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Original Article

STUDY OF ARACHIDONIC ACID RELEASING STATUS IN DIABETIC RATS TREATED WITH FLAXSEED OIL

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ABSTRACT

Objective: Diabetes mellitus increased inflammation through stimulation of arachidonic acid liberation from the cell membranes which is transformed into actively cellular mediators such as thromboxanes, prostaglandins, and leukotrienes. So, this study aimed to investigate the effect of flaxseed oil as a source of omega 3 fatty acids on releasing of arachidonic acid from erythrocyte membrane and also on possible reduction of hyperhomocysteinemia during experimental diabetes.

Methods: Forty male albino rats were used in this study and divided into four groups: control, diabetic, flaxseed oil and treated groups. After the experimental period, blood samples were collected to estimate different biochemical parameters in plasma and in the erythrocyte membrane.

Results: The data showed the elevation of membrane arachidonic acid along with the elevation of inflammatory and oxidative stress markers in the diabetic group while flaxseed oil supplementation significantly decreased these levels.

Conclusion: We concluded that flaxseed oil is a promising supplement during diabetes mellitus due to its powerful effect in reducing arachidonic acid release and inhibition of hyperhomocysteinemia.

Keywords: flaxseed oil, Arachidonic acid, Hyperhomocysteinemia, Cell membrane, HPLC

INTRODUCTION

During diabetes, hyperglycemia causes elevation of free radicals production such as reactive oxygen species (ROS) for all tissues from protein glycosylation and glucose auto-oxidation. The high levels of free radicals and decline of antioxidant systems can lead to the damage of enzymes and cellular organelles, increased lipid peroxidation, and development of diabetes mellitus [1].

In addition, Diabetes mellitus increased inflammation through stimulation of arachidonic acid releasing from the cell membranes which is transformed into actively cellular mediators such as thromboxanes, prostaglandins, and leukotrienes [2] in addition to elevation of homocysteine (Hcy) level. Hcy may be a good biomarker for increased risk of diabetes complications. Since retinopathy, nephropathy, neuropathy and cardiovascular disease have all been linked to higher Hcy levels [3].

Hyperhomocysteinemia has been documented in patients with vascular disorders; also increased homocysteine level is a well-defined risk factor of thrombosis and atherosclerosis [4]. Several studies indicated that omega-3 fatty acids can reduce inflammatory process activity [5].

Since natural products have played an important role throughout the world in both treatment and prevention of human from several diseases and flaxseed oil is documented as the best plant that contain high amount of polyunsaturated fatty acids especially omega-3 [6], so this study aimed to investigate the effect of flaxseed oil as a source of omega 3 fatty acids on releasing of arachidonic acid from erythrocyte membrane and also on possible reduction of hyperhomocysteinemia during experimental diabetes.

MATERIALS AND METHODS

Materials

The following chemicals were purchased from Sigma Chemicals Co. (Munih,Germany).

- Streptozotosin (STZ) (CAS Number: 18883-66-4).
- Homocysteine (Hcy) (CAS Number: 454-29-5).
- α–linolenic acid (ALA) (CAS Number 506-26-3).
- Arachidonic acid (AA) (CAS Number: 506-32-1).

Plant

Flaxseed oil was purchased from the local market.

Experimental animals

Forty male albino rats (180±10 g) were used in this study. Rats were obtained from the animal house of the National Research Centre (NRC) (Cairo, Egypt) and they were housed in stainless steel cages under controlled conditions. The temperature was 23-26 °C and the light/dark cycle was 12/12 h. The animals had free access to water and a standard rodent chow diet. All animals received human care in compliance with guidelines of the Ethical Committee of National Research Centre (NRC), Egypt and followed the National Institutes of Health Guide Recommendations Care and Use of Laboratory Animals. The number of ethical committee permission is (10/219).

Methods

Induction of diabetes mellitus

STZ was dissolved in sodium citrate (50 mM and PH was adjusted to 4.5) solution containing NaCl (150 mM). The solution (6.0 mg/0.5 ml/100g body weight) was subcutaneously injected into rats of group 3 and 4; after 3 d, fasting blood sugar was estimated to confirm the development of diabetes mellitus [7].

Experimental design

Forty male albino rats were used in this study and divided into four groups as follow:

Group I (control group): healthy rats received 1.2 ml corn oil.

Group II (flaxseed oil group): healthy rats received 1.2 ml flaxseed oil/kg b.w./day orally.

Group III (diabetic group): diabetic rats received 1.2 ml corn oil.

Group IV (treated group): diabetic rats received 1.2 ml flaxseed oil/kg b.w./day orally for 8 w [6].

After the experimental period, animals were kept fasting for 12 h before blood sampling, blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes and collected in heparinized tubes for biochemical parameters estimations. Blood was centrifuged at 2000 rpm for 10 min using cooling centrifuge. Plasma was separated; fasting blood glucose was estimated immediately using enzymatic colorimetric method. Centronic, Germany [8]. The rest of plasma was frozen. Packed RBCs were used for isolation of erythrocyte membrane.

The method used for erythrocyte ghost preparation was based on the hemolysis of RBCs in hypotonic solution for removal of hemoglobin [9].

Reduced glutathione (GSH) and malondialdehyde (MDA) were estimated in erythrocyte membrane according to the methods described before [10, 11] respectively.

Plasma insulin level was estimated by ELISA [12] using Bio Soure INS-EASIA Kit.

Erythrocyte membrane lipids were extracted with chloroform: methanol method modified from the method described previously [13].

Estimation of arachidonic and α–linolenic acids by HPLC

Erythrocyte membrane fatty acids were estimated by HPLC, Agilent technologies 1100 series, equipped with a quaternary pump (Quat. pump, G131A model).

Sample preparation

Erythrocyte membrane was homogenized in acetic acid (2 %): ethyl ether mixture (2:1) v/v. The solution was centrifuged at 3000 rpm, the organic phase was evaporated to dryness. The extract was then dissolved in 400 µl acetonitrile and filtered through hydrophilic PVDF 0.45 µ m.

HPLC condition

This method was carried out by reversed phase HPLC column (260 X 4.6, particle size 5µl) according to the method described previously [6], mobile phase was acetonitrile/water mixture (70/30) v/v by isocratic elution with flow rate 1 ml/min and UV detector was set at 200 nm. Serial dilutions of standards were injected onto HPLC and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the standard curve.

Determination of plasma homocysteine (hcy)

Homocysteine was estimated by high performance liquid chromatography (HPLC) according to the method described previously [14].

Sample extraction

200 µl plasma were treated with 16 µl TCA, mixed well and incubated in ice for 30 min to precipitate protein. After centrifugation for 20 min at 4000 rpm at 4 °C, supernatants were filtered through hydrophilic PVDF 0.45 µ m.

HPLC condition

30 µl from the solution were injected in HPLC; separation was achieved on reversed phase column (C18, 25, 0.46 cm i.d. 5 µ m). The mobile phase consisted of 40 mmol/l sodium phosphate monobasic monohydrate; 8 mmol/l heptane sulfonic acid and 18% (v/v) methanol adjusted to pH 3.1 by addition of phosphoric acid and filtered through a 0.45-µm membrane filter and was delivered at a flow rate of 1 ml/min at 40 °C. UV detector was performed at 260 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentrations in samples were obtained from the standard curve.

Statistical analysis

Results were expressed as mean±standard error. Data were analyzed by independent sample t test (SPSS) version 15 followed by (LSD) test to compare significance between groups. Difference was considered significant when P value<0.05.

RESULTS AND DISCUSSION

During diabetes, hyperglycemia causes elevation of reactive oxygen species (ROS) and increased inflammation through the stimulation of arachidonic acid releasing from the cell membrane. In this study, STZ-induced experimental diabetes significantly decreased erythrocyte membrane antioxidant enzyme (SOD) concomitant with an elevation of erythrocyte membrane MDA (table 2) in addition to the increase of inflammatory markers that increased through the releasing of arachidonic acid from the cell membrane. Thus in this study, homocysteine, IL-1 α, and TNF-α were increased (table3) concomitant with an elevation of erythrocyte membrane arachidonic acid and reduction of erythrocyte membrane α-Linolenic acid in diabetic group (table 4).

Table 1: Fasting blood glucose and insulin levels in different studied groups

Groups	Glucose (mg/dl)	Insulin (µIU/ml)
Control group	77.67±1.1	11.3±0.9
Flaxseed oil group	78.49±1.7 ♦	11.8±1.4
Diabetic group	252.66±2.2♥	8.6±1.3
Treated group	172.70±1.6♥♦	9.9±1.8

Results are expressed as mean±SE, n=10, ♥ significant difference compared to control group, ♦ Significant difference compared to diabetic group.

Table 2: Erythrocyte membrane oxidative stress parameters in different studied groups

Groups	MDA (nmol/ml RBCs)	SOD (U/ml RBCs)
Control group	0.89±0.18	466.5±2.3
Flaxseed oil group	0.88±0.20♦	489.7±3.1♦
Diabetic group	3.45±0.24♥	246.0±1.8♥
Treated group	2.53±0.19♥,♦	382.5±1.9♥,♦

Results are expressed as mean±SE, n=10, ♥ significant difference compared to control group, ♦ Significant difference compared to diabetic group.

Table 3: Inflammatory markers in different studied groups

Groups	Homocysteine µ mol/l	IL-1α Pg/ml	TNF-α Pg/ml
Control group	4.6±1.27	19±1.1	40.6±1.7
Flaxseed oil group	3.8±0.99♦	20.4±1.4♦	41.2±1.6♦
Diabetic group	22.7±1.20♥	42±1.4♥	66.9±2.0♥
Treated group	12.5±1.32♥,♦	27±1.6♦	47.7±1.8♦

Results are expressed as mean±SE, n=10, ♥ significant difference compared to control group, ♦ Significant difference compared to diabetic group.

Arguably; the most important polyunsaturated fatty acids in the cell membrane is the omega-6 family member AA. When the cells are activated by internal or external stimuli, arachidonic acid is immediately released from cell membranes and transformed into cellular mediators such as thromboxanes, prostaglandins and leukotrienes [15] hydroxy fatty acids, and lipoxins [16]. These mediators possess a range of activities, including activation of leukocytes and platelets, regulation of gastric secretions, induction of bronchoconstriction and signaling of pain in nerve cells and inflammation [17].

So, arachidonic acid metabolism is considered the target of non steroidal anti-inflammatory drugs (e. g., acetylsalicylic acid and ibuprofen), cyclooxygenase-2 (COX-2) inhibitors (e. g., rofecoxib and celecoxib) and leukotriene antagonists (e. g., montelukast and zafirlukast [18].

Table 4: Erythrocyte membrane α-Linolenic and arachidonic acids in different studied groups

Groups	α-Linolenic acid (mg/ml RBCs)	Arachidonic acid (mg/ml RBCs)
Control group	0.82±0.03	0.05±0.01
Flaxseed oil group	0.91±0.12 ♦	0.04±0.02♦
Diabetic group	0.41±0.13 ▼	0.11±0.02▼
Treated group	0.81±0.31 ♦	0.07±0.03♦

Results are expressed as mean±SE, n=10, ▼ significant difference compared to control group, ♦ Significant difference compared to the diabetic group.

Diet-induced changes in the polyunsaturated fatty acid composition of cell membranes have an important role in the cell's functions and activities, partially because these fatty acids represent a reservoir of molecules that perform important signaling or communication roles within and between cells.

In this study, supplementation of flaxseed oil to diabetic rats significantly decreased lipid peroxidation (MDA) in addition to antioxidant enzyme (SOD) elevation (table 2), Also decreased inflammatory markers (table 3).

Administration of flaxseed oil in this study as a source of omega-3 fatty acids (mainly α-linolenic acid) enhanced omega 3 fatty acids to compete with omega-6 family for incorporation into cell membranes [19, 20] resulting in a reduction of arachidonic acid liberation from the cell membrane as was found in our study, thus flaxseed oil supplementation significantly decreased arachidonic acid level and increased α-Linolenic acid in the cell membrane (table 4).

The reduction of arachidonic acid lead to a reduction of reactive oxygen species (ROS) and increasing SOD level in addition to the reduction of inflammatory markers. In the same line, the current data indicated a reduction of inflammatory markers in flaxseed oil treated group (table 2, 3) especially Hcy, the risk factor of diabetes complication.

CONCLUSION

In conclusion, these data proofed the potential effect of flaxseed oil as a plant source of omega 3 fatty acids in reducing inflammation as well as oxidative stress. The anti-inflammatory and antioxidant properties of flaxseed oil are related to its incorporation into the cell membranes and reducing arachidonic acid and homocysteine levels.

CONFLICT OF INTERESTS

Declare none

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