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Phytochemical Screening Toxicity and Anti-Hyperlipidemia of Punica Granatum Peel against Male Albino Mice

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ABSTRACT

Background: Hyperlipidemia (Dyslipidemia) is expressed by High levels of TC, TG, LDL, and decreased levels of HDL. Dyslipidemia as a threat agent for heart arteries complaints leads to an increase in the threat of cardiovascular mortality. Several herbal drugs have been suggested for the therapy of dyslipidemia like the one Punica Granatum peels. This research ambitious to clarify the thesis of using Punica Granatum peel extracts as an antihyperlipidemic agent.

Methodology: Three trials were done to clarify the hyperlipidemic thesis of Punica Granatum peels, screening of the phytochemical content by HPLC/GC; half of the lethal dose LD50 measurement, and, the prophylactic effect was analyzed in high lipid diet-fed male mice with the histopathological study.

The Results: 33 ingredients have been identified using HPLC/GC, the LD50 was 504mg/kg of the body weight, and the anti-hyperlipidemic result revealed the capability of the acetone aqueous extract to reduce Cholesterol, LDL, HDL, VLDL, TG, ALT, AST, GGT, and ALP. Furthermore, the reason the acetone aqueous extract was able to increase antioxidant enzymes, whereas it increased T. Chol. /HDL. Chol and total protein is poly-phenolic in the extract.

Conclusion: We suggest considering this study as a seeker for unborn studies n hyperlipidemia.

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Introduction

30% of global deaths around the world are attributed to cardiovascular CVD complaint, which encompasses a range of clinical conditions primary caused by a heart complaint, stroke, and other various cardiovascular conditions that are classified under endothelial dysfunction [1,2]. The main factor for CVD is hyperlipidemia HL 1, 2,3, which leads to an elevation in serum TG, TC, and LDL [3]. HL can be a result of genetic lipids metabolism disorder or unhealthy diet high in fats [4].

HL triger a series of cascading reactions begining with a chemical modification of LDL in arterial partitions via oxidation and non-enzymatic glycation subsequently leading to atherosclerotic [3]. Additionally, many studies have showon that LDL might also enter the intima or inner membrane via damaged vascular endothelium and form oxidized LDL, which is considered one of the main pathological agents of atheromatous development [2].

Recently numerous natural products obtained from plants have been reported as antihyperlipidemic agents including Punica granatum peels.

The pomegranate tree is inherent to North Africa and the Middle East, even though it is cultivated word wide. apart from fruit, the leaves, the roots, and the flowers have been utilized in traditional medicine due to their health properties [5]. These health effects of pomegranate fruit are particularly prominent in the peel that contains phenolic constituents, flavonoids, and proanthocyanidins [6-8]. Additionally, tannins, alkaloids, glycosides have been reported to be present in the juice, peel, pulp, and seed.

Several components of pomegranate have been utilized in medicinal applications for various, including cardiovascular diseases and diabetes mellitus [9]. Furthermore, Punica granatum has positive outcome against cancer, inflammation, allergies, and gastrointestinal and liver issues due to the presence of natural product [6]. Previous studies have indicated that pomegranate juice can reduce TG, and LDL-C ranges in blood samples, however, the impact of pomegranate peel extract on hyperlipidemia has not been extensively investigated [9]. Therefore, this research aims to explore the inherited Punica granatum peels in Libya and their potential prophylactic impact as antihyperlipidemic agent.

Materials and Methods

Plant Material

Punica granatum peels of good quality were obtained from market in Benghazi, Libya 2017; the plant species were separated and washed with distilled water for multiple times, and air-dried for fifteen days.

Taxonomical Investigation
The identification of the plant material was carried out with assistance of the herbarium at Department of botany, Faculty sciences, University of Benghazi, following the flora of Libya.

Method of Extraction
A continuous extraction method was employed to extract phenolic compounds from 100g of e Punica granatum peels. Acetone/water mixture was used as the solvent, and the extraction was performed at temperatures ranging from 40-60°C The solvent was evaporated using rotary vacuum evaporator.

HPLC Chromatography/ Mass Spectra
Thermo Scientific, Trace HPLC Ultra & ISQ Single Quadruple MS system, along with DB-5 bonded-phase fused-silica capillary column.

Experimental Animals
Ninety-five male albino mice weighing between 25 and 35 gwere utalized for this study, the mice were kept in animal facility at the Biochemistry laboratory,University of Benghazi, under standard conditions of temperature (22 ± 3C◦), relative humidity not exceeding 70%, and natural dark-light cycle. The animals were provided with a standard diet and had access to ad libitum water, which were withdrawn 3 hours before experimentations.

Induction of Hyperlipidemia
Hyperlipidemia was induced in male mice by administering a cholesterol/cholic acid mixture (3:1) mixed with the synthetic diet. The amount of the mixture given to each mouse was calculated to ensure adaiy intake of 0.5g/kg body Weight for a period of3 weeks. The diet used for inducing hyperlipidemia contained 10% saturated fat instead of corn oil, and 50% of sucrose was included to accelerate the development of hyperlipidemia.

The Prophylactic Effect of the Acetone Aqueous Extract against Hyperlipidemia
The study adhered to the ethical guidelines for the care and use of laboratory animals, as outlined in the AVMA guidelines [10].

- **Determination of LD50:** The LD50 of the acetone aqueous extract of Punica granatum peels was evaluated following the OECD guidelines for the testing of chemicals [11]. Fifty male mice were divided into groups, with each group receiving different oral dose of the extract "200, 300, 400, 500, 600 mg/kg b.w" orally the doses were selected based on previous studies, and administered according to OECD guidelines 425 [12,13]. Toxicity symptoms and mortality were observed at 2, 6, and 24 hours after the administration.
- **Calculation of (LD50)** The probit-log (dose) regression method as described by [14,15] with some modifications, was employed to calculate the LD50. The number of decreased mice was counted, and the % of mortality was calculated for each group. The probit values were plotted against log doses, and the dose corresponding to probit 5 was determined from the regression equation ($y = 7.2892x - 14.936$, $R^2 = 0.99$) to calculate the LD50 concentration.
- **Experimental Design:** The protective effect of the acetone aqueous extract against hyperlipidemia was studied in three groups of mice.
  Group 1: served the normal healthy control group (-ve).
  Group 2: mice were hyperlipidemic control group (+ve).
  Group 3: mice were orally administered acetone aqueous extract at a dose of 300 mg/kg b. w which was added to the hyperlipidemic diet added to the H.L.D.
- **Blood Sampling:** Blood samples were collected before and after the treatment. Retro-orbital veins were used for blood collection following the method described by Janet Hoff [16]. Serum and plasma samples were separated and stored frozen until used in the distinctive biochemical analyses.
- **Biochemical Analysis:** The biochemical tests were conducted at the medical center, while the antioxidant tests were performed in the university laboratory. Enzymatic method similar to those proposed by Allain et al. were used to determine TC and HDL-cholesterol [17]. LDL, VLDL, chylomicron fractions, and TG were determined by using technique described by David et al. [18] ALT and AST level were determined according to the International Federation of Clinical Chemistry (IFCC) guidline, and ALP levels were determined using the Bretaudiero and Vassault method [19,20]. TP was fixed according to the method described by Doumas & Biggs, and serum total TBIL was determined using the Tietz approach [21,22]. GGT and MDA levels were determined following the techniques by Shaw et al. and Ohkawa et al. respectively [23,24]. SOD, GR, GPx, and CAT activites were determined using the Goldberg and Spooner, Paglia and valentine and Aebi techniques respectively [25-27]

Results and Discussion
High-Performance Liquid Chromatography Coupled Mass Spectroscopy: HPLC revealed the presence of 33 fractionated peaks of the acetone aqueous extract as represented in Table (1).

Table 1: The Chemical Constituent of Acetone Aqueous Extracted from the *Punica Granatum Peels*

Peak	R. Time	Component Name	Area%
1	3.849	Furfural	4.87
2	6.320	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1.43
3	7.12	2-Thiazolamine, 4,5-dihydro-	0.95
4	7.48	Pyrogallol	4.25
5	8.04	Ethanamine, N-ethyl-N-nitroso-	0.17
6	8.33	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl	2.78
7	8.488	Pyrimidine, 4-chloro-5-ethoxy-2-methyl-	0.57
8	8.611	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	0.32
9	8.823	2,3-Dihydrooxazole, 2-t-butyl-3-pivaloyl-	0.25

10	9.173	Gallic acid	44.0
11	9.860	Cyclohexane, 1,4-diethoxy-, trans-	0.69
12	10.42	Punicalin -	1.32
13	10.70	Thiamine	3.12
14	10.921	Hexadecaneperoxoic acid, 1,1-dimethyl-3-[(1- oxohexadecyl)oxy] propyl ester	1.72
15	11.737	D-Allose	3.57
16	13.964	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.49
17	14.903	Hexanedioic acid, dioctyl ester	0.28
18	15.147	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.38
19	15.798	Ascorbic acid	3.16
20	15.999	3-Pentanamine, N,N'-1,2-ethanediylidenebis[2,4- dimethyl	1.28
21	16.277	Quercetin	12.3
22	17.322	1-Tetradecanol, 14-chloro-	1.0
23	17.630	Oleic Acid	4.52
24	17.881	Octadecanoic acid	0.93
25	18.991	Hexanedioic acid	1.04
26	20.849	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.32
27	20.947	1,2-Benzenedicarboxylic acid, diisooctyl ester	0.77
28	21.489	2-Furaldehyde azine	0.82
29	22.175	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.88
30	22.693	3-(2-Fluoro-benzylsulfanyl)-1H-[1,2,4] triazole	0.50
31	23.044	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23- hexamethyl-, (all-E)-	0.18
32	23.779	Cyclopropylpyrrol4-[3-(1Himidazolel-4-yl)propoxy] phenylmorphomethanone oxime	0.48
33	25.841	gamma-Sitosterol	3.77

RT = retention time; Conc. (%) based on peak area integration.

The HPLC/MS analysis results obtained in this study are consistent with the finding reported by reference, which also identified Punicalin, Gallic acid, Quercetin, flavan-3-ols, and other compounds in punica grantam peels [28]. Additionally, reference Other major phenolic compounds present in the extract such as have also been described by [29] has described the presence of major phenolic compounds such as Pyrogallol, Gallic acid, Ascorbic acid, Punicalin, and Quercetin, in the extract These phenolic compounds have been shown to exhibit antioxidant activity by chelating of ions and scavenge the free radicals as mentioned in references [30,31]. This antioxidant activity may contribute to anti-hyperlipidemic effect of the extract as suggested in reference [31]. The analysis revealed the presence of fatty acids, aldehydes, and carbohydrates. Plant carbohydrates can regulate have been reported to regulate lipid metabolism by reducing levels of TG and Cholesterol [32]. According to the data obtained from HPLC coupled with mass chromatography, several prominent polyphenolic compounds were identified in the extract from pomegranate peels. These compounds exhibit antioxidant activity and contribute to various health benefits. Punicalin, a hydrolyzed tannin, possesses both anti-inflammatory and antioxidant properties [33]. Gallic acid and ellagic acid display antioxidant, anti-inflammatory, and anticancer activities [34]. Quercetin, similar to gallic acid, exhibits antioxidant and anti-inflammatory effects, with an additional cardioprotective effect [35]. Additionally, pyrogallol was found to have antioxidant properties [34].

In our study, we investigated the antioxidant properties of these compounds present in pomegranate peels, shedding light on their mechanisms of action against oxidative stress. The antioxidants in the peels have the ability to neutralize free radicals, thereby preventing cellular damage. Furthermore, the compounds in the peels can chelate or bind to metal ions, such as iron and copper, which can catalyze the production of free radicals. By chelating these metal ions, the compounds inhibit their participation in the formation of reactive oxygen species (ROS), effectively reducing oxidative stress. Moreover, our research revealed that the compounds in the peels can influence the activity of enzymes involved in oxidative stress, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes are integral to the cellular antioxidant defense system, and the antioxidants in

the peels were found to upregulate their expression or enhance their activity, thereby enhancing the cellular defense against oxidative damage.

In addition to their antioxidant properties, some of these compounds have also been studied for their potential antihyperlipidemic effects. Punicalin has been shown to reduce total cholesterol, triglyceride levels, and LDL cholesterol levels, while increasing HDL cholesterol levels in animal models. Gallic acid, quercetin, and ellagic acid have also demonstrated antihyperlipidemic activity, reducing total cholesterol, triglyceride levels, LDL cholesterol levels, and increasing HDL cholesterol levels in various studies [36]. However, it is important to note that further research is needed to establish the efficacy and safety of these compounds in human subjects, as most of the evidence comes from preclinical and animal studies

Lethal Dose 50%

The results revealed that the lethal dose of 50% of Punica granatum peel is 504mg/kg b. w, which means half of the animals were dead at this dose as shown in table (2).

Table 2: The lethal dose LD50 of acetone aqueous extract from Punica granatum peel after oral ingestion in male albino mice (n = 5).

Group	Dosage (mg/Kg)	Log Dosage	%Dead	Correlation	Probits
1	200	2.301	0%	2.5	3.04
2	300	2.477	0%	2.5	3.04
3	400	2.602	20%	20	4.16
4	500	2.699	40%	40	4.75
5	600	2.778	60%	60	5.25

These data were compared with the data performed by [12] for the aqueous extract (500 mg/kg) and the results were approximately similar even though the different solvents used for the extraction, and the area of study. The difference between Ld50 in this study and A. Vidal et al. study (731 mg/kg b.w.) may be due to the use of the entire fruit rather than the peels as the amount and type of constituents will vary according to the part used from the plant [13]. The aqueous and EtOH extracts showed a lethal dose of 50 of 1321 ± 15 mg/kg and 160 mg/kg respectively in two different previous studies [37,38]. There are many factors that leads to differences in this numerical. Animal species and strain, age, sex, diet, food deprivation before dosing, temperature, caging, season, experimental procedures, etc., are considered variation factors for LD50 Values [39].

Biochemical Studies of Prophylactic Effect of Punica Peels Acetone Aqueous Extract Experiment

In this experiment the serum lipid profiles of three groups were analyzed: group I (negative control), group II (positive control) control, and group III (treatment group receiving 300mg/kg of Punica peels acetone aqueous extract). The results shown in table (3), revealed significant finding Group II, positive control, exhibited a substantial and statically significant increase ($P < 0.001$) in serum total cholesterol and TG level after 14 days of the experiment Conversely, group III, the treatment group, demonstrated a highly significant decrease ($P < 0.01$) in this level. The negative control group, group I, displayed no significant difference of ($P > 0.1$) in comparison to the normal range.

Regarding HDL, LDL, and VLDL, the positive control group (group II) experienced a significant increase ($P < 0.01$) by the end of the experiment. In contrast, the treatment group (group III) showed significantly lower levels ($P < 0.01$) of these lipoproteins. Furthermore, the ratio of T. Chol. /HDL Cholesterol, exhibited a high significant increase compared to the treated control group, and it was also higher than the control group.

Table 3: Prophylactic Effect on Lipid Profile Variants and % Variation from the Corresponding

	Animal Groups	Time Intervals (Days)		
		0	14	% Var.
S. T. Cholesterol	Control (G1)	56.1 ± 5.01	55.6± 5.06 †	0.89↓
	Positive control (G2)	55.9± 4.22	85.7± 4.91***	54.13↑[a]
	Treated group (G3)	56.7± 5.38	73.5± 2.89**	14.23↓[b]
HDL-Cholesterol	Control (G1)	34.2 ± 3.33	35.0 ± 4.06 †	2.33↑
	Positive control(G2)	33.8 ± 4.01	19.6 ±2.64***	44↓[a]
	Treated group (G3)	33.1 ± 4.75	27.5± 3.10**	40.30↑[b]
T. Chol./HDL. Chol	Control (G1)	1.90±0.03	1.58±0.01†	32↓
	Positive control (G2)	1.65±0.01	4.37±0.04**	176↑[a]
	Treated group (G3)	1.98±0.05	2.67±0.02**	40↓[b]

S. LDL-Cholesterol	Control (G1)	9.2±2.06	8.6±1.71†	6.52↓
	Positive control (G2)	10.32±1.98	46.82±5.76**	444↑[a]
	Treated group (G3)	11.94±2.11	31.26±4.75**	32.51↓[b]
VLDL-Cholesterol	Control (G1)	12.1±1.07	12±2.02†	0.83↓
	Positive control (G2)	11.78±2.11	19.28±2.53**	60.67↑[a]
	Treated group (G3)	11.66±1.38	14.74±2.09**	23.55↓[b]
Triglycerides	Control (G1)	60.5 ± 4.22	60.0 ± 4.29 †	0.83↑
	Positive control (G2)	58.9±5.82	96.4±6.50***	60.67↑[a]
	Treated group (G3)	58.3 ± 5.33	73.7 ± 6.32**	23.55↓[b]

† Non-significant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; * * highly sig. difference at P < 0.01; *** Very highly sig. the difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with the control group; b compared with the positive group.

The study provided evidence of the prophylactic effect of Punica peels acetone aqueous extract against hyperlipidemia. to the extract effectively reduced S. T. Cholesterol, T. Chol. / HDL. Chol, S. LDL, VLDL, and TG. Additionally, it increased the levels of S.HDL. These finding are consistent with previous studies [31,40]. Various studies have shown that the use of drugs or diets containing constituents that can regulate cholesterol levels can significantly reduce mortality and morbidity associated with CVD [41].

In summary, the results indicate that the Punica peels acetone aqueous extract had a beneficial effect on the serum lipid profiles by reducing total cholesterol, triglyceride levels, and the levels of LDL and VLDL. Additionally, it increased the levels of HDL cholesterol, thereby improving the overall lipid profile compared to the positive control group.

The table (4) demonstrate the impact of the acetone aqueous extract from Punica granatum peel on the liver enzyme, protein, and urea level in groups I, II and III. In group II, there was a significant increase in the liver enzymes ALT, AST, ALP, and GGT, whereas in -group III treated with the acetone aqueous extract, there was a notable decrease in these enzymes. When compared to the control group, the results indicate that the acetone aqueous extract of Punica granatum peel has the potential to reduce the liver enzyme level. Furthermore, the total protein was lower in both groups I and II compared to the negative control. Conversely, the urea was increased in groups II and III.

Table 4: Prophylactic Effect on the Liver Enzyme, Protein and Urea and % Variation

	Animal Groups	Time Intervals (Days)		
		0	14	% Var.
ALT	Control (G1)	35.6 ± 6.11	36.1 ± 5.33 †	1.37↑
	Positive control (G2)	33.8±5.30	57.4±5.06**	59.00↑[a]
	Treated group (G3)	34.2 ± 4.87	43.7± 3.95*	23.870.↓[b]
AST.	Control (G1)	65.8± 6.33	66.0 ± 6.86 †	0.33↑
	Positive control (G2)	64.1±6.01	90.1 ± 7.22**	36.52↑[a]
	Treated group (G3)	64.8 ± 6.20	79.7±5.40*	11.54↓[b]
Gama-Glutamyl transferase S. GGT	Control (G1)	11.6± 2.00	11.5 ± 1.08†	0.86↓
	Positive control (G2)	11.8 ± 2.06	25.9 ± 3.86**	125↑[a]
	Treated group (G3)	11.3 ± 1.40	17.5 ± 2.22*	32.43↓[b]
ALP.	Control (G1)	33.6 ± 4.97	33.9 ± 5.07 †	0.89↑
	Positive control (G2)	34.9±5.02	78.4±4.52**	131.27↑[a]
	Treated group (G3)	33.0 ± 5.28	59.6 ± 4.65**	23.98↓ [b]
T. Proteins	Control (G1)	6.0 ± 0.12	6.4 ± 0.03†	6.67↑
	Positive control (G2)	6.8 ± 0.14	5.0 ± 0.01*	21.88↓[a]
	Treated group (G3)	6.3± 0.11	5.8 ± 0.06†	16↑[b]
Bl. Urea	Control (G1)	26.1± 3.81	26.6 ± 2.22†	1.92↑
	Positive control (G2)	26.4 ± 3.05	32.5 ± 3.85*	22.18↑[a]
	Treated group (G3)	25.9 ± 2.87	28.2 ± 3.11†	13.23↓[b]

† Non-significant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; * * highly sig. difference at P < 0.01; *** Very highly sig. the difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with the control group; b compared with the positive group.

The study conducted by A Sadeghipour et al also demonstrated a reduction in ALT, ALP, GGT, ALP, and Bl. Urea level in the treated group, along with the increase in T [31]. protein compared to the positive control group. This highlights the importance of measuringliver enzyme as the liver plays a crucial role in lipid and lipoprotein metabolism, and elevated levels of these enzymes serve as biomarker for hepatic dysfunctions [42]. Furthermore, GGT has been linked to heart disease and strokes, while ALT and AST are connected to CVD mortality [42,43].

Table (5) present the impact of the acetone aqueous extract from Punica granatum peel on antioxidant enzymes in groups I, II, and III. The antioxidant enzymes P. SOD, GR, GPX, and S. CAT, which play a crucial role in combating hyperlipidemia, were significantly reduced in group I. However, in group II, there was a notable increase in these enzymes. As for MDA, its levels were increased in group II and reduced in-group III.

Table 5: Prophylactic Effect on Antioxidant Enzymes and % Variation

	Animal Groups	Time Intervals (Days)		
		0	14	% Var.
P. SOD	Control (G1)	8.32 ± 1.42	8.34 ± 1.99†	0.24↑
	Positive control (G2)	8.27 ± 1.22	4.62 ± 0.63**	44.60↓[a]
	Treated group (G3)	8.21 ± 1.85	6.23 1.21*	34.85↑[b]
MDA	Control (G1)	10.6 ± 2.08	10.6 ± 1.87†	Zero
	Positive control (G2)	10.7 ± 1.44	26.9 ± 4.02**	153.77↑[a]
	Treated group (G3)	10.4 ± 2.06	16.2 ± 2.11*	39.77↓[b]
GR	Control (G1)	18.7 ± 2.06	18.0 ± 1.11 †	3.74↓
	Positive control (G2)	18.4 ± 1.97	9.8 ± 0.97**	45.55↓[a]
	Treated group (G3)	18.0 ± 1.77	12.5 ± 2.09*	27.55↑[b]
GP_X	Control (G1)	32.0± 4.88	32.1 ± 4.06 †	0.31↑
	Positive control (G2)	32.0 ± 5.02	18.9 ± 1.89**	41.12↓[a]
	Treated group (G3)	32.6± 4.10	26.2 ± 3.72*	38.62↑[b]
S. CAT	Control (G1)	38.7 ± 2.40	39.0 ± 3.17†	0.78↑
	Positive control (G2)	40 ± 4.22	23.9 ± 3.56**	38.72↓[a]
	Treated group (G3)	39.5 ± 3.85	31.4 ± 3.11*	31.38↑[b]

† Non-significant difference from the corresponding control at $P > 0.1$; * Significant difference at $P < 0.05$; * * highly sig. difference at $P < 0.01$; *** Very highly sig. the difference at $P < 0.001$; ↓ Decrease; ↑ Increase; a compared with the control group; b compared with the positive group.

According to reference [44] the antioxidant enzymes P. SOD, GR, GPX, and S. CAT exhibited an increased in the treated group following their initial reduction in the hyperlipidemic mice. this increase in enzyme activity resulted in an enhanced capacity to scavenge free radicals in hyperlipidemic animals. Such an elevation in antioxidant enzyme levels supports the body's defense system, safeguarding cells and vital organs from damage and lipolysis [44].

Conclusion

To summarize, Punica granatum peels have shown therapeutic potential attributed to their antioxidative activity, which can be attributed to the flavonoids and polyphenolic compounds. The identification of specific compounds such as Pyrogallol, Gallic acid, Ascorbic acid, Punicalin, and Quercetin had been achieved using HPLC coupled with GC. The prophylactic effect of the extract against Hyperlipidemia has been established through the administration of 300 mg/kg of the body weight dosage of Punica granatum peels acetone aqueous extract, which is below the LD50 dose (504 mg/kg). However, further research is necessary to comprehensively understand the intricate effects of Punica granatum peels extracts on lipid metabolism pathways in metabolic disorders.

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Conflict of Interest: All contributing authors who are involved in various kinds of conflicts certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data Availability: The authors confirm that the data supporting the findings of this study are available within the article

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