Egypt. J. Food. Sci. **Vol. 50,** No.1, pp. 157-171 (2022)

Egyptian Journal of Food Science
http://ejfs.journals.ekb.eg/

Studying The Effect of Pomegranate Seeds and Wheat Germ Oils as Protective Against Hyperlipidemia in Rats

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HYPERLIPIDEMIA is one of the most public health problems in the modern era that increase the risk of several comorbidities such as cardiovascular disease and cancer. So, this study was investigated the effect of pomegranate seeds and wheat germ oils as protective agents against hyperlipidemia in rats. A total of 36 male albino rats weighing 120-130 g. were used. Rats were divided into six equal groups; the first group kept as a control negative group and was fed on basal diet, while the second group was fed high fat diet for 4 weeks as a positive control group .The third and fourth groups were fed on high fat diet and given oral administration of pomegranate seed oil (0.4) and (0.8) mL/kg. B. Wt. for 4 weeks as a protective agent, respectively. The fifth and sixth groups were fed on high fat diet and given oral administration of wheat germ oil (1) and (1.5) mL /kg. B. Wt. for 4 weeks as protective agents, respectively. At the end of the experimental period (4 weeks), the feed intake (FI), body weight gain (gm.), feed efficiency ratio (FER) and relative liver weight were calculated. Serum liver enzymes (ALT, AST and ALP), total cholesterol, triglycerides, HDLc and LDLc and histopathological changes of heart were examined. Total antioxidant and total polyphenols were performed in pomegranate seeds and wheat germ oils. The obtained results concluded that using pomegranate seeds and wheat germ oils improved the aforementioned biological and biochemical parameters. The best results found by using high doses of pomegranate seeds and wheat germ oils. According to the results, pomegranate seeds and wheat germ oils could be used for improving lipid profile and liver functions in hyperlipidemic rats.

Keywords: Hyperlipidemia, Vegetable oils, Triglyceride, Total cholesterol, Liver enzymes.

Introduction

Hyperlipidemia refers to a range of metabolic conditions in which lipid levels are abnormally high that include cholesterol, cholesterol esters, phospholipids and triglycerides (Karam et al., 2018). Lipid disorders are caused by the distribution of total serum cholesterol among different lipoproteins, not only the total amount (Thirumalai et al., 2014). Atherogenic dyslipidemia, central obesity and cardiovascular disease, are caused by a high fat diet (Lasker et al., 2019). Medicinal plants are a primary part of treatment for around eighty percent of the world's population (Shorinwa & Monsi, 2020).

Pomegranate *(Punica granatum)* is an edible fruit from the Punicaceae family that is native to Iran and widely cultivated across the world. Pomegranate juice, peel, and seed oil all contain high levels of antioxidants (Acar et al., 2018). Pomegranate seed oil (PSO) has a high concentration of conjugated fatty acids, including punicic acid, linoleic acid, and linolenic acid, which contributes to its antioxidant properties (Gram et al., 2018).

Wheat germ is one of the richest natural sources of tocopherols. Wheat germ protein is high in amino acids, particularly important amino acids like lysine, methionine, and threonine,

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Received: 9/3/2022; accepted: 24/7/2022
DOI: 10.21608/EJFS.2022.126382.1125

which are lacking in many cereals (Sakhawat et al., 2013). Wheat germ oil (WGO) is a good source of vitamin E and important polyunsaturated fatty acids. Wheat germ oil has been shown to lower plasma and liver cholesterol, reduce oxidative stress, and improve lipid metabolism in rats (Soliman et al., 2020). As a result, the purpose of this study was to assess the protective effect of administrating pomegranate seeds and wheat germ oils against hyperlipidemia in experimental rats.

Materials and Methods

Materials

Plant materials

Pomegranate seed oil and wheat germ oil were purchased from The Local Company for Herbs and Medicinal Plants, Cairo Governorate, Egypt.

Experimental animals

Thirty six adult male albino rats (Sprague-Dawley strain) were purchased from the animal colony, Helwan farm, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt.

Chemicals

All essential chemicals were obtained from El Gomhouria Company for Trading Drugs, Chemicals and Medical Appliances, Cairo, Egypt.

Methods

Plant sample preparation

Pomegranate seed and wheat germ oils were kept in a cool, dark location until used. The oils were used after evaluation their active constituents and their biological activities.

Determination of the antioxidant activity of Pomegranate seeds and wheat germ oils

Antioxidant activity determined by stable free radical diphenylpicrylhydrazyl (DPPH) Method to estimate the activity of antioxidants according to Ardabili (2010).

Determination of fatty acids composition of Pomegranate seeds and wheat germ oils

Fatty acid methyl esters (FAME) were made according to the instructions provided by Joseph & Ackman (1992). FAMEs were transferred into a separating funnel and four millilitres of hexane added at room temperature, the contents were briskly shaken and allowed to stand the hexane layer was removed, and then the aqueous layer was extracted once more. To eliminate any acid, the hexane fractions were combined together and rinsed with 3-4 parts of distilled water. Dehydration was achieved by adding anhydrous sodium sulphate. The filtrate was concentrated by bubbling it in nitrogen gas, and then 0.5 mL was fed into the GC. The technique was performed for all of the samples, including injecting the standard solutions. The technique was repeated for all the samples, including injecting the reference solutions according to AOAC (2000).

Experimental diets

The basal diet was composed of 10 % protein as 12 g of casein; 10 g of corn oil (10% fat) ; 4 g of minerals mixture (4 % minerals); 1g of vitamins mixture(1% vitamins); 4 g cellulose (4% fiber); and 69 g of corn starch (69 % starch) according to Jerome et al. (2002) as shown in Table 1.

Hyperlipidemic diet

Hyperlipidemic diet was prepared per 100 g according to Rashwan (1994); the diet had the following composition fat 20% (sun flower1%+sheep tallow19%),sugar 10%, salt mixture 4%,vitamin mixture1%,cholinechloride 0.2%, casein (14%), methionine 0.3%, and corn starch up to 100 g.

TABLE 1. Composition of basal diet.

INGREDIENTS	Quantity (g/100g)
Casein (10% protein)	12
Corn oil	10
Minerals mixture	4
Vitamins mixture	1
Fiber	4
Corn starch	Up to100%
Total	100%

Experimental design

Animals were kept in clean wire cages under hygienic conditions. Feed was introduced (*ad libitum*) to the rats in special food containers to avoid scattering. Similarly, fresh water was be provided *ad-libitum* and checked daily. Adaptation was continued for one week. After that, rats were randomly assigned to6 equal groups as follows:

The first group: fed on basal diet for 4 weeks as a negative control group.

The second group: fed high fat diet f or 4 weeks as a positive control group.

The third group: fed on high fat diet and pomegranate seed oil (0.4 mL) orally for 4 weeks as a protective agent.

The fourth group: fed on high fat diet and pomegranate seed oil (0.8mLl) orally for 4 weeks as a protective agent.

The fifth group: fed on high fat diet and wheat germ oil (1 mL) orally for 4 weeks as a protective agent.

The sixth group: fed on high fat diet and wheat germ oil (1.5 mL) orally for 4 weeks a protective agent.

Animals were weighed, fasted overnight, and then sacrificed at the end. Each rat's blood was collected from the hepatic portal vein. Blood samples were centrifuged at 3500 rpm (round per minute) for 15 min at room temperature to extract serum, which was then transferred to dry, clean ebendorf tubes and kept frozen at -20 °C for further analysis. The liver and heart of rats were carefully dissected, cleaned in 0.9 percent saline solution, dried using filter paper, and weighed separately.

Biological evaluation

Feed intake was recorded every day for the duration of the experiment (28 days), and body weight was measured every week. Body weight gain percent (BWG percent) and feed efficiency ratio (FER) were calculated according to Chapman et al. (1959).

Biochemical analysis of serum

Serum was analyzed for various biochemical parameters like lipid profile, Total cholesterol, Triglycerides and HDL-C were evaluated on the authority of Allain et al. (1974); Trinder & Ann (1969) and Lopes-Virella et al. (1977) but LDL-C and VLDL-C calculated according to Friedwald et al. (1972). The atherogenic indices were calculated according to Tilvis & Miettinen (1986). Antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GPx) and Malondialdehyde (MDA) level as a parameter for the lipid peroxidation were determined according to Kakkar et al. (1984), Ellman (1959) and Draper et al. (1993). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkalinephosphatase (ALP) were measured according to Bergmeyer et al. (1986) and Roy (1970). The concentration of glucose in the blood was determined using the method outlined by Trinder (1959).

Histopathological examination

The heart was promptly fixed in 10% buffered neutral formalin. Following that, the fixed tissues were processed for histopathological tests as described by Carleton (1979).

Statistical analysis

The average and standard deviation are used to express the results. The significance of differences in means between groups was examined using a one-way analysis of variance (ANOVA) followed by Duncan's test, with a probability value of 0.05 or less considered significant. The least significant differences test (LSD) was used to compare mean values according to Snedecor & Cochran (1967) using SPSS (version 20).

Results and Discussion

Hyperlipidemia is a risk factor for cardiovascular disease, particularly atherosclerosis, which is one of the leading causes of death worldwide. Tocopherols, sterols, phenolic compounds, coenzymes, and polyunsaturated fatty acids are all natural antioxidants found in vegetable oils, which give nutritional value, organoleptic qualities, and considerably delay or prevent lipid oxidation(Al-Suwaiegh et al. 2020).

Proximate analysis

The total phenols contents in pomegranate seed and wheat germ oils were (329.64 ppm 176.72 ppm, respectively). While, the amount of total antioxidant capacity in pomegranate seed and wheat germ oils was (300.36, 439.94 ppm, respectively) as illustrated in Table 2 .Also, pomegranate and wheat germ seed oil contain essential and non-essential fatty acids ,as recorded in Tables 3 & 4. The highest average was linoleic

acid. Our result agrees with Acar et al. (2018) who reported that Pomegranate juice, peel, and seed oil contain high levels of antioxidants, making them potential candidates for use as a nutritional supplement in animal feed. Pomegranate seed oil was used in medicine and cosmetics due to presence of punicic acid an omega-5 fatty acid, which exhibited anti-inflammatory properties. Also, Al Juhaimi et al. (2017) who showed that the total phenol content of pomegranate seed oil was 329.64 ppm as Gallic acid equivalent. While, antioxidant activity of oil samples varied 300.36 ppm as ascorbic acid equivalent ($p < 0.05$) and punicic acid is the most common fatty acid found in oils, Oleic acid, ɣ-tocopherol. Also Gram et al. (2018) reported that pomegranate seed oil (PSO) contains a high concentration of conjugated fatty acids composition containing high levels of punicic acid, linoleic acid, and linolenic acid which attributes its antioxidant effects. Pomegranate seed oil is predominantly made up of the rare conjugate linolenic acid (punicic acid), which has been shown to have anti-inflammatory properties in a variety of in vivo studies according to Verardo et al. (2014). Also, Szymczyk's et al. (2016) indicated that PSO is rich in polyphenols, such as ellagitannins, anthocyanins, ascorbic acid, and punicic acid, which are known for their powerful antioxidant properties. Also, our results consistent with Yanping et al. (2018) who demonstrated that Wheat germ oil contains a high concentration of nutrients, including vitamin E and total phenolics, total tocopherols and β-carotenoids. Also, Lian et al. (2011) reported that the total phenol content of wheat germ oil was 176.72 ppm (as gallic acid equivalent) while, antioxidant activity contents was439.94 ppm (as ascorbic acid equivalent) ($p < 0.05$). Also, Soliman (2020) reported that wheat germ oil is high in essential and polyunsaturated fatty acids, as well as vitamin E. It is one of the richest natural sources of tocopherol.

TABLE 2. Total phenols and total flavonoid contents in Pomegranate seed and wheat germ oils.

Sample type	Total phenols	Total antioxidant capacity
pomegranate seed oil	329.64 ppm as Gallic acid equivalent	300.36 ppm as ascorbic acid equivalent
wheat germ oil	176.72 ppm as Gallic acid equivalent	439.94 ppm as ascorbic acid equivalent

TABLE 3. The averages of pomegranate seed oil and essential and non-essential fatty acids .

Fatty acids	Nam	Relative% distribution
C11:0	Undecanoic acid	0.33%%
C12:0	Lauric acid	0.295
C13:0	Tridecanoic acid	0.26%
C14:0	Myristic acid	0.65%
C15:0	Pentadecanoic acid	0.96%
C16:0	Palmitolic acid	14.93%
C16:1w7	Palmitoleic acid	0.53%
C18:0	Stearic acid	2.30%
C18:1w9	Oleic acid	23.66%
C18:1w7	Vaccinic acid	1.34%
C18:2w6	Linoleic acid	46.48%
C18:2w4		1.66%
C18:3w4		0.57%
C18:3w3	Linolenic acid	2.81%
C20:0	Arachidic acid	0.29%
C20:1w11	Gadolic acid	0.32%
C20:1w9	Gondoic acid	1.00%
C22:1w11	Docosenoic acid	0.27%
C22:1w9	Erucic acid	0.47%
Non identified fatty acids		0.88%

TABLE 4. The averages of wheat germ oils essential and non-essential fatty acids.

Fatty acid	Name	Relative distribution
C16:0	Palmitic acid	8.14%
C17:0	Heptadecananoic acid	0.20%
C18:0	Stearic acid	3.81%
C18:1w9	Oleic acid	24.46%
C18:1w7	Vaccinic acid	0.90%
C18:2w6	Linoleic acid	57.83%
C18:2w4		0.65%
C18:3w4		0.31%
C18:3w3	Linolenic acid	2.17%
C20:0	Arachidic acid	0.27%
C20:1w11	Gadolic acid	0.12%
C20:1w9	Gondoic acid	0.28%
C22:0	Behenic acid	0.52%
Non identified fatty acids		0.34%

Effects on body weight gain and feed intake

The effect of pomegranate seed and wheat germ oils on feed intake (FI), body weight gain (BWG %) and feed efficiency ratio (FER) in hyperlipidemic rats are recorded in Table 5. Results showed that previous mentioned parameters recorded increase in a positive control group (C +ve) as compared to negative control group (C-ve). All the treated groups with pomegranate seed and wheat germ oils (0.8mL/kg, 1.5mL/kg) showed a significant decrease ($P<0.05$) as compared to C +ve.

In this respect, our results agree with Mohamed & Fayed (2020) who reported that Long-chain saturated fatty acids, which are the most toxic lipids due to buildup in adipose tissue, are the most harmful lipids caused by hyperlipidemic diets. In harmony with these findings, de Melo et al. (2016) who evaluated the effect of pomegranate seed oil (PSO) supplementation, on the lipid profile in the gastrocnemiusmuscle and adipose tissues of healthy rats. The results showed that Supplementation with PSO significantly reduced total food intake but did not affect body weight gain or muscle or fat tissues. This result matching with Mohamed & Fayed (2020) concluded that PSO reduced body weight gain. Anti-obesity benefits make PSO is an effective combo for preventing or treating obesity. Also, wheat germ oil is rich in essential fatty acids and includes vitamins B1, B2, B6, and other biologically active compounds, which improve health status according to Nagib et al. (2018). These data are confirmed with the study of Fouad et al. (2014). Additionally Saleh et al. (2010) who showed that a wheat germ oil-supplemented diet improved food consumption, body weight gain and FER in rats.

Effects on relative organs weight

The effects of pomegranate seed (0.4-0.8 mL/kg) and wheat germ oils (1-1.5 mL/kg) on relative organs weight in hyperlipidemic rats were showed in Table 6. Relative liver and heart weight value showed a significant increase in C +ve as compared to the C-ve group. All treated groups indicated significant decrease as compared to C +ve. The best result was found in the treated group with wheat germ oil (1.5 mL) and closed to the normal group. The relative heart weight value showed a significant increase in the C +ve as compared to the C –ve , while treated groups with wheat germ and pomegranate seed oils showed non- significant decrease in relative heart weight. These results in agreement with Raffaele et al (2020) who proved that increasing relative liver weight of hyperlipidemia male rats

could be a consequence of the higher fat content on liver. PSO help to promote weight loss while also having a direct effect on the liver by reducing hepatic steatosis. It can also help to reduce hepatic oxidative stress, which can help to improve hepatic lipid metabolism and insulin resistance. Also Sakhawat et al. (2013*)* who reported that feeding hyperlipidemic rats' wheat germ oil with diet significantly reduced liver weight compared to control positive group. This action may be due to the presence of hepatoprotective properties of wheat germ oil. Our results agree with Akool (2019) who showed that WGO protect against hepatotoxicity caused by a number of different oxygen radical producers. Because WGO has a significant amount of vitamin E, the most powerful natural antioxidant, it is thought to play a vital function in liver disease.

TABLE 5. Effect of feeding with pomegranate seeds and wheat germ oils on feed intake (FI), body weight gain (BWG %) and feed efficiency ratio (FER) in experimental rats (n= 6 rats).

parameters / Groups	FI (g/day)	BWG%	FER
Control – ve	16.06±0.01^{f}	37.8±0.18^{c}	0.013±0.00^{2}d
Control + ve	19.8±0.01^{a}	62.95±0.37^{a}	0.035±0.005^{a}
Wheat germ oil 1mL/ kg	18.05±0.01^{e}	53.89±0.647^{ab}	0.021±0.002^{b}
Wheat germ oil 1.5mL/ kg	18.8±0.036^{c}	47.92±0.60^{bc}	0.027±0.003^{bc}
Pomegranate seed oil 0.4 mL/ kg	18.4±0.01^{d}	50.08±0.58^{b}	0.029±0.003^{bc}
Pomegranate seed oil 0.8 mL / kg	19.45±01^{b}	45.058±0.85^{bc}	0.015±0.003^{cd}
LSD	0.012	10.95	0.004

Means in the same column with different superscript letters are significantly different at p ≤ 0.05

TABLE 6. Effect of feeding with pomegranate seeds and wheat germ oils on relative organs weight (liver and heart) in experimental rats (n= 6 rats).

Parameters / Groups	Liver (g/100g B .Wt)	Heart (g/100g B. Wt)
Control – ve	2.6±0.59^{c}	0.38±0.066^{a}
Control + ve	4.3±0.59^{a}	0.34±0.039^{a}
Wheat germ oil 1mL/kg	4.07±0.41^{a}	0.284±0.142^{a}
Wheat germ oil 1.5 mL/kg	2.62±0.408^{c}	0.301±0.043^{a}
Pomegranate seed oil 0.4 mL/kg	3.5±0.44^{b}	0.37±0.060^{a}
Pomegranate Seed oil 0.8 mL/kg	3.59±0.44^{b}	0.39±0.067^{a}
LSD	0.54	0.09

Means in the same column with different superscript letters are significantly different at p ≤ 0.05

Effects on serum lipid profile

The effects of wheat germ (1 & 1.5mL/ kg), and pomegranate seed oils (0.4-0.8 mL/kg) on total cholesterol (T.C) and triglycerides (T.G) in hyperlipidemic rats were illustrated in Table 7. The result revealed that, a significant increase in total cholesterol and triglyceride in the C +ve as compared to the C–ve , while, these parameters decreased in all treated groups especially treated group with (Pomegranate seed oil 0.8 mL / kg) which closed to normal group .Also, the effect of wheat germ oil (1&1.5mL/ kg), and pomegranate seed oils (0.4-0.8 mL/ kg) on lipid profile (HDL, LDL, VLDL) and atherogenic index (AI) in hyperlipidemic rats was recorded in table 8 . The result revealed that, a significant decrease in (HDL) in the C +ve as compared to the C – ve, while, this parameter (HDL) recorded a significant increase in treated groups with wheat germ oil (1&1.5mL/ kg) and pomegranate seed oil (0.4-0.8 mL/kg) which recorded the best result and closed to the normal group as compared to the C +ve. While, (LDL, VLDL and AI) parameters recorded a significant increase in the C +ve as compared to the C–ve, while, these parameters decreased in all treated groups especially treated group with (pomegranate seed oil 0.8 mL) which recorded the best result and closed to the normal group.

Our results revealed that, improving in lipid profile levels in treated groups with Pomegranate seed oil and germ wheat oil. Our result agree with Renuka (2014) proved that HFD in (positive control group) significantly ($p< 0.05$) decreased HDL-c level compare with control negative group. Administration of high fat diet showed a significant increase in blood glucose, insulin, total and low density lipoprotein cholesterol and triglycerides while a decrease in high density lipoprotein cholesterol. Our result in line with Al-Attar et al. (2018**),** who evaluated the influence of pomegranate seed oil on levels of some physiological parameters in male rats, glucose, triglycerides, cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), atherogenic index (AI), atherogenic coefficient (AC), glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MAD), the findings revealed that these oils have antioxidative properties that protect against toxicity. PSO also contains a lot of conjugated linolenic acid (9-cis, 11-trans, and 13-cis), octadecatrienoic acid, and punicic acid (main bioactive ingredient). Previous research has shown that n-3 polyunsaturated fatty acids are a hypolipidemic agent according to Faghihimani et al. *(*2016).

Also, Awadin (2015) proved that the considerably ($p>0.05$) raised blood lipid profile, MDA levels, and significantly lowered insulin hormone and HDL-C levels are all corrected by WGO. These findings are in agreement with Nagib (2018) reported that Wheat germ oil contains a variety of other nutritional and health benefits, including a high vitamin E and phytosterol content which may be the reason of its decrease triglyceride

Soliman *(*2020) indicated that administration of WG /WGO to diets of rats caused a marked reduction in TC, LDL-C, VLDL-C, TG and MDA.The antioxidant defense system and other elements may play a role in this beneficial impact. The mechanism action is may be due to Wheat germ oils are high in linolenic acid, which helps to promote cholesterol synthesis and turnover by increasing cholesterol secretion into the bile and depletion of the intra-hepatic pool of cholesterol. Wheat germ oil may also help to minimize hepatic lipid buildup by promoting -oxidation while inhibiting fatty acid production. WGO essential fatty acids have an antioxidant impact by blocking particular enzymes that facilitate the formation of free radicals, resulting in a reduction in the amount of free radicals produced. WGO's anti-atherosclerotic action may also be mediated via oxidative stress inhibition. Also, the presence of monounsaturated fatty acids, vitamin E, and phytosterol may cause WGO to reduce triacylglycerol. It could also be related to an increase in membrane permeability and fluidity, as well as pancreatic lipase inhibition and reduced triacylglycerol lipolysis.

Effects on Serum antioxidant enzymes and MDA

The effect of wheat germ oil (1 & 1.5mL/ kg) and pomegranate seed oil (0.8 & 0.4mL/ kg) on serum antioxidant enzymes in hyperlipidemic rats were shown in Table 9. The mean value of SOD and GPx value showed a significant decrease in C +ve as compared to the C -ve. All treated groups indicated a significant increase as compared to the C +ve. The best result was found in the treated groups with pomegranate seed oil (0.8 mL /kg) and wheat germ oil (1.5mL/ kg). In contract, value of MDA showed a significant increase in the C +ve as compared C -ve. All treated groups indicated a significant decrease as compared to the C +ve. The best result was found in the treated groups with pomegranate seed oil (0.8 mL /kg) and wheat germ oil (1.5 mL/ kg).

TABLE 7. Effect of feeding with pomegranate seeds and wheat germ oils on total cholesterol and triglycerides in experimental rats (n= 6 rats).

Parameters / Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Control – ve	61.3±5.24^{e}	76.3±9.60^{d}
Control + ve	127.3±19.93^{a}	162.16±35.07^{a}
Wheat germ oil 1mL/kg	105.00±12.60^{b}	117.67±13.74^{b}
Wheat germ oil 1.5 mL/kg	81.3±7.47^{cd}	95.8±10.26^{bcd}
Pomegranate seed oil 0.4 mL/kg	90.50±8.35^{c}	103.3±15.38^{bc}
Pomegranate seed oil 0.8 mL/kg	70.50±7.84^{de}	84.56±8.47^{cd}
LSD	13.36	21.11

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

TABLE 8. Effect of feeding with pomegranate seeds and wheat germ oils on lipoprotein fractions and AI in experimental rats (n= 6 rats).

Parameters / Groups	HDL-C (mg/ dl)	LDL-C (mg/ dl)	VLDL-C (mg/ dl)	AI
Control – ve	43.8±3.92^{bc}	2.2±0.56^{e}	15.3±1.92^{d}	0.398±0.03^{e}
Control + ve	34.0±5.93^{d}	60.9±9.73^{a}	32.4±7.01^{a}	2.76±0.33^{a}
Wheat germ oil 1 mL/kg	38.2±5.27^{cd}	43.3±6.734^{b}	23.5±2.75^{b}	1.76±.174^{b}
Wheat germ oil 1.5 mL/kg	50.2±3.60^{a}	13.5±2.67^{d}	19.2±2.05^{bcd}	0.61±0.07^{d}
Pomegranate seed oil 0.4 mL/kg	40.8±4.26^{c}	29.0±1.789^{c}	20.6±3.07^{bc}	1.23±.09^{c}
Pomegranate seed oil .08 mL/kg	47.7±5.43^{ab}	6.8±1.16^{e}	16.9±1.67^{cd}	0.48±.054de
LSD	5.67	5.9	4.2	0.19

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

This study agreed with Abbasi et al. (2015) reported that there was a considerable increase in serum MDA levels, as well as a decrease in SOD and GP_X activity in hypercholesterolemic rats. These results agreement with Tapias et al. (2014) who showed that pomegranate seed oil can increase the level of GPx and SOD in hyperlipidemic rats but decrease the level of MDA. Also Nuncio-Jáureguia et al. (2015) who suggested that Pomegranate seed oil is rich in flavonoids and phenols, both of which have antioxidant capabilities. Phenolic chemicals are known to act as antioxidants because of their ability to provide hydrogen molecules and to block lipid oxidation. Our result in line with Hussein et al. (2015) who showed that feeding rats with high fat-diet supplemented with (1% and 1.5%), Wheat germ oil altered serum MDA and increased the activities of GP_X and SOD enzymes compared to the feeding rats with high fat-diet only wheat germ oil is an antioxidant that lowers MDA levels while raising plasma SOD and GP_X activity. Wheat germ oil has been shown to improve serum biochemical parameters, as well as the enzymatic and non-enzymatic antioxidant defense systems in the liver.

Effects on serum liver enzymes

The effect of wheat germ oil and pomegranate seed oil on liver enzymes in hyperlipidemic rats was recorded in Table 10. Serum AST value showed a significant increase in the C +ve as

compared to the C -ve. All treated groups indicated a significant decrease as compared to the C +ve. The best result was found in the treated group with (pomegranate seed oil 0.8 mL and closed to the normal group. Also, the mean value of serum ALT and ALP showed significant increase in C +ve as compared to C -ve. All treated groups recorded significant decrease as compared to C +ve. The best result found in treated groups with pomegranate seed oil 0.8 mL /kg and wheat germ oil 1.5 mL/ kg.

The current study found that feeding rats a high fat diet resulted in a considerable rise in the activity of liver enzymes (AST, ALT, and ALP). These increases in liver enzymes could be due to their release from the cytoplasm into the bloodstream following a plasma membrane rupture or cellular injury that approved by McGill (2016).

Our results showed that pomegranate seed oil 0.8 mL/kg)and Wheat germ oil (1.5mL/ kg) can help to improve liver enzymes(AST, ALT , ALP), these results agree with Gram et al. (2018) reported that PSO treatment reduced the activities of AST, ALT, ALP, hyperglycemia, total cholesterol, and MDAPSO has been demonstrated to scavenge free radicals, reduce lipid peroxidation, and inhibit the enzyme lipoxygenase, which is a critical mediator in the inflammatory process. Punicic acid, ellagic acid, sterols, and fatty acids have been identified as the primary antioxidant components in PSO.

Our results agree with Nagib et al. (2018) who found that WGO decrease the oxidative stress by change the liver enzymes activity. WGO had the ability to safeguard the liver. Rats feeding WGO recorded significantly lower injury and betterment in liver functions. These results were in agreement with Abdou et al. (2017) demonstrated that the supplementation of vitamin E and WGO caused an improvement AST and ALT activities.

Effects on serum glucose

The effect of wheat germ oil and pomegranate seed oil on serum glucose in hyperlipidemic rats was illustrated in Table 11. Serum glucose showed a significant increase in the C +ve as compared to the C –ve. All treated groups indicated a significant decrease as compared to the C +ve. The best result was found in the treated group with (pomegranate seed oil (0.8 mL) and closed to the normal group. These results agree with Cano et al. (2012) who proved that the daily pattern of many hormones and adipocytokines may be disrupted by a high-fat diet that causes insulin resistance and symptoms of inflammation, indicating that obesity has a substantial impact on the circadian structure of neuroendocrine and immunological responses. Also, Nekooeian (2014) who reported that The major ingredient of pomegranate seed oil, punicic acid, has been proven to lower plasma glucose and have antioxidant properties. Also, Nekooeian et al. (2014) showed Pomegranate seed oil increased insulin secretion without

TABLE 9. Effect of feeding with pomegranate seeds and wheat germ oils on antioxidant enzymes and MDA in experimental rats (n= 6 rats).

Parameters / Groups	SOD (U/L)	GP_X (ng/mL)	MDA (m mol/gm)
Control – ve	51.3±4.88^{a}	98.06±5.5^{a}	9.1±1.64^{d}
Control + ve	32.26±2.828^{d}	56.2±5.7^{e}	22.0±1.53^{a}
Wheat germ 1 mL/kg	35.03±1.88^{d}	68.3±5.4^{d}	17.5±1.62^{b}
Wheat germ 1.5 mL/kg	42.13±3.97^{c}	90.7±4.1^{b}	13.7±0.98^{c}
Pomegranate seed oil 0.4 mL/kg	39.13±2.01^{c}	79.03±3.9^{c}	18.467±1.72^{b}
Pomegranate seed oil 0.8 mL/kg	47.30±2.84^{b}	95.4±5.1^{ab}	10.767±2.62^{d}
LSD	3.8	5.9	2.07

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

affecting fasting blood glucose levels. It could be linked to the upregulation of PPAR-responsive genes, as punicic acid, the major component of PSO, has been proven to do so. Also, our results agree with Awadin et al. (2015) who proved that WGO's potential anti-diabetic impact could be attributed to the active components, specifically vitamin E. (tocopherols).

Histopathological results

The changes in the cardiac cells are illustrated in Fig. 1 where the microscopic examination of cardiac sections showed normal crossly sectioned cardiac muscle fibers in control -ve group (A). Meanwhile, Cardiac sections from +ve group showed hyaline degeneration of many cardiac muscle fibers having pyknotic nuclei (black arrows) with presence of multiple large sarcoplasmic vacuoles (arrowheads) in many other cardiac muscle fibers (B). Cardiac sections from group received 1 mL wheat germ oil showed some degenerated cardiac muscle fibers having small sarcoplasmic vacuoles (arrowheads) (C). Cardiac sections from group received 1.5 mL wheat germ oil showed very few degenerated cardiac muscle fibers having small sarcoplasmic vacuoles (arrowheads) (D). Cardiac sections from group received 0.4 mL pomegranate oil showed much fewer degenerated cardiac muscle fibers having small sarcoplasmic vacuoles (arrowheads) (E). Cardiac sections from group received 0.8 mL pomegranate oil showed normal histological picture (F). Low magnification X: 100 bar 100 and high magnification X: 400 bar 50.

TABLE10. Effect of feeding with pomegranate seeds and wheat germ oils on liver enzymes in experimental rats (n= 6 rats).

Parameters / Groups	AST(U/L)	ALT (U/L)	ALP (U/L)
Control – ve	116.3±17.53^{e}	34.0±5.876^{e}	216.3±16.598^{e}
Control + ve	287.0±24.7^{a}	84.0±4.65^{a}	314.0±19.91^{a}
Wheat germ oil 1mL/kg	237.0±25.044^{b}	68.67±10.06^{b}	286.67±15.908^{b}
Wheat germ oil 1.5mL/kg	172.0±18.35^{d}	47.0±4.98^{d}	251.3±12.796^{cd}
Pomegranate seed oil 0.4 mL/kg	203.3±20.402^{c}	60.0±4.98^{c}	270.0±12.923^{bc}
Pomegranate seed oil0.8 mL/kg	128.3±19.44^{e}	43.3±4.23^{d}	234.67±19.4398^{de}
LSD	24.9	7.2	19.5

Means in the same column with different superscript letters are significantly different at p ≤ 0.05

TABLE 11. Effect of feeding with pomegranate seeds and wheat germ oils on serum glucose in experimental rats (n= 6 rats).

parameters / Groups	Serum Glucose (mg/dl)
Control – ve	73.5±7.92^{d}
Control + ve	135.83±17.9^{a}
Wheat germ oil 1mL/kg	100.5±11.26^{b}
Wheat germ oil 1.5mL/kg	88.3±10.172^{bcd}
Pomegranate seed oil 0.4 mL/kg	90.83±14.85^{bc}
Pomegranate seed oil0.8 mL/kg	80.0±6.63^{cd}
LSD	14.27

Means in the same column with different superscript letters are significantly different at p ≤ 0.05

Our result agree with Gram et al. (2018) reported that histopathological examination of liver tissues in the control and PSO groups showed normal crossly sectioned cardiac muscle fibers in control -ve group .Also, Our result agree with Rezq & Mahmoud (2011) showed that high dose of WGO corrected the histopathological picture in the liver of control negative and hypercholesterolemic rats cardiac sections showed normal crossly sectioned cardiac muscle fibers in control -ve group.

Conclusion

In conclusion, this study evaluated the effect of pomegranate seed oil and wheat germ as anti-hyperlipidemic agents in experimental rats. Pomegranate seed oil and wheat germ oil contain an amount of phenol compounds and flavonoids, which may play an important role as natural antioxidants. Consumption of pomegranate seed oil and wheat germ oil can be used to improve the lipid profile and liver Function and protection of the risk factor for hyperlipidemia in experimental rats.

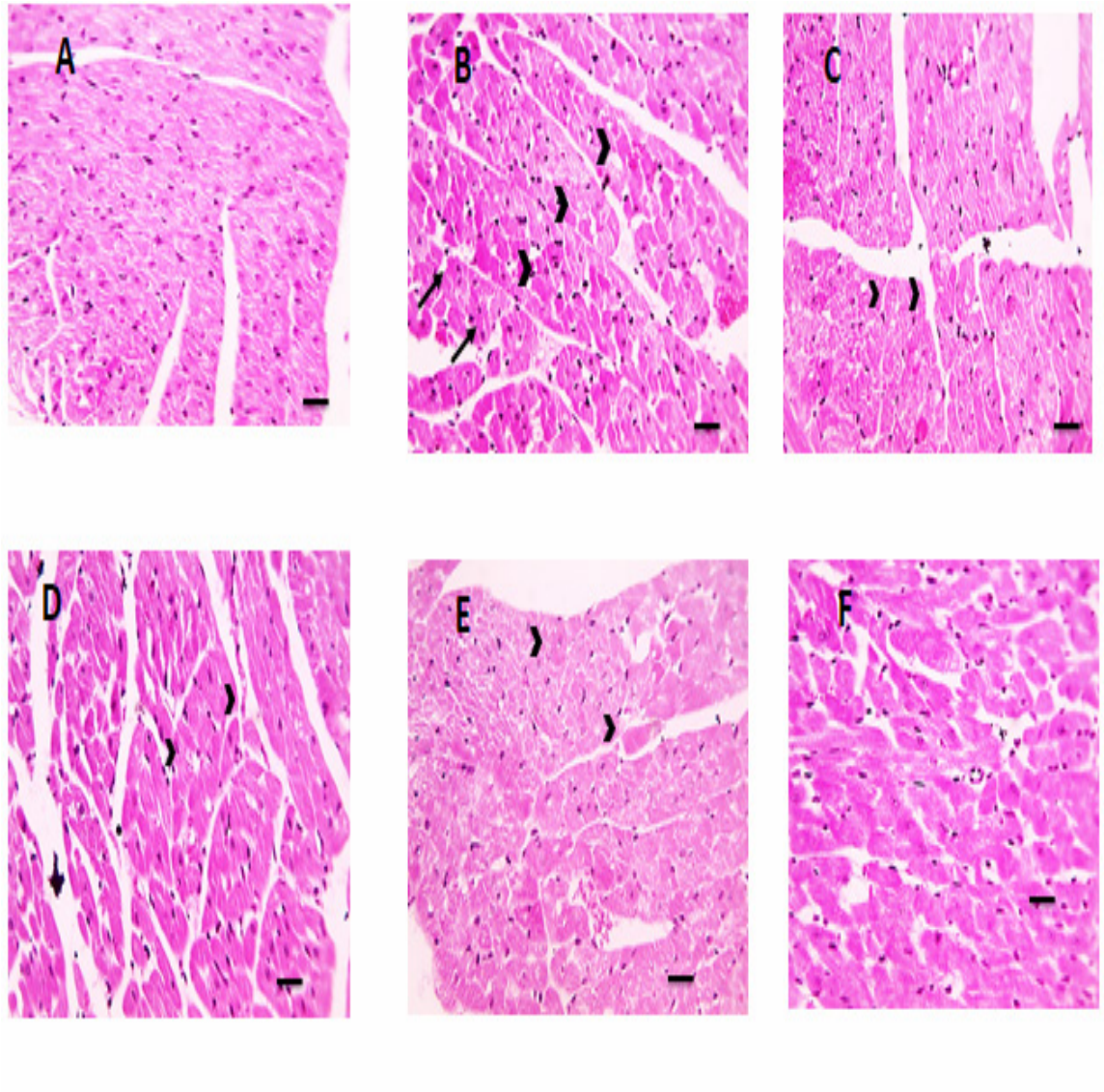

Fig. 1. (A) Negative control group. (B) Positive (+ control) group. (C) Wheat germ oil (low dose) (D) Wheat germ oil (high dose). (E) Pomegranate seed oil (low dose). (F) Pomegranate seed oil (high dose).

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دراسه تأثير زيوت بذور الرمان وجنين القمح كعوامل وقائيه ضد ارتفاع دهون الدم في الجرذان

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يعتبر ارتفاع دهون الدم من أكثر مشاكل الصحة العامة في العصر الحديث والتي تزيد من خطر الإصابة بالعديد من الأمراض المصاحبة مثل أمراض القلب والأوعية الدموية والسرطان. لهذا السبب؛ تمت دراسة تأثير كلا من زيت بذور الرمان و جنين القمح في الوقاية من ارتفاع دهون الدم في الفئران. تم استخدام ستة وثلاثين من الفئران الذكور (سلالة سبراجيو داولي) بوزن ١٢٠ ± ١٠ جم. و تم تقسيمهم إلى ست مجموعات متساوية . المجموعة الأولى مجموعة ضابطة سالبة وتم تغذيتها بالغذاء الأساسي . المجموعة الثانية تغذت على نظام غذائي عالي الدهون لمدة ٤ أسابيع كمجموعة ضابطة موجبة ، المجموعة الثالثة والرابعة تغذت على نظام غذائي عالي الدهون وتم اعطائها زيت بذور الرمان (٠,٤ و ٠,٨ مل / كجم من وزن الجسم) عن طريق الفم لمدة ٤ أسابيع كعامل وقائي . المجموعة الخامسة والسادسة تغذت على نظام غذائي عالي الدهون وتم اعطائها زيت جنين القمح (١ و ١,٥ مل/ كجم من وزن الجسم) عن طريق الفم لمدة ٤ أسابيع كعامل وقائي.

في نهاية فترة التجربة تم حساب كلا من المأخوذ الغذائي ، وزن الجسم المكتسب بالجرام ، معدل كفاءة الغذاء والوزن النسبي للكبد . كذلك تم فحص انزيمات الكبد والكوليسترول الكلي ، الدهون الثلاثية، البروتين الدهني المرتفع الكثافة والبروتين الدهني منخفض الكثافة وكذلت التغيرات النسيجية للكبد تم فحصها .

كذلك تم تحليل إجمالي مضادات الأكسدة الكلية والبولي فينول والفلافونويدات في بذور الرمان وزيت جنين القمح وخلصت النتائج التي تم الحصول عليها إلى أن استخدام زيوت بذور الرمان وجنين القمح أدى إلى تحسن في جميع التحاليل البيولوجية والبيوكيميائية. .

وكانت أفضل النتائج التي تم التوصل إليها باستخدام الجرعات العالية لزيوت بذور الرمان وجنين القمح. وفقًا للنتائج ، يمكن استخدام زيوت بذور الرمان وجنين القمح لتحسين دهون الدم في الفئران المصابة بارتفاع دهون الدم . .

الكلمات المفتاحية : دهون الدم ، الزيوت النباتيه ، جلسريدات ثلاثية– كوليستيرول كلي، إنزيمات الكبد، مضادات الأكسدة الكلية، الفلافونويدات.