Influence of Flaxseed on Lipid Profiles and Expression of LXRα, in Intestine of Diabetic Rat

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Abstract
The aim of this study is to examine the effect of flaxseed on lipid profiles in diabetic rats, focusing on intestinal LXRα. Animals were randomly divided into 3 groups of 8 rats each. group1: rats + chow diet (control), group 2: diabetic rats + chow diet (diabetic control), and group 3: diabetic rats + chow diet + 4% flaxseed (w/w) (flaxseed group). After one-month rats were sacrificed, blood was collected; lipid profiles were determined enzymatically as well as mRNA and protein levels of SR-BI were determined by RT-PCR and westernblot respectively. Compared with diabetic control (group 2), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides, and very low density lipoprotein cholesterol (VLDL-C) (all of them P < 0.01) significantly decreased in flaxseed group (group 3). Intestinal LXRα mRNA was significantly increased (P < 0.001) in flaxseed group treatment compared with diabetic animals (group 2). Levels of intestinal regulatory protein of LXRα significantly increased in flaxseed group (P < 0.05). In conclusion, flaxseedsignificantly reduced TC, LDL-C, TG, VLDL-C and atherogenic index, as compared with the diabetic rats (group 2). On the other hand flaxseed led to up-regulation of LXRα in the intestine of rats.

Keyword: flaxseed, cholesterol, LXRα, diabetes, Iran

1. Introduction
Dyslipidaemia is a major forecaster of cardiovascular disease (CVD) events and diabetic patient’s mortality. High plasma concentration of total cholesterol and low-density lipoprotein (LDL) cholesterol are major risk factor for cardiovascular diseases (Vafa, 2012; Manhas, 2004). Therefore, attentive control of lipid profiles is vital. Diabetic patient have two-four fold higher risk of developing coronary heart disease than people without diabetes, and CVDaccounts for 65-75 percent of diabetic patient deaths (Mohammed, 2010).

The restriction of nutritional cholesterol has been used as the principal primary therapeutic modality for the prevention and also treatment of dyslipidemia. Consequently, investigating nutritional components that can markedly improve dyslipidemia is important.

The livers X receptors (LXRα and LXRβ) are vital regulators of lipid and carbohydrate homeostasis that belong to the nuclear receptor superfamily. Two isotypes of LXR have been known in mammals including LXRα that is mainly expressed in the liver, intestine, spleen, kidney and adrenals, and LXRβ that is expressed universally
LXRα by regulating of cholesterol transporters in the intestine plays important role in whole blood cholesterol. Flaxseed is recognized as a richest source of alpha-linolenic acid (ALA), lignans, phytoestrogen, and soluble fiber. Studies showed that flaxseed markedly reduced plasma cholesterol and suggested as a useful plant in prevention and treatment of cardiovascular disease (Pellizzon, 2007).

We have evaluated the hypolipidemic effects of flaxseed in diabetic rats. We also have measured expression of LXRα in rat intestine.

2. Method

2.1 Animals

Male Wistar rats of same age with mean body weight of 200-250g were used for this experiment. The animals were bred and kept in the animal house. The animal house was maintained under standard hygienic condition, at a room temperature of 25 ± 1 °C and relative humidity 55 ± 5% with 12 h light/dark cycle (Hajianfar, 2013). After one week of acclimation, rats were randomly divided into 3 groups of 8 rats each. Group1: rats + chow diet (control), group2: diabetic rats + chow diet (diabetic control), and group3: diabetic rats + chow diet + 4% flaxseed (w/w) (flaxseed group). The procedure of this study has been approved by the Animal Research Ethic Committee of Tehran Payamenoor University (Tehran, Iran).

2.2 Diabetic Rats

After an overnight fast, diabetes was induced in animals by a single intraperitoneal (i.p.) injection of streptozotoc in (70 mg/kg in 10 mM citrate buffer, pH 4.5). Diabetes was recognized by polyuria, polydipsia and by evaluate of non-fasting serum glucose levels after one week. Rats with blood glucose levels greater than 300 mg/dl were considered diabetic (Abbasi-Oshaghi, 2012).

2.3 Preparing Samples

After one month, the rats were anesthetized with diethyl ether and killed by decapitation. Blood samples of each rat were collected by cardiac puncture into glass tubes and afterward centrifuged at 3000 rpm, for 15 min at 4 °C. The serum was conveyed to a clean glass tube and stored at -20 °C (Mohammadi, 2012; Esfahani, 2013).

2.4 Biochemical Analysis

Amount of TG, TC and HDL-C were measured enzymatically using kits (Pars Azmoon, Iran). Levels of LDL-C and VLDL-C were calculated according to the Friedewald formula. Non-HDL-cholesterol concentration was calculated by subtracting HDL-C from total cholesterol (Ghamar-Chehreh, 2012).

2.5 Semiquantitative RT-PCR

RNA from intestine was extracted with Accuzol Reagent (Bioneer, Korea) according to the manufacturers’ protocol. Synthesis of cDNA was performed according to the manufacturer’s protocol (Fermentas, Lithuania). For semiquantitative RT-PCR reaction 13 µl PCR Master Mix (Cinnagen, Iran), 1 µl forward primer (P mol/µl), 1 µl reverse primer (P mol/µl), 2 µl cDNA and 8 µl deionized water were added into a sterile tube on ice, and centrifuged for a short time. Thirty five cycles of PCR amplification were achieved with denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 30 s using a PCR machine. All reactions were completed with a single additional cycle at 72 °C for 5 minutes. The samples were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. The primers that used in this studywere fallowing: β-actin : upper, 5’-TGG AAT CCT GTG GCA TCC ATG AAA C-3’, lower primer, 5’-TAA AAC GCA GCT CAG TAA CTC GTG GAC ATC CCA GAT-3’ LXR alpha : pper primer, F: 5’-GCG TCC ATT CAG AGC AAG TGT-3’, lower primer, 5’-TCA CTC GTG GAC ATC CCA GAT-3’ (Mohammadi, 2012).

2.6 Western Blotting

About 50 mgmofintestine samplewas homogenized in 500 µl of RIPA buffer which containing 1% protein inhibitor cocktail (Santa Cruz, USA) and 1 µM PMSF. The homogenate then was centrifuged at 14,000 rpm for 15 min at 4 °C. Fifty microgram of protein was separated on a 12.5% SDS-PAGE gel and transferred to a Polyvinylidenedifluoride (PVDF) membrane (Roche Applied Science). The PVDF membrane was blocked (2 hr) with 3% non-fat dried milk in Tris-buffered saline with Tween 20 (TBS-T, Roche Applied Science) at room temperature. The membrane then was probed with rabbit anti-LXR alpha antibody (1:300 dilutions, Santa Cruz), and rabbit polyclonal β-actin antibody (1:2000 dilutions, Novus Biological) for 1.5 hr at room temperature. After washing in TBS-T (three times, 15 min), the blots were incubated for 1.5 hr sat room temperature with a hors eradish peroxidase-conjugated secondary antibody (1:10000, Roche Applied Science). The complexes of
antibody and antigen were exposed to ECL western blotting detection reagents (Roche Applied Science) for 30 s and the film was developed. Lab Work analyzing software (UVP, UK) was used to analyze Band densities. Data are expressed as the percent ratio of the LXR to β-Actin (Abbasi-Oshaghi, 2012; Mohammadi, 2012; Chen, 2009).

2.7 Statistical Analysis

The data were analyzed using one-way analysis of variance with ANOVA followed by Tukey. Results were expressed as mean values ± SD. The differences between groups were considered significant when \( P < 0.05 \).

3. Results

3.1 Effect of Flaxseed Treatment on Blood Lipid Levels

The effect of flaxseed treatment on the blood lipid profiles of the tested rat groups is given in Table 1. The levels of TG \( (P < 0.001) \), VLDL-C \( (P < 0.001) \), TC \( (P < 0.01) \) and LDL-C \( (P < 0.01) \) significantly reduced in the flaxseed treatment rats compared with diabetic control.

The high levels of TC, LDL-C and TG were brought down markedly after one month treatment period. A fall of 36% in TC, 63% in LDL-C and 55% in TG was observed in flaxseed treated group and there was also an increase of 7% HDL-C in the treated group compared with diabetic control.

Table 1. Lipid profiles in different treatment groups

<table>
<thead>
<tr>
<th>Biochemical factors</th>
<th>diabetic rat+ Flaxseed</th>
<th>diabetic rat (diabetic control)</th>
<th>chow diet (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC  ( 75.10 \pm 5.29^b )</td>
<td>119.40 ± 9.16</td>
<td>71.15 ± 4.03^b</td>
<td></td>
</tr>
<tr>
<td>TG  ( 62.93 \pm 5.53^c )</td>
<td>142.17 ± 11.2</td>
<td>164.35 ± 6.82^c</td>
<td></td>
</tr>
<tr>
<td>LDL-C  ( 17.15 \pm 2.06^b )</td>
<td>45.89 ± 2.04</td>
<td>16.22 ± 1.08^b</td>
<td></td>
</tr>
<tr>
<td>HDL-C  ( 49.70 \pm 5.50^c )</td>
<td>45.81 ± 5.31</td>
<td>43.75 ± 5.60</td>
<td></td>
</tr>
<tr>
<td>VLDL-C  ( 12.52 \pm 0.35^c )</td>
<td>28.34 ± 0.61</td>
<td>13.08 ± 0.45^c</td>
<td></td>
</tr>
</tbody>
</table>

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 \( ^c P < 0.001 \) Considered as significant compared with diabetic control (diabetic rats + chow diet).

3.2 Protein and mRNA Levels

Intestinal LXRα protein significantly increased in flaxseed treated group in comparison with diabetic rats \( (P < 0.05) \) (Figure 1).

LXRα mRNA significantly increased \( (P < 0.05) \) in flaxseed group in comparison with diabetic rats (Figure 2).
Figure 1. Expression of LXR protein in intestine of flaxseed, diabetic and control rats (n = 8)
LXR protein expression significantly increased in flaxseed group. *p < 0.05 considered as significant compared with diabetic rats. Data are presented as means ± SD. LXR alpha band from left to right; lane 1: flaxseed group (diabetic rats + chow diet + 4% flaxseed), 2: control (rats + chow diet), 3: diabetic control (diabetic rats + chow ).

Figure 2. Expression of LXR mRNA in intestine of flaxseed, diabetic and control rats (n = 8)
LXR mRNA expression significantly increased in flaxseed group. ***p < 0.001 considered as significant compared with diabetic rats. Data are presented as means ± SD. LXR alpha band from left to right; lane 1: flaxseed group (diabetic rats + chow diet + 4% flaxseed), 2: control (rats + chow diet), 3: diabetic control (diabetic rats + chow ).

4. Discussion
4.1 Biochemical Markers
Prevalence of diabetes and cardiovascular disease are growing quickly in the world. Optimal control of lipid profiles in diabetic patient is very important to prevention of cardiovascular disease and also growing health is a matter in the world (Manhas, 2004). Diabetic patients frequently have higher total cholesterol, TG and LDL-C.
This combination participates markedly to their cardiovascular risk. In the diabetic patients, and LDL-C is a powerful risk factor for cardiovascular (Brown, 2008).

In this study LDL-C significantly reduced by flaxseed. Studies have shown that rate of cardiovascular event is reduced by nearly 1% for every 1% decrease in LDL-C, and by at least 1% for every 1% raise in HDL-C (Brown, 2008).

We observed that the diabetic animals which fed flaxseed had the lowest plasma cholesterol concentration compared with the diabetic animals. However, high plasma cholesterol levels are related to coronary artery disease (CAD) (Manhas, 2004; Brown, 2008).

4.2 Gene Expression

Homeostasis of plasma cholesterol is controlled by a complex interaction of intestinal absorption, biliary clearance, faecal excretion and denovo synthesis. Absorption of cholesterol from intestinal has been established to be a main determinant of plasma lipid profiles.

The liver X receptor (LXR) is a part of the nuclear receptor family of transcription factors liver X receptors (LXRs) are vital regulators of cholesterol, triglyceride, and glucose metabolism. Two isoforms of LXR have been recognized including LXRα and LXRβ. LXRα expression is limited to liver, intestine, adipose tissue, kidney, lung, macrophages and spleen, while LXRβ is expressed in approximately all tissues. LXRα by regulating expression of many genes such as Niemann-Pick C1 Like 1 (NPC1L1) and heterodimer of ATP binding cassette transporter G5 and G8 (ABCG5 and ABCG8) leads to reduction of intestinal cholesterol absorption (Kruit, 2006; Zhoa & Dahlman-Wright, 2010).

Activation of LXR increases both ABCG5 and ABCG8 expression, which transfer absorbed cholesterol back to the intestinal lumen. Studies have shown that administration of LXR agonists significantly decreases intestinal net cholesterol absorption in animals (Zhoa & Dahlman-Wright, 2010).

NPC1L1 (Niemanna Pick C1 Like 1) has been recognized as a main cholesterol transporter which have important role in the cholesterol absorption. This protein is expressed in the brush border of enterocytes (Altmann, 2004). It was recently suggested that activation of LXR downregulates NPC1L1 expression.

Elevation of plasma cholesterol is related to atherosclerotic coronary heart disease. By reducing plasma cholesterol levels, NPC1L1 inhibition and activation of ABCG5 and ABCG8 should have useful effects on atherosclerosis (Davis, 2001). Treatment with flaxseed significantly reduces lowers total cholesterol probably with inhibition of cholesterol absorption, and also may inhibit the development and progression of atherosclerosis in diabetic rats.

Flaxseed is rich in alpha-linolenic acid (ALA), lignans, phytoestrogen, soluble fiber, and these substances are likely antioxidant and hypolipidemic effects. Thus, intake of flaxseed in diabetic rats enhanced antioxidant free radical scavenging capacity and significantly reduced lipid profiles. On the other hand, flaxseed with reduction of NPC1L1 and raise of ABCG5, ABCG8 and LXRα significantly reduced plasma LDL-C and total cholesterol. This research provides a rationale to examine these effects in diabetic patient.

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