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Research paper

Antihyperlipidemic effect of *Punica granatum* mesocarp extract (PGME) in rats

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Abstract

Hyperlipidemia is an umbrella term that refers to any disorder of elevated level of lipids circulating in the blood; and is considered the most significant risk factor contributing to the prevalence of cardiovascular, hepatic, and other diseases. The present study was designed to evaluate the possible antihyperlipidemic potential *Punica granatum* mesocarp extract (PGME) in albino rats using high-fat diet model of hyperlipidemia. Forty-two albino rats were utilized in this experiment arranged randomly in seven groups, six rats each, of different treatments. Hyperlipidemia model was induced by incorporating coconut oil (1.5% w/w) and cholesterol (1.5 % w/w) in diet supplied to rats, for 6 weeks (+ve control); test group rats received PGME at escalating doses of 100 or 200 mg/kg, orally, daily for 6 weeks with keeping on high-fat diet; standard group rats received Rosuvastatin at dose of 2 mg/kg, orally, daily for 6 weeks along with high-fat diet; further 2 groups of rats received only PGME at the same dose levels with keeping on normal diet; while rats of -ve control group received only the vehicles of the used agents. Blood samples were picked out at the end of the experimental course for different assays. Clinicochemical analyses revealed that PGME exhibited dose-dependent protection against hyperlipidemia indicated by improved biomarkers, including, lipid profile parameters, namely cholesterol, triacylglycerols and lipoproteins; enzymatic hepatic parameters, namely, AST, ALT, and GGT; and non-enzymatic parameters, namely, total protein, albumin, globulins, total bilirubin, conjugated bilirubin, unconjugated bilirubin. The mechanism of the obtained lipid profile improvement of PGME may be based on the phytochemical principals Tannins, Phenolics and Flavonoids, indicated by preliminary detection tests. Data of the present study may suggest PGME as a good natural source for promising antihyperlipidemic remedies.

Keywords: *Punica granatum; Mesocarp; Hyperlipidemia; Atherosclerosis; Phytomedicine.*

1. Introduction

The term hyperlipidemia refers to any of disorders whether genetic or acquired that result in a pathologically high level of lipids, including, triglycerides, cholesterol, and other fats circulating in the blood. These lipids can penetrate the walls of arteries and other tissues increasing risk of developing atherosclerosis, diabetes, kidney failure, heart failure and hypertension (Mushtaq et al. 2016). Epidemiologic data shows that the prevalence of dyslipidemia in Chinese adults aged 18 and above is 18.6%, which is to say the number of dyslipidemia patients has reached 160 million. It is also registered that nearly 12 million people die of cardiovascular disorders each year all over the world. Thus, it is important to pay attention to early-stage control and prevention of hyperlipidemia in a comprehensive way (Hossain et al. 2010). More than 3 million people have this disorder, genetically, in Europe and United States, and is extremely common for those who live in advanced countries and follow a high-fat diet (Drechsler et al. 2010).

Monotherapy is effective in treating hyperlipidemia, but combination therapy may be required for a complete course. Currently, antihyperlipidemic drugs have five major classes that include bile acid-binding resins (Cholestyramine), nicotinic acid derivatives (Niacin), statins (Rosuvastatin), fabric acid derivatives (Fenofibrate), and drugs that inhibit cholesterol absorption (Ezetimibe) (Shattat 2015). However, there are many adverse effects of chemical drugs being used in the treatment of hyperlipidemia such as myopathy, myalgia and dizziness, headache, and rhabdomyolysis after statins (Ramkumar et al. 2016); Gastrointestinal disturbances, including, constipation, indigestion, and osteoporosis due to calcium loss on long-term use, nausea, bloating, and flatulence after bile acid sequestrants (Scaldaferri et al. 2013); intense cutaneous flush, headache, itching, abdominal discomfort and nausea, elevating liver enzymes and hyperuricemia which precipitates a gout attack and promotes glucose intolerance after nicotinic acid derivatives (Guyton 2004).

The use of natural medicine has recently increased in an attempt, to find effective alternative therapies to avoid the adverse effects of chemical drugs.

Punica granatum belongs to the *Punicaceae* family. Its common name is Pomegranate that is derived from “Pomuni granatum,” in which Pomum means apple and granatus means grainy, which is also considered as “seeded apple (Kushwaha et al. 2020). Pharmacological studies have been conducted on various types of extracts of various parts of the plant indicated its potential as anti-infective (Sharifiyan et al. 2016), hepatoprotective (Sharifiyan et al. 2016), antioxidants (Gaikwad et al. 2018), analgesic (Guerrero-Solano et al. 2020), and antidiabetic (Jandal & Naji 2021).

The aim of the present study was to evaluate the antihyperlipidemic properties of PGME in a hypercholesterolemic rat model. To achieve this aim, the following objectives have been investigated: lipid profile markers in plasma as cholesterol, triacylglycerols and lipoproteins;

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liver enzymatic markers in plasma as AST, ALT, GGT; liver non-enzymatic parameters in plasma as proteins and bilirubins; cardiac markers as CK-MB and Troponin-I; and renal markers as urea, creatinine, and uric acid.

2. Materials and methods

2.1. Plant part and its identification

Punica granatum was identified by Dr. Mustafa Hamza Mohamed, Assistant professor of vegetable crops, Horticulture Department, Faculty of Agriculture, Benha University (Voucher number 1011a 2021). The whole fruit was obtained from a local market, Qaliobia, Egypt. The mushroom was obtained in December 2021 (Fig. 1).

Fig. 1: The mesocarp (the white enclosing the seeds) part of *Punica granatum* used in extraction and its identification.

2.2. Preparation of the plant extract (PGME)

The mesocarp of *Punica granatum* was picked out the fruit, washed by bi-distilled water then dried and sliced to small slices. The extraction was done by cold maceration of a weighed amount of mesocarp (500 gm) in a known volume (4 liters) of 70% v/v ethanol/distilled H_2O for 72 hours in refrigerator with occasional mixing. The extract was strained with muslin mesh and then filtrated by Whatman paper #1. The filtrate was concentrated on a shaking water bath at 65° C till semisolid crude extract was obtained. The semisolid extract was weighed and kept in refrigerator (4° C) till preparation for administration to rats.
The concentrated yielded extract (79.5 gram from the macerated 500) was re-constituted by dissolving 4 g in 100 ml of isosaline (0.9%) for high dose PGME and dissolving 2 g in 100 ml of isosaline (0.9%) for the low dose. The extracts were always kept in dark air-tight bottles in refrigerator. The concentrations of the prepared extracts were 40 mg/ml and 20 mg/ml which were equivalent to PGME high dose (200 mg/kg) and small dose (100 mg/kg), respectively, if a rat weighing 200 g received 1 ml of the corresponding extract (Harborne 1998). Percentage of yield was determined according to the formula: Yield % = (weight of extract) / (weight of plant material) × 100

2.3. Chemicals, reagents, and kits

Rosuvastatin: It is an inhibitor of cholesterol synthesis used as a standard antihyperlipidemic in the present study. It was produced by Future Pharmaceutical Industries, 1st Industrial zone, 4G, Badr city, Egypt, under the commercial name ROSITOR®. It is formulated as tablets of 40 mg of the drug. The average dose prescribed for humans is 40 mg/day was converted to an equivalent dose for rats according to Paget & Barnes (1964), where a rat dose is equivalent to a human dose multiplied by 0.018 as a conversion factor considering body surface area and body weight as well.
Preparation of Rosuvastatin for rats: Rosuvastatin was suspended in distilled water (0.4 mg/ml; 10 mg tablet in 25 ml) and each rat was administered 1 ml of the prepared suspension daily using a gastric tube. This amount is equivalent to the dosage rate of 2 mg/kg, daily (Gao et al. 2011) as average dose between the two doses computed considering body weight on one hand and body surface area on the other hand.
Kits: The kits for estimating plasma biomarkers of lipid and organ functions were purchased from Spectrum® (Hannover, Germany), Genesis® (Málaga, Spain), and Diamond ® (Hannover, Germany).
Reagents: The reagents, chemicals and solutions used for phytochemical analysis and all other bench procedures were available at our laboratory and all were of analytical grade.

2.4. Experimental animals

Forty-two male albino rats aging 6 weeks of approximate weights 150 ± 10 g were used in this study. Animals were supplied by Animal house, Faculty of Veterinary Medicine, Benha University. Rats were kept in separate cages and allowed to plenty water and diets (normal or high-fat) at room temperature. After a week of acclimatization, rats were randomly grouped and received different treatments as mentioned in the study design below.

2.5. Experimental design

2.5.1. Grouping

The acclimatized rats were divided into seven groups, each consisting of six rats, in a parallel study design. To achieve the aim of the present work, groups were treated differently as described below:
Group (1): Rats were fed on normal diet and received 1 ml saline, orally, once daily, for 6 weeks, and kept as negative control.
Group (2): Rats were fed on high-fat diet (1.5% cholesterol + 1.5% coconut oil) and received no drugs and kept as positive control.
Group (3): Rats were fed on high-fat diet and received Rosuvastatin® at a dose of 2 mg/kg orally once daily for 6 weeks and kept as standard group.
Group (4): Rats were fed on a high-fat diet and received small dose (100 mg/kg) of PGME, orally, once daily, for 6 weeks, and kept as SD-positive treated group.

Group (5): Rats were fed on a high-fat diet and received high dose (200 mg/kg) of PGME, orally, once daily, for 6 weeks, and kept as HD-positive treated group.
Group (6): Rats were fed on normal diet and received small dose (100 mg/kg) of PGME, orally, once daily, for 6 weeks, and kept as SD-negative treated group.
Group (7): Rats were fed on normal diet and received high dose (200 mg/kg) of PGME, orally, once daily, for 6 weeks, and kept as HD-negative treated group.
The research protocol treatments, administration and sampling procedures were ethical to animals and performed in a merciful and humane manner in accordance with the committee of experimental animal care and procedure, Faculty of Veterinary Medicine, Benha University, Egypt, Ethical approval number "BUFVTM 02-02-22".

2.5.2. Sampling

Blood for plasma was collected on the 42nd day from the start of the experiment. Samples were collected from the retrobulbar venous plexus located at the medial canthus of the eye using heparinized capillary tubes, under light Isoflurane inhalation anaesthesia (Sedico®, 6th of October City, Egypt). Blood samples were harvested into sampling tubes containing lithium heparin as anticoagulant at room temperature, then centrifuged at 900Xg for 5 minutes to collect plasma for clinicochemical analysis. The plasma samples were kept frozen at -20 °C till analysed.

2.5.3. Assessments

Clinicochemical measurements were conducted spectrophotometrically using specific kits, following the instructions of the manufacturer with minor modifications. Total cholesterol was determined according to principles described by Ellefson & Caraway (1976), Richmond (1992),and Vega & Grundy (1994).Triacylglycerols were determined according to the method described by Bucolo & David (1973), Fossati & Prencipe (1982), and Program (2002). Low –density lipoprotein-cholesterol was determined according to the method described by Tietz et al. (1995), and Young & Friedman (2001). High-density lipoprotein-cholesterol was determined according to the method described by Lopes-Virella et al. (1977), Friedewald et al. (1972), and Warnick & Wood (1995). The plasma very-low-density lipoprotein-cholesterol value has been calculated using the formula described by Bauer & Covert (1984) as follows:
Cholesterol = Triglycerides / 5 = mg/dl.
Enzymatic activities of AST of ALT in plasma were determined according to Schumann et al. (2010), and Chen et al. (2012). Plasma GGT was determined according to the method described by Szasz (1974), Persijn & van der Slik (1976), and Moss et al. (1987). Total protein and albumin were according to Tietz et al. (1995), and Burtis (1999); while total globulins fraction was calculated mathematically by subtracting the value of plasma albumin from the value of total protein (Hrubec et al. 2000); Albumin/Globulin ratio was calculated using the formula described by Zhou et al. (2016). Total and conjugated bilirubins were measured according to Tietz et al. (1995), and Burtis (1999); while unconjugated one was calculated mathematically by subtraction (Liman & Atawodi 2015).
The plasma CK-MB was determined according to the method described by Horder et al. (1990), Young (1995), and Burtis (1999). The plasma cardiac Troponin-I Assay (cTnI) was determined according to the method described by Bodor et al. (1992), Adams et al. (1993), and Mair et al. (1994).
The plasma creatinine, uric acid and urea were determined according to Burtis (1999), Tietz (1990), and Kaplan (1984), respectively.
Phytochemical screening of PGMEs for the presence of active principal groups, namely, tannins/phenols and flavonoids was conducted using qualitative basic tests. All tests were performed as triplicates and given marks from (-) to (+++) according to the negativity or positivity of the colour or precipitate that appeared.
Detection of Tannins:
About 2 g of the air-dried powder of the plant were extracted with ethanol (50 %) and tested for the presence of tannins and/or other Phenolic compounds using the following tests:
Ferric Chloride test: One-two drops of ferric chloride solution (1%) were added to 2 ml of the prepared extract, the appearance of bluish or greenish-black coloration indicates the presence of pyrogallol or catechol tannins, respectively (Tamilselvi et al. 2012).
Lead acetate test: Fifty mg of the plant extract were dissolved in distilled water and to this, 3 ml of 10% lead acetate filtered clear solution is added. A bulky white precipitate indicates the presence of tannin and/or phenolic compounds (Tamilselvi et al. 2012).
Vanillin test: Five ml of the alcoholic extract of the studied plant were mixed with 2 mL vanillin hydrochloric acid solution if a precipitate was formed; this indicates the presence of Gallic acid (Deshpande & Cheryan 1987).
Detection of Flavonoids:
Shinoda's test: One mL of 10% ethanolic extract of pomegranate mesocarp was mixed with 0.5 ml of hydrochloric acid (10%) and a few mg of magnesium metal. Appearance of a reddish colour indicates presence of flavonoids (Jamdade et al. 2020).
Lead Acetate test: One mL of ethanolic extract of mesocarp was taken in a test tube and a few drops of lead acetate solution were added. The formation of a yellow precipitate indicates the presence of flavonoids (Santhi & Sengottuvel 2016).
Alkaline reagent test: One mL of an aqueous mesocarp extract was treated with 10% Ammonium Hydroxide solution; yellow fluorescence indicates the presence of flavonoids (Lallianrawna et al. 2013).

2.6. Statistical analysis

All data are represented as mean ± SE of 6 observations. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests were done using GraphPad Prism v. 6 software to determine the significant differences among groups. Values were considered significant at P value ≤ 0.05. Phytochemical tests were done as triplicates.

3. Results

3.1. Lipid profile parameters

Results of the effects of PGME on lipid profile parameters are presented in Table 1.

Table 1: The effect of PGME (100 and 200 mg/kg, orally, daily) and Rosuvastatin (2 mg/kg, orally, daily) on plasma lipids in normal and a high – fat diet rats after 6 weeks of different treatments

Group / Parameter	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)
Control (normal diet, ad lib. + saline, PO, daily)	79.58 ± 6.51[d]	99.17 ± 5.83[c]	80.33 ± 6.28[c]	59.83 ± 3.48[b]	19.83 ± 1.17[c]
Diseased (high – fat diet 1.5 % cholesterol + 1.5 % coconut oil), ad lib.	297.5 ± 12.76[a]	245.0 ± 18.39[a]	180.0 ± 5.92[a]	26.83 ± 1.72[b]	49.00 ± 3.68[a]
Standard (high – fat diet + Rosuvastatin, 2 mg /kg, PO, daily	150.3 ± 9.17[c]	146.7 ± 7.27[b]	122.5 ± 5.59[b]	51.00 ± 2.13[b]	28.67 ± 1.71[b]
Diseased administered small dose (100 mg/kg) of PGME, PO, daily	271.8± 13.01[a]	213.3 ± 7.27[a]	161.2 ± 3.25[a]	35.83 ± 1.30[b]	42.67 ± 1.45[a]
Diseased administered high dose (200 mg/kg) of PGME, PO, daily	229.5 ± 9.57[b]	158.3 ± 7.03[b]	146.7 ± 3.74[a]	45.33 ± 1.61[b]	31.67 ± 1.41[b]
Control administered small dose (100 mg/kg) of PGME, PO, daily	81.67 ± 7.17[d]	91.67 ± 4.78[c]	74.67 ± 6.59[c]	61.17 ± 1.20[b]	18.33 ± 0.95[c]
Control administered high dose (200 mg/kg) of PGME, PO, daily	57.92 ± 3.56[d]	85.50 ± 4.50[c]	56.83 ±5.81[c]	77.50 ± 2.99[a]	17.10 ± 0.90[c]

Values are presented as means ± SE of 6 rats per group. Means within a column with different letter superscripts are significantly different ($P \leq 0.05$, ANOVA followed by Tukey's post-hoc).

3.2. Hepatic function parameters

Results of the effects of PGME on non-enzymatic hepatic function parameters are presented in Table 2-A; while its effects on enzymatic ones are presented in table 2-B.

Table 2-A: The effect of PGME (100 and 200 mg/kg, orally, daily) and Rosuvastatin (2 mg/kg, orally, daily) on plasma nonenzymatic hepatic parameters in normal and a high – fat diet rats after 6 weeks of different treatments

Group / Parameter	Total protein (g/dl)	Albumin (g/dl)	Globulins (g/dl)	T. bilirubin (mg/dl)	Con. Bilirubin (mg/dl)	Uncon. bilirubin (mg/dl)
Control (normal diet, ad lib. + saline, PO, daily)	7.17 ± 0.18[a]	3.98 ± 0.120[a]	3.2 ± 0.047[a]	0.68 ± 0.083[c]	0.20 ± 0.018[a]	0.48 ± 0.075[c]
Diseased (high – fat diet 1.5 % cholesterol + 1.5 % coconut oil), ad lib.	4.57 ± 0.20[c]	2.35 ± 0.290[b]	2.7 ± 0.076[a]	2.70 ± 0.120[a]	0.33 ± 0.015[a]	2.30 ± 0.130[a]
Standard (high – fat diet + Rosuvastatin, 2 mg /kg, PO, daily	6.12 ± 0.11[b]	3.12 ± 0.053[b]	3.1 ± 0.052[a]	1.00 ± 0.042[c]	0.23 ± 0.0099[a]	0.82 ± 0.036[c]
Diseased administered small dose (100 mg/kg) of PGME, PO, daily	4.97 ± 0.22[c]	2.55 ± 0.230[b]	2.8 ± 0.097[a]	2.00 ± 0.013[b]	0.32 ± 0.018[a]	1.70 ± 0.110[b]
Diseased administered high dose (200 mg/kg) of PGME, PO, daily	5.25 ± 0.22[c]	2.90 ± 0.230[b]	3.0 ± 0.076[a]	1.30 ± 0.110[c]	0.27 ± 0.011[a]	0.98 ± 0110[c]
Control administered small dose (100 mg/kg) of PGME, PO, daily	7.85 ± 0.15[a]	4.17 ± 0.088[a]	3.3 ± 0.037[a]	0.62 ± 0.077[c]	0.19 ± 0.017[a]	0.44 ± 0.070[c]
Control administered high dose (200 mg/kg) of PGME, PO, daily	8.37 ± 0.13[a]	4.37 ± 0.088[a]	3.4± 0.045[a]	0.54 ± 0.074[c]	0.14 ± 0.015[a]	0.40 ± 0.067[c]

Values are presented as means ± SE of 6 rats per group. Means within a column with different letter superscripts are significantly different ($P \leq 0.05$, ANOVA followed by Tukey's post-hoc).

Table 2-B: The effect of PGME (100 and 200 mg/kg, orally, daily) and Rosuvastatin (2 mg/kg, orally, daily) on plasma enzymatic hepatic parameters in normal and a high – fat diet rats after 6 weeks of different treatments

Group / Parameter	ALT (U/L)	AST (U/L)	GGT (U/L)
Control (normal diet, ad lib. + saline, PO, daily)	34.17 ± 2.45[c]	30.33 ± 3.43[d]	29.83 ± 3.16[c]
Diseased (high – fat diet 1.5 % cholesterol + 1.5 % coconut oil), ad lib.	92.83 ± 6.81[a]	87.33 ± 3.21[a]	68.83 ± 3.61[a]
Standard (high – fat diet + Rosuvastatin, 2 mg /kg, PO, daily	59.00 ± 3.76[b]	45.00 ± 2.38[c]	45.83 ± 2.54[b]
Diseased administered small dose (100 mg/kg) of PGME, PO, daily	87.17 ± 3.63[a]	68.50 ± 4.30[b]	66.67 ± 3.86[a]
Diseased administered high dose (200 mg/kg) of PGME, PO, daily	61.00 ± 3.51[b]	65.50 ± 2.59[b]	50.33 ± 2.73[b]
Control administered small dose (100 mg/kg) of PGME, PO, daily	30.33 ± 2.50[c]	26.17 ± 2.86[d]	25.83 ± 3.04[c]
Control administered high dose (200 mg/kg) of PGME, PO, daily	19.00 ± 2.03[c]	23.17 ± 2.85[d]	23.50 ± 2.86[c]

Values are presented as means ± SE of 6 rats per group. Means within a column with different letter superscripts are significantly different ($P \leq 0.05$, ANOVA followed by Tukey's post-hoc).

3.3. Cardiac and renal parameters

Results of the effects of PGME on biomarkers of renal and cardiac functions are presented in Table 3.

Table 3: The effect of PGME (100 and 200 mg/kg, orally, daily) and Rosuvastatin (2 mg/kg, orally, daily) on plasma biomarkers of cardiac and renal functions in normal and a high – fat diet rats after 6 weeks of different treatments

Group / Parameter	CK-MB (IU/L)	Troponin-1 (ng/ml)	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control (normal diet, ad lib. + saline, PO, daily)	125.2 ± 7.1[b]	0.85 ± 0.13[b]	1.03 ±0.063[b]	27.67 ± 2.80[b]	5.60 ± 0.41[b]
Diseased (high – fat diet 1.5 % cholesterol + 1.5 % coconut oil), ad lib.	215.8 ± 5.7[a]	2.0 ± 0.10[a]	1.85 ±0.090[a]	59.17 ± 2.97[a]	8.50 ± 0.32[a]
Standard (high – fat diet + Rosuvastatin, 2 mg /kg, PO, daily	198.8 ± 5.2[a]	1.7 ± 0.066[a]	1.55 ±0.048[a]	49.67 ± 2.97[a]	7.93 ± 0.31[a]
Diseased administered small dose (100 mg/kg) of PGME, PO, daily	212.3 ± 5.3[a]	1.9 ± 0.130[a]	1.73 ±0.090[a]	56.50 ± 2.76[a]	8.32 ± 0.31[a]
Diseased administered high dose (200 mg/kg) of PGME, PO, daily	204.8 ± 5.0[a]	1.8 ± 0.120[a]	1.65 ±0.086[a]	54.17 ± 3.19[a]	8.05 ± 0.29[a]
Control administered small dose (100 mg/kg) of PGME, PO, daily	114.2 ± 6.5[b]	0.74 ± 0.13[b]	0.90 ±0.070[b]	25.33 ± 2.72[b]	5.35 ± 0.39[b]
Control administered high dose (200 mg/kg) of PGME, PO, daily	102.5 ± 5.9[b]	0.65 ± 0.11[b]	0.83 ±0.054[b]	22.83 ± 2.70[b]	5.05 ± 0.39[b]

Values are presented as means ± SE of 6 rats per group. Means within a column with different letter superscripts are significantly different ($P \leq 0.05$, ANOVA followed by Tukey's post-hoc).

3.4. Phytochemical analysis

Results of the preliminary phytoanalysis of PGME are presented in Table 4.

Table 4: Detection of Phenols and Flavonoids in PGME

Active group	Test	Result
	Gelatin	+++
Tannin	Lead acetate	+++
	$FeCl_3$ test	+++
Gallic acid	Vanillin test	--
	Shinoda's test	±
Flavonoids	Lead acetate	++
	Alkaline reagent	++

Detection tests were performed as triplicates and given marks from (-) to (+++) according to the strength of the colour or precipitate that appeared.

4. Discussion

Hyperlipidaemia is a condition of elevated lipid levels in the blood. It is a major cause of atherosclerosis and atherosclerosis-related conditions like coronary heart disease (CHD), ischemic cerebrovascular disease, peripheral vascular disease, and pancreatitis. The increase in lipids like LDL, cholesterol, and triglycerides are mainly responsible for this condition. These lipids are associated with blood plasma proteins in the form of lipoproteins and remain distributed in the blood. The primary reason for hyperlipidaemias is a defect in lipid metabolism which is caused by the defect in lipoprotein lipase activity or the absence of the surface Apo protein C-II. Other causes of hyperlipidaemias include genetic, nutritional and environmental factors (Samoo et al. 2018).
Plants, herbs, and spices used in traditional medicine have been accepted currently as one of the main sources of chemo-preventive drug discovery and development (Aruoma 2003). Punica granatum is one of the oldest known medicinal plants. It is mentioned in the Ebers papyrus of Egypt written in about 1550 BC (Ross & Harker 1976).
Punica granatum juice contains approximately 5 mmol/L of total polyphenols in comparison to other fruit juices which contain approximately 1.3 to 4.0 mmol/L of total polyphenols (Heber et al., 2006). Phenols and flavonoids are very important plant constituents because of their antioxidant activity (Annegowda et al. 2010). The antioxidant activity of phenolic compounds is mainly based on their redox properties as free radical scavengers, quenchers of singlet oxygen, reducing agents, and chelators of pro-oxidant metals (Mustafa et al. 2010).
In the present study we have evaluated the extract of mesocarp of Punica granatum as an antihyperlipidemic drug. The mesocarp was selected because of the little number of studies conducted on it comparing to juice and leaves.
Findings of the present study revealed that long-term feeding of rats with a high-fat diet resulted in significant increases in plasma levels of TCh, TAGs, LDL-Ch, and VLDL-Ch accompanied by a significant decrease in HDL-Ch level. Administration of PGME showed marked lipid profile improving effects when given to high lipid diet-fed rats. PGME decreased plasma TCh, TAGs, LDL-Ch, and VLDL-Ch, while increased plasma HDL-Ch levels in high lipid diet-fed rats in comparison to saline-treated rats. These effects could be attributed to phenols, tannins and flavonoids which are considered the main active constituents of Punica granatum and have detected obviously in mesocarp in the present study (Table 4). The data may be supported with those of Li et al. (2009), who found that pomegranate peel polyphenols can reduced cholesterol aggregation by decreasing cholesterol intake and promoting cholesterol efflux. Consistent with our findings, different parts of Punica granatum especially the leaves have been reported to decrease the dyslipidemia of obesity and cardiovascular risk factors (Lei et al. 2007). In addition, flower of Punica granatum has been demonstrated to ameliorate hyperlipidemia and decrease excess cardiac lipid accumulation in Zucker diabetic fatty rats via modulation of cardiac endothelin-1 and nuclear factor-kappaB pathways (Huang et al. 2005), and to attenuate atherosclerosis in Apolipoproteins E deficient mice (Aviram et al. 2008).
The levels of plasma triglycerides were found to be significantly reduced in the PGME-treated animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis. Moreover, phenols and flavonoids have been reported to inhibit the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase in vitro (Costamagna et al. 2016) and/or reduce intestinal cholesterol absorption (Ricketts & Ferguson 2018).
Regarding plasma LDL-Ch, VLDL-Ch and HDL-Ch levels, the current results could be explained, at least partly, as PGME may inhibit the activity of CETP cholesteryl ester transfer protein activity in hyperlipidemic rats. CETP is a plasma protein that mediates the movement of cholesteryl esters from HDL to LDL or VLDL in an exchange of equivalent triglycerides (Lam et al. 2008).
In the current study, the obtained hyperlipidemia was associated with elevated biomarkers of liver, heart, and kidney functions. The liver is the main organ responsible for a multitude of essential functions and plays an essential role in the metabolism of foreign compounds entering the body. AST (formerly SGOT) is found in cytosol and mitochondria of hepatocytes and other cells throughout the body organs. ALT (formerly, SGPT) is found in the cytosol of liver cells mainly, thus a more specific indicator of liver injury than AST. GGT is a membrane-bound enzyme that catalyzes the transfer of γ–glutamyl groups of peptides such as glutathione to other amino acids, and its level is increased in cholestasis and hepatocellular disease (Kirtipal et al. 2022) & (Suri et al. 2021). Actually, the effect of high-fat diet on AST and ALT is a matter of debate. Mölgaard et al. (1989) reported that there were no changes in the levels of AST and ALT. On the other hand, Prasad (2010) & Lu et al. (2007) reported that high-cholesterol diet has moderately elevated levels of ALT and AST in rats. This discrepancy might be attributed to the degree and duration of hypercholesterolemia (Lu et al. 2007). Recently, high-fat diet-fed rats, as mentioned above, showed increased plasma cholesterol and triglycerides, which finally develop lipotoxicity of the liver. Fat accumulation leads to fatty liver disease, insulin resistance, oxidative stress and inflammation which lead to leakage of liver markers from liver cells to plasma (Ulla et al. 2017).. In the present study, PGME have been considered beneficial to protect liver cells against pathological effect of high-fat diet stress indicated by decreased elevated liver markers with percentage of 94.1 (ALT), 50 (AST), and 82.6 (GGT) when standardized against rosuvastatin. Statins are known to inhibit HMG-CoA reductase that is responsible for cholesterol biosynthesis. This may indicate a statin-like mechanism for PGME, at least partly, where other mechanisms could not be excluded. Lipid-intoxicated liver can not synthesize albumin and/or conjugate bilirubin, which are also evident in the present study. Decreased albumin leads consequently to decreased total protein level; while decreased ability to conjugate bilirubin leads to elevated unconjugated and total bilirubins.

In the present study, plasma specific cardiac parameters CK-MB and troponin-I have been increased in upon supplementing rats with high-fat diet. The markers are increased after cardiac myocyte injury caused by elevated cholesterol level (Zeng et al. 2021). Also, Vargas & Vásquez (2017) showed that there is a direct relationship between the intake of a high-fat diet and obesity, which in turn can induce cardiac changes, supporting the hypothesis of the relationship between high-fat diet and cardiovascular risk factors. Co-administration of PGME significantly decreased these elevated cardiac parameters based on the improved lipid profile proved in the present study. Our cardiac finding may be consistent with those of Dong et al. (2012) who found that pomegranate with its polyphenolic content protected the cardiac function of rats with ischemia/reperfusion injury, probably in context of enhancing oxygen free radical scavenging activity and decreasing lipid peroxidative damage of the cardiac cells.
Lastly, plasma creatinine, urea and uric acid which are biomarkers for extent of renal function have been elevated in high-fat-fed rats in the present study. The data may be supported with those of Sun et al. (2020) who reported that long-term high-fat-diet feeding causes kidney injury as a result of tissue lipid accumulation, increased oxidative stress, and mitochondrial dysfunction, which promote excess programmed cell death. We have found that PGME could protect against such renal dysfunction indicated by decrement of these parameters. These data may be consistent with those reported by Hua et al. (2022) who found that punicalagin ameliorated renal injury induced by high-fat diet in mice via the gut-kidney axis.

5. Conclusion

In conclusion, the data achieved by this study revealed that *Punica granatum* mesocarp extract (PGME) is effective protecting agent against high-fat-induced metabolic syndrome in rats, and, thus, would be exploited as a potential natural nutraceutical for hyperlipidemia and associated risks.

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7. Declaration

Conflict of interest: There is no actual or potential conflict of interest in relation to this article.

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