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Jilan A. Nazeam *

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Research Article

Linum Usitatissimum Oil Fraction Reverse Cardiac Remodeling at Molecular Level: Suppressing Mirna-29b And Mirna 1 Genes in Isoproterenol in Vivo Model

Sylvia A Boshra, Jilan A. Nazeam*

October 6 University, 6th of October City, Egypt.

***Corresponding Author:** Jilan A. Nazeam, October 6 University, 6th of October City, Egypt.

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Abstract

Linum usitatissimum (flaxseed) produce one of the oldest commercial oils which use traditionally as a functional food for lowering cholesterol level. Nevertheless, to date, there is no scientific evidence to assess the role of flaxseed oil in cardiac remodeling management. The study aimed to clarifying the underlying mechanism of standardized oil to restore cardiac remodeling in a heart toxicity rat model induced by isoproterenol (ISO). Oil fraction was purified, and major components were identified by gas-chromatography-mass spectrometry (GC-MS). The in vivo tests were conducted by ISO (85 mg/kg/ twice subcutaneously) with 24 hours between each dose. The rats were treated with flaxseed oil fraction (100 mg/kg orally) and the same dose was used for omega 3 as a positive control group. GC- MS revealed that α-linolenic acid (24.6%), oleic acid (10.5%), 6-octadecenoic acid (Z), 2,3 dihydroxypropyl ester (9.0%), 2,3-dihydroxypropyl elaidate (7.0%), n-propyl 9,12,15-octadecatrienoate (6.0%) are the major components. After 4 weeks of oil uptake, the results revealed an improvement in cardiac function, a decrease in apoptosis, and simultaneous prevention of myocardial fibrosis. The levels of BNP, NT-pro-BNP, endothelin-1, Lp-PLA2, and MMP2, and cTnI and cTn were significantly decreased, and a higher plasma level of Topo 2B was observed, moreover, miRNA − 1 and 29b were downregulated. Current evidence provide insight into the mechanism of flaxseed oil to restore cardiac remodeling, which supports its future application as cardioprotective against heart diseases.

Keywords: flaxseed oil; miRNA-1; ISO; CVD; MMP9; CTNI

Introduction

Cardiovascular disease (CVDs) is a leading cause of disability and premature mortality throughout the world [1]. The prevalent case of total CVDs was shifted from 271 million cases in 1990 to 523 million cases in 2019. The global trend of years lived with a disability doubled from 17.7 million to 34.4 million over that period [2]. According to WHO estimates, 17.9 million people died from CVDs in 2019, accounting for 32% of all fatalities. Heart attack and stroke deaths accounted for 85% of these fatalities. Hence, there is an urgent need to focus on adopting existing low-cost public health programs to promote healthy aging throughout the lifetime and minimize disability and premature death from CVD [3].

Frequently cardiovascular remodeling leads to myocardial fibrosis, which can cause heart failure disease (HFD) [4]. Myocardial fibrosis is brought on by elevated myofibroblast activity and increased extracellular matrix deposition. Various cells and substances are involved in this process, and they may serve as targets for potential future medicinal therapies [5]. Proptosis is a kind of inflammation with programmed cell death, it causes cell expansion, rupture of the plasma membrane, the release of cell contents, and sends pro-inflammatory signals to adjacent cells which promote inflammatory responses [6]. According to reported literature, proptosis can cause cardiac remodeling and myocardial dysfunction. Therefore, targeting proptosis has a good prospect of improving cardiac remodeling in HFD [7].

Linum usitatissimum L. (flaxseed or linseed) reaches back to prehistoric times that directly refers to historical significance and wide applications, the generic name Linum derives from the Celtic word line, which means thread, and the species name usitatissimum, means very useful [8, 9]. The geographical origin of the plant has been attributed to the Mediterranean and Southwest Asia [10]. Flaxseed is one of the oldest crops, that has been cultivated in Egypt and Samaria since at least 5000 BC, and the ancient Egyptians utilized plant in medicine and food [11]. The flax therapeutic properties are observed in Hippocrates, Qantas, and Discords works [12].

According to archeological evidence, flax was initially used for fiber and continues to be widely grown for oil [13]. The flax-based products represent the highest potential for growth in the functional food business [14]. Since it has been reported to assist in improving human health and alleviating

symptoms associated with a wide range of human ailments, such as cardiovascular, gastrointestinal, and neural disorders [15, 16]. The value of global linseed production represented 1.8 and 8.7 million tons, worldwide in 2011 and 2016 [17]. https://www.statista.com/statistics/916996/linseed-production-global/.

Flaxseed is the richest source of essential omega-3-fatty acid; α-linolenic acid (ALA, 57%) and it is gaining recognition as a functional food [18, 19]. Previous reports indicated that flaxseed supplements can suppress atherosclerosis and have a hypo cholesterol emic effect [20, 21].

The Food Directorate of Health Canada found that a claim relating the intake of ground whole flaxseed with blood cholesterol reduction was acceptable based on the weight of evidence from human clinical studies available prior to 2011 [22]. Hence, clinical studies have revealed that flaxseed can lower serum total and low-density lipoprotein cholesterol, inhibit inflammation markers, and raise serum levels of eicosatetraenoic acid [23]. Flaxseed fiber was also added to bread to lower cholesterol in diabetic patients [24]. Moreover, in young healthy adults, the plasma LDL cholesterol was lowered by 8% after the consumption of 50 g flaxseed/day for four weeks [25].

Flaxseed oil increasing demand in traditional consumption as a value-added functional food for lowering cholesterol level [26]. In a clinical trial for 50 hypercholesterolemic men, the C-reactive protein and serum amyloid A levels were reduced after daily consumption of one tablespoon of flax oil for 12 weeks [27, 28]. A clinical trial study for 30 healthy adults taking flaxseed oil implied that ALA could activate endogenous neuroprotective and neurorestorative pathways in 30 healthy adults taking flaxseed oil (ALA for 500 mg/day) [29].

The raw oil is used traditionally as insecticidal and astringent [30]. It has been aborted significant therapeutic antiulcer [31], antiarthritic [32], and anti-inflammatory [33], antidiabetic [34] properties along with anti-esophagitis effect [35]. By detecting the therapeutic effect of flaxseed oil, it inhibited fat accumulation and inflammation response in HFD-induced Δ-6 desaturase knock-out mice. Also, suppression of inflammatory factors; TNF, IL-6, MCP-1, VCAM-1, and NADPH oxidase were observable. According to previous study, the flaxseed oil regulated the lipid metabolism-related genes (HMGCR, PPARα, and SREBPs) and improved atherosclerosis in HFD-induced rats [36].

Flaxseed oil restore autophagic flux and inhibited the production of mitochondrial reactive oxygen species in spontaneously hypertensive and Ang II-induced hypertensive rats [37]. The oil with a concentration of 35% ALA prevented hepatic steatosis in ethanol-induced mice by suppressing fatty acid uptake and triglyceride synthesis in the liver and promoting the expression of adiponectin and AdipoR2 [38].

The research on the impact of flaxseed oil on the health-related efficacy against cardiac disorder and the action mechanism is still debated. A previous report has also suggested that more human trials are needed to confirm the protective role of flaxseed products against coronary artery disease [39].

Before extensive work can be undertaken in clinical populations, the cardioprotective molecular mechanism and genetic modulation effect of flaxseed oil need to be assessed. The current study advocates a potential pleiotropic effect of flaxseed oil beyond the more conventional cholesterol-lowering actions of most cardiovascular agents. The study aimed to investigate the effect of flax oil to restore cardiac remodeling as well as identify the related pharmacological mechanism, to verify the popular and clinical claim of its use against cardiac disorder.

Materials and Methods

Extraction and fractionation of flaxseed oil

The flaxseed (500 g) was grounded to about 0.8 mm particle size (18–20 mesh) and macerated with dichloromethane: methanol (1:1) for 72 hours. The filtrate was concentrated using a rotary evaporator and 250 ml oil was collected and dried over anhydrous sodium sulfate. The fixed oil was fractionated over the Diaion column (5×50 cm) and the methanol fraction was transferred to amber-colored vials, sealed, and stored in a refrigerator until required.

Analysis of essential oils (EOs)

The fixed oil fraction was analyzed by GC-MS (Shimadzu GCMS-QP 2010, Koyoto, Japan) equipped with Rtx-5MS capillary column (30 m length × 0.25 mm i.d. × 0.25 µm film thickness) (Restek, Bellefonte, PA, USA). The oven temperature was kept at 50 ◦C for 2 min (isothermal) and programmed to 300 ◦C at 5 ◦C/min and kept constant at 300 ◦C for 10 min (isothermal); the injector temperature was 280 ◦C. Helium was used as a carrier gas with a constant flow rate set at 1.37 mL/min. Diluted samples (1% v/v) were injected with a split ratio of 30:1, and the injected volume was 1 µL. Ion source temperature: 300 ◦C; EI mode: 70 eV; scan range: 35–500 amu. Each sample was analyzed in triplicate. The mass spectrum of every chemical constituent was compared with the corresponding reported spectrum (in NIST, Wiley Mass Spectral Database − 1995, and ADAMS-2007 libraries) for GC-MS and published references. Identification of compounds was also confirmed by comparing their retention indices (RI) relative to n-alkanes (C8-C20) with reported values in the literature including Adamís library.

Animal treatments and experiments

Forty mature albino rats, each weighing 160 ± 10 g, were provided from Cairo University, National cancer institute. Animals were housed in polypropylene cages with a natural light-dark cycle and humidity levels that were set to industry standards. Animals were given unlimited access to regular pellets and water. As shown in Table 1, the rats were divided into four groups of ten rats each at random.

	Group	Treatment description
(I)	Normal control	A typical diet over the period of four weeks
(II)	ISO	ISO (85 mg/kg) on day 29 and 30
(III)	ISO + Flaxseed	ISO (85 mg/kg) on day 29 and 30 and oil (100 mg/kg) orally for 30 days
(IV)	ISO + Omega 3	ISO (85 mg/kg) on day 29 and 30 and Omega 3 (100 mg/kg) orally for 30 days

Table 1: Experimental design of treated groups.

According to Gorriti et al., the LD50 of flaxseed oil is above 37 g/kg of body weight, 100 mg/kg was used as flaxseed dose (3×10− 3 of lethal dose) for in vivo model, the same dose was used for omega 3 as standard drug [40]. The fasted rats were decapitated 24 hours after the last ISO doses, and blood was obtained using sodium fluoride as an anticoagulant. Fresh plasma was then used to estimate BNP, NT-pro-BNP, CTNI, and CTNT according to the kit manufacturer's instructions (Abcam, Cambridge, UK).

Endothelin-1 and Topo 2B were measured using rat ELISA kits in accordance with the manufacturer's instructions from BG Medicine, Waltham, Massachusetts, and Jiangsu Microplate Biotechnology Company in Jiangsu, China, respectively. According to ELISA techniques Kangerke

Biotech Co., Ltd., Tianjin, China, and Immunoassay, Atlanta, GA30338, USA, respectively, at 450 nm, plasma levels of Lp-PLA2 and MMP9 were estimated.

Quantitative real-time PCR

Following the manufacturer's recommendations, total RNA was isolated from cardiac tissues using a Sepasol-RNA1Super (Nakarai Tesque), and sections (10–15 g) of the obtained RNA were subjected to real-time quantitative PCR testing. A two-step RT-PCR was used to quantify gene expression. Quantitative real-time PCR was used to measure the levels of miRNA-1 and miRNA29b.PCR buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, and 0.4 M of specific primers made up the PCR reaction mixture (Table 2). In 50 ml of the single-plex reaction mixture, assays were carried out. Forty cycles of 95 oC for 15 s and 60 o C for 1 min each made up the reaction conditions, which also included a pre-incubation at 50 o C for 2 min and 95 o C for 10 min. The measurements were automatically taken down. Data from quantitative RT-PCR are displayed as a percentage of the control. The internal check was done using U6 mRNA.

Gene	Primer sequence	Amplicon Size
miRNA1	F: 5′-ACACAGAGAGGGCTCCGGCA-3′ R:5′-ACACGACCGTCCACCAACGC-3′	342 bp
miRNA 29b	F: 5′- -GCT GAG TGTGGCATCCATTT-3 R: 5′- CCACTTCACAAAGCTTTGCAC − 3	141bp
U6 (internal control for qRT-PCR)	F: ‘5- GCTTCGGCAGCACATATACTAAAAT − 3’R: 5′- CGCTTCACGAATTTGCGTGTCAT − 3’.	84 bp

Table 2: Primers used in real-time PCR.

Statistical analysis

Ten independent determinations for spectrophotometric and ELISA measurements, three separate determinations for analysis of gene expression, and the obtained data were expressed as mean SD. One-way analysis of variance (ANOVA) and the Bonferroni multiple comparison tests were both used by SPSS/20 Software to evaluate the data. Statistics were considered significant when P 0.01 was present.

Results

Chemical composition of extracted oil

Characterization and standardization of purified oil fraction by GC-MS analysis revealed the presence of 83 compounds (Table 3), the major constituents were 9,12,15-octadecatrienoic acid, (Z, Z, Z)- (24.69%), oleic acid (10.57%), 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (9.04%), 2,3-dihydroxypropyl elaidate (7.05%), n-propyl 9,12,15-octadecatrienoate (6.05%), 9-octadecenoic acid, methyl ester (E) (4.45%), and n-hexadecanoic acid (4.02%).

Peak	R. Time	Area%	Name	Base m/z
1	6.246	0.01	Ethanol, 2-butoxy-	57.10
2	9.019	0.02	Furan, 2-pentyl-	81.05
3	9.237	0.02	3-Methyl-but-2-enoic acid, 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl ester	83.10
4	13.112	0.01	Octanoic acid, methyl ester	74.10
5	18.459	0.01	Rhodium, [1,2-bis(.eta.2-ethenyl)-4-ethenylcyclohexane]di-.mu.-chlorodi-	121.10
6	18.523	0.02	Furan, 2-hexyl-	81.05
7	20.894	0.01	n-Caprylic acid isobutyl ester	127.15
8	21.471	0.03	Nonanoic acid, 9-oxo-, methyl ester	111.15
9	22.743	0.07	Cycloheptanone, 3-butyl-	111.15
10	24.584	0.02	Nonanedioic acid, dimethyl ester	111.15
11	25.555	0.07	Nonanedioic acid, dimethyl ester	111.15
12	26.557	0.03	Imidazole-5-pentanoic acid	95.10
13	27.449	0.05	2-n-Heptylfuran	81.05
14	28.156	0.05	Azelaaldehydic acid, butyl ester	109.15
15	29.276	0.01	Heptadecanoic acid, 16-methyl-, methyl ester	87.10
16	30.011	0.02	Tetradecanoic acid	129.15

Peak	R. Time	Area%	Name	Base m/z
17	30.748	0.02	Nonanedioic acid, dimethyl ester	125.15
18	32.569	0.02	Imidazole-5-pentanoic acid	95.10
19	33.638	1.50	Hexadecanoic acid, methyl ester	87.10
20	34.493	4.02	n-Hexadecanoic acid	73.10
21	35.020	0.01	Decanoic acid, 2,4,6-trimethyl-, methyl ester	88.10
22	36.850	2.07	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	81.10
23	36.933	2.94	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	79.10
24	37.053	4.45	9-Octadecenoic acid, methyl ester, (E)-	97.15
25	37.126	0.22	9-Octadecenoic acid, methyl ester, (E)-	97.15
26	37.589	2.55	Methyl stearate	87.10
27	37.995	24.69	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
28	38.095	10.57	Oleic Acid	83.15
29	38.419	2.63	Octadecanoic acid	43.10
30	38.734	2.71	Hexadecanoic acid, butyl ester	56.10
31	38.860	0.05	Heptadecanoic acid, ethyl ester	88.10
32	39.985	0.06	Ether, (2-ethyl-1-cyclodecen-1-yl)methyl methyl	125.15
33	40.138	0.07	(Z,Z,Z)-6,9,15-Octadecatrienoic acid methyl ester	95.15
34	40.216	0.09	9-Octadecenoic acid, 12-hydroxy-, methyl ester, (Z)-	95.10
35	40.685	0.05	cis-Methyl 11-eicosenoate	97.15
36	40.874	0.27	9,12-Octadecadien-1-ol, (Z,Z)-	81.10
37	40.951	0.42	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	79.10
38	41.026	0.51	Octyl cis-vaccenate	97.15
39	41.140	0.03	5,5,8a-Trimethyldecalin-1-one	111.15
40	41.209	0.07	Eicosanoic acid, methyl ester	87.10
41	41.274	0.05	13-Docosenamide, (Z)-	72.10
42	41.598	3.22	9-Octadecen-1-ol, acetate, (Z)-	81.10
43	41.689	6.05	n-Propyl 9,12,15-octadecatrienoate	79.10
44	41.771	7.05	2,3-Dihydroxypropyl elaidate	83.10
45	41.846	0.29	cis-9-Octadecenoic acid, propyl ester	97.15
46	42.236	1.89	Octadecanoic acid, butyl ester	56.10
47	42.689	0.03	Cedrol	95.10
48	43.352	0.01	Glycidyl (Z)-9-nonadecenoate	129.10
49	43.780	0.97	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	98.10
50	43.935	0.10	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	98.10

Peak	R. Time	Area%	Name	Base m/z
51	44.063	0.06	6-Octadecenoic acid, methyl ester, (Z)-	97.15
52	44.407	0.18	Bis(2-ethylhexyl) phthalate	149.05
53	44.520	0.11	Heptadecanoic acid, 16-methyl-, methyl ester	87.10
54	44.606	0.09	4H-Cyclopentacycloocten-4-one, decahydro-	95.10
55	44.754	0.05	2-Buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-	121.15
56	45.000	0.07	cis-13-Eicosenoic acid	97.15
57	45.454	0.12	Arachidic acid, butyl ester	56.10
58	46.641	9.04	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	98.15
59	46.722	0.98	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	95.15
60	47.011	0.64	Hexadecanedioic acid, dimethyl ester	98.15
61	47.145	0.09	Octadecanoic acid, 2,3-dihydroxypropyl ester	98.15
62	47.604	0.05	Tetracosanoic acid, methyl ester	87.10
63	48.062	0.11	Erucic acid	97.15
64	48.460	0.06	Docosanoic acid	56.10
65	49.033	0.02	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	81.10
66	49.621	0.03	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	95.15
67	49.996	0.02	1-Heptacosanol	97.15
68	50.467	0.02	Heptadecanoic acid, 16-methyl-, methyl ester	87.10
69	51.459	0.02	(-)-Globulol	81.10
70	52.675	0.02	5-.alpha.-Androst-2-en-17-.beta.-ol, 17-methyl-	105.10
71	53.383	0.03	Andrographolide	109.15
72	54.152	0.07	Retinoic acid, methyl ester	107.15
73	54.325	1.40	Ergost-5-en-3-ol, (3.beta.)-	107.10
74	54.788	0.48	Humulenol-II	83.15
75	55.414	0.03	Andrographolide	105.10
76	55.765	2.50	gamma.-Sitosterol	107.10
77	55.939	0.18	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	105.10
78	56.238	0.20	6.beta.Bicyclo[4.3.0]nonane,5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-	109.15
79	56.601	0.02	6.beta.Bicyclo[4.3.0]nonane,5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-	121.15
80	56.954	2.27	6.beta.Bicyclo[4.3.0]nonane,5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-	95.10
81	57.344	0.01	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1. alpha)	107.10
82	57.936	0.02	Pregn-4-ene-3,20-dione, (9. beta.,10.alpha.)-	124.10
83	58.060	0.03	6.beta. Bicyclo [4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-	95.15

Table 3: GC-MS of purified flaxseed oil fraction.

The result indicates that 9,12,15-octadecatrienoic acid, (Z, Z, Z); alpha-linolenic acid (ALA) is represented as 9.8% of extracted crude oil and 4.8% of total flaxseed weight. ALA (18:3n-3) is an 18-carbon atoms carboxylic acid with three cis double bonds and consider an essential fatty acid indispensable to the human body. It is characterized by anti-inflammatory, anti-obesity, neuroprotection, anticancer, antioxidant, and anti-metabolic syndrome. It can convert into eicosapentaenoic acid and docosahexaenoic acid in the body. However, this conversion is limited and affected by many factors such as gender, dose, and disease [41].

In vivo study

The synthetic catecholamine and b-adrenergic agonist isoproterenol (ISO), also known as 1-(3, 4-dihydroxyphenyl)-2-isopropylamino ethanol hydrochloride, causes a large amount of oxidative stress in the myocardium and causes infarct-like necrosis of the heart muscle [42]. Through autooxidation, it produces highly cytotoxic free radicals that speed up the peroxidation of membrane phospholipids and seriously damage the heart membrane [43].

The findings indicates that group II animals had plasma BNP and NT-pro-BNP levels that were significantly increased to 207.34% and 372.51%, respectively ($p < 0.01$) compared to group I (Figure 1). Additionally, when compared to group II, the administration of flaxseed oil caused a significant decrease in plasma BNP and NT-pro-BNP levels by 32.02% and 52.14%, respectively ($p < 0.01$). However, when compared to group II, the BNP and NT-pro-BNP levels in group IV significantly dropped by 42.38% and 57.3%, respectively ($p < 0.01$).

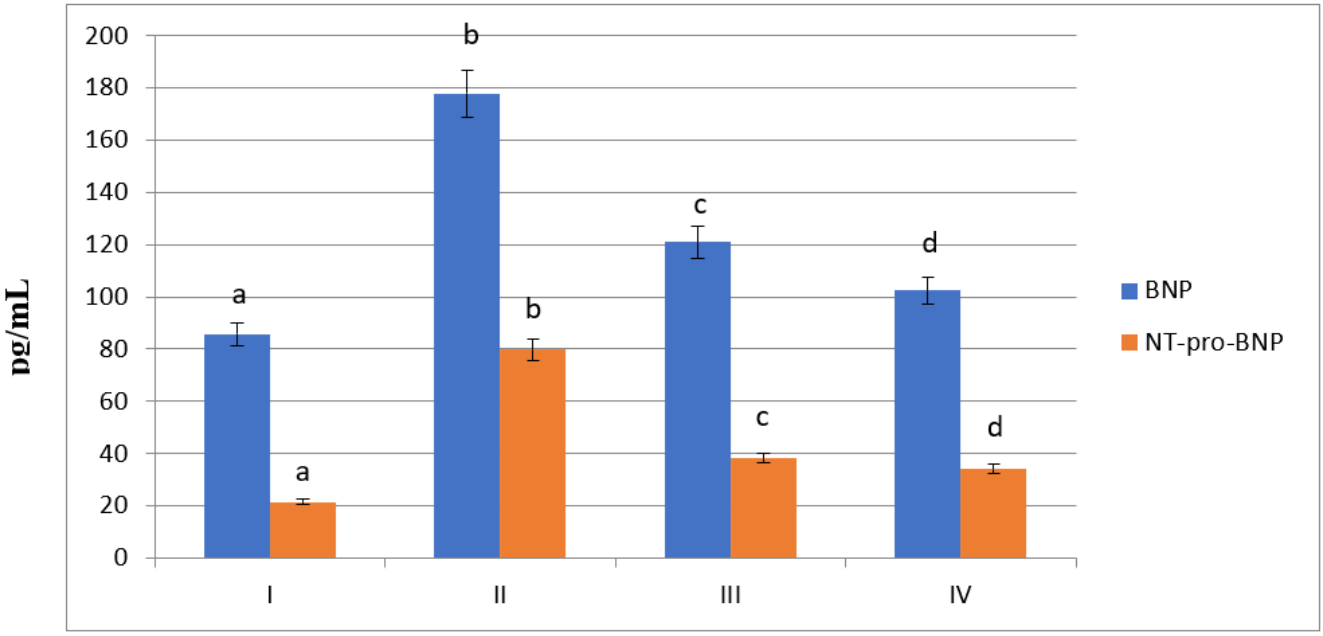

Figure 1: Effect of flaxseed oil on plasma BNP and NT-pro-BNP in isoproterenol treated rats. Data are represented as mean value (pg/mL). Data followed by the same letter within the same parameter are not significantly different at $P \leq 0.05$.

In contrast to the group I, Group II had significantly increased plasma levels of endothelin-1, Lp-PLA2, and MMP9 to 248.33%, 148.50%, and 179.33% as well as significantly lower plasma levels of topo 2B by 63.9% ($p < 0.01$) (Figure 2).

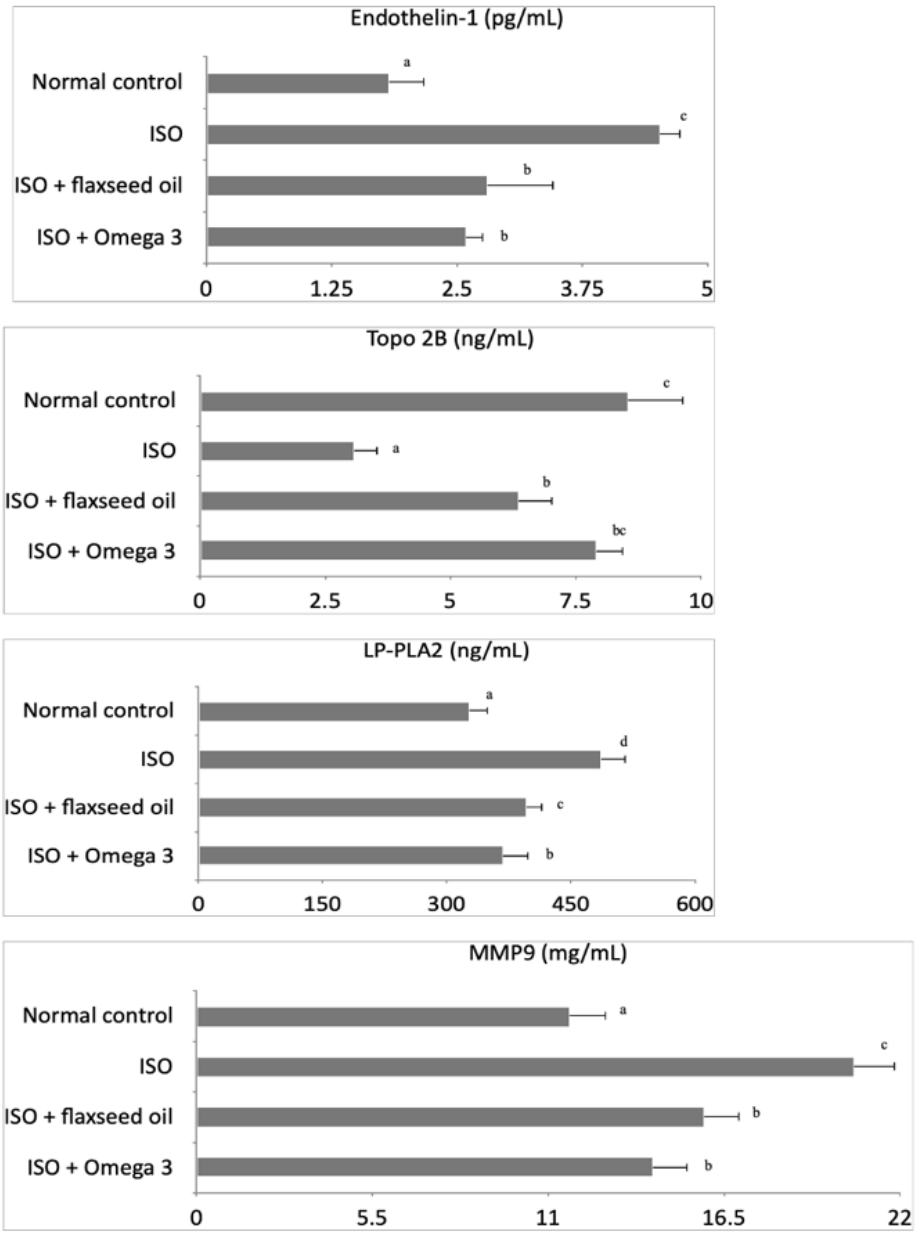

Figure 2: Effect of flaxseed oil on plasma Endothelin-1, Topo 2B, Lp-PLA2 and MMP9 in isoproterenol treated rats. Data shown are mean ± standard deviation of number of observations within each treatment. The tested flaxseed oil was orally given daily for 4 weeks at 100 mg/kg. Data followed by the same letter are not significantly different at $P \leq 0.05$.

Additionally, when compared to group II, the administration of flaxseed oil significantly increased plasma levels of topo 2B to 206.84% ($p < 0.01$) while significantly decreasing plasma endothelin-1, Lp-PLA2, and MMP9 levels by 38.05%, 18.51%, and 22.85%, respectively. In group IV endothelin-1, Lp-PLA2, and MMP9 levels considerably decreased by 42.69, 24.20, and 30.64%, respectively, as well as a significantly increase of plasma topo 2B level to 257.33% ($p < 0.01$) was observed.

According to the findings, group II animals had plasma cTnI and cTnT levels that were significantly higher than those of group I by 211.76 and 196.42%, respectively (p 0.01). Additionally, when compared to group II, the administration of flaxseed oil caused a substantial drop in plasma cTnI and cTn T levels by 43.05 and 33.63%, respectively ($p < 0.01$). When compared to group II, the levels of cTnI and cTn T in group IV significantly dropped by 47.22 and 37.30%, respectively (p 0.01) Figure 3.

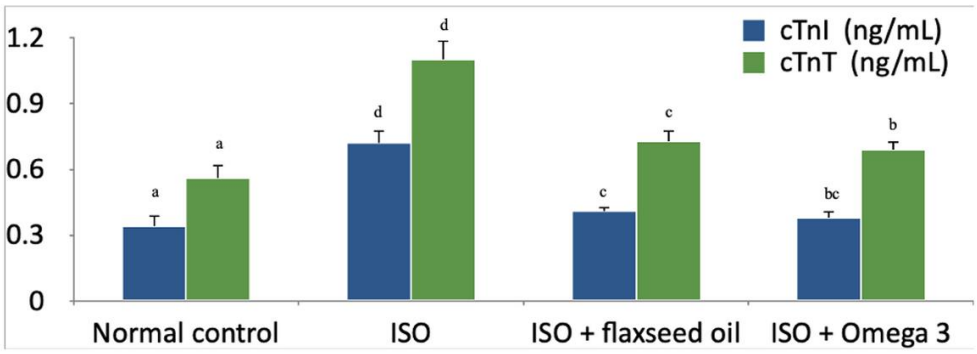

Figure 3: Effect of flaxseed oil on cardiac troponin I (cTnI) and troponin T (cTn T) in isoproterenol treated rats. Data shown are mean ± standard deviation of number of observations within each treatment. The tested flaxseed oil was orally given daily for 4 weeks at 100 mg/kg. Data followed by the same letter are not significantly different at $P \leq 0.05$.

In Figures 4 and 5, a significant increase in plasma miRNA1 and miRNA29b expression levels was detected obviously in group II animals in contrast to the normal group I (211.81% and 379.09%, respectively). However, group III had a significant decrease in plasma miRNA1 and miRNA29b gene expression levels ($p < 0.01$), by 41.17% and 45.08%, respectively as compared to group II. On the other hand, miRNA1 and miRNA29b gene expression levels decreased significantly by 47.06% and 50.6%, respectively in group IV compared to group II ($p < 0.01$).

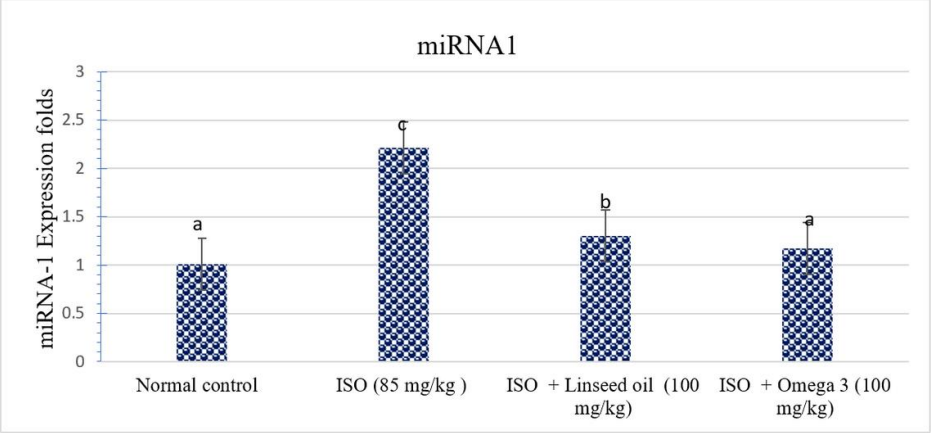

Figure 4: Effect of flaxseed oil on plasma miRNA1 gene expression in isoproterenol treated rats. Data followed by the same letter are not significantly different.

Discussion

In the recent decade, essential fatty acids (EFAs) have become the primary focus of scientific research with substantial research, as a crucial metabolite in the field of cardiac medicine [44]. Despite this progress, the molecular mechanisms of action, and their exact physiological ability that prevents associated cardiovascular diseases are still unanswered [45].

Kaithwas et al., reported that flax oil comparable to aspirin where it exhibits dose-dependent inhibition of protein exudation vascular permeability, and leukocyte migration in pleural exudates [30]. As the eicosanoids, EPA derived from linolenic acid metabolism account for the production of lets vasodilatory (PGE3) and chemotactic (LTB5). The current study describes the primary biological mechanisms and genetic modification of how flaxseed oil-rich ALA, could have a cardiac protective effect against cardiac remodeling in an ISO-induced vivo model.

BNP and NT-proBNP are commonly used as key indicators for the clinical diagnosis of HF and cardiac dysfunction [46, 47]. Previous studies have demonstrated the use of BNP and NT-proBNP as postmortem biomarkers in forensic medicine to identify cardiac dysfunction. These investigations used comprehensive animal experiments and postmortem tissues to represent the cardiac activity of the deceased before death [48, 49]. This result revealed that flaxseed oil-rich ALA inhibits BNP, and NT-pro-BNP, and reduces apoptosis, and cardiac fibrosis.

Increased levels of endothelin-1, Lp-PLA2, and MMP9 have been linked to different heart conditions, including hypertension, interferon-induced chronic active myocarditis, cardiomyopathy, streptozotocin-induced diabetic cardiomyopathy, pulmonary artery banding-induced HF in both the left (RV) and right (LV) heart [50, 51]. In the present study, elevation levels of Endothelin-1, Lp-PLA2, and MMP9 as well as depletion of plasma Topo 2B have been observed in heart disease animal models after ISO administration. Moreover, compared to controls, ISO-treated rats showed an increase in ET-1, Lp-PLA2, and MMP9 plasma levels, which indicates that ISO induces cardiac tissue inflammation. It is tempting to speculate that these mediators' increased expression is accessible to myocardial toxicity and can promote inflammation and activation of vascular smooth muscle cells. This result is in accordance with the Kaithwas et al., a study that reported the anti-inflammatory activity of flaxseed oil (57.38%, ALA) [33].

According to early studies examining Top2a and Top2b's differential expression, Kondapi, and colleagues discovered that Topo 2B lower expression is associated with aging in an adult rat's whole brain preparation when compared to its younger counterpart [52]. More particular, the cerebellum and cerebellar area showed a decrease in protein expression. This

was further supported by the observation that Top2B activity declined with age in sheep neural cell preparation [53, 54].

Aging and sex hormone deprivation is considered the primary factor of cardiac remodeling, while the heart "shrinking" with an increase in heart rate, smaller ventricular volumes, and changes in LV morphology are observed [55]. Hence, the investigation of Topo 2B levels in ISO-treated rats was important. Our results showed a reversible correlation between Topo 2B and cardiac inflammation while plasma Topo 2B levels were significantly decreased in ISO-treated rats. The administration of flaxseed oil increased the level of plasma Topo 2B with subsequent inhibition of inflammation and improvement of cardiac tissue. Flaxseed oil delivery raises plasma levels of cTnI and cTnT compared to the treated group with ISO. These outcomes were consistent with the previous results that referred to the inhibition of cTnI and cTnT plasma protein expression in the CVD animal model treated with ISO [56, 57]. Hence, the reported anti-inflammatory effect of flaxseed oil [33] could be attributed to its biological modifying effect on cTnI and cTnT receptor cells.

The biological functions of miR-1 and miR-29 in ISO-induced cardiac damage are indicated in the current study. According to earlier research, MiR-1 and miR-29 were found to be involved in heart morphogenesis in a mouse model [58]. The upregulation of miR-29 level has been shown by investigations on liver, lung, and kidney fibrosis disease [59]. Indeed, it has been reported that ISO-treated groups have higher levels of miR-1 and miR-29 expression than normal rats [60, 61]. Moreover, all major types of cardiovascular cells, including vascular smooth muscle cells (VSMCs) [62], endothelial cells [63], cardiomyocytes [64], and cardiac fibroblasts [65, 66] have been reported to have significant levels of miR-1 and miR-29 expression. Contrary, according to the current study, ALA-rich flaxseed oil protected the heart by decreasing the levels of miRNA-1 and miRNA-29b genes expression.

Conclusion

The current study provides experimental evidence that the standardized flaxseed oil fraction can improve cardiac function after ISO-induced cardiotoxicity. The finding clarified the molecular mechanism as well as the benefit of oil as a cardioprotective drug candidate against cardiac remodeling for lowering the incidence of cardiovascular risk factors and mortality. The flaxseed oil downregulated BNP, NT-pro-BNP, endothelin-1, Lp-PLA2, and MMP2, as well as cTnI and cTn plasma levels and upregulated Topo 2B. It also down-regulated the expression of miRNA-1 and miRNA-29b genes. Future research for preparation proper formulation and clinical investigation are recommended to ascertain the impact of bioavailability, dose, gender, and other diseases on the pharmacokinetics of flaxseed-rich ALA oil.

Abbreviations

ALA	Alpha-linolenic acid	MCP-1	Monocyte chemoattractant protein-1
BNP	Brain natriuretic peptide	MMP9	Matrix metalloproteinase 9
cTnI	Cardiac troponin I	NADPH oxidase	Nicotinamide adenine dinucleotide phosphate oxidases
cTnT	Cardic troponin T	NT-pro-BNP	N-terminal pro–B-type natriuretic peptide
CVDs	Cardiovascular disease	PGE3	Prostaglandin E3
ELISA	Enzyme-linked immunoassay	PPARα	Peroxisome proliferator-activated receptor alpha
ET-1	Endothelin 1	RT-PCR	Reverse transcription polymerase chain reaction
GC-MS	Gas chromatography mass spectrometry	SREBPs	Sterol regulatory element binding proteins
HFD	Heart failure disease	TNF	Tumor necrosis factor
HMGCR	Hydroxy Methyl Glutaryl Coenzyme A Reductase	Top2a	DNA Topoisomerase II Alpha
IL-6	Interleukin − 6	Top2b	Topoisomerase 2 beta
ISO	Isoproterenol	VCAM-1	Vascular cell adhesion molecule-1
Lp-PLA2	Lipoprotein-Associated Phospholipase A2	VSMCs	Vascular smooth muscle cells
LTB5	Leukotrienes B5	WHO	World Health Organization

Declarations

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Additional Declarations

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