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Chia seeds oil enriched with phytosterols and mucilage as a cardioprotective dietary supplement towards inflammation, oxidative stress, and dyslipidemia

Doha A. Mohamed*, Shaimaa E. Mohammed, Ibrahim M. Hamed

Nutrition and Food Sciences Department, National Research Centre, Dokki, Cairo, Egypt

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ABSTRACT

Introduction: Non-communicable diseases are a cluster of metabolic diseases, which include type-2 diabetes, cancer, and cardiovascular diseases (CVDs). The aim of the current research was to incorporate dietary fibers (mucilage) and phytosterol for enriching chia seeds oil for producing new dietary supplements for cardio-protection from oxidative stress, inflammation, and dyslipidemia.

Methods: Fatty acids profile, phytosterols, and phenolic compounds content of the prepared dietary supplement were assessed. The cardioprotective potency of the dietary supplement was evaluated in rats fed on a high-fat diet for a month. Biochemical parameters related to inflammation, oxidative stress, lipid profile, cardiac enzymes, and kidney function were determined in all rats.

Results: The results revealed that dietary supplement was rich in omega-3 fatty acids. Beta-sitosterol and campesterol were the major phytosterols in chia seeds oil dietary supplement. Phenolic compounds were present by 25.9 ± 1.202 mg gallic acid equivalent (GAE)/g dietary supplements. Rats fed on the high-fat diet showed significant elevation ($P<0.05$) in inflammatory markers, oxidative stress, dyslipidemia, and cardiac enzymes in association with the elevation of kidney function compared with normal rats. Administration of both doses of dietary supplement significantly ($P<0.05$) improved all the studied biochemical parameters. The high dose of the dietary supplement was promising in the reduction of inflammatory markers, oxidative stress, and improved dyslipidemia in accordance with the reduction of all cardiac enzymes and kidney function.

Conclusion: Dietary supplements investigated in the current research showed cardioprotective potency through its anti-inflammatory and dyslipidemic activities, which may be attributed to the presence of phenolic compounds, omega-3 fatty acids, phytosterols, and soluble dietary fibers.

Implication for health policy/practice/research/medical education:
Dietary supplements containing chia seeds oil enriched with phytosterols and mucilage might be served as potential protective agents against cardiovascular disease through its anti-inflammatory and dyslipidemic activities.

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Introduction

Non-communicable diseases such as cancer and cardiovascular diseases (CVDs) are probably responsible for 70% of global deaths, therefore representing the leading cause of mortality (1). CVDs such as coronary heart disease, myocardial infarction, stroke, and heart failure are the leading cause of worldwide death, and the prevalence is rising in low- and middle-income countries (2). Etiologies of CVDs include inflammation and oxidative stress associated with hypercholesterolemia as the main risk factors for developing cardiovascular events (3,4). Hyperlipidemia is a metabolic disorder involving high levels of cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C) in association

***Corresponding author**: Doha Abdou Mohamed,
Email: dohamohamed@yahoo.com

with a reduction in high-density lipoprotein cholesterol (HDL-C) (5). Moreover, the development of CVDs depends on the amount of oxidized lipoproteins (oxidized LDL, Ox-LDL) existing in the arterial wall (6). Unhealthy dietary habits are one of the leading causes of CVDs (7). Some dietary components are less consumed than recommended such as dietary fibers (gums, mucilages, pectins, oligosaccharides), which leads to many health problems. Dietary fibers (soluble and insoluble) consumption exhibited health benefits such as reduction of postprandial glucose concentration, prevention from cancer and lowering the risk of CVDs (8,9). Mucilage is an insoluble fiber containing 80% soluble polysaccharides and forming-gel in water (10). Phytochemicals extracted from plant sources (phenolic compounds, phytosterols) exhibited promising preventing activities against chronic diseases such as rheumatoid arthritis, type 2 diabetes, CVDs and cancer (11-15). Agriculture by-products such as rice bran and wheat germ, which are produced during the cereals refining process (16) contain bioactive phytochemicals possessing many beneficial activities for the management of chronic diseases (17,18). Wheat germ oil is one of the richest sources of phytosterols (19). Previously phytosterols showed anti-inflammatory activity and LDL-C lowering activity (17,20). It was reported that the recommended daily intake of phytosterols is 2 g/d for lowering the risk of chronic diseases (21). Chia seeds (*Salvia hispanica* L.) are the richest source of dietary fibers and omega-3 fatty acids, also rich in protein, minerals, and vitamins (22). Dietary fibers (mucilaginous polysaccharide/gum) are represented by 34%-36% in chia seeds, which is higher than many fruits, vegetables, or cereals (23). Chia seed gum/mucilage (soluble fiber) constitutes 4%–6% of the seed dry mass, forming a gel when dissolved in water. It can absorb water up to 12 times its weight (21,24,25). Timilsena et al (24) reported xylose, glucose, arabinose, galactose, glucuronic acid, and galacturonic acid as monosaccharide units in the chia seeds gum. Chia seeds dietary fibers (soluble and insoluble) are responsible for reducing blood glucose and lowering glycemic response (23) also showed anti-inflammatory activity (13). Chia seeds oil is rich in omega-3 fatty acids, and the essential fatty acids are in a ratio of 3:1 represented by 64% alpha-linolenic acid (18:3 omega-3 fatty acid) and 21% linoleic acid (18:2 omega-6 fatty acid), respectively (26). This ratio has been recommended for the prevention of metabolic diseases (27,28). Omega-3 fatty acids exhibited affirmative potential on the metabolism of fat, inflammation, oxidative, vascular and arrhythmogenic factors implicated in CVDs (29). So, the current research aimed to incorporate dietary fibers (chia seeds mucilage) and phytosterol (unsaponifiable matter of wheat germ oil) for enriching chia seeds oil for producing new dietary supplements for cardio-protection from oxidative stress, inflammation, and dyslipidemia.

Materials and Methods

Materials

Plant materials

Chia seeds (*Salvia hispanica* L.) were purchased from local markets, Cairo, Egypt.

Animals

Male Sprague Dawley rats 170.7 ± 6.411 as mean ± SD were used in the present study. Animals were obtained from the Animal House of National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel metabolic cages; water and food were given *ad libitum*. This study has been carried out as a part of internal project No. 12050203 in the NRC.

Animal's diets

Two diets were prepared and fed to animals all the experimental period. A high-fat diet was prepared according to Mohamed et al (11) and Gao et al (5), which contained 45% of calories from fat, 1% cholesterol and 0.25% cholic acid. The balanced diet consisted of 12% casein, 10% corn oil, 68.5% carbohydrate, 1% vitamin mixture, and 3.5% salt mixture. Salt and vitamin mixtures were prepared according to AIN-93 (30).

Methods

Extraction of chia seeds oil and mucilage

Chia seeds were crushed, then pressed with laboratory type of Carver hydraulic press under 10.000 Ib/in (pic) pressure for 1 hour at room temperature according to the method of Üstun et al (31). The produced oil was filtered and kept in dark bottles in deep freeze until used. Chia seeds mucilage was extracted from whole chia seeds according to the method of Segura-Campos et al (32) and Mohamed et al (13). Seeds of chia were submitted to mucilage extraction with water at a 1:20 ratio (w/v) for 30 minutes and at a 50°C temperature under constant stirring. After that, the suspension was milled in a mixer, and then it was boiled again at 50°C under stirring for 15 minutes. The crude mixture containing water, gum, and seeds was frozen at -20°C for 12 hours and then freeze-dried. The material dried was sprayed and separated on a sieve using No. 20 ASTM (0.849 mm) meshes.

Extraction of unsaponifiable matter from wheat germ oil

The wheat germ was soaked in pure n-hexane for 24 hours. The miscella was collected and filtered. This process was repeated three times using fresh solvent each time. The solvent was evaporated under vacuum at 40°C in a rotary evaporator. The oil was dried over anhydrous sodium sulfate, filtered, stored in dark brown bottles without any further purification, and then kept in deep freeze until used (33). The unsaponifiable matter was separated from the wheat germ oil according to the method described in Association of Official Analytical Chemists (AOAC) (33).

Preparation of dietary supplement

Chia seeds oil, chia seeds mucilage, and unsaponifiable matter of wheat germ oil were mixed in ratio 2:1:1 for preparation of dietary supplement. The dietary supplement was emulsified in water using Tween 80 as an emulsifying agent. The dietary supplement was orally given to rats in two different doses of 150 mg and 300 mg/kg RBW/day for a month.

Extraction and determination of phenolic compounds in the prepared dietary supplement

Phenolic compounds were extracted from the prepared dietary supplement according to the method of Oliveira-Alves et al (34) with minor modification using a mixture of hexane and methanol 80%. Phenolic compounds' content was determined using the Folin-Ciocalteu reagent (35). Absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g extract. The results were expressed as mean ± SD.

Determination of fatty acids profile and phytosterols of the prepared dietary supplement

Fatty acid methyl esters and phytosterols of the prepared dietary supplement were prepared according to methods of AOAC (33) to be subjected to GLC analysis of fatty acids and phytosterols. Identification and assessment of the fatty acids methyl ester and phytosterols were carried out by the same condition used in Mohamed et al (18).

Evaluation of the cardioprotective role of chia seeds oil dietary supplement

Twenty-four male rats were allowed acclimatizing for 7 days in their cages before starting the experiment. Rats were divided into four groups (6/group) and fed on balanced or high fat diet for a month. Group one was served as the normal control group, while group two was hypercholesterolemic control. The third and fourth groups were fed on the high-fat diet and given dietary supplements orally on daily basis; at 150 and 300 mg/kg rat body weight, respectively for a month. During the experiment, body weight and food intake were recorded weekly. At the end of the experimental study, total food intake, body weight gain, and food efficiency ratio were calculated. Blood samples were collected from all rats after an overnight fast for determination of plasma total cholesterol (TC), HDL-C, LDL-C and triglycerides (TG) using colorimetric kits. TC/HDL-C ratio was calculated as cardiac risk ratio. Also the ratio of HDL-C to LDL-C was calculated as cardioprotective index, while atherogenic index was calculated according to the equation Atherogenic index = (total cholesterol–HDL cholesterol)/HDL cholesterol. Plasma malondialdehyde (MDA) was assessed using colorimetric kit and Ox-LDL was assessed using ELISA kit (Catalogue # SL0554Ra, Sunlong®) the indicators of lipid peroxidation. Plasma catalase (CAT) was determined as an antioxidant enzyme using a colorimetric kit. C-reactive protein (CRP) and tumor necrosis factor (TNF-α) were determined as inflammatory markers using ELISA kits (Catalogue # SL0202Ra Sunlong® and Catalogue # SL0722Ra, Sunlong®, respectively). Plasma activities of cardiac marker enzymes aminotransferase (ALT & AST), lactate dehydrogenase (LDH), and creatine kinase (CK) were measured using colorimetric and kinetic kits. Plasma creatinine and urea were estimated using colorimetric and kinetic kits as kidney function indicators. After animal anesthesia and scarification, the heart was dissected and weighed. Relative heart weight percent was calculated as follows:

Relative heart weight%= Absolute heart weight (g) × 100/final body weight (g) (14).

Statistical analysis

Results of the animal experiments were expressed as mean ± SEM (standard error of mean). Different groups were compared statistically using one-way analysis of variance (ANOVA) followed by Duncan's test (Using SPSS, version 22). In all cases, $P<0.05$ was used as the criterion of statistical significance.

Results

Fatty acids, phytosterols and total phenolic compounds content of chia seeds oil dietary supplement

The fatty acids profile of the chia seeds oil dietary supplement revealed that it contained high amounts of unsaturated fatty acids, especially linolenic acid (59.2%) as an omega-3 fatty acid. Linoleic acid as omega-6 fatty acid was present by 19.1%. Chia seeds oil dietary supplement contained perfect proportion of omega-6/omega-3 fatty acids 1:3.1. Phytosterols were present by 71.6%; β-sitosterol (51.6%) was the major phytosterol followed by campesterol (16.4%). Stigmasterol (3.6%) was the minor phytosterol present in the chia seeds oil dietary supplement. Total phenolic compounds content was present by 25.9 ± 1.202 as mg GAE/g dietary supplement (Table 1).

Studying the cardioprotective effect of chia seeds oil dietary supplement

Table 2 represents the nutritional parameters and relative-heart weight % of the different experimental groups. Initial and relative heart weight % of the different experimental groups showed non-significant changes. Final, body weight gain and food efficiency ratio showed a significant reduction in the rats group given the high dose dietary supplement ($P<0.05$). The rats, which were given both doses of chia seeds oil dietary supplement showed a significant decrease in food intake compared with the normal and hypercholesterolemic control groups.

The rats fed on the high fat diet for a month showed a significant elevation of plasma total cholesterol,

Table 1. Fatty acids, phytosterols, and total phenolic compounds content of chia seeds oil dietary supplement

Parameters	Dietary supplement
Fatty acids (as a percentage of total fatty acids)	
Palmitic acid (C16:0)	9.1
Oleic acid (C18:1)	7.8
Linoleic acid (C18:2) (ω-6)	19.1
Linolenic acid (C18:3) (ω-3)	59.2
Total saturated fatty acids	9.1
Total unsaturated fatty acids	86.1
Ratio of ω-6: ω-3	1:3.1
Phytosterols (as a percentage of total unsaponifiable matter)	
β-Sitosterol	51.6
Stigmasterol	3.6
Campesterol	16.4
Total phytosterols	71.6
Total phenolic compounds (mg GAE/g)	25.9±1.202

GAE: Gallic acid equivalent.

triglycerides, LDL-C, non-HDL-C, and VLDL-C in association with a significant reduction in plasma HDL-C and cardioprotective index (HDL-C/LDL-C) in the hypercholesterolemic control group compared with the normal control group (Table 3). Hypercholesterolemia was associated with a significant elevation of the atherogenic index (Tch–HDL-C)/HDL-C) and cardiac risk ratio (TC/HDL-C). Administration of chia seeds oil dietary supplement in low and high doses showed significant improvements in all the studied lipid profile parameters with different degrees (Table 3). The high dose of chia seeds oil was the most promising in this concern.

In the present study, MDA and Ox-LDL, as indicators of lipid peroxidation, were significantly higher in hypercholesterolemic rats in comparison with the normal rats, while CAT as an antioxidant enzyme reduced significantly in the hypercholesterolemia control group compared with the normal control group (Table 4).

The increments in the lipid peroxidation markers in association with reduction of CAT as antioxidant enzyme are indicators for oxidative stress. Significant increases were noticed in the inflammatory markers (CRP and TNF-α) in hypercholesterolemia control in comparison with normal control (Table 4). Administration of chia seeds oil dietary supplement in low and high doses exhibited a significant reduction in MDA and Ox-LDL as lipid peroxidation markers in the association of elevation of CAT as an antioxidant enzyme, which indicated that dietary supplement improved oxidative stress. A significant reduction in the inflammatory markers CRP and TNF-α was observed in the groups given an oral dose of chia seeds oil dietary supplement in low and high doses. The high dose (300 mg/kg BW) of chia seeds oil dietary supplement was superior.

Table 2. Nutritional parameters and relative heart % of different experimental groups

Parameters	Normal control	Hypercholesterolemia control	Dietary supplement low dose	Dietary supplement high dose
Initial body weight (g)	$170.8^{a} \pm 3.269$	$170.8^{a} \pm 2.868$	$170.33^{a} \pm 1.174$	$170.67^{a} \pm 3.343$
Final body weight (g)	$233.7^{b} \pm 1.994$	$233.0^{b} \pm 2.221$	$229.5^{b} \pm 1.431$	$221.5^{a} \pm 2.459$
Body weight gain (g)	$62.8^{b} \pm 2.749$	$62.2^{b} \pm 1.166$	$59.2^{b} \pm 1.222$	$50.8^{a} \pm 1.701$
Food intake (g)	$504^{b} \pm 6.026$	$508.3^{b} \pm 7.922$	$480.8^{a} \pm 7.479$	$464^{a} \pm 7.557$
Food efficiency ratio	$0.125^{b} \pm 0.005$	$0.123^{b} \pm 0.004$	$0.123^{b} \pm 0.002$	$0.109^{a} \pm 0.005$
Relative heart %	$0.353^{a} \pm 0.008$	$0.359^{a} \pm 0.009$	$0.357^{a} \pm 0.007$	$0.358^{a} \pm 0.007$

In the same raw: the similar letters mean non-significant difference within groups at $P \leq 0.05$.

Table 3. Plasma lipid profile of different experimental groups

Parameters	Normal control	Hypercholesterolemia control	Dietary supplement low dose	Dietary supplement high dose
TC (mg/dL)	$69.9^{a} \pm 1.969$	$156^{d} \pm 3.224$	$112^{c} \pm 3.51$	$98^{b} \pm 2.206$
TG (mg/dL)	$78.8^{a} \pm 2.212$	$106.8^{d} \pm 2.663$	$93^{c} \pm 1.183$	$89.2^{b} \pm 1.077$
HDL-C (mg/dL)	$42.5^{a} \pm 0.957$	$26.5^{a} \pm 0.922$	$36.5^{b} \pm 0.764$	$41^{a} \pm 0.966$
LDL-C (mg/dL)	$20^{a} \pm 0.577$	$94^{d} \pm 2.38$	$58.7^{c} \pm 1.202$	$46.5^{b} \pm 1.543$
Non-HDL-C (mg/dL)	$27.4^{a} \pm 2.259$	$129.5^{d} \pm 3.621$	$75.5^{c} \pm 3.844$	$57^{b} \pm 2.695$
VLDL-C (mg/dL)	$15.8^{a} \pm 0.442$	$21.4^{d} \pm 0.533$	$18.6^{c} \pm 0.237$	$17.8^{b} \pm 0.215$
Cardiac risk ratio (TC/HDL-C)	$1.7^{a} \pm 0.063$	$5.9^{d} \pm 0.268$	$3.1^{c} \pm 0.133$	$2.4^{b} \pm 0.089$
Cardioprotective index (HDL-C/LDL-C)	$2.133^{d} \pm 0.074$	$0.283^{a} \pm 0.012$	$0.624^{b} \pm 0.021$	$0.887^{c} \pm 0.038$
Atherogenic index (Tch–HDL ch)/HDL-C	$0.650^{a} \pm 0.063$	$4.928^{d} \pm 0.268$	$2.078^{c} \pm 0.133$	$1.399^{b} \pm 0.089$

TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglycerides.
In the same raw: the similar letters mean non-significant difference within groups at $P < 0.05$.

Table 4. Oxidative stress and inflammatory markers of different experimental groups

Parameters	Normal control	Hypercholesterolemia control	Dietary supplement low dose	Dietary supplement high dose
MDA (nmol/mL)	6.2^{a}±0.230	12.3^{c}±0.419	7.4^{b}±0.190	6.7^{a}±0.128
Ox-LDL (pg/mL)	25.92^{a}±0.808	53.3^{d}±3.928	34.8^{c}±1.188	31.5^{b}±1.103
CAT (U/L)	448.4^{d}±6.163	327.1^{a}± 6.402	374.3^{b}±7.951	396.7^{c}±4.409
TNF-α (pg/mL)	19.3^{a}±0.494	31.7^{d}±0.666	23.3^{c}±0.667	21.4^{b}±0.735
CRP (ng/mL)	2.63^{a}±0.161	5.55^{d}±0.154	4.6^{c}±0.124	3.8^{b}±0.128

MDA: malondialdehyde, Ox-LDL: oxidized-LDL, CAT: catalase, CRP: C-reactive protein, TNF-α: tumor necrosis factor-alpha.
In the same raw: the similar letters mean non-significant difference within groups at $P < 0.05$.

In the present study, cardiac enzymes LDH, CK, AST, and ALT appeared a significant elevation ($P<0.05$) in the hypercholesterolemia control rats in comparison with normal rats (Table 5). Kidney function parameters (urea and creatinine) showed significant rise in the hypercholesterolemic rats when compared with normal rats. The rats which were given low or high doses of chia seeds oil dietary supplement showed a significant reduction in cardiac enzymes in association with reduction of kidney function parameters. The high dose of chia seeds oil dietary supplement was the best treatment.

Discussion

Globally CVDs are the major reason for death and responsible for about 30% of all deaths, and it is increased obviously in low- and middle-income countries (20). Dyslipidemia is an important determinant of CVD (36). Reducing plasma levels of cholesterol is one of the important targets in the treatment of CVDs and improving its outcomes (37). In the present research, oral administration of the low and high doses of chia seeds oil dietary supplements reduced total food intake, while high dose also reduced final and body weight gain significantly compared with normal and hypercholesterolemic rats' group. These results are in accordance with the results of Mohamed et al (13). The reduction in food intake and body weight observed in the present study may be attributed to the presence of dietary fibers, which elevated satiety through increasing the transit time and decelerating digestion (10-13).

In the present research, hypercholesterolemia was induced by feeding the rats on the high-fat diet, which led to the elevation of plasma TC, TG, and LDL-C in association with the reduction of HDL-C in hypercholesterolemia control group (Table 3). The atherogenic index and cardiac risk ratio were elevated in the hypercholesterolemia control in association with the reduction of the cardioprotective index (Table 3). The present results are in accordance with many previous studies (11,13,38,39). Higher levels of plasma lipid peroxidation markers (MDA and Ox-LDL) and elevation of inflammatory markers (CRP and TNF-α) were observed in rats of the hypercholesterolemia control group in association with the reduction of CAT as an antioxidant enzyme (Table 4). These results indicated the presence of oxidative stress and inflammation in hypercholesterolemic rats. It was reported previously that hypercholesterolemia is associated with inflammation and oxidative stress (3). Production of inflammatory cytokines such as TNF-α and elevation of oxidative stress (Ox-LDL) are some of the leading causes of atherosclerosis (3,40).

Hypercholesterolemia leads to significant changes in the cardiac enzymes (LDH, CK, AST, and ALT) (Table 5). LDH is also released during heart tissue damage resulting from hypercholesterolemia (42,43). Elevation of kidney function parameters (urea and creatinine) (Table 5) observed in the present research is in agreement with the finding of Mohamed et al. (11). Hypercholesterolemia seems to be a threat factor for kidney injury and chronic kidney disease due to high levels of LDL-C (44).

Administration of chia seeds oil dietary supplement in low and high doses showed significant improvement in plasma lipid profile, reduction of oxidative stress, and inflammation with different degrees. Enhanced cardiac enzymes in association with decline of the rise of kidney

Table 5. Cardiac-enzymes and kidney function of different experimental groups

Parameters	Normal control	Hypercholesterolemia control	Dietary supplement low dose	Dietary supplement high dose
CK (U/l)	41.2^{a}±1.78	44^{a}±0.894	41.4^{a}±0.699	41^{a}±0.532
LDH (U/l)	242.7^{a}±4.371	340.3^{c}±5.269	285.5^{b}±3.547	246^{a}±4.725
AST (IU/L)	47.9^{a}±0.749	63.2^{c}±0.354	49.7^{a}±0.673	48^{a}±0.872
ALT (IU/L)	17.3^{a}±0.306	24.3^{c}±1.373	18.2^{a}±0.278	17.4^{a}±0.317
Urea (mg/dL)	23.6^{a}±0.716	30.99^{b}±1.435	25.1^{a}±1.067	24.7^{a}±0.962
Creatinine(mg/dL)	0.608^{a}±0.035	0.818^{b}±0.021	0.646^{a}±0.027	0.633^{a}±0.035

CK: creatine kinase, LDH: lactate dehydrogenase, AST: aspartate aminotransferase, ALT: alanine aminotransferase.
In the same raw: the similar letters mean non-significant difference within groups at $P < 0.05$.

function parameters were noticed in rats given chia seeds oil dietary supplements orally in low and high doses. Also, all nutritional parameters of rats given the high dose of chia seeds oil dietary supplements reduced, significantly.

The improvement in plasma lipid profile, oxidative stress, inflammation, cardiac-enzymes and kidney function due to administration of chia seeds oil dietary supplement in low and high doses may be attributed to the presence of phenolic compounds, mucilage (soluble fibers), phytosterols, and omega-3 fatty acids as observed from the analysis (Table 1).

Soluble fibers are characterized by gel-forming, which increases food transit time and decelerates digestion. Soluble fibers are fermented in the large intestine by bacteria to short-chain fatty acids (SCFA) (10,45). Absorption of SCFA led to the reduction of cholesterol synthesis in the liver, so reduced cholesterol in blood. Also, SCFA increases the excretion of bile acids as a result of acidification of the colon environment (45). It was reported previously that mucilage from different sources (Flaxseed, fenugreek seeds, and okra) exhibited a cholesterol-lowering activity and cardioprotective potency in an association with the reduction of CRP as an inflammatory marker and reduced the synthesis of VLDL by liver cells (18,46). Chia seeds mucilage possessed anti-inflammatory and cholesterol-lowering activities in obese and non-obese arthritic rats (18).

Wheat germ oil phytosterol showed promising anti-inflammatory, anti-arthritic, cholesterol-lowering activities (17,47). The cholesterol-lowering activity of phytosterols is through the reduction of-absorption of cholesterol in the intestine (47).

The role of omega-3 fatty acids towards the protection of cardiovascular takes considerable interest. Previous studies reported an reverse relation between dietary intake of omega-3 fatty acids and the incidence of cardiovascular events (29). Omega-3 fatty acids supplementation exhibited beneficial activities in preventing CVD through lipid lowering, inflammation, and oxidative stress (reduced reactive oxygen species) (48). A previous research reported cardiovascular beneficial effects of supplementation with 1 g/d of omega-3 fatty acids (49). Chia seeds oil dietary supplement prepared in the present study contains high levels of omega-3 fatty acids (59.2% as α–linolenic acids). Chia seeds oil possessed anti-inflammatory and cholesterol-lowering activities (18). Chia seeds attenuated postprandial blood glucose, increased satiety, increased weight reduction, and reduced inflammatory markers and cardiovascular risk factors due to the presence of omega-3 fatty acids and dietary fibers (22).

Conclusion

Chia seeds oil dietary supplement investigated in the current research showed cardioprotective potency through its anti-inflammatory, antioxidant, and dyslipidemia activities, which may be attributed to the presence of phenolic compounds, omega-3 fatty acids, phytosterols, and soluble dietary fibers (mucilage). The high dose of the dietary supplement is superior to others as a cardioprotector dose.

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Authors' contributions

DM was the PI of the project, designed all the experimental works, prepared oil, mucilage, and unsaponifiable matter, contributed in the analysis of blood samples, analyzed all the phytochemicals (fatty acids profile, phenolic compounds, and phytosterol), wrote the final manuscript, and the final reviewing of the manuscript. SM has done all animal intervention, animal experiment, contributed in the analysis of blood samples, and contributed in writing the manuscript. IH made the statistical analysis of the results, prepared the final tables of the manuscript, and contributed in writing the manuscript. The paper has been read and approved by all authors for publication.

Conflict of interests

There are no conflicts of interest.

Ethical considerations

Ethical issues including plagiarism, misconduct, data fabrication, falsification, double publication or submission have been carefully checked by authors.

Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and approved by the ethics committee at Cairo University. This study has been carried out as a part of internal project No. 12050203 in the National Research Centre, Cairo, Egypt. This project was approved by the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt with approval number 19176.

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