Egypt. J. Chem. **Vol. 68**, No. 8 pp. 211 - 226 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Long-Term Overconsumption of Chia Seed Oil (Salvia hispanica L.) and Black Seed Oil (Nigella Sativa) in Relation to Metabolic and Inflammatory Markers Expressions in Rats

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Abstract

Introduction: Notwithstanding their positive health effects, excessive usage of vegetable oils like chia seeds oil (CSO) and black seeds oil (BSO) could lead to an unbalanced intake of n-3 and n-6 fatty acids. This study assessed the impact of long-term consumption of CSO and BSO alone or blended at amounts of 1:1, 4: 1, and 1: 4, representing ratios of n-6 to n-3 fatty acids of 1.2:1, 1:1.8, and 4:1, respectively, on oxidative stress, inflammation, liver function, blood lipids, and relevant hepatic genes expression.

Methods: Seventy-two adult rats were segregated into six groups (12 rats each). Rats in group one were provided with a standard diet. Rats in the group (2) were supplied with a CSO-containing diet. The third group of rats received a BSO-containing diet. In groups four through six, rats received diets containing blended CSO and BSO at ratios 1:1, 4:1, and 1:4, respectively. Rats were fed their corresponding diets for 12 weeks.

Results: Chemical analysis revealed that CSO has lower n-6/n-3 fatty acids, more active constituents, and more free radicals scavenging activity and reducing power than BSO. The in vivo study showed that long-term dietary over-consumption of BSO elicited an upsurge in the activity of COX-2 and serum values of PGE-2, nitric oxide, and IL-1β, along with a reduction in serum IL-10 levels. BSO over-supplementation also compromised the anti-oxidant status, evidenced by the decline in the serum content of GSH, SOD, Nrf2, and HO-1, together with marked lipid peroxidation leading to dyslipidemia, elevation in liver enzymes, downregulation in hepatic PPARγ, MAPK, UCP2, and Adipor2, and upregulation in NF-κB and SREBF1 hepatic gene expression. Conversely, CSO consumption reinforced the anti-inflammatory capacity, enhanced the antioxidant status, reduced lipid peroxidation, blood lipids, and liver enzyme activities, and regulated the transcription of genes implicated in the inflammation reaction. Furthermore, feeding the blended oils favorably enhanced these inflammatory oxidative and metabolic variables compared to feeding BSO or standard diets. These effects were more prominent when feeding a higher chia oil than black seed oil.

Conclusion: Prolonged over-consumption of BSO, which has a very high n-6/ n-3 FA ratio, significantly instigated dyslipidemia, elevation in liver enzymes, oxidative stress, increase in serum COX-2 activity, and PGE-2 production, an upsurge in inflammatory cytokines and dysregulation in the expression of critical genes implicated in the inflammatory response. Feeding CSO either alone or blended with BSO favorably enhanced these metabolic, oxidative, and inflammatory consequences. Therefore, the diet's background of n-6/n-3 fatty acid content is crucial, especially when consuming vegetable oils like CSO and BSO for a long time.

Keywords: Chia seeds oil; Nigella Sativa Seeds Oil; n-6/n-3 fatty acid ratio, Rats

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Received Date: 14 December 2024, Revised Date: 29 December 2024, Accepted Date: 06 January 2025

DOI: 10.21608/ejchem.2025.344357.10985

1- Introduction

Consuming foods enriched with functional ingredients could promote mental and physical health and, minimize the incidence of chronic illnesses [1]. Therefore, vegetable oils with beneficial properties, like chia seed oil (CSO) and black seed oil (BSO), for human health have garnered much attention [2]. Chia, *Salvia hispanica* L. is an herb that is one of the species of the Lamiaceae genus [3]. It is cultivated for its seeds and used as a functional food owing to its high protein content and biologically active components, including phenolic compounds, carotenoids, phytosterols, and tocopherols [4], which could potentially mitigate lots of illnesses and also boost human health [2,3]. However, chia seeds' substantial amount of alpha-linolenic acid (ALA, C18:3 n-3) is the main factor for its popularity, as it is the most affluent known plant-based omega-3 fatty acid source, reaching up to 68 percent, compared to linseed (51%), rapeseed (8%), soybean (7.5%) and sunflower (1.9%) oils [5]. The seed oil of Nigella sativa (Ranunculaceae), commonly mentioned as black seed oil (BSO), has also garnered much attention and is significant for human nutrition and health. BSO is composed chiefly of polyunsaturated fatty acids (PUFAs, 80%), of which linoleic acid (C18:2 n-6, LA 57.49%) is the most abundant essential n-6 PUFA [6]. The most prominent bioactive substances in BSO are thymoquinone and thymol, which have demonstrated pharmacological and antibacterial characteristics [7].

Chia and black seed oil's chemical diversity and functional qualities, along with their advantageous combination of tocopherols, phytosterols, and unsaturated fatty acids, make their actions regarding the risk factors for many diseases intriguing [8]. Therefore, these oils are considered functional foods for human nutrition with remarkable positive effects on health, causing growing popularity. Nevertheless, there is a risk of excessive supplementation. Over-consumption of these oils may disrupt the balance of the dietary essential fatty acids, specifically, the n-6/n-3 fatty acids ratio, leading to deleterious impacts in immunoregulation, which leads to life-threatening severe illnesses [9]. The body systems and general health may suffer from an inconsistency between the n-6 and n-3 fatty acid ratios [10]. According to Alshatwi et al. [10], over-consumption of n-6 fatty acids promotes lipid storage, triggers inflammation reactions, and amplifies inflammatory damage that raises the incidence of cardiovascular ailments. Moreover, LA is a precursor of pro-inflammatory cytokines, including interleukin1β ((IL-1β) and leukotriene-B4 (LTB4). Elevations in these cytokines contribute to aging, depression, cancer, insulin insensitivity, and ischemic heart disorders [11]. Contrariwise, n-3 PUFAs, like docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and ALA, have anti-inflammatory properties that might control inflammatory and autoimmune diseases [9]. Accordingly, the amount of n-6 to n-3 profoundly impacts the secondary protection against immunomodulatory and chronic metabolic disorders [12]. Chronic low-grade inflammatory responses can be triggered by an unbalanced n-6 to n-3 PUFA ratio in the diet, promoting inflammation [9].

Considering the justifications mentioned above, the upsurge in the dietary consumption of CSO and BSO might affect the balance of n-6 to n-3 fatty acids and could have metabolic and inflammatory consequences. Therefore, the current study aimed to assess how long-term dietary consumption of CSO and BSO, separately or blended in various proportions, affects liver function, blood lipids, and several key inflammatory and oxidative stress indicators in rats.

2. Materials and Methods

2.1. Seeds and oils extraction

Chia (Salvia hispanica L.) and black (Nigella sativa L.) seeds were acquired from the Egyptian Agriculture Ministry in Cairo. The impurities were removed by sieving and sorting. Then, oils were extracted using cold-pressing methods. The produced oils were preserved coldly in dark glass bottles until needed [13].

2.2. In vitro investigation

2.2.1. Peroxide, acid, iodine, saponification values, free fatty acid percentage (FFA%), and un-saponification matter assessments in oil samples

All the above parameters were determined and complied with the established protocols of ISO [14-17].

2.2.2. Evaluation of fatty acid content using gas chromatography

The composition of fatty acids was ascertained in compliance with EEC/2568/91 regulation [18]. Prior to analysis, fatty acids were first transformed into fatty acid-methyl esters (FAMEs) by agitating an arrangement of sixty mg oil, three mL hexane, and 0.3 mL 2 N methanolic form of potassium hydroxide for twenty-five minutes. FAMEs were characterized using a Varian chromatograph model CP-3800 (Varian Inc.) configured with an FID. Each fatty acid's relative percent in the sample was used to express the results.

2.2.3. Evaluation of total phenolic content and the reducing power of the tested oils

The entire phenolic composition in the sample oils was ascertained using the Foline-Ciocalteu technique [19]. The oils' capacity to scavenge free radicals was assessed by applying the 1, 1-diphenyl-2-picryl-hydrazyl radical (DPPH) procedure [20]. The reducing capacities of the oils under investigation were identified by the ferric-reducing antioxidant-power (FRAP) assay. [21].

2.3. In vivo experiment

2.3.1. Animals

Seventy-two adult male Sprague-Dawley rats weighed between 120 - 130 g resided in wire cages with adequate ventilation in the animal house of the Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University, Cairo, Egypt. Before the twelve-week trial began, the rats were permitted to adjust for seven days. Rats were given a regular diet and full access to tap water and were exposed to twelve-hour alternating cycles of light and dark. The animal trial went ahead pursuant to the institutional animal ethics committee's rules and regulations. The Ethical Committee of the Faculty of Women for Arts, Science, and Education, Ain Shams University, validated it (Approval-number: Sci-1532410002).

2.3.2. Diet

A balanced diet was established to comply with the American Institute of Nutrition's AIN-93M standards, revised by Reeves et al. [22]. Chia and black seed oils, or a combination of both oils in 1:1, 1:4, and 4:1 ratio, were utilized instead of corn oil. According to Simopoulos [23], these oil ratios were chosen based on the oil content of n-6 and n-3 fatty acids. The composition of the experimental diets is recorded in Table (1).

Table 1 Composition of experimental diets

Ingredients	Composition of diet g/Kg					
	Standard diet	CSO	BSO	1 CSO:1 BSO	4 CSO:1BSO	1 CSO: 4 BSO
Casein	170	170	170	170	170	170
Methionine	3	3	3	3	3	3
Choline chloride	20	20	20	20	20	20
Vitamin mixture*	10	10	10	10	10	10
Mineral mixture*	40	40	40	40	40	40
Corn oil	75	-	-	-	-	-
Chia seed oil	-	75	-	37.5	60	15
Black seed oil	-	-	75	37.5	15	60
Sucrose	50	50	50	50	50	50
Cellulose	50	50	50	50	50	50
Starch	582	582	582	582	582	582

*AIN93 vitamin and mineral mixture [22]

2.3.3. Experimental Design

Seventy-two rats were categorized into six groups of twelve each, as outlined subsequently:

1. Standard diet-fed group (SD): Rats were provided with a balanced diet
2. Chia seeds oil-fed group (CSO): rats were given a chia oil-containing diet
3. Black seeds oil-fed group (BSO): rats were fed a black seed oil-containing diet
4. 1: 1 Chia seeds and black seed oils fed group (1 CSO:1 BSO): Rats were administered a diet containing a 1:1 mixture of the two oils.

5. 4:1 Chia seeds and black seed oils fed group (4 CSO:1BSO): Rats received a diet containing a mixture of the two oils in a 4:1 ratio.
6. 1: 4 Chia seeds and black seed oils fed group (1 CSO: 4 BSO): Rats were supplied with a diet containing a 1:4 ratio of the two oils.

All rat groups were fed on their assigned diet, ad libitum, for twelve weeks.

2.3.4. Samples collection

Upon completion of the study duration, rats were euthanized by decapitation under isoflurane anesthesia. Blood samples were obtained, and serum was becoming isolated. Each rat's liver was promptly removed, washed with a buffered saline (0.9% sodium chloride) solution, dried with filter paper, and processed to create tissue homogenate for further examinations.

2.3.5. Biochemical analysis

Serum superoxide-dismutase enzyme (SOD) activity, reduced-glutathione (GSH), serum nitric oxide, and malondialdehyde (MDA) levels were determined colorimetrically using Biodiagnostics kits, Egypt [24-27]. Serum nuclear-factor erythroid-2-related factor 2 (Nrf2) and heme-oxygenase-1 (HO-1) values were determined by MyBioSource, enzyme-linked immunosorbent (ELISA) kits (San Diego, USA). Liver function tests, serum alanine-aminotransferase (ALT), and aspartate-aminotransferase (AST) enzyme activities were determined by the colorimetric methods using the Diamond Diagnostic kit, Egypt [28]. Serum β-hydroxy β-methyl-glutaryl-CoA reductase (HMG-COAR) enzyme activity was recorded using a My BioSource rat ELISA kit [29]. The enzymatic colorimetric standard kits were utilized to quantify the levels of triacylglycerols (TAG), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) in serum. The formula [LDL-C=TC-(HDL-C + TAG/5)] was used to compute the serum low-density lipoprotein-cholesterol (LDL-C) values [30-33]. The inflammatory condition was evaluated by measuring serum cyclooxygenase-2 (COX-2) enzyme activity, prostaglandin E2 (PGE2), interleukin-1 beta (IL-1β), and interleukin-10 (IL-10) levels using ELISA kits explicitly designed for rats and provided by MyBioSource (San Diego, USA).

2.3.6. Hepatic gene expression analysis

Hepatic tissues were treated with the Rneasy-Plus Mini-kit to extract the entire RNA (Qiagen, USA) in compliance with the product directions. The value of RNA was evaluated using a Nano-Drop-1000 colorimeter set to 260 nm (Thermo-Fisher Scientific, Waltham, USA). RNA pureness was confirmed via the 260/280 nm absorption ratio, ranging from 1.7 to 2.0. The reliability of the RNA data was validated through electrophoresing on an ethidium-bromide-stained agarose gel. MBI Fermentas's First Strand cDNA Synthesis Kit was used to reverse-transcribe the extracted entire RNA into complementary DNA (cDNA). PPARγ, NF-κB, MAPK, SREBF1, Adipor2, and UCP2 mRNA expression was assessed by quantitative polymerase chain reaction (qPCR) for reverse transcription in a 96-well optic plate applying universal cycling conditions (ten min at 95 °C; forty cycles of 15 seconds at 95 °C and 60 seconds at 60 °C) and using the PRISM, which 7500-fast sequence detection system (Applied-Biosystems, Carlsbad, CA). Each gene's PCR primer pair sequences are displayed in Table 2. The ABI Spectrum sequence detector system software was used to interpret the data, and PE-Biosystems' v1·7 sequence software for detection (Foster City, CA) was employed to quantify the results. The comparative cycle threshold approach was used to compute the relative expression of the genes under study. Every value was expressed as a change in fold from the control after being normalized to the β-actin gene [34].

2.4. Statistical analysis

Using the 20.0 version of SPSS (SPSS, Chicago, IL, USA) software, the collected data were statistically evaluated and stated as mean ± standard deviation (mean ± SD). The least significant difference (LSD) was used for comparison across several groups after a one-way analysis of variance. A statistically significant level was specified as less than 0.05 [35].

Table 2: Forward and reverse primer sequences used in qRT-PCR

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)
PPARγ	TGTGGGGATAAAGCATCAGGC	CCGGCAGTTAAGATCACACCTAT
NF-κB	GACGACACCTCTACACATAGCAG	TTCTTCTCCAGCCTTCTCCCA
MAPK	AGGGCGATGTGACGTTT	CTGGCAGGGTGAAGTTGG
Srebf1	TCTGCCTTGATGAAGTGTGG	AGCAGCCCCTAGAACAAACA
UCP2	AGCAGTT CTACACCAAGGGC	AGAGGTCCCTTTCCAGAGGC
Adipor2	CATGTTTGCCACCCCTCAGTA	ATGCAAGGTAGGGATGATTCCA
β-actin (internal control for qRT-PCR)	GATTACTGCTCTGGCTCCTGC	GACTCATCGTACTCCTGCTTGC
18s rRNA (internal control for qRT-PCR)	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

3. Results:

3.1. Chemical characteristics of chia and black seed oils

Table 3 lists the results of the CSO and BSO chemical analyses. BSO displayed a peroxide value of 3.62±0.33 meq O_2/kg, acid value14.18±0.65 mg KOH/g, iodine value of 123.57±2.76 (g I_2/100 g), and saponification value of 186.92±3.69 (mg KOH/g). The free fatty acids in BSO were established as 6.83±0.28% (oleic acid), while the unsaponifiable matter was 0.80±0.02 g/kg. On the other hand, CSO recorded lower peroxide (1.86±0.2) and acid (2.21±0.26) values. Furthermore, CSO recorded higher iodine (220.24±2.67) and saponification (196.37±4.23) values than BSO. The unsaponifiable matter in CSO was recorded as1.54±0.19 g/kg, and the free fatty acids% was found to be 1.54±0.19.

Table (3): Peroxide, acid, iodine, saponification values, free fatty acid percent (FFA%), and unsaponifiable matter in black seed and chia seed oil samples

Parameter	Black seeds oil	Chia seeds oil
Peroxide-value (meq oxygen/kg)	3.62±0.33	1.86±0.20
Acid-value (mg KOH/g)	14.18 ±0.65	2.21±0.26
Iodine-value (g I_2/100 g)	123.57 ±2.76	220.24±2.67
Saponification-value (mg KOH/g)	186.92 ±3.69	196.37±4.23
FFAs% (oleic acid)	6.83 ±0.28	1.54±0.19
Unsaponifiable matter (g/kg)	0.80 ±0.02	0.91±0.14

The values are displayed as the three measurements' average± standard deviation.

Fatty acid patterns of the BSO and CSO are represented in Table 4. Gas chromatography analysis of the methyl ester of fatty acids (FAMEs) of the tested oils revealed that BSO possessed appreciable amounts of myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid. On the other hand, it was found that the order of fatty acids abounding in chia seed oil was α-linolenic, linoleic, oleic, palmitic, and stearic acids.

Data presented in Table 4 and Figure 1 also showed that the total saturated: total unsaturated fatty acid ratio was (1:3.87) in BSO and (1:7.24) in CSO, respectively. On the other hand, the ratio of ω-6 to ω-3 fatty acid levels was considerably higher in black seed oil (195:1) than in chia seed oil (1:3.41). In the investigated oils, ω-3 PUFAs were in the form of α-linolenic acid (ALA, C18:3, ω-3), while linoleic acid (LA, C18:2, ω-6) was the representative of the ω-6 PUFAs group.

As indicated in Figure 2A, BSO had total polyphenol and flavonoid amounts of 1.1±0.2 (mg gallic acid eq/g oil) and 1.4±03 (mg quercetin eq/g oil), respectively. Meanwhile, the phenolic quantity in CSO was 2.74±0.4 (mg gallic acid eq/g oil), and the flavonoid was 1.8±0.3 mg quercetin eq/g oil. On the other hand, Figure 2B demonstrates that the free radical scavenging activities against DPPH for black and chia seed oils were 310±5.9 and 418±10.3 (mg Vit. C eq/g oil), respectively. The reducing power of black and chia seed oils was 338±6.8 and 397±9.1 µg/g, respectively.

Table (4): Fatty acid characteristics of tested oils

Fatty Acid Composition	Black Seed Oil	Chia Seed Oil
	(Amount of total fatty acids per 100 grammes)	
Myristic-acid, C14:0	7.9 ± 0.11	0.03 ± 0.01
Palmitic-acid, C16:0	10.21 ± 0.20	6.79 ± 0.31
Palmitoleic-acid, C16:1	0.20 ± 0.10	0.08 ± 0.02
Stearic-acid, C18:0	2.10 ± 0.09	5.26 ± 0.31
Oleic-acid, C18:1	20.78 ± 0.12	8.33 ± 0.34
Linoleic-acid, C18:2	58.53 ± 0.06	17.88 ± 1.16
Linolenic-acid, C18:3	0.30 ± 0.13	60.93 ± 2.98
Arachidic-acid, C20:0	0.51 ± 0.08	0.35 ± 0.03
Total saturated fatty acids%[1]	20.72	12.08
Total monounsaturated fatty acids%[2]	20.98	8.41
Total polyunsaturated fatty acids%[3]	59.04	78.81
Total Unsaturated fatty acids%[4]	80.02	87.22
Saturated: unsaturated fatty acids ratio	1: 3.87	1: 724
Total ω-3 fatty acids%[5]	0.3	60.93
Total ω-6 fatty acids%[6]	58.53	17.88
ω-6: ω-3 fatty acids ratio	195: 1	1:3.4

[1] The total amount of saturated fatty acids equals the sum of myristic acid, palmitic acid, stearic acid, and arachidic acid. [2] Total monounsaturated fatty acids equals the total quantity of palmitoleic acid and oleic acid. [3] The total polyunsaturated fatty acids is the overall sum of linoleic and linolenic acids. [4]Total amount of unsaturated fatty acids equals the sum of palmitoleic acid, oleic acid, linoleic acid, and Linolenic acid. [5] Total ω-3 fatty acids correspond to linolenic acid. [6] Total ω-6 fatty acids are equivalent to linoleic acid. The values are displayed as the three measurements' average± standard deviation.

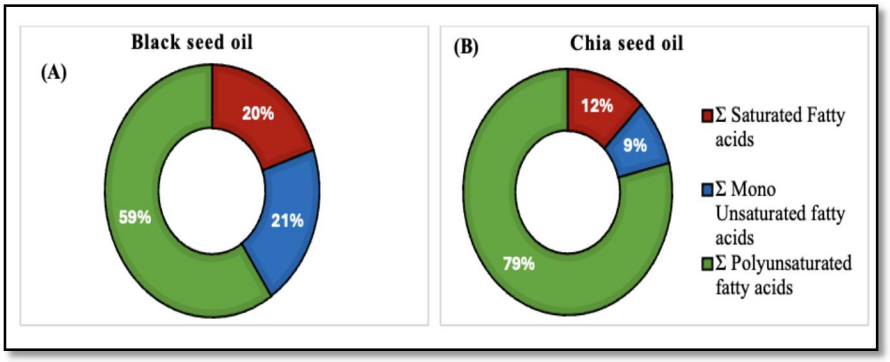

Fig. 1: Saturated, mono-unsaturated, and poly-unsaturated fatty acids content percent in **(A)** black seed oil and **(B)** chia seed oil.

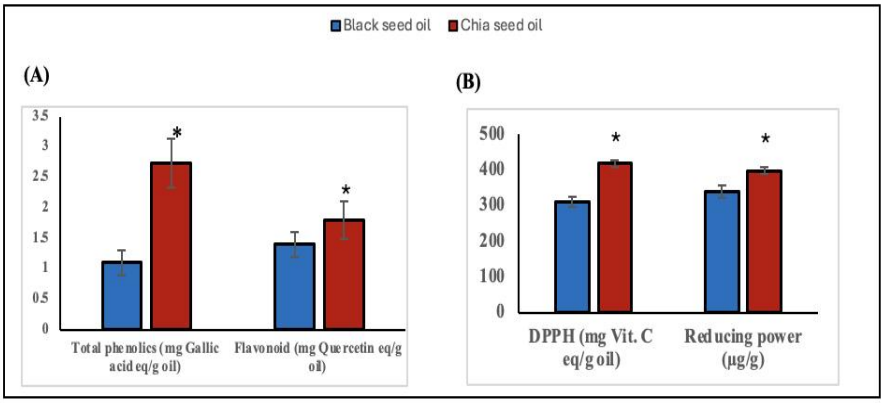

Fig. 2. (A) Phenolic and flavonoid contents **(B)** Free radicals scavenging activity and the reducing power of the black seed and chia seed oils. The values are displayed as the mean ± the standard deviation of the three measurements. *Demonstrate statistical significance from the values of black seed oil at a P-value lower than 0.05.

3.2. Effects of consumption of chia and black seed oils by different ratios on the prostaglandin synthesis and some inflammatory and anti-inflammatory cytokines in rats

Figure (3) shows that BSO consumption significantly (P<0.05) surged serum COX-2 activity (32.2%) and COX-2-derived pro-inflammatory eicosanoids, PGE2 (31.45%), as compared to feeding the standard diet.

Furthermore, BSO dietary intervention provoked an inflammatory response, as indicated by the remarkable (P<0.05) elevation in serum inflammatory cytokines, NO (11.9%), and IL-1β (15.98%) concomitant with a noticeable drop in the anti-inflammatory cytokine IL-10 (31.3%) compared to the control. Contrariwise, CSO intake substantially (P <0.05) diminished the serum COX-2 activity (78.5%) and the arachidonic acid COX-derived PG, PGE2 (54.56%). Additionally, CSO consumption presented a beneficial significant effect (P<0.05) on reducing these major pro-inflammatory cytokines, NO (37.43%), and IL-1β (72.5%), and increasing the anti-inflammatory cytokine IL-10 (54%) compared to the rats that received the standard diet or BSO diet. Consistently, an increment in the ratio between CSO and BSO in the diet had a more pronounced effect on improving the inflammatory status when compared with feeding rats on a diet supplemented with BSO or the standard diet.

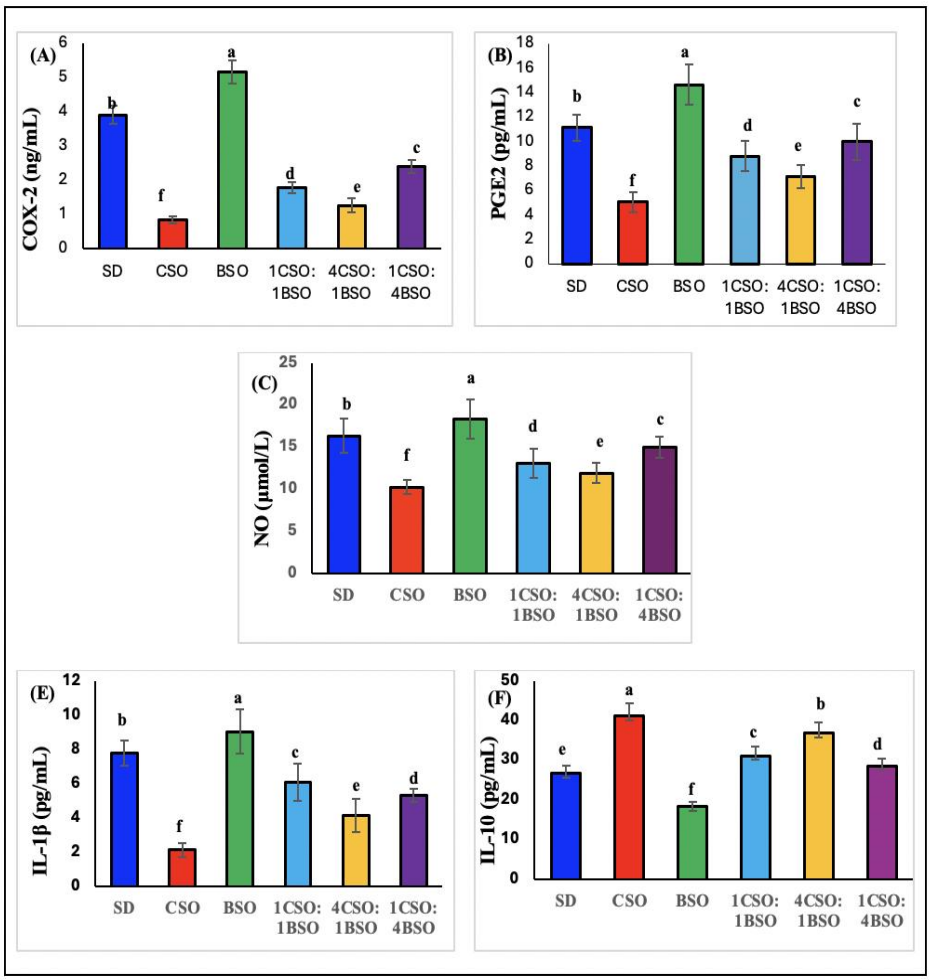

Fig. 3. (A) COX-2, **(B)** PGE-2, **(C)** NO, **(D)** IL-1β, and **(E)** IL-10 levels on rats fed on standard diet (SD), chia seed oil diet (CSO), black seed oil diet (BSO), 1 chia seed oil: 1 black seed oil (1CSO: 1BSO), 4 chia seed oil: 1 black seed oil (4 CSO: 1 BSO), and 1 chia seed oil: 4 black seed oil (1 CSO: 4 BSO). The values are displayed as the mean ± the standard deviation for 12 rats. Superscript variations through bars point out a significant difference at a P-value lower than 0.05.

3.3. Effects of chronic intake of black and chia seed oils at different ratios on the oxidative status in rats

The results illustrated in Figure (4) revealed that BSO overconsumption elicited oxidative stress demonstrated by the noticeable (P<0.05) elevation in serum lipid peroxidation product (57.7%) along with a marked (P<0.05) decline in serum levels of GSH (6.5%), SOD activity (48.5%), NrF2 (47.8%), and HO-1 (40.4%) in comparison with feeding the standard diet. Contrariwise, feeding of rats on a diet supplemented with chia seed oils for twelve weeks significantly enhanced the oxidative status, evidenced by the marked (P<0.05) increase in serum GSH (18.45), SOD activity (46.9%), Nrf2 (2.49-folds), and HO-1 (1.89-folds) levels accompanied by a substantial (P<0.05) reduction in the serum MDA (80%) levels as compared with feeding the standard diet. Furthermore,

data presented in Figure 3 also revealed that the groups fed on diets containing the blended oils (chia and black seed oils at ratios 1:1, 4:1, and 1:4) displayed better oxidative status than those fed on the standard diet or black seed oil diet. In addition, as the ratio of chia oil to black seed oil in the diet increased, the oxidative status in rats was improved.

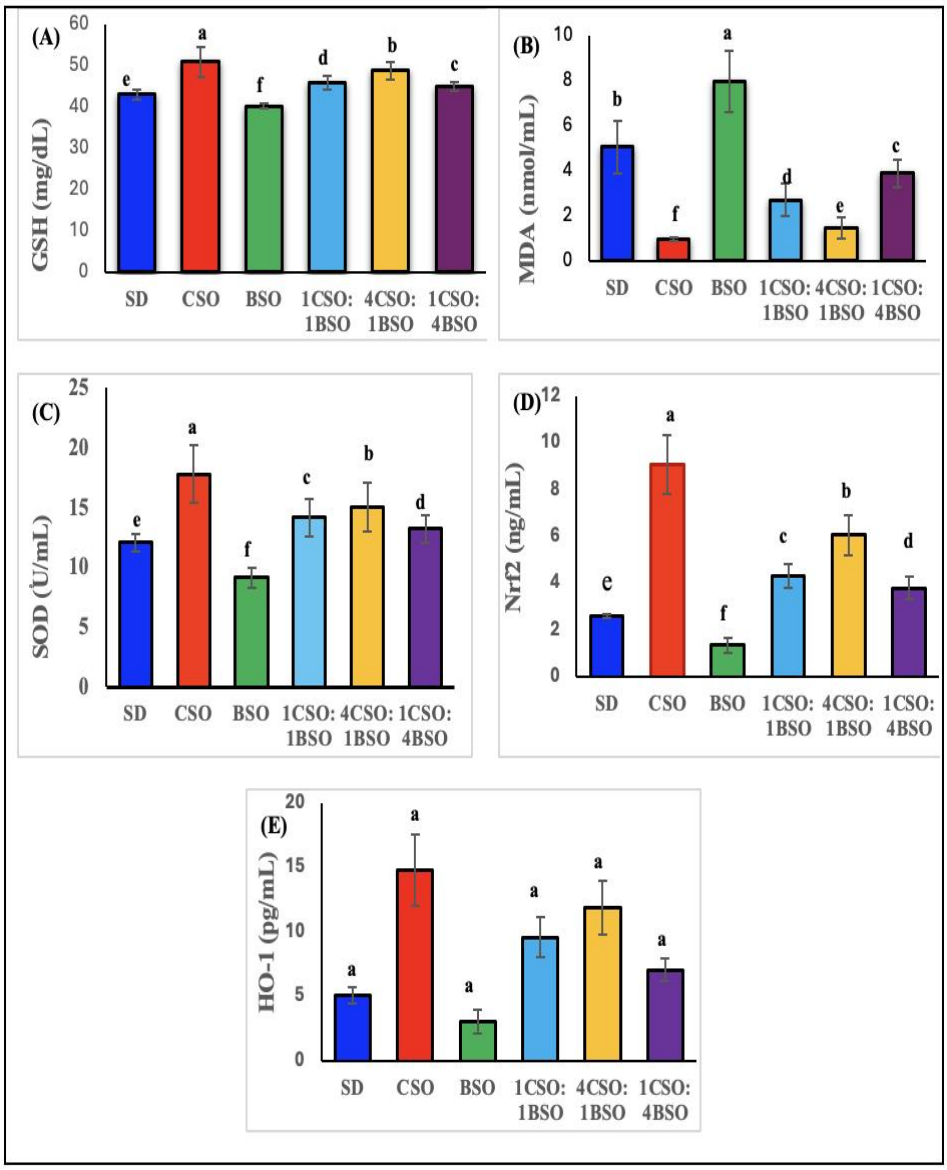

Fig. 4. (A) Blood GSH, **(B)** serum MDA, **(C)** blood SOD activity, **(D)** serum Nrf2, and **(E)** serum HO-1 levels on rats fed on standard diet (SD), chia seed oil diet (CSO), black seed oil diet (BSO), 1 chia seed oil: 1 black seed oil (1CSO: 1BSO), 4 chia seed oil: 1 black seed oil (4 CSO: 1 BSO), and 1 chia seed oil: 4 black seed oil (1 CSO: 4 BSO). The values are displayed as the mean ± the standard deviation for 12 rats. Superscript variations through bars point out a significant difference at a P-value lower than 0.05.

3.4. Gene expression

The results illustrated in Figure 5 demonstrate the alterations in the expression of relevant genes reported to be implicated in the inflammatory response and oxidative stress. Black seed oil consumption instigated dramatic over-expression ($P<0.05$) in hepatic NF-κB (25.5%) and SREBF1 (46.3%) and considerable ($p<0.05$) down-regulation in PPARγ (25.8%), Adipor2, and MAPK (62%) gene expression compared to the control group. In contrast, CSO consumption elicited noticeable down-regulation ($P<0.05$) of the NF-κB (76.5%) and SREBF1 (55.2%) genes and up-regulated PPARγ (2.68-fold), MAPK (1.98-fold), and Adipor2 genes. Feeding rats on diets supplemented with the blended CSO and BSO had pronounced positive effects on the up-regulation of the genes

involved in the anti-inflammatory reactions (PPARγ and MAPK) and down-regulation of the genes related to inflammatory responses (NF-κB and SREBF1). These effects were more potent when the ratio of CSO to BSO was raised in the diet.

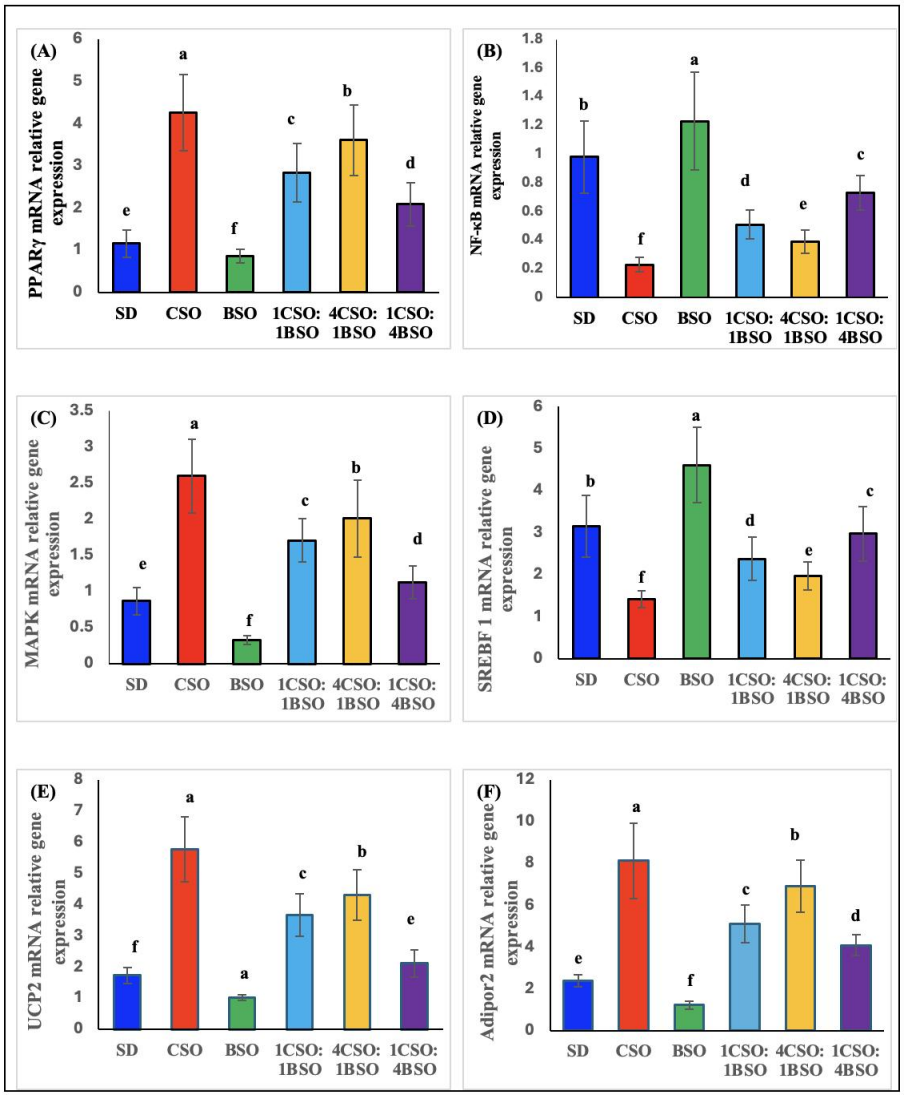

Fig. 5. Hepatic mRNA gene expression levels of **(A)** PPARγ, **(B)** NF-κB, **(C)** MAPK, **(D)** SREBF 1 **(E)** UCP2 and (F) Adipor 2 in rats fed on standard diet (SD), chia seed oil diet (CSO), black seed oil diet (BSO), 1 chia seed oil: 1 black seed oil (1CSO: 1BSO), 4 chia seed oil: 1 black seed oil (4 CSO: 1 BSO), and 1 chia seed oil: 4 black seed oil (1 CSO: 4 BSO). The values are displayed as the average ± standard deviation for 12 rats. Superscript variations through bars point out a significant difference at a P-value lower than 0.05.

Results presented in Table 5 demonstrate the effects of chronic intake of CSO and BSO on serum liver enzymes. Chronic intake of BSO significantly ($P<0.05$) elevated serum ALT and AST as compared to rats fed on the standard diet (21.7% and 11.8%, respectively) or the diet enriched with CSO (1.51- and 0.94-fold, respectively). Meanwhile, feeding CSO reduced ($P<0.05$) ALT (51.6%) and AST (40.9%) serum activity compared to the control. In the groups fed on diets containing CSO and BSO, ALT and AST levels were significantly $(P<0.05)$ reduced as the ratio of CSO increased.

Data in Table 5 further revealed that feeding BSO for twelve weeks significantly ($P<0.05$) impaired serum lipid profile, evidenced by the significant elevation in serum total cholesterol (25.1%), triacylglycerols (39.1%), and LDL-cholesterol (2.1-fold) concomitant with a marked decline ($P<0.05$) in serum HDL-cholesterol (41,7%). On the contrary, CSO consumption markedly ($P<0.05$) improved serum lipid profile as total cholesterol, triacylglycerols, and LDL-cholesterol levels were reduced by 52.7%, 66.21%, and 61.97%, respectively. In

contrast, the levels of HDL-cholesterol increased ($P<0.05$) by 2.21-fold as compared with the control group. Similarly, the serum levels of the cholesterol-synthesizing enzyme HMG-COA reductase were dramatically elevated when the rats were fed the black seed oil diet, reaching 25.14% higher than those of the rats fed the standard diet. In contrast, it was reduced by feeding chia seed oil as compared to the control. Furthermore, it was observed that the increment in consumption of chia seed oil instead of black seed oil is more effective in reducing serum liver enzymes and improving blood lipids.

Table (5): Effects of chronic intake of black and chia seed oils at different ratios on serum liver enzymes, HMG-COA reductase, and lipid profile

Parameters	Standard Diet	Chia seed oil	Black seed oil	1CSO: 1BSO	4CSO: 1BSO	1CSO: 4BSO
ALT (U/L)	21.18 ±1.18^{b}	10.2 ±0.48^{f}	25.79 ±1.43^{a}	15.6 ±0.72^{d}	13.20 ±0.51^{e}	18.39 ±0.63^{c}
AST (U/L)	28.54 ±2.01^{b}	16.3 ±0.81^{f}	31.9 ±2.14^{a}	22.47 ±1.62^{d}	19.06 ±1.27^{e}	26.54 ±1.81^{c}
HMG-COA reductase (ng/mL)	9.15 ±1.03^{b}	5.4 ±0.29^{f}	13.2 ±0.85^{a}	6.9 ±0.36^{e}	7.8 ±0.53^{d}	8.09 ±0.42^{c}
TC (mg/dL)	21.4±2.61^{b}	10.1±0.58^{f}	26.7±3.14^{a}	15.17±1.96^{d}	12.90±1.48^{e}	19.48±2.37^{c}
TAGs (mg/dL)	0.74±0.14^{b}	0.2±0.04^{f}	1.03±0.27^{a}	0.49±0.07^{d}	0.37±0.05^{e}	0.60±0.11^{c}
HDL-C (mg/dL)	0.91±0.33^{e}	3.1±0.81^{a}	0.5±0.20^{f}	2.10±062^{c}	2.79±0.69^{b}	1.45±0.42^{d}
LDL-C (mg/dL)	0.71±0.19^{b}	0.27±0.04^{f}	2.2±0.97^{a}	0.51±0.11^{d}	0.39±0.08^{e}	0.60±0.16^{c}

The values are displayed as the mean ± the standard deviation for 12 rats. Means with distinct superscripts throughout the row differ significantly at a P-value less than 0.05.

4. Discussion

This study hypothesized that the upsurge in the dietary consumption of CSO and BSO might impact the n-6 to n-3 ratio, which might lead to metabolic and inflammatory complications. Therefore, this research aimed to assess the consequences of these oils' upsurging and prolonged consumption on liver function, blood lipids, and specific oxidative stress and inflammation indicators.

As expected, prolonged over-consumption of BSO significantly instigated inflammation, as evidenced by the exacerbated COX-2 activity and PGE2 production and elevation in cytokines that trigger inflammation, including NO and IL-1β, and a significant drop in the anti-inflammatory cytokine IL-10. Furthermore, BSO excessive intake substantially suppressed PPAR-γ, MAPK, UCP2, and Adipor-2 genes and over-expressed NF-kappa and SREBF1 genes compared to the standard diet group. Contrariwise, CSO consumption had a notable anti-inflammatory response and favourably affected these indices.

The observed effects of CSO and BSO on the inflammatory process in this study might be explained by the type of PUFAs they have. In conformity with previous studies, the gas chromatophore analysis in the current research revealed that the most dominant fatty acid in BSO is LA and that it has a very high n-6 to n-3 ratio of about 195:1. On the contrary, the most predominant fatty acid in CSO is ALA; it has a ratio of n-6 to n-3 FA of about 1:3.4 [2]. Dietary fat may affect the fatty acid kind in tissues [36]. Valenzuela et al. [37] found that giving rats meals with greater ALA levels accelerates its accumulation into multiple tissues and its metabolism to n-3 long-chain PUFA (EPA and DHA), which are also built up in a variety of tissues of the animals. Similarly, long-term consumption of BSO could result in a high level of linoleic acid in rat tissues and its transformation to n-6 long-chain PUFAs like AA [11]. The cytochrome-P450 (CYP450), lipoxygenases (LOX), and cyclo-oxygenases (COX) metabolize polyunsaturated fatty acids (PUFAs) into oxy-lipins, which can function as endogenous mediators. AA and other n-6 fatty acids are sources of pro-inflammatory and pro-thrombotic eicosanoids, including the 4-series leukotrienes, thromboxane, and the 2-series prostaglandins [38]. Consequently, the effects of chia and black seed oils on the inflammatory process might be explained by the ability of n-6 and n-3 fatty acids to modulate the balance of pro- to anti-inflammatory lipid mediators, particularly eicosanoid production. Our findings align with Kokke et al. [39], who reported that plant oil with a high n-6/n-3 FA ratio elevated prostaglandin synthesis, promoting negative consequences in chronic inflammation. In the same context, studies of many cell types conducted in vitro reveal that n-3 fatty acids control inflammatory reactions. NF-κB activity is hampered in the monocytic cell line by n-3 fatty acids [40]. Similarly, Clinical investigation has demonstrated

that EPA and DHA ethyl esters supplemented at moderate or high doses augment n-3-PUFA-based anti-inflammatory oxylipins while hampering n-6 FA-based eicosanoids in human plasma [38].

The current study's findings proved that consuming chia seed oil suppressed the NF-kappa and SREBF1 genes while up-regulating the PPAR-γ, MAPK, UCP2, and Adipor-2 genes. On the other hand, black seed oil over-consumption negatively impacted the expression of these inflammatory genes. Therefore, the effects of PUFA on the inflammatory mediators' gene expression may be an additional possible mechanism responsible for the observed effects of tested oils on regulating the inflammatory response. Former investigations suggested that n-3 fatty acids could mitigate inflammation by preventing PPARγ from being phosphorylated and so maintaining a higher level of PPARγ activity [9]. Moreover, Previous work exhibited that when n-3 fatty acids activated PPARγ, they inhibited the pro-inflammatory transcription factor nuclear factor, (NF)-κB, and boosted the production of adiponectin and AMP-activated protein kinase (AMPK) [41]. Furthermore, through PPARγ activation, a supplement of n-3 fatty acids inhibited the NF-κB pathway from producing monocyte chemoattractant protein-1 (MCP-1) [42]. NF-kB is a modulatory transcription element that controls the expression of numerous genes that contribute to inflammation, such as TNF-α and IL-1[43].

Furthermore, the present study's results proved that prolonged consumption of a diet over-supplemented with black seed oil provoked oxidative stress, as evidenced by an apparent spike in serum lipid peroxidation and a remarkable reduction in serum levels of GSH, SOD activity, NrF2, and HO-1 when compared to feeding the standard diet or the diet supplemented with chia seed oil. The aforementioned elevated inflammatory response brought on by the high intake of n-6 fatty acids could explain the sparked oxidative stress in the rats fed the black seed oil diet. Prolonged inflammation may cause certain cellular afflictions, thereby increasing oxidative and endoplasmic reticulum (ER)-stress [44]. On the contrary, consuming chia seed oil augmented the antioxidant state and mitigated oxidative stress in rats by boosting GSH, SOD activity, NrF2, and HO-1 levels, along with lowering lipid peroxidation. Thus, chia seed oil might protect from oxidative stress-induced insults by targeting Nrf2/HO-1 signaling. Nrf2 is the transcriptional principal modulator of genes encoding for detoxifying anti-oxidant, anti-inflammatory protein [45-46]. Nrf2-dependent genes and related proteins, like heme oxygenase-1 (HO-1), partly mirror the main associated with Nrf2. HO-1 and its metabolites provide positive outcomes through their ability to mitigate oxidative damage, manage apoptosis, alleviate inflammation, and stimulate angiogenesis [47]. They also control the Nrf2 and NF-κB pathways that are triggered by oxidative stress [48]. The antioxidant effect of chia seed oil could be explained by the presence of either natural antioxidants in chia oil or by PUFA action, primarily ALA, which increases cell membrane mobility and, thus, the antioxidants' membrane accessibility [49]. These findings agree with those reported by Alam et al. [50], who noted that oral ALA administration can impede neuroinflammation and oxidative stress in the cortex of Cd-injected mouse brains. ALA is abundant in chia seed oil [51], and prior studies using the exact fatty acid from other sources verify the current findings. It was demonstrated that dietary consumption of ALA, produced from linseed oil, alleviated oxidative stress induced by organic mercury in rats' liver, kidneys, and blood by limiting lipid peroxidation and boosting antioxidants [52].

Additionally, the current study's results revealed that prolonged consumption of black seed oil (high n-6/n-3 FA ratio) elicited dyslipidemia, with a substantial elevation in the activity of the cholesterol-synthesizing enzyme HMG-COA and a considerable elevation in gene expression sterol-regulatory element-binding protein 1 (SREBP1), transcription factors that involved in fatty acid and cholesterol biosynthesis. Dyslipidaemia is a profound adverse effect of inflammatory reactions linked to metabolic disorders [53]. Thus, dyslipidemia observed in the rats fed on black seed oil might be influenced by the inflammatory consequences of high n-6 fatty acids consumption. Our data further show that feeding the rats with chia seed oil reduced blood lipids and HMG-COA and down-regulated SREBF-1, which agrees with the previous studies, which showed that feeding chia seed oil decreased blood lipids in rats compared to the control group. The hypolipidemic effect of chia seed oil was attributed to the enhancement of omega-3 unsaturated fatty acid and omega-6 fatty acid levels by CSO [36, 37]. These fatty acids inhibit the production of cholesterol in multiple ways, including reducing its reabsorption from the small intestine, restricting the action of the enzymes beta-hydroxymethylglutaryl-CoA and 7 alpha-hydroxylase, which transform cholesterol to bile acids, and hindering the creation of cholesterol by transforming mevalonate into squalene [54]. Furthermore, the presence of natural antioxidants in chia oil could reduce cholesterol levels. This study verified these suggestions, as our results demonstrated that feeding the chia seed oil-containing diet decreased the cholesterol-synthesizing enzyme HMG-COA activity. The impacts of n-3 PUFA supplementation on lowering blood triglyceride concentrations are well established. Supplementing with n-3 FAs was therefore suggested as a therapeutic approach to lower plasma triacylglycerols via promoting triglyceride

oxidation, enhancing lipoprotein lipase (LPL) activity, reducing hepatic lipogenesis, and facilitating VLDL and chylomicrons catabolism [55]. Omega-3 long-chain PUFAs suppress the hepatic production and secretion of VLDL-triglycerides by reducing transcription factors that regulate the expression of triglyceride assembly enzymes [55, 56]. Former studies have shown that lower triglyceride production in animal models after a fish oil diet is associated with the down-regulation of SREBP 1. Our results align with previous animal studies, which showed reduced HMG-CoA reductase expression or activity following n-3 PUFA intervention [56].

Data from the current research demonstrated an elevation in liver enzyme activities, AST and ALT, in rats fed an excessive black seed oil. The discharge of ALT and AST from hepatic cells into the circulation represents hepatocellular injury or death. This hepatocellular damage may be due to hepatic ectopic fat accumulation [57]. The ectopic fat buildup in the liver increases the liver's susceptibility to the flow of multiple pathological elements that may trigger increased hepatic injury, inflammation, and fibrosis [58]. The elevation of liver enzymes observed in rats fed black seed oil could be attributed to the over-consumption of n-6 PUFAs and the under-consumption of n-3 PUFAs that provoked inflammation and pro-inflammatory cytokine production [59]. Excessive consumption of n-6 PUFA may raise the liver's synthesis of oxidized linoleic acid metabolites, which could be a risk factor for developing fatty liver [60]. On the other hand, chia seed oil reduced serum levels of ALT and AST. In line with our results, Da Silva et al. [61] reported that chia seeds inhibited the buildup of cytosolic lipid aggregates in the hepatocytes of a healthy, non-obese Wistar rat model, altering the susceptibility to NAFLD. In another study, the chia seed impeded the hepatocyte buildup of cytosolic lipid vesicle arrangements in diet-induced obese rat models [62]. Chia seeds contain many bioactive and antioxidant substances that might protect hepatic cells against oxidative injury. Furthermore, n-3 fatty acids in chia seed oil may reduce hepatic lipogenesis and inflammation [63]. Likewise, Khadge et al. [64] found that fish oil supplementation significantly improved Western diet-induced fish oil administration markedly ameliorated Western diet-induced hyperlipidemia, liver enzyme elevation, inflammatory infiltration, liver steatosis, and fibrosis in male Sprague–Dawley rats.

Finally, this study demonstrated the effect of the excessive, long-term dietary administration of chia and black seed oils in rats. The most prominent findings of this study were that prolonged over-consumption of BSO, which has a very high n-6/ n-3 FA ratio, significantly instigated dyslipidemia, elevation in liver enzymes, oxidative stress, increased serum COX-2 activity, the production of PGE 2, and an upsurge in inflammatory cytokines and dysregulation in the expression of critical genes implicated in the inflammatory response. In contrast, CSO consumption significantly reduced blood lipids and liver enzyme activities, enhanced the antioxidant status, attenuated lipid peroxidation, decreased serum inflammatory cytokines, and regulated the expression of genes involved in the inflammatory response. Additionally, feeding the blended oil prepared from both oils in different proportions favorably enhanced these metabolic, oxidative, and inflammatory consequences compared to rats fed on BSO or standard diets. These effects were more prominent when feeding a higher CSO proportion than BSO.

Conclusion

The upsurge consumption of functional oils, such as chia and black seed oils, could affect the diet's n-6/ n-3 FA balance. An unbalanced omega-6 to omega-3 ratio favoring omega-6 PUFAs is highly proinflammatory, contributing to chronic inflammatory disease progression. Therefore, when adopting a dietary habit with excessive functional oils such as chia or nigella sativa seed oils for therapeutic or health-promoting purposes, the diet's overall n-6/n-3 fatty acid content is crucial.

Declarations

Ethics approval

All methods in the current research were approved by the Ethics Committee in Women's College, Ain Shams University, Egypt (approval code: Sci-1532410002).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no specific grant from any funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

A.M.S.G.: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing-original draft. MMM: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing-original draft, Validation, Writing-review. G.M.M: Conceptualization, Investigation, Methodology. All authors read and approved the final manuscript.

References

1. Hashempour-Baltork, F., Farshi, P., Alizadeh, A. M., Azadmard-Damirchi, S., & Torbati, M. (2022). Nutritional Aspects of Vegetable Oils: Refined or Unrefined? *European Journal of Lipid Science and Technology*, *124*(12), 2100149. https://doi.org/10.1002/ejlt.202100149
2. El-Feky, A. M., Elbatanony, M. M., Aboul Naser, A. F., Younis, E. A., & Hamed, M. A. (2022). *Salvia hispanica L.* seeds extract alleviate encephalopathy in streptozotocin-induced diabetes in rats: role of oxidative stress, neurotransmitters, DNA and histological indices. *Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals*, *27*(5), 427–440. https://doi.org/10.1080/1354750X.2022.2051072
3. Melo, D., , Machado, T. B., , & Oliveira, M. B. P. P., (2019). Chia seeds: an ancient grain trending in modern human diets. *Food & function*, *10*(6), 3068–3089. https://doi.org/10.1039/c9fo00239a
4. Mohamed, D. A., Mohamed, R. S., & Fouda, K. (2020). Anti-inflammatory potential of chia seeds oil and mucilage against adjuvant-induced arthritis in obese and non-obese rats. Journal of basic and clinical physiology and pharmacology, 31(4), /j/jbcpp.2020.31.issue-4/jbcpp-2019-0236/jbcpp-2019-0236.xml. https://doi.org/10.1515/jbcpp-2019-0236
5. Muñoz, L. A., Cobos, A., Diaz, O., & Aguilera, J. M. (2013). Chia Seed (*Salvia hispanica*): An Ancient Grain and a New Functional Food. *Food Reviews International*, *29*(4), 394–408. https://doi.org/10.1080/87559129.2013.818014
6. Kiralan, M., Özkan, G., Bayrak, A., & Ramadan, M. F. (2014). Physicochemical properties and stability of black cumin (Nigella sativa) seed oil as affected by different extraction methods. *Industrial Crops and Products*, *57*, 52-58. https://doi.org/10.1016/j.indcrop.2014.03.026
7. Trigui, I., Yaich, H., Zouari, A., Cheikh-Rouhou, S., Blecker, C., Attia, H., & Ayadi, M. (2021). Structure-function relationship of black cumin seeds protein isolates: Amino-acid profiling, surface characteristics, and thermal properties. *Food Structure*, *29*, 100203. https://doi.org/10.1016/j.foostr.2021.100203
8. Kabir, Y., Akasaka-Hashimoto, Y., Kubota, K., & Komai, M. (2020). Volatile compounds of black cumin (*Nigella sativa* L.) seeds cultivated in Bangladesh and India. *Heliyon*, *6*(10), e05343. https://doi.org/10.1016/j.heliyon.2020.e05343
9. Wang, C., Lee, C., Wu, D., Chen, S., & Lee, T. (2022). Differential effects of EPA and DHA on PPARγ-mediated sympathetic innervation in infarcted rat hearts by GPR120-dependent and -independent mechanisms. *The Journal of Nutritional Biochemistry*, *103*, 108950. https://doi.org/10.1016/j.jnutbio.2022.108950
10. Jump D. B. (2011). Fatty acid regulation of hepatic lipid metabolism. *Current opinion in clinical nutrition and metabolic care*, *14*(2), 115–120. https://doi.org/10.1097/MCO.0b013e328342991c
11. Alshatwi, A. A., & Subash-Babu, P. (2018). Effects of increasing ratios of dietary omega-6/omega-3 fatty acids on human monocyte immunomodulation linked with atherosclerosis. Journal of Functional Foods, *41*, 258-267. https://doi.org/10.1016/j.jff.2017.12.020
12. Bhol, N. K., Bhanjadeo, M. M., Singh, A. K., Dash, U. C., Ojha, R. R., Majhi, S., Duttaroy, A. K., & Jena, A. B. (2024). The interplay between cytokines, inflammation, and antioxidants: mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine compounds. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *178*, 117177. https://doi.org/10.1016/j.biopha.2024.117177

13. Mohamed M.M. (2024). Sesamum Indicum L. seed oil synergistically enhances the effectiveness of acetylcholine esterase inhibitor in managing scopolamine-induced neurological dysfunction in rats 10.21608/ejchem.2024.290877.9736
14. AOCS (American Oil Chemists Society), 5th ed. Official Methods and Recommended Practices of the American Oil Chemists Society, "Calculated iodine value", AOCS Press, Champaign, IL, p. Cd 1c-8, 1999.
15. ISO 3960:2007. Animal and vegetable fats and oils. Determination of Peroxide value, p. 9.
16. ISO 660:2009. Animal and vegetable fats and oils. Determination of acid value and acidity, p. 5.
17. ISO/FDIS 3657:2001. Animal and vegetable fat and oils. Determination of Saponification value Commission of the European Communities. Regulation 2568/91 on the characteristics of olive oil and olive-residues and on relevant methods of analysis.Off. J. Eur. Commun., L248 (2003), pp. 1-109.
18. Mohamed M.M. & Farid A.O. (2024). Gastro-Protective, Antioxidant, and Anti-Inflammatory Potential of Three Vegetable Oils Against Indomethacin-Induced Gastric Ulceration in Rats: in Vivo and in Vitro Study. Egyptian Journal of Chemistry, 67, 341-359. 10.21608/ejchem.2024.262460.9181
19. Swain, T. and Hillis, W.E. (1959) The Phenolic Constituents of Prunus domestica. I.—The Quantitative Analysis of Phenolic Constituents. Journal of the Science of Food and Agriculture, 10, 63-68. http://dx.doi.org/10.1002/jsfa.2740100110
20. Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, *11*(8), 2248. https://doi.org/10.3390/pr11082248
21. Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 239, 70e76.
22. Reeves, P. G., Nielsen, F. H., & Fahey, G. C., Jr (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *The Journal of nutrition*, *123*(11), 1939–1951. https://doi.org/10.1093/jn/123.11.1939
23. Simopoulos A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Experimental biology and medicine (Maywood, N.J.), 23, 674–688. https://doi.org/10.3181/0711-MR-311
24. Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of biological chemistry*, *247*(10), 3170–3175.
25. Beutler, E., Duron, O. and Kelly, B.M. (1963) Improved Method for the Determination of Blood Glutathione. Journal of Laboratory and Clinical Medicine, 61, 882-88
26. Montgomery, H.A.C. and Dymock, J. (1961) The determination of nitrite in water. Analyst, 86, 414-416.
27. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
28. Reitman, S.; Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 1957, *28*, 56–63.
29. Lakshmanan, M. R., Dugan, R. E., Nepokroeff, C. M., Ness, G. C., & Porter, J. W. (1975). Regulation of rat liver beta-hydroxy-beta-methylglutaryl coenzyme. A reductase activity and cholesterol levels of serum and liver in various dietary and hormonal states. *Archives of biochemistry and biophysics*, *168*(1), 89–95. https://doi.org/10.1016/0003-9861(75)90231-3
30. Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical chemistry*, *20*(4), 470–475.
31. Fossati, P., & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical chemistry*, *28*(10), 2077–2080.
32. Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), 499–502.
33. Steele, B. W., Koehler, D. F., Azar, M. M., Blaszkowski, T. P., Kuba, K., & Dempsey, M. E. (1976). Enzymatic determinations of cholesterol in high-density-lipoprotein fractions prepared by a precipitation technique. *Clinical chemistry*, *22*(1), 98–101.
34. Mohamed, M. M., Kamel, E. A., Ahmed, K. A., Rashed, L. A., & Ismail, S. H. (2024). The potential efficacy of *Artemisia anuua* L. extract nanoparticles in mitigating obesity-related-metabolic complications in hypercaloric diet-fed rats. *Egyptian Journal of Basic and Applied Sciences*, *11*(1), 183–212. https://doi.org/10.1080/2314808X.2024.2331903
35. Levesque, R. (2007): SPSS programming and data management: A Guide for SPSS and SAS user.3rd Edition, United States of America.
36. Ali, Y. M., Kadir, A. A., Ahmad, Z., Yaakub, H., Zakaria, Z. A., & Hakim Abdullah, M. N. (2011). Free radical scavenging activity of conjugated linoleic acid as single or mixed isomers. *Pharmaceutical Biology*, *50*(6), 712–719. https://doi.org/10.3109/13880209.2011.621714
37. Valenzuela R, Barrera C, Ayala JM, Sanhueza J, Valenzuela A. (2014). Vegetable oils rich in alpha linolenic acid allow a higher accretion of n-3 LCPUFA in the plasma, liver and adipose tissue of the rat. Grasas Aceites 65 (2): e026. doi: http://dx.doi.org/10.3989/gya.110113
38. Elajami, T. K., Colas, R. A., Dalli, J., Chiang, N., Serhan, C. N., & Welty, F. K. (2016). Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *30*(8), 2792–2801. https://doi.org/10.1096/fj.201500155R

39. Kokke, K. H., Morris, J. A., & Lawrenson, J. G. (2008). Oral omega-6 essential fatty acid treatment in contact lens associated dry eye. *Contact Lens and Anterior Eye*, *31*(3), 141-146. https://doi.org/10.1016/j.clae.2007.12.001
40. Huang, C. H., Hou, Y. C., Yeh, C. L., & Yeh, S. L. (2015). A soybean and fish oil mixture with different n-6/n-3 PUFA ratios modulates the inflammatory reaction in mice with dextran sulfate sodium-induced acute colitis. *Clinical nutrition (Edinburgh, Scotland)*, *34*(5), 1018–1024. https://doi.org/10.1016/j.clnu.2014.11.008
41. Mocellin, M. C., Camargo, C. Q., Nunes, E. A., Fiates, G. M. R., & Trindade, E. B. S. M. (2016). A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer. *Clinical nutrition (Edinburgh, Scotland)*, *35*(2), 359–369. https://doi.org/10.1016/j.clnu.2015.04.013
42. Fang, I. M., Yang, C. H., & Yang, C. M. (2014). Docosahexaenoic acid reduces linoleic acid induced monocyte chemoattractant protein-1 expression via PPARγ and nuclear factor-κB pathway in retinal pigment epithelial cells. *Molecular nutrition & food research*, *58*(10), 2053–2065. https://doi.org/10.1002/mnfr.201400196
43. Patterson, W. L., 3rd, & Georgel, P. T. (2014). Breaking the cycle: the role of omega-3 polyunsaturated fatty acids in inflammation-driven cancers. *Biochemistry and cell biology = Biochimie et biologie cellulaire*, *92*(5), 321–328. https://doi.org/10.1139/bcb-2013-0127
44. Wang, H., Blumberg, J. B., Chen, C. Y., Choi, S. W., Corcoran, M. P., Harris, S. S., Jacques, P. F., Kristo, A. S., Lai, C. Q., Lamon-Fava, S., Matthan, N. R., McKay, D. L., Meydani, M., Parnell, L. D., Prokopy, M. P., Scott, T. M., & Lichtenstein, A. H. (2014). Dietary modulators of statin efficacy in cardiovascular disease and cognition. *Molecular aspects of medicine*, *38*, 1–53. https://doi.org/10.1016/j.mam.2014.04.001
45. Harris, J. I., Hibbeln, J. R., Mackey, R. H., & Muldoon, M. F. (2004). Statin treatment alters serum n-3 and n-6 fatty acids in hypercholesterolemic patients. *Prostaglandins, leukotrienes, and essential fatty acids*, *71*(4), 263–269. https://doi.org/10.1016/j.plefa.2004.06.001
46. Moi, P., Chan, K., Asunis, I., Cao, A., & Kan, Y. W. (1994). Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(21), 9926–9930. https://doi.org/10.1073/pnas.91.21.9926
47. Loboda, A., Damulewicz, M., Pyza, E., Jozkowicz, A., & Dulak, J. (2016). Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: An evolutionarily conserved mechanism. *Cellular and Molecular Life Sciences: CMLS*, *73*(17), 3221-3247. https://doi.org/10.1007/s00018-016-2223-0
48. Kang, J. X., & Weylandt, K. H. (2008). Modulation of inflammatory cytokines by omega-3 fatty acids. *Sub-cellular biochemistry*, *49*, 133–143. https://doi.org/10.1007/978-1-4020-8831-5_5
49. Hernández-Rodas, M. C., Valenzuela, R., Echeverría, F., Rincón-Cervera, M. Á., Espinosa, A., Illesca, P., Muñoz, P., Corbari, A., Romero, N., Gonzalez-Mañan, D., & Videla, L. A. (2017). Supplementation with Docosahexaenoic Acid and Extra Virgin Olive Oil Prevents Liver Steatosis Induced by a High-Fat Diet in Mice through PPAR-α and Nrf2 Upregulation with Concomitant SREBP-1c and NF-kB Downregulation. *Molecular nutrition & food research*, *61*(12), 10.1002/mnfr.201700479. https://doi.org/10.1002/mnfr.201700479
50. Alam, S. I., Kim, M. W., Shah, F. A., Saeed, K., Ullah, R., & Kim, M. O. (2021). Alpha-Linolenic Acid Impedes Cadmium-Induced Oxidative Stress, Neuroinflammation, and Neurodegeneration in Mouse Brain. *Cells*, *10*(9), 2274. https://doi.org/10.3390/cells10092274
51. Ayerza (h), R. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *J Am Oil Chem Soc* **72**, 1079–1081 (1995). https://doi.org/10.1007/BF02660727
52. Pal, M., & Ghosh, M. (2012). Prophylactic effect of α-linolenic acid and α-eleostearic acid against MeHg induced oxidative stress, DNA damage and structural changes in RBC membrane. Food and Chemical Toxicology, 50(8), 2811-2818. https://doi.org/10.1016/j.fct.2012.05.038
53. Chen, L. Z., Jing, X. B., Wu, C. F., Zeng, Y. C., Xie, Y. C., Wang, M. Q., Chen, W. X., Hu, X., Zhou, Y. N., & Cai, X. B. (2021). Nonalcoholic Fatty Liver Disease-Associated Liver Fibrosis Is Linked with the Severity of Coronary Artery Disease Mediated by Systemic Inflammation. *Disease markers*, *2021*, 6591784. https://doi.org/10.1155/2021/6591784
54. Alagawany, M., Nasr, M., Abdulaziz Al-Abdullatif, Alhotan, R. A., Azzam, M. M., & Reda, F. M. (2020). Impact of dietary cold-pressed chia oil on growth, blood chemistry, haematology, immunity and antioxidant status of growing Japanese quail. *Italian Journal of Animal Science*, *19*, 896–904. https://doi.org/10.1080/1828051X.2020.1807420
55. Lima Rocha, J. É., Mendes Furtado, M., Mello Neto, R. S., da Silva Mendes, A. V., Brito, A. K. D. S., Sena de Almeida, J. O. C., Rodrigues Queiroz, E. I., de Sousa França, J. V., Silva Primo, M. G., Cunha Sales, A. L. C., Gomes Vasconcelos, A., Felix Cabral, W., Souza Kückelhaus, S. A., de Souza de Almeida Leite, J. R., Fortes Lustosa, A. K. M., Lucarini, M., Durazzo, A., Arcanjo, D. D. R., & Martins, M. D. C. C. E. (2022). Effects of Fish Oil Supplementation on Oxidative Stress Biomarkers and Liver Damage in Hypercholesterolemic Rats. *Nutrients*, *14*(3), 426. https://doi.org/10.3390/nu14030426
56. Notarnicola, M., Messa, C., Refolo, M. G., Tutino, V., Miccolis, A., & Caruso, M. G. (2011). Polyunsaturated fatty acids reduce fatty acid synthase and hydroxy-methyl-glutaryl CoA-reductase gene expression and promote apoptosis in HepG2 cell line. *Lipids in health and disease*, *10*, 10. https://doi.org/10.1186/1476-511X-10-10
57. Day C. P. (2006). From fat to inflammation. *Gastroenterology*, *130*(1), 207–210. https://doi.org/10.1053/j.gastro.2005.11.017
58. Medeiros, G. C. B. S., Azevedo, K. P. M., Mesquita, G. X. B., Lima, S. C. V. C., Silva, D. F. O., Pimenta, I. D. S. F., Gonçalves, A. K. D. S., Lyra, C. O., & Piuvezam, G. (2019). Red meat consumption, risk of incidence of cardiovascular disease and cardiovascular mortality, and the dose-response effect: Protocol for a systematic review and meta-analysis of longitudinal cohort studies. *Medicine*, *98*(38), e17271. https://doi.org/10.1097/MD.0000000000017271

59. Simopoulos A. P. (2016). An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients*, *8*(3), 128. https://doi.org/10.3390/nu8030128
60. Bogl, L. H., Kaprio, J., & Pietiläinen, K. H. (2020). Dietary n-6 to n-3 fatty acid ratio is related to liver fat content independent of genetic effects: Evidence from the monozygotic co-twin control design. *Clinical nutrition (Edinburgh, Scotland)*, *39*(7), 2311–2314. https://doi.org/10.1016/j.clnu.2019.10.011
61. da Silva, B. P., Dias, D. M., de Castro Moreira, M. E., Toledo, R. C., da Matta, S. L., Lucia, C. M., Martino, H. S., & Pinheiro-Sant'Ana, H. M. (2016). Chia Seed Shows Good Protein Quality, Hypoglycemic Effect and Improves the Lipid Profile and Liver and Intestinal Morphology of Wistar Rats. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *71*(3), 225–230. https://doi.org/10.1007/s11130-016-0543-8
62. Poudyal, H., Panchal, S. K., Waanders, J., Ward, L., & Brown, L. (2012). Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. *The Journal of nutritional biochemistry*, *23*(2), 153–162. https://doi.org/10.1016/j.jnutbio.2010.11.011
63. Yan, J. H., Guan, B. J., Gao, H. Y., & Peng, X. E. (2018). Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. *Medicine*, *97*(37), e12271. https://doi.org/10.1097/MD.0000000000012271
64. Khadge, S., Sharp, J. G., Thiele, G. M., McGuire, T. R., Klassen, L. W., Duryee, M. J., Britton, H. C., Dafferner, A. J., Beck, J., Black, P. N., DiRusso, C. C., & Talmadge, J. (2018). Dietary omega-3 and omega-6 polyunsaturated fatty acids modulate hepatic pathology. *The Journal of nutritional biochemistry*, *52*, 92–102. https://doi.org/10.1016/j.jnutbio.2017.09.017