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Original Article

Flaxseed oil ameliorated the expression level of the IL-1β/NLRP3/NRF-1/Sirt-1of diabetic heart conditions

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Abstract

Background: Inflammation and cytokine storms due to cardiovascular diseases (CD). Herbals can be the primary source of compounds with anti-inflammatory properties. Furthermore, herbals agents may consider as a complementary medicine. Bioactive compounds and phytochemicals have potential strategies to halt or manage CD. Flaxseed have antioxidant and anti-inflammatory activity.

Method: In this study, we used H9c2 cell lines of rat embryonic cardiomyocytes. To induce CD we used hyperglycemic and hyperlipidemic conditions. Real-Time-PCR performed to evaluated the relative expression of the genes.

Results: We found that flaxseed could decreased the expression level of the IL-1β and NLRP3. Moreover, the expression level of the NRF-1 and Sirt-1 increased by flaxseed.

Conclusion: Our data proposed that flaxseed could have potential approaches to manage and prevent CD.

Keywords: cardiovascular diseases, Flaxseed, H9c2, genes

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Introduction

Cardiovascular diseases (CD) could induce hypertension and vasodilation. (1). Moreover, immense epidemiology studies have indicated that CD is the primary cause of mortality (2). Several signaling pathways could modulate heart function, leading to the progression of new therapeutic strategies (3-6). Increasing numbers of deaths and diseases are currently ascribed to chronic inflammation. The inflammatory process could stimulate the response to external and endogenous factors such as tissue damage, traumas, and disease and may thus play an essential part in tissue repair (7, 8). Proinflammatory cytokines, including NLR family pyrin domain containing 3 (NLRP3) and interleukin one beta (IL-1β), macrophage buildup, and their involvement in this process (9).

Oxidative stress, apoptosis, and inflammation have been related to the genesis of heart failure, resulting in dysregulated cardiac tissue pathophysiology. Hyperglycemia and hyperlipidemia may enhance oxidative stress and inflammation, contributing to myocardial dysfunction, mitochondrial dysfunction, and the loss of cardiac stem cells. (9). At the molecular level, the expression or activity of various proteins and genes involved in maintaining or regulating intracellular homeostasis may also play a role in these modifications. (10). Several signaling pathways and genes implicating environmental variables in the etiology of heart failure have been identified in heart failure. (9). As an important target in oxidative heart stress and inflammation processes, the NLRP3-IL-1β-Sirt-1-NRF-1 pathway has been recognized as a vital gene in the molecular mechanism of diabetic heart disease.

Flaxseed may be an effective treatment for a variety of illnesses and diseases. (11). The majority of studies revealed that flaxseed and flaxseed oil diets could attenuate hyperglycemia in type 2 diabetes (T2D) and improve cardiovascular disease (CVD) symptoms.

We intend to reveal the effect of flaxseed in adherent H9c2 cell lines of rat embryonic cardiomyocytes.

Material and methods

2.1. Preparation of flax seed and oil extraction

The acquisition of brown flaxseeds (Linum Usitatissim L.) from Isfahan medicinal plants, Iran. The flaxseeds were washed and ground in a room-temperature electric mill. Then, 50 grams of flaxseed powder was extracted with 500 milliliters of hexane. The defatted meal was extracted using 200 ml of 100% methanol, 100% ethanol, and 80% ethanol after 12

hours of evaporation at room temperature in a fume hood. The replication extraction was completed in 12 hours at room temperature with a 750 rpm magnetic stirrer. Using Whatman No. 1 filter paper, the recovered supernatant solvent was separated from the sediment. Using a rotary evaporator, the extracts were concentrated to dryness at 45°C and under a vacuum. We determined the yield by weighing the extracts before dissolving them in a small volume of the initial solvent and storing them at -18°C.

Cell culture

In this investigation, adherent H9c2 rat embryonic cardiomyocyte cell lines were utilized (ATCC, Manassas, VA, USA). To prevent contamination, H9c2 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Capricorn Scientific DMEM-LPXA) supplemented with 10% fetal bovine serum (FBS) (Capricorn Scientific FBS-11A) at 37 °C and 5% CO2. To avoid contamination, 100 U/mL penicillin and 100 g/mL streptomycin were added. A concentration of 1106 cells/mL was utilized and distributed across 10 5-mL-volume Petri plates for cell culture. Several vials contained 10 to 12 cell passages, and cells were examined at 70% confluence. Control cells were grown in DMEM with a glucose concentration of 5.5 mM, whereas hyperglycemic cells were cultured in DMEM with a glucose concentration of 50 mM. (G 25 mM and G 33 mM). As a hyperlipidemic medium, 500 M Palmitic Acid (PA, PO500 Sigma-Aldrich) was coupled with 1% bovine serum albumin (BSA, A3675 Sigma-Aldrich). Mixing 0.5 mL of a 100 mM PA solution in 0.1 M NaOH with 9.5 mL of a 10.5 m/v BSA solution in distilled water yielded a five mM PA/10% BSA stock solution. The mediums of the hyperglycemic (25 mM or 33 mM glucose) and hyperlipidemic (25 mM or 33 mM glucose) were produced by adding 1/10 of the stock solution 5 mM PA/%10 BSA to reach a final concentration of 500 M/%1 BSA.

Relative Expression assay

Trizol was used to isolate total RNA from the H9c2 cell line of rat embryonic cardiomyocytes using RNase-free procedures (Thermo Scientific, USA). In this investigation, we employed the DNase1 endonuclease according to the DNase I Treatment Kit (TaKaRa, Japan) methodology to boost the purity of extracted total RNA samples and eliminate DNA. cDNAs were also generated using reverse transcriptase (RT) enzyme (TaKaRa, Kusatsu, Shiga Prefecture, Japan). To determine the relative expression of genes

in Mortlake, Australia, we utilized SYBR Green dye (TaKaRa, Kusatsu, Japan) and quantitative real-time PCR (Rota-Gene 6000 apparatus, Corbett Life Science). As a reference gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utilized to determine the normalization of gene expression data. This work calculated the fold-change analysis of mRNA expression using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

We used GraphPad Prism Software (Version 9, Graph Pad Software Inc., La Jolla, CA) for statistical analysis. In addition, the data were assessed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to control for multiple comparisons. In all analyses, differences were judged significant at p0.05. Moreover, data are presented using the mean and standard deviation (SD).

Result:

Flaxseed improved the relative expression of the NLRP3-IL-1β-Sirt-1-NRF-1 pathway

In the H9c2 line's hyperglycemic and hyperlipidemic circumstances, the expression levels of Il-1β and NLRP3 were significantly higher than in the Control group. (Figure1 a and b). NRF-1 and Sirt-1 levels were lower in the H9c2 line with hyperlipidemia and hyperglycemia compared to the Control group. (Figure1 c and d). We discovered that flaxseed had anti-inflammatory and antioxidant characteristics in our study. The 24 h treatment with flaxseed lowered the relative expression of Il-1 and NLRP3 in the hyperlipidemic and hyperglycemic H9c2 line exposed to flaxseed, compared to the CD group. Furthermore, in the HF+ flaxseed group, the level of NRF-1 Sirt-1and expression was much higher than in the HF group.

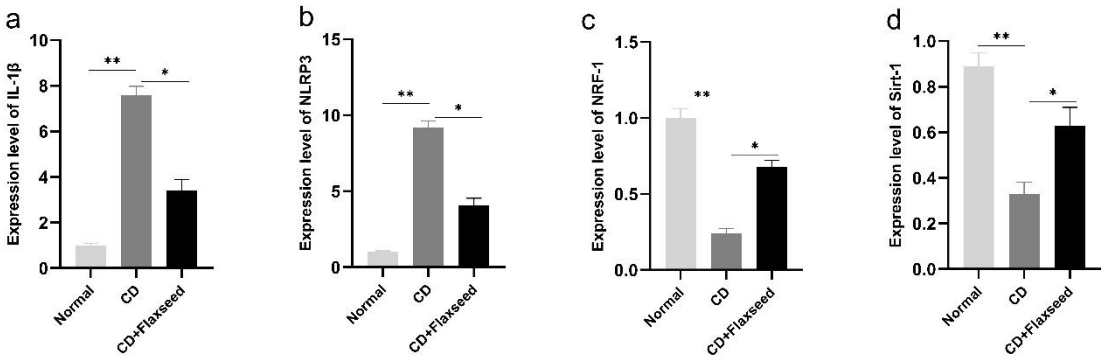

Figure (1) Flaxseed modulated the expression level of the NLRP3-IL-1-Sirt-1-NRF-1. (a-d) The expression level of the NLRP3-IL-1-Sirt-1-NRF-1.

Discussion

Based on the expression of the hub gene, biological processes might detect cardiovascular disease (CD) relative to healthy physiological circumstances. Analysis of bioinformatics data indicated that the NLRP3-IL-1-Sirt-1-NRF-1 genes are major hub genes with differential expression levels in CD situations vs. normal states. Evidence indicated the effect of flaxseed on the Hypercholesterolemic indices and inflammatory marker TNF- in a rabbit model of atherosclerosis with hypercholesterolemia. Consuming 8 g/kg BW of crude flaxseed for 12 weeks has been shown to reduce atherosclerotic factors, lipid indices, and inflammation (12). A comprehensive and meta-analysis research revealed that flaxseed supplementation might reduce circulating CRP, VCAM-1, and IL-6 in adults (13). SIRT1 promotes deacetylation and significantly affects the function of many proteins, including those involved in oxidative stress, metabolism, cell proliferation, and genomic integrity. Regulatory processes are implicated in the association between gene expression and translation and metabolic disorders, cardiovascular illnesses, malignancies, neurodegenerative diseases, and aging. According to Zhao Zhong Chong et al., SIRT1 might offer new avenues for the treatment of cardiovascular disease and suggest the possibility of protection in the etiology (14). Another study discovered that NRF-1 is a master transcription factor found in most tissues and plays a crucial role in amplifying antioxidant pathways associated with enzymes produced in heart tissue (15).

Chronic inflammation may progress to rheumatoid arthritis, diabetes, cardiovascular and neurological problems, and asthma if the inflammatory response is not controlled appropriately. In addition, the computational system biology test identified NLRP3-IL-1-Sirt-1-NRF-1 as a druggable candidate protein, and enrichment of hub genes suggested that SIRT1 and NRF-1 may function as signaling pathway initiating factors. Alternatively, the enrichment of hub genes indicated the major molecular signaling pathways implicated in diabetic cardiomyopathies, including TNF- signaling, inflammatory response, IL-6/JAK/STAT signaling, Wnt/B-catenin signaling, and apoptosis. Due to CD being the most frequent noncommunicable disorder with a high mortality rate worldwide, numerous therapeutic techniques for the prevention and treatment of cardiovascular disease hallmarks were developed. Combining complementary medicine with commercial/conventional pharmaceutical medication may impact the treatment of cardiovascular disorders. Herbal medicine is one of the most often utilized forms of complementary and alternative medicine.

It is commonly believed that flaxseed is a healthful food having anti-tumor, anti-inflammatory, anti-thrombotic, anti-diabetic, and cardioprotective properties (11, 16-18). Flaxseed as an oilseed contains several bioactive compounds, including lignans, linolenic acid, linoleic acid, essential amino acids, cyclopeptide, cyanogenic glycosides, alkaloids, and poly-carbohydrates, according to the results of prior chemical studies. In light of this, we investigated the anti-inflammatory and antioxidant potential of flaxseed bio compounds as a new perspective on complementary and alternative medicine strategies in an in-vitro model of adherent H9c2 cell line of rat embryonic cardiomyocytes exposed to a low-grade inflammation scenario. In addition, our experimental findings suggested that the flaxseed compound might enhance the function and myogenesis of H9c2 cardiac cells under hyperlipidemic and hyperglycemic circumstances. Researchers might utilize these findings to direct future studies into developing flaxseed-based anti-inflammatory and antioxidant medicines. To evaluate the anti-inflammatory potential of phytochemicals, an adherent H9c2 cell line of rat embryonic cardiomyocytes was employed to imitate the inflammatory, and oxidative milieu observed in clinically important chronic inflammatory diseases. Based on in-vitro modeling, we exposed H9c2 cardiac cells to hyperglycemic and hyperlipidemic conditions to assess the effect of flaxseed on inflammation, antioxidant status, function, and myogenesis.

Conclusion

Experimental findings indicated that flaxseed phytochemicals can be used to treat, prevent, and manage inflammation and related illnesses.

Declarations

-Ethics approval

Not applicable

-Consent for publication

Not applicable.

-Availability of data and materials

The data and materials supporting this study's findings are available from the corresponding author upon reasonable request.

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