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Influence of Flaxseed on Some Biochemical Factors, Antioxidant Activity and Expression of ABCG5 and ABCG8 Genes in the Liver of Diabetic Rat

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Authors' contributions

This work was carried out in collaboration between all authors. AM designed the study and manuscript. Author ABO carried out the molecular genetic studies, authors NN, KH, RAT, FO and ABO participated in biochemical analysis and drafted the manuscript. Author NM performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of this experiment is to study the effect of flaxseed on lipid profile, antioxidant activity and ATP-cassette binding proteins G5 and G8 (ABCG5 and G8)

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levels in the liver of diabetic rat.
Place and Duration of Study: Department of Biochemistry and Physiology Research Centre, Afzalipour School of Medicine, Kerman University of Medical Sciences(Kerman, Iran), between 2008 and 2011.
Methodology: rats randomly were divided into three groups: diabetic rat + flaxseed (treatment group), diabetic rat (control group I), healthy rat (control group II). Afterward one month Serum lipid parameters and also Super oxide dismutase (SOD) activity, reduced glutathione (GSH) and malondialdehyde (MDA) levels were measured. ABCG5 and ABCG8 levels were determined by RT-PCR.
Results: Flaxseed markedly reduced malondialdehyde (MDA), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C) (all of them $P<0.05$). GSH, SOD ($P<0.05$) as well as liver ABCG5 and ABCG8 were significantly increased ($P<0.01$) in flaxseed treated-animals compared with diabetic group.
Conclusion: The results of this experiment showed that flaxseed has antioxidant and ani-atherogenic effect. This plant reduced cholesterol levels may be via ABCG5 and ABCG8 transporters in diabetic rats.

Keywords: Brackets; morphological evaluation; surface characteristics; as received; in vivo.

1. INTRODUCTION

Cardiovascular disease (CVD) has known as a chief reason of mortality and morbidity in the world. The causes of CVD are very diverse and most of them including high levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and hypertension. Moreover, high amount of serum lipids have been established the main cause of CVD [1,2].

Herbal medicines are generally preferred and suitable in traditional medicine for treatment of many diseases on all over the world. The recent report of World Health Organization showed that around 80% of individuals in developing countries choice traditional medicine for their primary health care, and around 85% of such traditional medicine includes the usage of herbal medicine [3]. In this respect, many studies showed that flaxseed has many useful effects for treatment of CVD. For example, flaxseed is accepted as a major source of cholesterol lowering agents including lignans, phytoestrogen, alpha-linolenic acid (ALA), and fiber [4]. The data of prior experiments have revealed that flaxseed declined both total cholesterol (TC) and LDL-C. Some compartment of flaxseed such as lignans of flaxseed also is recognized to have hypocholesterolemic effect and regressed formation of atherosclerotic plaque [5].

The exact molecular mechanisms of intestinal lipid absorption in the intestine are not completely understood. Nevertheless, several proteins such as ATP-binding cassette transporter G5/G8 (ABCG5/G8), scavenger receptor BI (SR-BI), Niemann-Pick C1-like 1 (NPC1L1) and aminopeptidase N have been suggested as candidates of intestinal cholesterol transporters [6]. The both transporters of ABCG5 and ABCG8 have been identified as a cholesterol efflux which work together and propelled free cholesterol and plant sterols from the enterocyte back into lumen of intestinal for excretion and also increased cholesterol excretion from liver to the bile duct. Therefore ABCG5 and ABCG8 with excretion of cholesterol & plant sterols and rise of bile secretion play important role in cholesterol homeostasis and have been proposed as target of atherosclerosis treatment [7]. In this

experiment we have evaluated the anti-atherogenic and antioxidant properties of flaxseed as well as ABCG5 and ABCG8 expression in diabetic rats.

2. MATERIAL AND METHODS

2.1 Animals

Male Wistar rats weighing 200-250g were used for this experiment. Animals maintained under 12/12 h light/dark cycles at room temperature of 25±1°C and humidity of 55±5%. After about one week of acclimation, Wistar rats divided into 3 groups (n=8): Diabetic rat + 4% of flaxseed (w/w, which flaxseed added to animal's diet) (treatment group), diabetic rat (control group I), healthy rat (control group II).

2.2 Diabetic Rats

For generation diabetic model, 70 mg/kg body wt, streptozotocin (STZ) was injected intraperitonealy (streptozotocin dissolved in citrate buffer, pH 4.5) following fasting for overnight. One week after STZ administration, animals with fasting blood glucose above 300 mg/dl were recognized as a diabetic [8].

2.3 Serum Lipid Parameters

Later than one month of different treatment anesthetized rats were sacrificed. Blood samples of each rat was collected and then centrifuged at 3000 rpm, for 15 min at 4 °C [9]. Amount of lipid profile including triglyceride, total cholesterol and HDL-C levels were measured enzymatically according manufacturers' protocol (Pars Azmoon, Iran). LDL-C and VLDL-C levels were calculated according to the Friedewald formula [10].

2.4 Antioxidant and Lipid Peroxidation Assay

Super oxide dismutase (SOD) activity, reduced glutathione (GSH) and malondialdehyde (MDA) levels were measured by the method of Misra, et al. Beutler E, et al. and Ohkawa, et al. respectively [11].

2.5 Semiquantitative RT-PCR

Total RNA from the liver of each Wistar rat was extracted with Accuzol Reagent according to the manufacturer stated protocol (Bioneer, Korea). After that, synthesis of cDNA was organized according to the manufacturer stated protocol (Fermentas, Lithuania). Thirty five cycles of PCR amplification were achieved and PCR product were electrophoresed on a 2% agarose gel and then immediately visualized by staining with ethidium bromide [12,13]. The following primers were used in this study; mouse *β*-actin primer; forward:5'-TGGAATCCTGTGGCATCCATG AAAC-3' and reverse primer: 5'-TAAAACGCAGCTCAGTAACAGTCCG-3', ABCG5 forward primer, 5'-TGCCCTTTCTGAGTCCAGAG-3', and reverse primer, 5'-TGCTCTTTCAATGTTCTCCAG-3', ABCG8 forward primer, 5'-ATGAGCTGG AAGACGGGCTG-3', and reverse primer, 5'-GCCAGTGAGAGCAAGGCTGA-3'.

2.6 Statistical Analysis

All data of this experiment are presented as mean ± SD. Statistical analysis was done by analysis of variance (ANOVA) and Tukey test Post-Hoc). After analysis, the P<0.05 were established statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effect of Flaxseed Treatment on Blood Lipid Levels

Table 1 shows lipid profiles diabetic, control and flaxseed-treated groups. There was no difference in body weight between the 3 groups.The levels of total cholesterol (P<0.01), triglyceride (P<0.001), VLDL-C (P<0.001), LDL-C (P<0.01) and non-HDL-C (P<0.01) significantly reduced flaxseed-treated animal in comparison with diabetic rats. Ratio of and LDL/HDL (P<0.001) and atherogenic index (P<0.01) markedly reduced in flaxseed-treated rat in comparison with diabetic group. The activity of SOD was significantly increased in flaxseed-treated animals (p<0.05) compared with diabetic animals. GSH level was also increased significantly in flaxseed-treated rats (p<0.05) compared with diabetic group. The MDA levels reduced markedly in flaxseed-treated rats compared with diabetic group (p<0.05) (Figs. 1-3).

3.2 Gene Expression

Analysis of reverse transcriptase PCR showed significant up-regulation of both ABCG5 and ABCG8 mRNA levels in flaxseed treated rat in comparison with diabetic rats (*P*<0.01) (Figs. 4 and 5).

Table 1. Lipid profiles in different treatment groups

Serum lipid parameters	Diabetic Rat + Flaxseed	Diabetic Rat	Healthy Rat
TC (mg/dl	75.10±5.29 [b]	119.40±9.16	71.15±4.03[b]
TG (mg/dl)	62.93±5.53[c]	142.17±11.21	64.35±6.82[c]
LDL-C(mg/dl)	17.15±2.06 [b]	45.89±2.04	16.22±1.08 [b]
HDL-C (mg/dl)	49.70±5.50	45.81±5.31	43.75±5.60
VLDL-C(mg/dl)	12.52±0.35 [c]	28.34±0.61	13.08±0.45[c]

TC: Total cholesterol,
TG: Triglyceride,
LDL-C: Low-density lipoprotein cholesterol,
HDL-C: High-density lipoprotein cholesterol,
VLDL-C: Very low-density lipoprotein
Cholesterol, Data represent as mean ± SD (n = 8).
[a] P< 0.05, [b]P < 0.01 and [c]P < 0.001
compared with diabetic control rats.

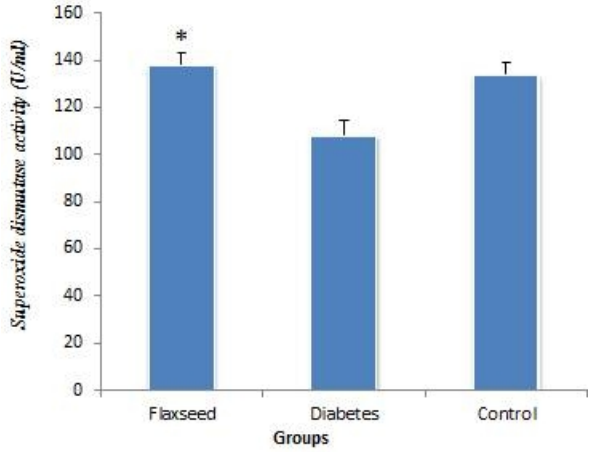

Fig. 1. Superoxide dismutase (SOD) activity in different groups. Data are expressed as mean±SD. * SOD significantly increased compared to diabetic control ($p < 0.05$).

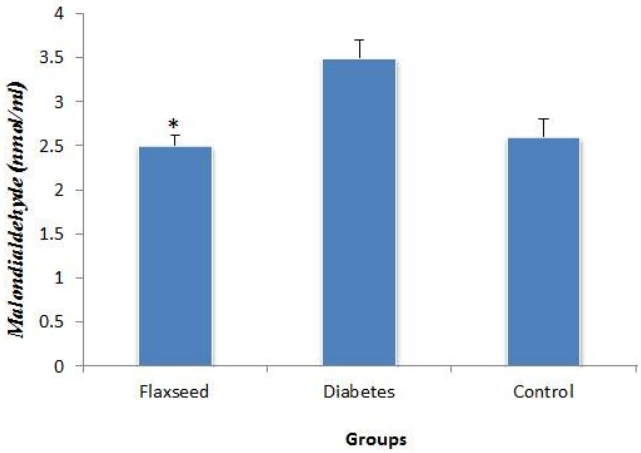

Fig. 2. Malondialdehyde (MDA) levels in different groups. Data are expressed as mean±SD. MDA significantly reduced compared to diabetic control ($p < 0.05$).

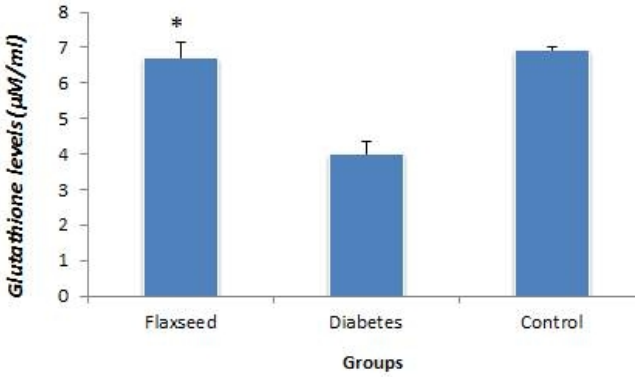

Fig. 3. Gutathione (GSH) levels in different groups. Data are expressed as mean±SD. GSH significantly increased compared to diabetic control ($p < 0.05$).

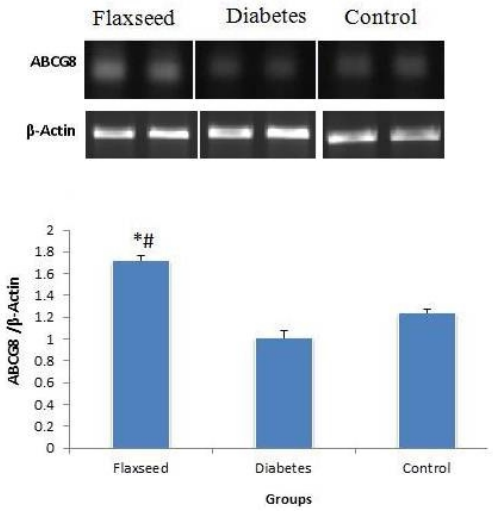

Fig. 4. Expression of ABCG8 mRNA in the liver of different treated rats (n = 8). ABCG5 mRNA levels significantly increased in flaxseed group. *p < 0.01 compared with diabetic group. #p < 0.05 compared with chow group. Data are presented as means±SD.

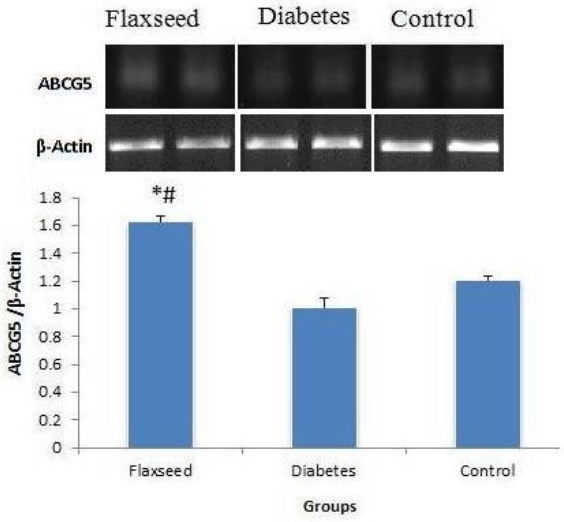

Fig. 5. Expression of ABCG5 mRNA in liver of different treated rats (n = 8). ABCG5 mRNA levels significantly increased in flaxseed group. *p < 0.01 compared with diabetic group. #p < 0.05 compared with chow group. Data are presented as means±SD.

Animal model of some disorder such as diabetes and cardiovascular disease have been broadly used to understanding of the pathophysiology and development of new medicine for its treatment. Streptozotocin (STZ) is usually used for induction of diabetes in animal models. In this study we used STZ for produce of diabetic model [14].

Flaxseed is an herbal medicine which has many useful effects [15]. In this experiment flaxseed increased the levels of HDL-C by 7% compared with diabetic animals. Studies have established that CVD risk reduced by 2%-3% by raise of every 1mg/dl in HDL-C levels while CVD event is reduced by 1% for every 1mg/dl reduction in LDL-C levels [16]. In this experiment flaxseed markedly reduced LDL-C levels (63%). Higher levels of HDL commonly mean lower risk of CVD [17]. Flaxseed-treated animals had the low levels TC compared with the diabetic rats.

Internal antioxidants, including catalase, superoxide dismutase, peroxidase glutathione and peroxidase are existent in organisms and keep cells from many oxidant attacks. Internal and external antioxidants[18-20] play vital role in keeping health and normal cellular activity before free radicals devastating cellular components [21].Current studies have revealed that oxidative stress and peroxidation of lipid are involved in the pathogenesis of cardiovascular disease especially atherosclerosis. Peroxidation of lipid has known as an initial event in atherosclerosis. Lipid peroxidation is happened through in vitro LDL oxidation (oxLDL) and stimulated by several oxidants in vivo [22]. Lipid peroxidation in the vessel wall is responsible of wide range of cellular dysfunctions [23]. The MDA levels are identified as a positive sign of lipid peroxidation. Compared to controls, in flaxseed-treated animals, MDA were markedly reduced.

Homeostasis of cholesterol is controlled by a several ways including: intestinal absorption, de novo synthesis, biliary clearance and also faecal elimination of cholesterol. Biliary clearance has been recognized to be one of the main ways of cholesterol elimination [6].

The Liver X receptors (LXRs) are a part of the nuclear receptor family which are controlled lipid carbohydrate metabolism. Studies have shown that treatment of animal with liver X receptor agonist improved elimination of sterol, reduced cholesterol absorption and increased the biliary cholesterol excretion, which is mediated by ABCG5 and ABCG8 [24].

ABCG5 and ABCG8 expressed mainly in liver and intestine and are shape heterodimer for functional transporter. Lacking of these transporters that happened in sitosterolemia patient is associated with decline in biliary sterols exertion with increase of cholesterol absorption [24].

In this experiment, flaxseed markedly increased expression of ABCG5 and ABCG8 genes in liver of diabetic rats. Linqing Yu, et al. [25] reported that upregulation of ABCG5 and ABCG8 in the liver and intestinal lead to notably rise of biliary cholesterol secretion and decrease of cholesterol absorption. Overexpression of human ABCG5 and ABCG8 transgenes in mice lead to a 50% reduction in the fractional absorption of dietary cholesterol which associated with rise of biliary cholesterol excretion [7].

In mice, defect in ABCG5 and ABCG8 increases sitosterol and declined bile cholesterol, while overexpression of these transporters increases excretion of biliary cholesterol and declined intestinal cholesterol absorption. Co-administration of a statin with ABCG5/ABCG8 activator agent is anticipated to have a synergistic effect in reduction of cholesterol and LDL-C [26,27]. Ikeda et al. reported that soy protein with increasing of ABCG5 and ABCG8 led to reduction of cholesterol accumulation in the liver and plasma [28]. We recently showed that flaxseed significantly increased LXR in the rat intestine [29]. In another study we revealed that flaxseed significantly increased ABCG5 and ABCG8 genes in the intestine [30]. Raise in LXR levels leads to up-regulation of ABCG5 and ABCG8 genes and consequently reduction of cholesterol and also rise of biliary cholesterol secretion. Basso F, et al. showed increased

biliary cholesterol secretion when coupled with inhibition of intestinal cholesterol absorption significantly reduces apoB-lipoproteins and atherosclerosis [31]. We suggest that flaxseed with increase of ABCG5 and ABCG8 in the liver and intestine have important role in control of whole body cholesterol levels.

4. CONCLUSION

Flaxseed is used as a herbal medicine which has high amount of lignans, phytoestrogen, soluble fiber and alpha-linolenic acid. Many studies have shown that these composites have hypolipidemic and antioxidant properties. Flaxseed with increase of intestinal and liver ABCG5/ABCG8 significantly reduced plasma whole body cholesterol. This experiment is suggesting the treatment of diabetic patient with herbal medicine. However, more experiment need to study and accept therapeutic properties of flaxseed in diabetic patient.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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