.13 ± 0.02	468587.6	499911	484249.3 ± 15661.71
C18:3	8.46	8.39	8.425 ± 0.035	78174.09	79003.56	78558.83 ± 414.735
C20:0	0.02	0.02	0.02 ± 0	265.89	200	232.945 ± 32.945
Others	0.04	0.07	0.055 ± 0.015	-	-	-
SFA	9.98	10.03	10.005 ± 0.025	-	-	-
MUFA	22.28	22.3	22.29 ± 0.01	-	-	-
PUFA	67.61	67.5	67.555 ± 0.055	-	-	-

Tocopherols: According to the results, γ-Tocopherol (539.15 ppm) and α-Tocopherol (16.57 ppm) accounted for the highest and lowest amounts of tocopherols in the extracted oil, respectively. Furthermore, δ-Tocopherol was found to be 54.84 ppm in the extracted walnut oil. It is noteworthy that β-Tocopherol was not detected in the extracted oil.

Sterol profile: Table 2 depicts sterol compounds in the extracted walnut oil. As seen in this table, the extracted oil does not contain cholesterol. In addition, β-sitosterol is the highest amount of sterol compounds (around 86%), which is indicative of high nutritional and health value of the extracted oil. After β-sitosterol, the highest sterol compounds in the extracted oil were Δ5-avenasterol and campesterol.

Table 2: Sterol profile of extracted oil from walnut

Sterol	Percentage			ppm		
	1st	2nd	Mean ± SD	1st	2nd	Mean ± SD
Cholesterol	ND (a)	ND	-	ND	ND	-
Campesterol	5.6	5.84	5.72 ± 0.12	64.476	62.408	63.442 ± 1.034
Stigmasterol	0.8	0.8	0.8 ± 0	9.254	8.513	8.8835 ± 0.3705
Clerosterol	0.61	0.68	0.645 ± 0.035	6.995	7.252	7.1235 ± 0.1285
β-sitosterol	86.17	85.53	85.85 ± 0.32	991.38	914.111	952.74 ± 38.6345
Δ5-avenasterol	6.7	7	6.85 ± 0.15	77.078	74.863	75.97 ± 1.1075
7-Δ Stigmastenol	0.12	0.15	0.135 ± 0.015	1.311	1.63	1.4705 ± 0.1595
Total	-	-	-	1150.496	1068.777	1109.63 ± 40.85

(a) Not Detected

Hematological parameters

Hematological parameters in control and experimental samples are shown in Figure 1. According to the figure, gavage of pure walnut oil significantly reduced blood glucose compared to control sample ($p<0.05$). In addition, fortification of the extracted oil with β-sitosterol and gavage of the fortified oil to the STZ-induced diabetic rats resulted in significant decrease in blood glucose compared to the rats receiving pure oil ($p<0.05$). Moreover, increased concentration of β-sitosterol in the extracted oil led to significant decrease in blood glucose in the diabetic rates ($p<0.05$). Same patterns were detected in the results of assays measuring cholesterol, triglycerides, AST, ALT, ALP, and total bilirubin (Figure 1). Although low concentration of β-sitosterol added in the extracted oil did not lead to significant reduction in blood HDL in the diabetic rats ($p>0.05$), HDL significantly decreased in the rats when they received walnut oil fortified with medium or high concentrations of β-sitosterol ($p<0.05$).

As seen in Figure 1, the highest rate of direct bilirubin belongs to control sample which is significantly higher than that in other samples ($p<0.05$). Fortification with low concentration of β-sitosterol did not render any significant change in direct bilirubin compared to those receiving walnut oil without β-sitosterol fortification ($p>0.05$). However, fortification of walnut oil with medium or high doses of β-sitosterol resulted in significant decrease in direct bilirubin in blood samples ($p<0.05$). In addition, significant difference was detected in direct bilirubin of the rats receiving medium and high concentration of β-sitosterol in walnut oil ($p<0.05$).

The highest amount of albumin was detected in the blood of control rats which was significantly higher than that in the blood of other samples ($p<0.05$). Furthermore, albumin was found to be significantly higher in the blood of the rats receiving low concentration of β-sitosterol ($p<0.05$). It can also be seen that β-sitosterol in walnut oil led to significant decrease in blood albumin of diabetic rats ($p<0.05$).

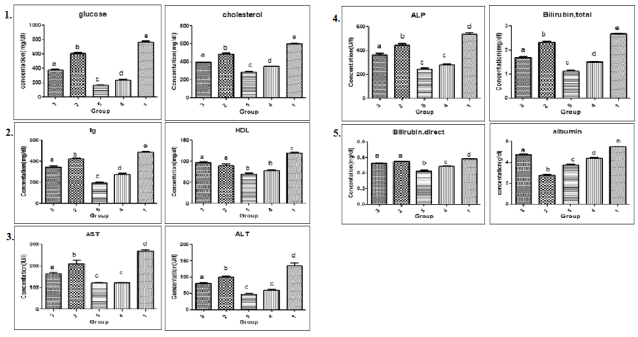

Figure 1: Hematological parameters in blood samples of diabetic rats: 1) Gavage of normal saline (control); 2) Gavage of pure walnut oil without fortification with β-sitosterol; 3) Gavage of walnut oil with low dose of β-sitosterol; 4) Gavage of walnut oil with medium dose of β-sitosterol; 5) Gavage of walnut oil with high dose of β-sitosterol (The letters a-e indicate significant differences among the samples)

Histological results

Figure 2 depicts the results of histological studies carried out through three staining protocols, i.e. H&E, trichrome, and TUNEL. As seen in this figure, in control rats, there is an extensive inflammation around central vein and inside sinusoids and there is evident sinusoidal dilatation and inflammation. Furthermore, there are several irregularities in Remark cordons in addition to accumulation of lymphocytes. Rather similar trend is seen in the histological results of the STZ-induced diabetic rats fed with non-fortified walnut oil. After gavage of fortified walnut oil with low concentration of β-sitosterol, inflammation around central vein and inside sinusoids decreased whereas there was still a little dilatation and inflammation in sinusoids. In addition, although there are few irregularities in Remarks codons, there is no accumulation of lymphocytes. Feeding rats with walnut oil fortified with medium or high concentrations of β-sitosterol, however, led to considerable reduction or disappearance of inflammation around central vein and inside sinusoids and to disappearance of irregularities in Remark codons and of inflammation of lymphocytes. Moreover, the results obtained from staining through TUNEL protocol revealed that STZ-induced diabetic rats fed with walnut oil fortified with medium and high concentrations of β-sitosterol had very few apoptotic cells compared to control rats and rats receiving walnut oil without or with low concentration of β-sitosterol.

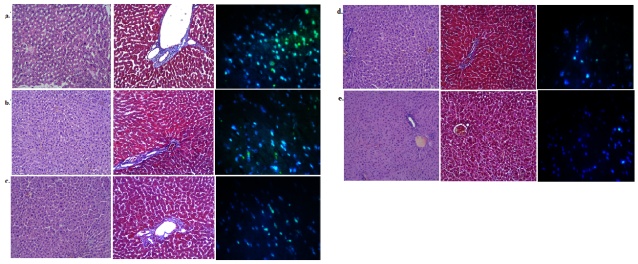

Figure 2: Microscopy of liver samples in diabetic rats; (a) Gavage of normal saline (control); (b) Gavage of pure walnut oil without fortification with β-sitosterol; (c) Gavage of walnut oil with low dose of β-sitosterol; (d) Gavage of walnut oil with medium dose of β-sitosterol; (e) Gavage of walnut oil with high dose of β-sitosterol (The letters a-e indicates significant differences among the samples) Left: H&E (20 µm); middle: trichrome (20 µm); right: TUNEL (magnification: 400 ×)

DISCUSSION

Diabetes mellitus is a chronic disorder caused by problem in production, secretion, or function of insulin [16]. The key factor in outbreak of diabetes mellitus are malfunction of glucose-regulating endocrine organs or of genes involved in metabolism and transport of carbohydrates, proteins, and lipids [17]. Chronic studies showed that hepatic disorders are the main cause of fatality in diabetic patients [18] and therefore, finding solutions to stop or ameliorate these disorders could be a promising way to save lives in diabetes mellitus. One way to do so is through modification of eating habits [19].

Some plants, such as walnut, contain very low concentration of saturated fatty acids (SFA) and they, in

turn, have high level of mono- and polyunsaturated fatty acids (MUFA and PUFA, respectively) and they are composed of high concentrations tocopherols,, phytosterols, polyphenolic antioxidants, and fiber [20]. Research indicated that considering its high nutritional value, diets rich in walnut could improve lipid condition in diabetes mellitus [21]. The walnut oil extracted in the present study contained high level of PUFAs and tocopherols and did not have cholesterol. It also contained high level of β-sitosterol, which indicates high nutritional value of the extracted oil.

The results of the present study revealed that fortification of the extracted oil with β-sitosterol improved blood parameters in STZ-induced diabetic rats. For instance, gavage of walnut oil with different concentrations of β-sitosterol led to reduction in blood glucose, cholesterol, triglyceride, ASP, ALT, ALP, and bilirubin, which was consistent with previous studies [15,22,23]. Fink et al. [23] stated that quality and quantity of lipids in diets have substantial influence on liver lipid metabolites and blood circulation. They also mentioned that high absorption of walnut oil resulted in lower triglycerides in liver while triglycerides in blood serum in fasting state increased in fat rats. They found that decreased triglycerides in liver were concomitant with significant change in fatty acids in liver and reduction in Stearoyl-CoA Desaturase (SCD) activity index as well as yielding normal 6:3 in liver. Histological analysis also showed that fortification of walnut oil with β-sitosterol resulted in considerable decrease in inflammation around central vein and inside sinusoids and minimized irregularities in Remark codons and accumulation of lymphocytes in STZ-induced diabetic rats.

Treatment with β-sitosterol results in increased insulin in fasting state. Furthermore, β-sitosterol improves the results of oral glucose test and increases insulin secretion induced by glucose, which is similar to the effect of Glibenclamide, a hyperglycemic antidiabetic drug. Gupta et al. [24] showed that treating diabetic rats with β-sitosterol prevented the development of diabetes mellitus and ameliorated its adverse effects. They further stated that β-sitosterol increased glucose adsorption in adipocytes and stimulated adipogenesis in differentiating preadipocytes. In contrast, β-sitosterol induces lipolysis in adipocytes, which is not ameliorated with insulin and simultaneous incubation with epinephrine. Like insulin, β-sitosterol downregulates GLUT4 expression; however, it is to be determined whether increased glucose adsorption by β-sitosterol in adipocytes is related to GLUT4 and whether lipolysis is related to downregulation of Akt and PI3K. Although β-sitosterol is considered an important potential factor in diabetes mellitus due to its effects on regulation of glucose adsorption, adipogenesis, and lipolysis in adipocytes [25], it should be studied clinically and it should be found whether β-sitosterol has any role in insulin sensitivity and glucagon secretion [22].

CONCLUSION

The present study was formulated to determine protective effect of β-sitosterol in the oil extracted from walnut through cold press method in STZ-induced diabetic rats. The results of this study showed that fortification of walnut oil, especially at medium and high concentrations, resulted in significant decrease in blood parameters and improved liver tissue by reducing inflammation around central vein and inside sinusoids and preventing irregularities in Remark codons. Moreover, fortification of walnut oil with inflammation of lymphocytes considerably decreased inflammation of lymphocytes and prevented the formation of apoptotic cell. Therefore, use of walnut oil, especially the one fortified with β-sitosterol, is recommended in diets to prevent the consequences of diabetes mellitus and improve blood parameters and hepatic health.

CONFLICT OF INTEREST

The authors declared no conflicts of interests.

REFERENCES

1. Ghorbani Z, Hekmatdoost A, Mirmiran P, et al. Anti-hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent curcumin. Int J Endocrinol Metab 2014; 12.
2. Junejo JA, Rudrapal M, Nainwal LM, et al. Antidiabetic activity of hydro-alcoholic stem bark extract of Callicarpa arborea Roxb. with antioxidant potential in diabetic rats. Biomed Pharmacother 2017; 95:84-94.
3. Rocha DS, Casagrande L, Model JF, et al. Effect of yerba mate (Ilex paraguariensis) extract on the metabolism of diabetic rats. Biomed Pharmacother 2018; 105:370-6.
4. Feksa DL, Coelho RP, da Costa Güllich AA, et al. Extract of Citrus maxima (pummelo) leaves improves hepatoprotective activity in Wistar rats submitted to the induction of non-alcoholic hepatic steatosis. Biomed Pharmacother 2018; 98:338-46.
5. Alkan EE, Celik I. The therapeutics effects and toxic risk of Heracleum persicum Desf. extract on streptozotocin-induced diabetic rats. Toxicol Rep 2018; 5:919-26.
6. Zhang L, Liu Y, Ke Y, et al. Antidiabetic activity of polysaccharides from Suillellus luridus in streptozotocin-induced diabetic mice. Int J Biol Macromol 2018; 119:134-40.
7. Liang X, Chen D, Cao L, et al. Effects of pressed degreased walnut meal extracts on lipid metabolism in postnatally monosodium glutamate-induced mice and 3T3-L1 preadipocytes. J Funct Foods 2017; 31:89-96.
8. Mollica A, Zengin G, Locatelli M, et al. An assessment of the nutraceutical potential of Juglans regia L. leaf powder in diabetic rats. Food Chem Toxicol 2017; 107:554-64.

9. Cherbal A, Kebieche M, Yilmaz EM, et al. Antidiabetic and hypolipidemic activities of Algerian Pistachia lentiscus L. leaves extract in alloxan-induced diabetic rats. S Afr J Bot 2017; 108:157-62.
10. Asadi-Shekaari M, Kalantaripour TP, Nejad FA, et al. The anticonvulsant and neuroprotective effects of walnuts on the neurons of rat brain cortex. Avicenna J Med Biotechnol 2012; 4:155.
11. Willis LM, Shukitt-Hale B, Cheng V, et al. Dose-dependent effects of walnuts on motor and cognitive function in aged rats. Br J Nutr 2008; 101:1140-4.
12. Harandi S, Golchin L, Ansari M, et al. Antiamnesic effects of walnuts consumption on scopolamine-induced memory impairments in rats. Basic Clin Neurosci 2015; 6:91.
13. Haider S, Batool Z, Tabassum S, et al. Effects of walnuts (Juglans regia) on learning and memory functions. Plant Foods Hum Nutr 2011; 66:335-340.
14. Abam EO, Oladipo FY, Atasie VN, et al. Effect of walnut (Tetracarpidium conophorum)-oil on cadmium-induced alterations in lipid metabolism in male albino rats. Food Pub Health 2013; 3:169-175.
15. Rahimi P, Kabiri N, Asgary S, et al. Anti-diabetic effects of walnut oil on alloxan-induced diabetic rats. Afr J Pharm Pharmacol 2011; 5:2655-61.
16. Shaw JE, Sicree RA, Zimmet PZ, et al. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87:4-14.
17. Fatima SS, Rajasekhar MD, Kumar KV, et al. Antidiabetic and antihyperlipidemic activity of ethyl acetate: Isopropanol (1:1) fraction of Vernonia anthelmintica seeds in Streptozotocin induced diabetic rats. Food Chem Toxicol 2010; 48:495-501.
18. Cao H, Hua R, Wu ZY, et al. Association of nonalcoholic fatty liver disease with type 2 diabetes: Clinical features and independent risk factors in diabetic fatty liver patients. Hepatobiliary Pancreat Dis Int 2005; 4:389-92.
19. Riediger ND, Othman R, Fitz E, et al. Low n-6: n-3 fatty acid ratio, with fish-or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. Eur J Nutr 2008; 47:153-60.
20. Bolling BW, McKay DL, Blumberg JB, et al. The phytochemical composition and antioxidant actions of tree nuts. Asia Pac J Clin Nutr 2010; 19:117.
21. Wu H, Pan A, Yu Z, et al. Lifestyle Counseling and Supplementation with Flaxseed or Walnuts Influence the Management of Metabolic Syndrome–4. J Nutr 2010; 140:1937-42.
22. Sayeed BM, Karim S, Sharmin T, et al. Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: A plant-derived orphan phytosterol. Medicines 2016; 3:29.
23. Fink A, Rüfer CE, Le Grandois J, et al. Dietary walnut oil modulates liver steatosis in the obese Zucker rat. Eur J Nutr 2014; 53:645-60.
24. Gupta R, Sharma AK, Dobhal MP, et al. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. J Diabetes 2011; 3:29-37.
25. Chai JW, Lim SL, Kanthimathi MS, et al. Gene regulation in β-sitosterol-mediated stimulation of adipogenesis, glucose uptake, and lipid mobilization in rat primary adipocytes. Genes Nutr 2011; 6:181.