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Hypolipidemic Effects Of Red Radish (Raphanus Sativus) Seed Oil In Rat Fed High-Fat Diet: Its Phytochemical Characterization

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Abstract

Hyperlipidemia is a gear in a big cascade leading to fatal disease ending in death especially among elderly patients. The reduction in serum cholesterol may alter or save the human life away from cardiovascular risks. Due to what called "drug side effects" and the failure of modern medicine to control this issue. So, the use of natural products in remedy becomes the famous topic *Raphanus sativus* L. (Cruciferaceae), commonly known as radish. Radish, *Raphanus sativus L*., is an annual vegetable belonging to the family Cruciferae and is a traditionally important vegetable in many countries All plant parts are edible and it has been used in folk medicine. The present study was designed to evaluate phytochemical analysis and hypolipidemic activity of radish seed oil (RSO) in a hyperlipidemic rat model induced by a high-fat diet (HFD). GC/MS of the Radish oil seed fatty acid methyl esters revealed that the major fatty acids were unsaturated fatty acids, and saturated fatty acids (79%, 21 %) of total fatty acids respectively. Twenty-four rats were divided into three groups, group I as control, group II fed with high-fat diet (HFD), and group III fed with HFD and administered RSO orally (70 µl/Kg) for ten weeks. Results showed that *Raphanus sativus* seed oil significantly inhibits the body weight gain of rats induced by HFD, significantly reducing concentration levels of plasma cholesterol, triglycerides, and LDL- cholesterol in hyperlipidemic rats. Conclusion *Raphanus sativus* seed oil has good hypolipidemic effects on hyperlipidemic rats thus suggesting its beneficial effect in the treatment of cardiovascular diseases associated with hyperlipidemia.

Keywords: Raphanus sativus, Seeds oil; Fatty acids; Sterols; Hydrocarbons; High-Fat diet; Hypolipidemic; Lipid profile.

1. Introduction

Hyperlipidemia (dyslipidemia) is a gear in a big cascade leading to fatal disease ending to death especially among elderly patients, it is characterized by imbalance in serum total cholesterol and its major lipoprotein families (low density and high density lipoprotein) [1]. Hyperlipidemia is a major cause of cardiovascular disease (CVD) in both the developed and developing world and highly associated state with society's economic state [2]. Where obesity and High-Fat diet play an important role in incidence of hyperlipidemia [3]. Dyslipidemia is one of the most fatal disorders leading to severe insults, it represents as one third of total deaths around the world which may be due to atherosclerosis of the arterial blood supply of the vital organs [4,5]. Approximately the third of ischemic heart disease mainly is due to hyperlipidemia and also it is the main cause of atherosclerotic cardiovascular disease [6]. So the success of 10% reduction in serum cholesterol in men aged 40 years resulted in a 50% reduction in cardiovascular disease within 5 years [2]. Currently available synthetic hypolipidemic drugs have been associated with many side effects [7,8]. The search in nature revealed safe control to all of human kind insults, especially after failure of modern medicine in controlling the majority of diseases due to drugs toxicity [9,10].

Herbal medicines that are used for controlling hyperlipidemia and its related complications in most of patients [3,11], having some beneficial properties such as minimal side effects, economic, effective in reducing lipid levels [12,13]. It was reported that some untraditional vegetable oils showed hypolipidemic effect in hyperlipidemic rats through influence on lipoproteins [14]. Ethanolic seed extract of radish has a beneficial effect in the treatment

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of hypertension and cardiovascular diseases [15,16]. Currently, many research studies focus on the beneficial effects of bioactive phytochemicals of several medicinal plants for new hypolipidaemic agents with minimal side effects[16–18].

Red radish *Raphanus sativus* L. is a member of the Brassicaceae family. It is an essential vegetable that has various medicinal activities. Different parts of radish including roots, seeds and leaves are used for medicinal purposes [19]**.** Phytochemically, *Raphanus sativus* L. seeds are rich in active pharmacological compounds. including alkaloids, flavonoids, glycosides, phenols, sterols and tannins) [15,20]. The red radish contains significant amounts of anthocyanins [21]**.** Glucoraphenin (GRE) and glucoraphasatin (GRH) are the major glucosinolates found in the seeds and roots, and their derived isothiocyanates sulforaphene and raphasatin [22,23]. Sulforaphene, S-6-(methylsulfinyl) methyl-1,3-thiazinan-2-thione and O-ethyl N-(E)-4-(methylsulfinyl) but-3-enylcarbamothioate were isolated from the seeds [24,25] . Three 4-Methylthio-butanyl derivatives with four known compounds, 5-(methylsulfinyl)-4-pentenenitrile, 5-(methylsulfinyl)-pentanenitrile, sulforaphane, and sulforaphane were isolated from the most active $CHCl_3$-soluble fraction of the methanolic extract from *Raphanus sativus* seeds as anti-cancer and anti-inflammatory agents [26]**.** MeOH extract of seeds resulted in the isolation and identification of fifteen compounds including phenolic compounds. Among the isolates, three indole alkaloids showed good in vitro antiproliferative effects [27]. Extraction and characterization of the seed oil were investigated and the fatty acids compositions were determined with high erucic acid content [28–30]**.**

Raphanus sativus oils enhance hepatic and renal tissues regeneration in white mice [31]**.** Radish oil improved glucose tolerance, serum insulin level and metabolic pathways in diabetic rats [32]. Moreover, the seed extract of radish is used as expectorant, digestive, diuretic, laxative, carminative [33]. Aqueous extract of radish seed showed hepatoprotective and antioxidant potential on cadmium-induced hepatotoxicity and oxidative stress in mice [34]. Sulforaphene purified from radish seeds inhibited the growth of six cancer cell lines [35] and possesses broad antimicrobial activity [36]. Raphanin is a constituent present in seeds and leaves has already been reported to possess antibacterial and antifungal potential [37].

Likewise, crude ethanol of red radish seeds displays antibacterial [38] and antioxidant activities [39]. The active constituents of red radish seeds include sulfur compound [40] and machrolysin which is active against mycobacterium tuberculosis [41].

The current study aimed to assessment the phytochemical composition and hypolipidemic effects of Seed Oil of red radish

2. Experimental

2.1. Plant section

All solvents and chemicals were of analytical grade. n-Hexnae (40-60°), ether, were (>98%) obtained from Merck, Darmstadt, Germany. Kits for lipid parameters were purchased from Salucea Company, the Netherlands.

2.1.1 Oil extraction and fractionation

Raphanus. sativus (red radish) seeds were purchased from local market, the seeds were cleaned and were extracted using Soxhlet extraction. 500 g of crushed seeds were extracted by n- hexane for 6 h. The residue was evaporated at rotary evaporation at 40 °C till dryness. The total oil content of the samples was determined by AOAC (1990) standard methods, the process was implemented three times. The average value was obtained and the oil content was expressed as a percentage.

2.1.2 Isolation and identification of saponifiable and unsaponifiable matter of n-hexane extract.

About 2g of n-hexane extract was saponified with alcoholic KOH 10% (ethanol 95%) and the residue was fractionated into unsaponifiable and saponifiable matters according to the method of [42] , as well as, the fatty acids were liberated by acidification of the saponifiable matter, extracted with ether, dried over anhydrous Na2So4 and concentrated under reduced pressure. The fatty acid methyl esters were prepared according to the method adopted by [43]. Fatty acid methyl esters and unsaponifiable matter were analyzed by GC/MS and were identified based on fragmentation pattern of mass spectra data and a library database [Wiley (Wiley Institute, Los Angeles, CA), NIST (National Institute of Technology, Los Angeles, CA)] and by comparison with previous data. Quantitative determination was carried out on the basis of peak area measurements of the GC/MS chromatograms.

2.1.3 Gas Chromatography/Mass Spectrometry (GC/ MS) analysis of unsaponifiable matter GC-

MS analysis of unsaponifiable matter was carried out using gas chromatography-mass spectrometry instrument stands with the following specifications Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp.; USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d.; 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 50 °C for 3 min; rising at 5 °C /min to 300 °C and held for 20 min. The injector and detector were held at 280 °C. Diluted samples (1:10 hexane, v/v) of 0.2 µl of the mixtures were always

injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library) and by comparison with previous references and data.

2.1.4 Gas chromatographic-mass spectrometry (GC-MS) analysis of fatty acid methyl esters

GC-MS analysis of the fatty acid methyl esters was carried out using gas chromatography-mass spectrometry instrument stands with the following specifications Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp.; USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a PR-5MS column (30 m x 0.25 mm i.d.; 0.25 m film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:10 and the following temperature program: 50 °C for 3 min; rising at 4.0 oC/min to 260 °C and held for 6 min; rising at 6 °C /min to 300 °C and held for 1 min. The injector and detector were held at 200 °C. Diluted samples (1:10 hexane, v/v) of 0.2 µl of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library) and by comparison with previous data.

2.2. Biological activity

2.1.5 Animals and treatment

Adult Sprague-Dawley male rats (3 months age, weighing 145-160 g) were obtained from Animal House, of National Research Center (NRC), Dokki, Giza, Egypt. Rats were kept in conventional cages with free access to water ad libitum and standard rat feed with rodent pellet diet. All animals received human care in compliance with guidelines of Ethical Committee of National Research Center and followed the recommendations of The National Institute of Health Guide for care and use of Laboratory Animals (Eighth edition).

The animals were housed in polypropylene cages (each cage housing eight animals) and allowed to acclimatize to laboratory conditions for seven days prior to the experiment. Animals were maintained under controlled conditions of temperature (25oC± 1 oC), humidity (50±15%) and normal photoperiod (12–12 h light-dark cycles). All the experiments were performed in accordance with the guide for the care and use of laboratory animals, as adopted by Medical Research Ethics Committee, National Research Centre, Dokki, Egypt (Publication No. 85-23, revised 1985, Ethical approval-No 16216).

Twenty- four rats were sorted into three groups. Group I was a control group. Group II was hyperlipidemic group. Group III was hyperlipidemic group treated with seed oil of red radish orally at a dose level of 0.07ml /kg [44] daily for ten weeks. Blood samples were collected every two weeks in heparinized tubes withdrawn from the retro-orbital venous plexus under local anesthesia by diethyl ether and immediately mixed with ethylene diamine tetra acetic acid (EDTA) as anticoagulant.

Blood samples were centrifuged at 4000 rpm for 20 min at 4 ºC [45] to obtain plasma for the determination of cholesterol, triglycerides, total lipids, HDL and LDL using kits from Salucea Company, the Netherlands. Body weight of rats was recorded every two weeks.

Cholesterol and Triglycerides were determined using standard spectrophotometric methods according to[46]. LDL and HDL were measured in blood serum according to the method adopted by[46]. Total lipids were determined according to the method described by [47].

2.1.5.1 Induction of hyperlipidemia

The male rats were made hyperlipidemic by giving high-fat diet (HFD) for 30 days. The HFD contained 58% fat, 25% protein and 17% carbohydrate and 1% Cholic acid [48]. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats.

2.3. Statistical analysis

All results were presented as means ± SE Comparisons between groups were performed using one-way ANOVA with the LSD post hoc analysis. Differences were considered to be statistically significant when $P < 0.01$. Statistical analysis was performed with SPSS 17.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. Fatty Acid Composition

The fatty acids compositions of *Raphanus. sativus* seeds oil were identified by gas chromatography-mass spectrometry (GC/MS) as shown in Table 1. Eleven fatty acids at different quantities were detected, oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and erucic (C22:1) acids were the main unsaturated fatty acids, which accounted 78.597% of total fatty acids.

Saturated fatty acids such as octanoic acid (C8:0), undecanoic (C11:0), lauric (C12:0), pentadecanoic (C15:0), palmitic (C16:0), stearic (C18:0) and arachidic (C22:0) acids were detected and the seed oil contains appreciable amounts of arachidic acid (C22:0) saturated fatty acid which represented 12.702 % of the total fatty acids. Mono unsaturated fatty acids (oleic and erucic acids) accounted 66.583 % of the total fatty acids whereas polyunsaturated fatty acids (linoleic and linolenic acids) represented 12.014

% of the total fatty acids. It was also observed that the analysis of the fatty acids composition showed that the radish seed oil was rather poor in octanoic acid (C8:0), undecanoic acid (C11:0), lauric acid (C12:0), pentadecanoic acid (C15:0), palmitic acid (C16:0).

3.2. Unsaponifiable matter

The results of chromatographic analysis (GC/MS) of red radish seeds unsaponifiable matter are shown in Table 2.

Sterols from the unsaponifiable matter were isolated and identified by GC- MS comparing their MS fragments with authentic chemicals, Wiley spectral library collection and NSIT library and literature values and represented 30.46% from this fraction. It is apparent that the sterols of red radish seeds oil consisted mainly of β-sitosterol (18.12 %), campesterol (8.42 %) and Ergosta-7,22-dien-3-ol (3.92 %), among which β-sitosterol was the major sterol in fraction with 18.12 % , the next major sterol was campesterol (8.42 %).

Table (1) Fatty acids compositions of Raphanus. sativus (red radish) seeds oil

Fatty acids	Structural Formula	No. of Carbon atoms	RT	Relative Percentage (%)
Octanoic acid	$CH_3(CH_2)_6COOH$	$C_{8:0}$	18.45	0.597
Undecanoic acid	$CH_3(CH_2)_9COOH$	$C_{11:0}$	21.65	0.169
Lauric acid	$CH_3(CH_2)_{10}COOH$	$C_{12:0}$	25.40	0.343
Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$	$C_{15:0}$	33.92	0.221
Palmitic acid	$CH_3(CH_2)_{14}COOH$	$C_{16:0}$	37.99	1.929
Stearic acid	$CH_3(CH_2)_{16}COOH$	$C_{18:0}$	44.86	5.442
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	$C_{18:1}$	45.36	4.185
Linoleic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	$C_{18:2}$	45.62	6.225
γ-Linolenic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4COOH$	$C_{18:3}$	46.34	5.789
Arachidic acid	$CH_3(CH_2)_{18}COOH$	$C_{20:0}$	46.65	12.702
Erucic acid	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	$C_{22:1}$	47.92	62.398
Mono unsaturated fatty acids				66.583
Poly unsaturated fatty acids				12.014
Unsaturated fatty acids				78.597
Saturated fatty acids				21.403

Table(2) oil Unsaponifiable matter of Raphanus. sativus (red radish) seeds

RT	Relative Percentage (%)	Name	Molecular formula	Molecular weight
24. 10	4.6	Docosane	$C_{22}H_{46}$	310
26.94	7.6	Pentadecanoic acid, 14methyl, methyl ester	$C_{17}H_{34}O_2$	270
30.33	6.52	10- Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
30.62	21.81	Phytol	$C_{20}H_{40}O$	296
37.19	3.55	Nonacosane	$C_{29}H_{60}$	408
37.40	3.22	13-Docosenoic acid, methyl ester	$C_{23}H_{44}O_2$	352
43.21	22.24	Hentriacontane	$C_{31}H_{64}$	436
48.33	3.92	Ergosta-7,22-dien-3-ol	$C_{28}H_{46}O$	398
49.38	8.42	Campesterol	$C_{28}H_{48}O$	400
50.92	18.12	β-Sitosterol	$C_{29}H_{50}O$	414
Total				100
Total sterols				30.46
hydrocarbons				52.20
Methoxylated fatty acids				17.34 %

Unsaponifiable matter was found to be rich in hydrocarbons which represented 52.20 % of the fraction with phytol (21.81%) and hentriacontane (22.24 %) the main hydrocarbons whereas docosane

and nonacosane were detected at lower concentrations (Table 2).

3.3. Hypolipidemic activity of radish oil

The results of cholesterol, triglycerides and total lipids are presented in table 3. The levels of cholesterol, triglycerides and total lipids of HFD+ radish oil group showed a significant decrease ($p \leq 0.01$) at different time intervals as compared to HFD group. The High-Fat diet or oil had no significant influence on HDL (Table 4). On the other hand, the radish oil mitigates the increase of LDL level in hyperlipidemic rats, the level of LDL of HFD+ radish oil group is significantly less than those of HFD group.

Table 3: Effect of seed oil of *Raphanus. sativus* (red raddish) on plasma cholesterol, triglycerides and total lipids in rats.

Parameter treatment Time in week	Cholesterol (mg/dl)			Triglycerides (mg/dl)			Total lipids (g/dl)		
	Control	HFD	HFD +Oil	Control	HFD	HFD +Oil	Control	HFD	HFD +Oil
0	88.31±5.00	170.20±11.16	197.14±8.23	66.14±2.33	153.79±10.51	181.73±3.11	2.69 ±0.15	3.53±0.22	3.73±0.17
2	82.55±2.67	284.94 ±10.82	193.85±6.42*	63.14±3.00	170.23±11.29	145.21±12.60	2.64±0.12	4.50±0.30	2.74±0.13*
4	96.21±3.41	290.11±9.85	141.71±9.16*	61.46±2.75	199.66±8.94	91.81±3.64*	2.82±0.09	4.83±0.26	2.66±0.16*
6	90.00±1.66	298.35±7.82	138.15±6.35*	72.47±1.92	214.58±12.34	86.65±6.91*	2.55±0.17	5.11±0.26	2.56±0.15*
8	87.95±4.00	310.62±9.55	119.15±5.13*	75.08±4.93	220.67±9.22	84.62±3.32*	2.50±0.06	5.21±0.31	2.44±0.11*
10	94.86±2.54	305.41±10.46	89.34±3.66*	74.86±3.58	215.56±11.88	79.85±2.46*	2.47 ±0.14	6.00±0.38	2.36±0.10*

Each value is the mean ±SE, N=8
*Significant difference in comparison with HFD group ($P \leq 0.01$)

Table4: Effect of seed oil of *Raphanus. sativus* (red raddish) on plasma HDL and LDL levels and LDL/HDL ratio in rats.

Parameter treatment Time in week	HDL			LDL			LDL/HDL ratio		
	Control	HFD	HFD +Oil	Control	HFD	HFD +Oil	Control	HFD	HFD +Oil
0	41.00±1.42	42.30±1.43	40.46±1.38	29.37±1.54	83.47±4.19	69.53±5.39	0.84±0.02	1.97±0.05	1.72±0.03
2	38.13±2.00	45.10±2.70	47.20±4.01	30.94±2.62	120.92±6.51	69.37±5.80*	0.80±0.03	2.68±0.09	1.48±0.05*
4	39.46±1.09	43.10±1.20	46.60±3.41	36.07±2.00	145.36±7.85	57.61±3.52*	0.94±0.03	3.39±1.10	1.22±0.07*
6	35.58±1.62	50.26±3.14	49.60±2.55	33.86±1.55	149.78±11.82	47.50±2.75*	0.97±0.03	2.98±0.08	0.94±0.03*
8	40.11±2.03	48.60±2.41	56.32±4.20	37.00±2.04	168.83±6.41	49.10±3.32*	0.95±0.04	3.47±1.12	0.87±0.04*
10	48.00±1.37	53.48±4.62	58.45±2.01	39.00±2.89	160.42±5.38	41.52±2.40*	0.98±0.02	3.00±1.07	0.71±0.05*

Each value is the mean **±SE, N=8**
*Significant difference in comparison with HFD group ($P \leq 0.01$)

3.4. Weight reduction effect of radish oil

Table: 5. explained the significant increase in body weights of rats upon HFD feeding for ten weeks in comparison with normal group while these increments in the weight were reduced significantly ($P<0.001$) by the administration of radish oil (0.07ml /kg) for ten weeks in comparison with the HFD fed rats.

4. Discussion

The oil content of the seeds was determined according to AOAC [49] standard methods, the process was implemented three times . The average value was obtained and the oil content was 36.80 %, similar to those reported by [50]who found that radish seeds contained 37.00-43.00 % (w/w) oil whereas [51] reported that the seeds contained 43.12 ± 0.34% oil.
Our results of fatty acid composition analysis showed that radish seeds oil contained higher amounts of erucic (C22:1) acid which account for 62% of the total fatty acid similar to those reported by [28,30].

Table5: Effect of seed oil of *Raphanus. sativus* (red radish) on body weight (g) in rats.

Treatment Time in	Control	HFD	HFD +Oil
0	180.00±7.32	210.89±15.06	200.56±10.00
2	194.35±11.48	220.52±14.37	212.00±6.58
4	205.56±10.00	225.00±17.63	205.32±8.69
6	220.34±13.01	250.42±13.00	210.64±12.21
8	231.48±14.00	271.00±12.48	227.43±10.05*
10	250.75±9.67	325.00±11.00	261.32±8.27*

Each value is the mean **±SE, N=8**
* Significant difference in comparison with HFD group (P≤0.01)

Our result indicated that radish seeds oil rich in unsaturated fatty acids, accounted 78.597% of total fatty acids and our results are in agreement with previous several researchers, who reported that unsaturated fatty acids of *Raphanus Sativus* seed oil were represented over 80% of the total fatty acids. Linolenic acid, erucic acid and oleic oil were the main fatty acids detected [29]. In addition to [28] found that Raphanus Sativus seed oil contains oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and erucic (C22:1) acids as the main unsaturated fatty acids, which accounted for about 90 % of total fatty acids. Besides the saturated fatty acids such as palmitic (C16:0), stearic (C18:0) and docosanoic (C22:0) acids. According to [29,51,52] the main components of Raphanus Sativus seed oil are unsaturated fatty acids. The unsaturated fatty acids are octadecatrienoic acid, (α-linolenic acid) octadecadienoic acid (linoleic acid) and 13-docosenoic acid, (erucic acid). Likewise erucic (C22:1) acid was reported to be the main unsaturated fatty acid [28,30].

Our results indicated that Mono unsaturated fatty acids (oleic and erucic acids) accounted 66.583 % of the total fatty acids whereas polyunsaturated fatty acids (linoleic and linolenic acids) represented 12.014 % of the total fatty acids, which is in the agreement with [50] who reported that radish oil contained 64.55-69.26% monounsaturated fatty acids and 20.33-25.11% polyunsaturated. In contrast, in the present study oleic acid content was found to be low and erucic acid is apparently higher than that suggested by [53] who reported that seeds oil of Raphanus sativus was found richer in oleic acid (30.011%,) and erucic acid (16.411%) of the total oil . According to [53]the major fatty acid found in radish seed oil was erucic acid (30-33%). On the other hand, erucic acid content of radish seed oil contributed 40.83 ± 1.48% to the total fatty acids [51].

Unsaponifiable fraction has received very little attention by the researchers, despite the potential relevance that may have in phyto-medical technology, the discipline studying the plan derived pharmaceutical and nutraceutical compounds. The compositional and structural studies of the principal components of unsaponifiable matter were i.e., phytosterols, methoxylated fatty acids and hydrocarbons.

Methoxylated fatty acids pentadecanoic acid, 14-methyl, methyl ester, 10- octadecenoic acid, methyl ester, 13-docosenoic acid, methyl ester were detected comprising 17.34 % of the fraction. Methoxylated fatty acids have been identified from just a few natural sources [54–56].

Hyperlipidemia is a common metabolic disorder, that is considered a risk factor for many diseases, specifically atherosclerosis [57]. Dietary fats have a major role in the prevention and treatment of hyperlipidemia [58]. Both polyunsaturated fatty acids and monounsaturated fatty acids could affect lipoprotein metabolism with a hypocholesterolemic effect [59].

The current study reported that the levels of cholesterol, triglycerides and total lipids of HFD+ radish oil group showed a significant decrease ($p \leq 0.01$) at different time intervals as compared to HFD group, those is in the same way with previous scientific studies that indicated a direct relationship between LDL and atherosclerosis and The reduction of LDL will reduce coronary heart disease risk in men [60]. The hypolipidemic effect of radish oil may be due to its active principle, phytosterols. The phytosterols are of great interest due to their antioxidant activity and their impact on health. The analysis of sterols provides information about the quality of the oil. It was reported that phytosterols are of great interest due to their antioxidant activity [61] or lowering LDL cholesterol levels in hypercholesterolemic and diabetic patients, and healthy human volunteers. Many studies also showed that dietary intake of plant sterols is related to lower risk of myocardial infarction, lowering LDL cholesterol even when incorporated in non-fat matrices [62]. Animal studies suggest that phytosterols reduce atherosclerosis [63]. In addition, they may reduce biomarkers of oxidative stress and inflammation, and modulate atherosclerosis development [64,65]. Clinical studies have demonstrated that the dietary intake of phytosterols (as part of normal diet or as a supplement) effectively reduce LDL-cholesterol levels [66–68]. The high level of β-sitosterol in seeds oil could make it the most suitable and effective for lowering blood cholesterol and preventing coronary heart disease [69].

Moreover, our study indicated that the radish oil contained oleic, Linoleic, and γ-Linolenic acids. Oleic acid inhibited de novo fatty acid and cholesterol syntheses without affecting cell viability [70]. It is concluded that Peony seed oil rich in linolenic acid exhibited hypolipidemic activity via inhibition activities of HMG-CoA reductase (An enzyme responsible for cholesterol synthesis), and *fatty acid syntheses* (the key regulatory enzyme in lipogenesis) or via increase the expression of hepatic PPARα , a gene involved in fatty acid β-oxidation [71]. Also,

roselle seeds oil rich in linoleic and palmitic acids showed hypolipidemic effect via lowering LDL level [72].

The seed oil of radish lowered LDL/HDL ratio significantly in hyperlipidemic rats (Table 4). The ratio of LDL to HDL is thought to be an index of atherogenicity and continues to be a valuable and standard tool to evaluate cardio-vascular disease risk in all populations [73]. LDL/HDL cholesterol ratio had better predictor of cardiovascular disease than simple lipid parameters [74].The values of lipid parameters in radish seed oil + HFD group were almost as control values at the 10th week, which may indicate its powerful hypolipidemic effect.

explained the significant increase in body weights of rats upon HFD feeding for ten weeks in comparison with normal group while these increments in the weight were reduced significantly ($P<0.001$) by the administration of radish oil (0.07ml /kg) for ten weeks in comparison with the HFD fed rats. This may be attributed to increased thermogenesis and decreased lipogenesis [75] Our finding agreed with the study of [76,77].

5. Conclusion

In conclusion, the seed oil of *Raphanus Sativus* contains essential fatty acids that participate in hypolipidemic activity of the oil as oleic, Linoleic, and γ-Linolenic acids beside phytosterol.

The current study concluded that consumption seed oil of *Raphanus Sativus* decreased the blood lipid profiles, which precipitated to low risk of cardiovascular disease. Because the polyunsaturated fatty acids have been found to facilitate lipid transportation and metabolism, utilization of oils containing more unsaturated fatty acids like seed oil of *Raphanus Sativus* can be recommended to reduce the risk of developing cardiovascular diseases.

6. Acknowledgment

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The authors declare no conflict of interest

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9. References

[1] M.T. Cooney, A. Dudina, D. De Bacquer, L. Wilhelmsen, S. Sans, A. Menotti, G. De Backer, P. Jousilahti, U. Keil, T. Thomsen, P. Whincup, I.M. Graham, HDL cholesterol protects against cardiovascular disease in both genders, at all ages and at all levels of risk, Atherosclerosis. 206 (2009) 611–616. https://doi.org/10.1016/j.atherosclerosis.2009.02.041.

[2] who, Global Health Observatory (GHO) data, Raised cholesterol. Situation and trends, (2020). https://www.who.int/gho/ncd/risk_factors/cholesterol_text/en/.

[3] S.S. Mohamed, A.H.M. Fayed, Anti-obesity synergistic effect of pomegranate seed oil (PSO) and arabic gum (AG) in Albino Rats, Int. J. Vet. Sci. 9 (2020) 84–89.

[4] C. Ayata, H.K. Shin, E. Dileköz, D.N. Atochin, S. Kashiwagi, K. Eikermann-Haerter, P.L. Huang, Hyperlipidemia Disrupts Cerebrovascular Reflexes and Worsens Ischemic Perfusion Defect, J. Cereb. Blood Flow Metab. 33 (2013) 954–962. https://doi.org/10.1038/jcbfm.2013.38.

[5] T. Jørgensen, S. Capewell, E. Prescott, S. Allender, S. Sans, T. Zdrojewski, D. De Bacquer, J. de Sutter, O.H. Franco, S. Løgstrup, M. Volpe, S. Malyutina, P. Marques-Vidal, Ž. Reiner, G.S. Tell, W.M. Verschuren, D. Vanuzzo, Population-level changes to promote cardiovascular health, Eur. J. Prev. Cardiol. 20 (2013) 409–421. https://doi.org/10.1177/2047487312441726.

[6] R. Saravanan, N. Rajendra Prasad, K.V. Pugalendi, Effect of Piper betle Leaf Extract on Alcoholic Toxicity in the Rat Brain, J. Med. Food. 6 (2003) 261–265. https://doi.org/10.1089/10966200360716689.

[7] J.L. Parra, K.R. Reddy, Hepatotoxicity of hypolipidemic drugs, Clin. Liver Dis. 7 (2003) 415–433. https://doi.org/10.1016/S1089-3261(03)00024-2.

[8] O. Wiklund, C. Pirazzi, S. Romeo, Monitoring of Lipids, Enzymes, and Creatine Kinase in Patients on Lipid-Lowering Drug Therapy, Curr. Cardiol. Rep. 15 (2013) 397. https://doi.org/10.1007/s11886-013-0397-8.

[9] M.F. Abdelhameed, G.F. Asaad, T.I.M. Ragab, R.F. Ahmed, A.E.-N.G. El Gendy, S.S. Abd El-Rahman, A.M. Elgamal, A.I. Elshamy, Oral and Topical Anti-Inflammatory and Antipyretic Potentialities of Araucaria bidiwillii Shoot Essential Oil and Its Nanoemulsion in Relation to Chemical Composition, Molecules. 26 (2021) 5833. https://doi.org/10.3390/molecules26195833.

[10] S.S. El-Hawary, W.E. Hammam, M. El-Mahdy El-Tantawi, N.A.Z. Yassin, F.N. Kirollos, M.F. Abdelhameed, M.A.O. Abdelfattah, M. Wink, M. Sobeh, Apple leaves and their major secondary metabolite phlorizin exhibit distinct neuroprotective activities: Evidence from in vivo and in silico studies, Arab. J. Chem. 14 (2021).

https://doi.org/10.1016/j.arabjc.2021.103188.
[11] A.A. Salama, N.M. Ismael, M. Bedewy, Hypolipidemic and cardioprotective benifits of Garden cress seeds in rats compared to atorvastatin, J. Med. Plants. 7 (2019) 101–108.
[12] A.N.A. Rahman, Anti-obesity and Hypolipidemic Effects of GymnemaSyylvestre Leaves on Rats Fed on High Fat Diet, (n.d.).
[13] G. Maiolino, G. Rossitto, P. Caielli, V. Bisogni, G.P. Rossi, L.A. Calò, The Role of Oxidized Low-Density Lipoproteins in Atherosclerosis: The Myths and the Facts, Mediators Inflamm. 2013 (2013) 1–13. https://doi.org/10.1155/2013/714653.
[14] H. Zahran, M. Gad, H. Hassan, M. Fawzy, The role of different edible vegetable oils as hypolipidemic agents on experimental hyper-lipidemic rat model, Egypt. J. Chem. (2021) 0–0. https://doi.org/10.21608/ejchem.2021.83866.4111.
[15] M. Umamaheswari, M.P. Ajith, K. Asokkumar, T. Sivashanmugam, V. Subhadradevi, P. Jagannath, A. Madeswaran, In vitro angiotensin converting enzyme inhibitory and antioxidant activities of seed extract of Raphanus sativus Linn, Cent. Eur. J. Exp. Biol. 1 (2012) 11–17.
[16] I.-M. Choi, J.-H. Kim, I.-H. Kim, J.-Y. Lee, The Relationship between Consumption of Vegetables and Risk Factors for Cardiovascular Disease in Korean Adults: The Sixth Korea National Health and Nutrition Examination Survey (2013), Korean J. Fam. Pract. 7 (2017) 688–697. https://doi.org/10.21215/kjfp.2017.7.5.688.
[17] S. Ali, M. Farooq, W.A. Panhwar, Evaluation of hypoglycemic and hypolipidemic properties of Swertia chirata, J. Entomol. Zool. Stud. 5. (2017) 1448–1451.
[18] A.H. Garra, S.A. Eltomy, A.H. Aarrag, N.M. Ahmed, Antidiabetic, Antihyperlipidemic and Antioxidant Activities of Acacia albida in Streptozotoc in Induced Diabetes in Rats and its Metabolites, Egypt. J. Chem. 63 (2020) 337–348. https://doi.org/10.21608/ejchem.2019.18863.2160.
[19] R.M.P. Gutiérrez, R.L. Perez, Raphanus sativus (Radish): Their Chemistry and Biology, Sci. World J. 4 (2004) 811–837. https://doi.org/10.1100/tsw.2004.131.
[20] F. Ahmad, I. Hasan, D.K. Chishti, H. Ahmad, Antibacterial activity of Raphanus sativus Linn. Seed extract, Glob. J. Med. Res. 12 (2012) 25–34.
[21] T. Otsuki, H. Matsufuji, M. Takeda, M. Toyoda, Y. Goda, Acylated anthocyanins from red radish (Raphanus sativus L.), Phytochemistry. 60 (2002) 79–87. https://doi.org/10.1016/S0031-9422(02)00063-8.
[22] M.S. Malik, M.B. Riley, J.K. Norsworthy, W. Bridges, Glucosinolate Profile Variation of Growth Stages of Wild Radish (Raphanus raphanistrum), J. Agric. Food Chem. 58 (2010) 3309–3315. https://doi.org/10.1021/jf100258c.
[23] A. Papi, M. Orlandi, G. Bartolini, J. Barillari, R. Iori, M. Paolini, F. Ferroni, M. Grazia Fumo, G.F. Pedulli, L. Valgimigli, Cytotoxic and Antioxidant Activity of 4-Methylthio-3-butenyl Isothiocyanate from Raphanus sativus L. (Kaiware Daikon) Sprouts, J. Agric. Food Chem. 56 (2008) 875–883. https://doi.org/10.1021/jf073123c.
[24] X. Zhang, H.-B. Liu, J.-J. Jia, W.-H. Lv, Two novel sulfur compounds from the seeds of Raphanus sativus L., J. Asian Nat. Prod. Res. 12 (2010) 113–118. https://doi.org/10.1080/10286020903479691.
[25] P. Kuang, D. Song, Q. Yuan, R. Yi, X. Lv, H. Liang, Separation and purification of sulforaphene from radish seeds using macroporous resin and preparative high-performance liquid chromatography, Food Chem. 136 (2013) 342–347. https://doi.org/10.1016/j.foodchem.2012.08.082.
[26] K.H. Kim, E. Moon, S.Y. Kim, S.U. Choi, J.H. Lee, K.R. Lee, 4-Methylthio-butanyl derivatives from the seeds of Raphanus sativus and their biological evaluation on anti-inflammatory and antitumor activities, J. Ethnopharmacol. 151 (2014) 503–508. https://doi.org/10.1016/j.jep.2013.11.003.
[27] K.H. Kim, E. Moon, S.R. Lee, K.J. Park, S.Y. Kim, S.U. Choi, K.R. Lee, Chemical Constituents of the Seeds of Raphanus sativus and their Biological Activity, J. Braz. Chem. Soc. (2015). https://doi.org/10.5935/0103-5053.20150222.
[28] G. Zhao, Y. Ren, H. Ma, Extraction and characterization of Raphanus Sativus seed oil obtained by different methods, Trop. J. Pharm. Res. 15 (2016) 1381. https://doi.org/10.4314/tjpr.v15i7.5.
[29] D. HOU, R. HUI, T. LI, Q. DIAO, Determination of fatty acids and octadecadienoic acid in Schisandra chinensis, J. Anshan Norm. Univ. (2011).
[30] E.M. ALTUNER, T. ÇETER, M. GÜR, K. GÜNEY, B. KIRAN, H.E. AKWIETEN, S.I. SOULMAN, Chemical Composition and Antimicrobial Activities of Cold-Pressed Oils Obtained From Nettle, Radish and Pomegranate Seeds, Kastamonu Üniversitesi Orman Fakültesi Derg. 18 (2018) 236–247. https://doi.org/10.17475/kastorman.498413.
[31] A. Kamil, Eruca sativa and Raphanus sativus oils Enhance Hepatic and Renal Tissues Regeneration in White Mice, Al-Mustansiriyah J. Sci. 29 (2019) 27. https://doi.org/10.23851/mjs.v29i4.404.

[32] O.M. Ahmed, E.S. Abdel-Reheim, M.B. Ashour, H.I. Fahim, H.H. Mohamed, Efficacies of Eruca sativa and Raphanus sativus seeds' oils in streptozotocin-induced diabetic rats, Int. J. Clin. Endocrinol. Metab. 2 (2016) 34–43.

[33] S. Alqasoumi, M. Al-Yahya, T. Al-Howiriny, S. Rafatullah, Gastroprotective effect of radish``Raphanus sativus''L. on experimental gastric ulcer models in rats, FARMACIA-BUCURESTI-. 56 (2008) 204.

[34] Q. He, Y. Luo, P. Zhang, C. An, A. Zhang, X. Li, L. You, C. Liu, Hepatoprotective and antioxidant potential of radish seed aqueous extract on cadmium-induced hepatotoxicity and oxidative stress in mice, Pharmacogn. Mag. 15 (2019) 283.

[35] S. Lim, J. Ahn, E.J. Lee, Sulforaphene Isolated from Radish (Raphanus Sativus L.) Seeds Inhibits Growth of Six Cancer Cell Lines and Induces Apoptosis of A549 Cells, (2018).

[36] S. Lim, S.-W. Han, J. Kim, Sulforaphene identified from radish (Raphanus sativus L.) seeds possesses antimicrobial properties against multidrug-resistant bacteria and methicillin-resistant Staphylococcus aureus, J. Funct. Foods. 24 (2016) 131–141. https://doi.org/10.1016/j.jff.2016.04.005.

[37] S. Shukla, S. Chatterji, D.K. Yadav, G. Watal, Antimicrobial efficacy of Raphanus sativus root juice, Int. J. Pharm. Pharm. Sci. 3 (2011) 89–92.

[38] H.-W. Lim, K.-Y. Song, J.-W. Chon, D. Jeong, K.-H. Seo, Antimicrobial Action of Raphanus raphanistrum subsp. sativus (radish) Extracts against Foodborne Bacteria Present in Various Milk Products: A Preliminary Study, J. Milk Sci. Biotechnol. 37 (2019) 187–195. https://doi.org/10.22424/jmsb.2019.37.3.187.

[39] A.H. Khamees, Phytochemical and pharmacological analysis for seeds of two varieties of Iraqi Raphanus sativus, Int J Pharm Sci Rev Res. 43 (2017) 237–242.

[40] J. Jadoun, A. Yazbak, S. Rushrush, A. Rudy, H. Azaizeh, Identification of a New Antibacterial Sulfur Compound from Raphanus sativus Seeds, Evidence-Based Complement. Altern. Med. 2016 (2016) 1–7. https://doi.org/10.1155/2016/9271285.

[41] P. Singh, J. Singh, Medicinal and therapeutic utilities of Raphanus sativus, Int J Plant Anim Env. Sci. 3 (2013) 103–105.

[42] R.S. Farag, S.A.S. Hallabo, F.M. Hewedi, A.E. Basyony, Chemical evaluation of rapeseed, Fette, Seifen, Anstrichm. 88 (1986) 391–397.

[43] J.E. Kinsella, Metabolic patterns of the fatty acids of Periplanteta americana (L.) during its embryonic development, Can. J. Biochem. 44 (1966) 247–258.

[44] M.O. Elshazly, A.M. Morgan, M.E. Ali, E. Abdel-mawla, S.S. Abd El-Rahman, The mitigative effect of Raphanus sativus oil on chromium-induced geno- and hepatotoxicity in male rats, J. Adv. Res. 7 (2016) 413–421. https://doi.org/10.1016/j.jare.2016.02.008.

[45] A. Salama, M.F. Abdelhameed, S. Mostafa, S.A. Nada, H.S. Taha, A.A. Amer, Influence of extract derived cell cultures of broccoli against osteoporosis in ovariectomized rats, Egypt. J. Chem. 64 (2021) 3521–3539. https://doi.org/10.21608/ejchem.2021.64803.3419.

[46] S.H. Abo-Raya, N.A. Salama, S.E. Mohammed, A.-H.M. Fayed, A.A. Abdulhamid, Biochemical effect of palm oil fractions on rats, Plant Arch. (2020). http://www.scopus.com/inward/record.url?eid=2-s2.0-85083177684&partnerID=MN8TOARS.

[47] J. Folch, M. Lees, G.H.S. Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.

[48] T. Thirumalai, N. Tamilselvan, E. David, Hypolipidemic activity of Piper betel in high fat diet induced hyperlipidemic rat, J. Acute Dis. 3 (2014) 131–135. https://doi.org/10.1016/S2221-6189(14)60029-9.

[49] A. of O.A. Chemists, Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement, 1990.

[50] Q. BingJun, P. YuePing, C. Zhan, J. Pu, Phytochemical composition, antioxidant capacity and ACE-inhibitory activity of China-grown radish seeds., J. Appl. Bot. Food Qual. 90 (2017) 315–322.

[51] S. Uluata, N. Özdemir, Antioxidant activities and oxidative stabilities of some unconventional oilseeds, J. Am. Oil Chem. Soc. 89 (2012) 551–559.

[52] H.C. Kaymak, Profile of (n-9) and (n-7) Isomers of Monounsaturated Fatty Acids of Radish (Raphanus sativus L.) seeds, J. Am. Oil Chem. Soc. 92 (2015) 345–351. https://doi.org/10.1007/s11746-015-2600-0.

[53] A. Waheed, F.S. Hamid, B. Madiha, A. Seemab, A. Naveed, K. Nadia, A. Sohail, M. Saqib, G. Hina, GC-MS analysis of chemical components seed oil of Raphanus sativus L, MOJ Toxicol. 5 (2019) 112–118.

[54] N.M. Carballeira, M. Pagán, New Methoxylated Fatty Acids from the Caribbean Sponge Callyspongia f allax, J. Nat. Prod. 64 (2001) 620–623.

[55] J. Cho, Y.P. Gyawali, S. Ahn, M.N.A. Khan, I. Kong, Y. Hong, A methoxylated fatty acid isolated from the brown seaweed Ishige okamurae inhibits bacterial phospholipase A2, Phyther. Res. 22 (2008) 1070–1074.

[56] S. Lin, Y.-L. Zhang, M.-T. Liu, J.-C. Zi, M.-L. Gan, W.-X. Song, X.-N. Fan, S.-J. Wang, Y.-C.

Yang, J.-G. Shi, Methoxylated fatty acids from the bark of Fraxinus sieboldiana, J. Asian Nat. Prod. Res. 14 (2012) 235–243.
[57] Y.A. Carpentier, Dietary intake and hyperlipoproteinemias, Rev. Med. Brux. 18 (1997) 32–36.
[58] S. Abo-Raya, N. Salama, S. Mohammed, A.M. Fayed, BIOCHEMICAL EFFECT OF PALM OIL FRACTIONS ON RATS, n.d.
[59] M. Mancini, M. Parillo, Lipid Intake and Atherosclerosis, Ann. Nutr. Metab. 35 (1991) 103–108. https://doi.org/10.1159/000177689.
[60] B.A. Ference, H.N. Ginsberg, I. Graham, K.K. Ray, C.J. Packard, E. Bruckert, R.A. Hegele, R.M. Krauss, F.J. Raal, H. Schunkert, Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (2017) 2459–2472.
[61] Y. Yoshida, E. Niki, Antioxidant effects of phytosterol and its components, J. Nutr. Sci. Vitaminol. (Tokyo). 49 (2003) 277–280.
[62] S. Klingberg, L. Ellegård, I. Johansson, J.-H. Jansson, G. Hallmans, A. Winkvist, Dietary intake of naturally occurring plant sterols is related to a lower risk of a first myocardial infarction in men but not in women in northern Sweden, J. Nutr. 143 (2013) 1630–1635.
[63] S. John, A. V Sorokin, P.D. Thompson, Phytosterols and vascular disease, Curr. Opin. Lipidol. 18 (2007) 35–40.
[64] S. Montserrat-de la Paz, Á. Fernández-Arche, M. Ángel-Martín, M.D. García-Giménez, The sterols isolated from evening primrose oil modulate the release of proinflammatory mediators, Phytomedicine. 19 (2012) 1072–1076.
[65] J.M. Lou-Bonafonte, C. Arnal, M.A. Navarro, J. Osada, Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development, Mol. Nutr. Food Res. 56 (2012) 1043–1057.
[66] R.E. Ostlund, S.B. Racette, W.F. Stenson, Effects of Trace Components of Dietary Fat on Cholesterol Metabolism: Phytosterols, Oxysterols, and Squalene, Nutr. Rev. 60 (2002) 349–359. https://doi.org/10.1301/00296640260385793.
[67] A. de Jong, J. Plat, R.P. Mensink, Metabolic effects of plant sterols and stanols, J. Nutr. Biochem. 14 (2003) 362–369.
[68] R.E. Ostlund, S.B. Racette, A. Okeke, W.F. Stenson, Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans, Am. J. Clin. Nutr. 75 (2002) 1000–1004. https://doi.org/10.1093/ajcn/75.6.1000.
[69] S. Cheikh-Rouhou, S. Besbes, G. Lognay, C. Blecker, C. Deroanne, H. Attia, Sterol composition of black cumin (Nigella sativa L.) and Aleppo pine (Pinus halepensis Mill.) seed oils, J. Food Compos. Anal. 21 (2008) 162–168. https://doi.org/10.1016/j.jfca.2007.09.001.
[70] P. Priore, A. Gnoni, F. Natali, M. Testini, G. V. Gnoni, L. Siculella, F. Damiano, Oleic Acid and Hydroxytyrosol Inhibit Cholesterol and Fatty Acid Synthesis in C6 Glioma Cells, Oxid. Med. Cell. Longev. 2017 (2017) 1–10. https://doi.org/10.1155/2017/9076052.
[71] J. Su, C. Ma, C. Liu, C. Gao, R. Nie, H. Wang, Hypolipidemic Activity of Peony Seed Oil Rich in α-Linolenic, is Mediated Through Inhibition of Lipogenesis and Upregulation of Fatty Acid β-Oxidation, J. Food Sci. 81 (2016) H1001–H1009. https://doi.org/10.1111/1750-3841.13252.
[72] R.F.M. Ali, A.M. El-Anany, Hypolipidemic and Hypocholesterolemic Effect of Roselle (Hibiscus sabdariffa L.) Seeds Oil in Experimental Male Rats, J. Oleo Sci. 66 (2017) 41–49. https://doi.org/10.5650/jos.ess16126.
[73] M.L. Fernandez, D. Webb, The LDL to HDL Cholesterol Ratio as a Valuable Tool to Evaluate Coronary Heart Disease Risk, J. Am. Coll. Nutr. 27 (2008) 1–5. https://doi.org/10.1080/07315724.2008.10719668.
[74] P.M. Ridker, M.J. Stampfer, N. Rifai, Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein (a), and standard cholesterol screening as predictors of peripheral arterial disease, Jama. 285 (2001) 2481–2485.
[75] K. Gurpreet, S.K. Kulkarni, Antiobesity effect of a polyherbal formulation, OB-200G in female rats fed on cafeteria and atherogenic diets., Indian J. Pharmacol. 32 (2000) 294–299.
[76] G. Balamurugan, A. Shantha, Effect of Erythrina variegata seed extract on hyperlipidemia elicited by high-fat diet in wistar rats, J. Pharm. Bioallied Sci. 2 (2010) 350.
[77] C. Apparao, R. Kasetti, Pr. Sudarshan, M. Sampathkumar, S. Nabi, S. Swapna, Antihyperlipidemic and antiatherogenic activities of Terminalia pallida Linn. fruits in high fat diet-induced hyperlipidemic rats, J. Pharm. Bioallied Sci. 3 (2011) 449. https://doi.org/10.4103/0975-7406.84464.