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Research Article

Effect of *Punica Granatum* Juice on Lipid Profile of Hypercholesterolemic Albino Rats

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Abstract

Objectives: The present study was performed to investigate the effect of Punica granatum juice in plasma level of Total cholesterol, Triglycerides, High density lipoprotein cholesterol, Low density lipoprotein cholesterol of Hypercholesterolemic Albino Rats.

Material and Method: Hypercholesterolemia was inducted using cholesterol powder 5.5g/30 ml of Ghee (lipid formula) at a dose of 1 ml/kg b.wt, for one week. A total number of 56 rats were divided into 8 groups equally. All eight groups were fed on the normal diet and lipid formula for one week. These rats were distributed into groups A1, B1, C1 and D1 (stopped lipid formula after one week), A2, B2, C2 and D2 (continued lipid formula another two weeks). Groups A1, A2 represented positive control. Groups B1, B2 were administered 5 ml/kg b.wt of Punica granatum juice (PJ) orally for two weeks. Groups C1, C2 were administered 10 ml/kg b.wt of PJ orally for two weeks, while groups D1, D2 were administered 0.3 ml/kg b.wt of Atrovastatin orally for two weeks at a concentration of 20 mg/100 ml (for two weeks). Blood samples were collected from the Retro- Orbital plexus of rats' eyes at days 0, 7, 14 and 21 for determination of plasma level (TC, TG, HDL-c and LDL-c).

Result: The results revealed a significant increase (P. Value < 0.05) in plasma level of (TC, TG, HDL-c and LDL-c) at day 7. The levels of (TC, TG, HDL-c, and LDL-c) showed a significant decreased (P. Value < 0.05) after oral administration of Punica granatum juice and statin drug at days 14 and 21.

Conclusion: It can be concluded that PJ can be a potent hypolipidemic agent.

Keywords: *Albino Rats; Lipid profile; Hypercholesterolemia; Punica granatum; Lipid Formula*

Abbreviations: PJ: Punica granatum Juice; b.wt: Body weight;

Introduction

Dyslipidemias are disorders of lipoprotein metabolism that result in the abnormalities, associated with an increase in plasma concentrations of low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c) and/or a decrease in high density lipoprotein (HDL-c) . Modification of oxidation of LDL-c, is thought to play a key role during early atherogenesis i.e. Formation of atheroma inside the walls of blood vessels that finally lead to arteriosclerosis [1]. The prevalence of dyslipidemia varies with the population being studied, and is highest in patients with premature coronary heart disease (CHD), which can be defined as occurring before 55 to 60 years of age in men and before 65 years in women. In this setting, the prevalence of dyslipidemia is as high as (75-85%) compared to approximately (40-48%) in age-matched controls without (CHD) [2]. Hypercholesterolemia usually has no noticeable symptoms and tends to be discovered during routine examination or evaluation for atherosclerotic, cardiovascular diseases [3].

Hyperlipidemias may basically be classified as either familial caused by specific genetic abnormalities, or acquired when resulting from another underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism [4]. Acquired hyperlipidemias often mimic primary forms of hyperlipidemia and can have similar consequences. They may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome. The most common causes of acquired hyperlipidemia are :(diabetes mellitus), use of drugs such as diuretics, beta blockers, and estrogens. Other conditions leading to acquired Hyperlipidemia include: Hypothyroidism, Renal failure, Nephrotic syndrome and Alcohol consumption. Some rare endocrine disorders and metabolic disorders [5]. Dietary intake of antioxidants can delay the oxidation of susceptible cellular substrates so prevent oxidative stress. Therefore, it is important to enrich our diet with antioxidants to protect against many chronic diseases. Antioxidants also play an important role in food quality preservation due to their ability to prevent oxidative deterioration of lipids [5]. Many medicinal plants are used for lowering the level of blood lipids such as *Citrus aurantifolin*, *Cinanomum verum* and Cicer arientinum [6]. *Punica granatum* has an ancient history of use in each of the areas in which it grows. It is used as food, beverage source and medicine; it carries religious importance in many cultures [7].

Materials and Methods

Punica granatum: Ripe pomegranate fruits were purchased from a local market Khartoum Center of Fruits and Vegetables. Exported from Egypt.

Cholesterol powder: Was purchased from Lab Line Company in the form of white crystalline powder each containing 25g.

Rats: Adult albino rats both sex 6-8weeks old and weighted 160 ± 40 grams were used in this study, were obtained from Faculty of Pharmacy, Khartoum University.

Kits for Biochemical analysis: Were purchased from Biomed Trading Enterprises, Khartoum, production Germany. Commercial diagnostic kits for estimating plasma lipid profile (TC, TG, and HDL-c).

Induction of Hypercholesterolemia

All Rats were administered cholesterol powder (Lipid Formula) 5.5g dissolved in 30 ml of ghee, using an oro-gastric feeding tube for one week. The administration of lipid formula was stopped for groups A1, B1, C1 and D1, while groups A2, B2, C2 and D2 continued the lipid formula till week three [8].

Preparation of *Punica granatum* Juice

The fresh fruit were washed. The seeds were separated after manual peeling. Then the seeds were manually squeezed and filtered using a Sieve [9].

Collection of blood samples

Blood was collected from all rats after they have been anesthetized (using chloroform). Blood was collected from the Retro- Orbital plexus of rats' eyes using heparin zed capillary tubes [10].

Ethical Consideration

All rats received humane care according to the guidelines outlined by the Committee for the Purpose of Control and Supervision on Experiments on Animals [11]

Experimental Design

All Fifty-Six rats were kept under the same environmental conditions. The rats were distributed equally and randomly into eight groups with seven rats each. They were allowed free access to water and food and were kept without any interference for one week before starting the experiment as a period of adaptation. All eight groups were fed on the normal diet and lipid formula for one week. These rats were distributed into groups A1, B1, C1 and D1 (stopped lipid formula after one week), A2, B2, C2 and D2 (continued lipid formula another two weeks). Groups A1, A2 represented positive control groups. Groups B1, B2 were administered 5 ml/kg b.wt of PJ orally for

two weeks. Groups C1, C2 were administered 10 ml/kg b.wt of PJ orally for two weeks. While groups D1, D2 were administered 0.3 ml/kg b.wt Atorvastatin orally for two weeks at a concentration of 20 mg/100 ml (for two weeks). Blood samples were collected from the Retro- Orbital plexus of rats' eyes at days 0, 7, 14, 21 for determination of plasma level of (HDL-c, LDL-c, TC, and TG).

Analysis for lipid profile: Plasma samples were analyzed for presence of (TC, TG, HDL-c and LDL-c).according to Enzymatic method (Peroxidase method), using Spectrophotometer (Mindray BA–88A P.R.C). Determination of Plasma LDL-c for equation:

$$\text{LDL-c} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL-c}$$

Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) for Windows, Version 11 (SPSS Inc., Chicago, IL, USA). The obtained data were presented as mean ± standard deviation (SD). Statistical analysis of variance between mean values of different groups was performed using t-Test to determine the variance between all treatments, differences were considered significant at ($P < 0.05$) [12].

Results and Discussion

Effect of PJ on the level of (TC) of Hypercholesterolemic Rats

All rats showed a significant (P. Value < 0.05) increase in total cholesterol level at day 7 (after administration of lipid formula). Groups B2, C2 and D2 showed a high a significant reduction (P. Value < 0.05) at day 21 (after treatment). The most prominent effect was achieved by the Statin group D2, compared to the positive control groups A1, A2.

Groups	Cholesterol Levels (mg/Dl)			
	Before treatment (Mean ± SD)		After treatment (Mean ± SD)	
	DAY-0	DAY-7	DAY-14	DAY-21
A1	84.9 ± 8.3	237.7 ± 11.8	86.0 ± 3.9	71.4 ± 11.5
A2	86.0 ± 5.7	235.7 ± 10.9	121.8 ± 54.8	105.1 ± 46.6
B1	98.3.0 ± 10.4	240.1 ± 6.8	77.8 ± 3.7	69.0 ± 6.0
B2	73.3 ± 6.5	239.5 ± 6.1	122.6 ± 79.5	66.3 ± 54.8
C1	92.6 ± 5.8	236.4 ± 9.5	79.4 ± 3.9	67.9 ± 3.8
C2	69.1 ± 4.9	242.0 ± 5.9	169.4 ± 9.9	112.7 ± 6.7
D1	91.1 ± 6.3	266.6 ± 7.4	107.6 ± 4.6	76.4 ± 5.1
D2	82.3 ± 5.7	256.0 ± 5.4	161.5 ± 71.4	91.9 ± 40.9

Table 1: *Effects of PJ in Cholesterol levels in study groups.*
Data are presented as means ± standard deviation (P. Value < 0.05)
Key: -A1: Positive Control (Stop lipid formula) -A2: Positive Control (Continue lipid formula)
-B1: Dose of PJ 5 ml/Kg (Stop lipid formula) -B2: Dose of PJ 5 ml/Kg (Continue lipid formula)
-C1: Dose of PJ 10 ml/Kg (Stop lipid formula) -C2: Dose of PJ 10 ml/Kg (Continue lipid formula)
-D1: Dose of statin 0.3 ml/Kg (Stop lipid formula) - D1: Dose of statin 0.3 ml/Kg (continue lipid formula)

Effect of PJ on level of Triglyceride of Hypercholesterolemic Rats

All rats showed a significant increase (P. Value < 0.05) in TG level at day 7 (after administration of lipid formula).Groups A1, C1 and B1 showed a high significant increase (after treatment) at day 21 .The most prominent decrease was achieved by A2, C2, D1 and D2 groups as shown in Table (2)

Groups	Triglyceride (mg/dl)			
	Before Treatment (Mean ± SD)		After Treatment (Mean ± SD)	
	DAY-0	DAY-7	DAY-14	DAY-21
A1	103 ± 17.0	93.1 ± 7.2	32.0 ± 4.3	70.6 ± 4.8
A2	103.3 ± 6.8	91.1 ± 6.3	64.9 ± 32.0	32.6 ± 15.2
B1	98.3.0 ± 10.4	77.4 ± 8.7	24.6 ± 3.4	78.4 ± 5.7
B2	69.0 ± 6.1	76.9 ± 8.1	59.4 ± 37.0	58.8 ± 36.4
C1	101.0 ± 16.2	101.1 ± 6.2	37.1 ± 5.7	81.0 ± 4.2
C2	98.5 ± 5.2	100.9 ± 4.7	90.6 ± 3.6	80.6 ± 4.7
D1	89.9 ± 30.6	81.9 ± 9.9	70.7 ± 5.3	60.7 ± 4.3
D2	85.7 ± 5.8	82.0 ± 9.9	81.8 ± 36.4	60.0 ± 27.3

Table 2: *Effects of PJ in Triglyceride levels in study groups. Data are presented as means ± standard deviation (P. Value < 0.05). Effect of PJ on the level of HDL-c of Hypercholesterolemic rats: All rats showed a significant decrease (P. Value < 0.05) in HDL-c plasma level at days 7, 14, and 21, for all groups including control groups, as shown in table (3).*

Groups	HDL (mg/dl)			
	Before Treatment (Mean ± SD)		After Treatment (Mean ± SD)	
	DAY-0	DAY-7	DAY-14	DAY-21
A1	118.7 ± 17.5	87.0 ± 8.6	53.9 ± 9.7	30.1 ± 2.9
A2	100.5 ± 6.2	86.0 ± 7.2	41.0 ± 20.2	27.3 ± 12.8
B1	113.8 ± 7.7	81.0 ± 8.9	53.4 ± 6.6	40.3 ± 4.7
B2	99.3 ± 5.7	79.5 ± 8.4	40.8 ± 26.5	27.9 ± 17.5
C1	101.9 ± 10.1	107.6 ± 6.7	51.3 ± 5.8	43.7 ± 4.1
C2	89.5 ± 4.1	103.2 ± 6.3	48.1 ± 5.5	39.7 ± 5.2
D1	101.1 ± 11.1	97.9 ± 4.8	55.4 ± 7.5	44.6 ± 3.5
D2	88.9 ± 4.5	90.9 ± 4.5	38.7 ± 17.8	32.7 ± 14.9

Table 3: *Effects of PJ in HDL levels in study groups.*
Data are presented as means ± standard deviation (P. Value < 0.05).

Effect of PJ on the level of LDL-c of Hypercholesterolemic rats

All rats showed a significant increase (Value < 0.05) in LDL-c level at day 7 (after administration of lipid formula). Groups B2, C2, D1 and D2 showed a high significant reduction (P. Value < 0.05), at days 14, 21 (after treatment). The most prominent effect was achieved by the PJ 10 ml/kg b.wt in group C2 and Statin group D2 compared to the positive control groups, as shown in Table (4).

In this study the chemical analysis of PJ showed presence of Tannins, Cumarins, Saponins, Flavonoids and Triterpens. In another study, to investigate anti hyperlipidemic and hepatoprotective activity of Tannic acid in streptozotocin (STZ) induced diabetic rats, the diabetic rats showed a significant increased level of (TG, LDL-c, and VLDL-c) as well as decreased level of HDL-c. Whereas the Tannic acid treated diabetic rats maintained the liver weight as that of normal rats. Altered lipid profile, and other biochemical parameter were also maintained by the Tannic acid treated diabetic rats when compared to diabetic control rats. The anti hyperlipidemic and hepatoprotective activity of tannic acid was compared with standard drug glibenclamide [9]. Our finding agrees with the finding of this study and confirms that presence of Tannins in Punica granatum juice play an important role in reducing (TC, TG, LDL-c and HDL-c) [13].

Groups	LDL (mg/dl)			
	Before Treatment (Mean ± SD)		After Treatment (Mean ± SD)9	
	DAY-0	DAY-7	DAY-14	DAY-21
A1	54.6 ± 12.1	130.6 ± 7.5	27.2 ± 9.0	22.7 ± 4.1
A2	65.3 ± 8.1	127.3 ± 8.2	68.3 ± 30.4	69.9 ± 31.1
B1	25.0 ± 7.2	143.6 ± 11.4	19.4 ± 4.9	19.5 ± 2.4
B2	33.9 ± 5.4	138.2 ± 9.6	69.4 ± 45.7	36.0 ± 25.3
C1	28.7 ± 7.6	108.7 ± 10.3	20.4 ± 2.9	16.0 ± 2.1
C2	25.6 ± 6.7	100.1 ± 8.5	106.6 ± 4.9	50.9 ± 7.4
D1	23.4 ± 4.9	142.3 ± 5.4	38.0 ± 9.4	21.2 ± 4.2
D2	26.9 ± 5.3	140.9 ± 4.9	106.6 ± 47.6	47.7 ± 8.2

Table 4: *Effects of PJ in LDL levels in study groups.*

Data are presented as means ± standard deviation (P. Value < 0.05).

In evaluating the hypocholesterolemic activities of Saponin, in cholesterol- fed rabbits, it was reported that the mechanism of action of Saponin is due to decrease of the intestinal absorption (25–75 %.) [14].

This study interprets that the hypolipidemic activity of Punica granatum can be due to presence of saponin which reduces absorption of lipids from the intestine to the blood circulation, resulting in low blood lipids level.

The present study was performed to investigate the effect of different doses of PJ in plasma level of (TC, TG, LDL-c and HDL-c) of Hypercholesterolemic rats.

Result revealed that feeding rats lipid formula for one week resulted in a significant increase (P. value < 0.05) in plasma levels of (TC,TG, and LDL-c), while HDL-c was a significantly decreased (P. value < 0.05).

The levels of (TC, TG, and LDL-c) were a significantly decreased (P. value < 0.05) after treatment at days 14 and 21. This was accompanied by a significant reduction (P. value < 0.05) in HDL-C, (Table 3).

It was reported in a previous study that supplementation of pomegranate peels powder and its extract to obese hypercholesterolemic male rats produced a significant decrease on lipid metabolism, food consumption, body weight gain ratio and all tested lipid parameters except HDL-c compared to control positive group Hypercholesterolemic rats [15].

In preliminary laboratory research and clinical trials, juice of the pomegranate may be effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation.

In a previous study ,it was suggested that principal mechanisms of action of pomegranate juice is anti atherogenic and may include the following: lipid peroxidation, oxidized LDL-c uptake by macrophages, atherosclerotic, angiotensin converting enzyme activity and decreased systolic blood pressure[16].

Another study reported that Pomegranate juice exerts a direct effect on macrophage cholesterol metabolism by reducing cellular uptake of ox-LDL and inhibiting cellular cholesterol biosynthesis. Both of these processes eventually lead to a reduction in macrophage cholesterol accumulation and foam cell formation and attenuation of atherosclerosis development [17].

In accordance to our findings and the finding of [15-17] it is clear that Punica granatum juice has a lipid lowering activity.

The results of this current study showed that PJ act on reducing Total cholesterol, Triglycerides and LDL-c without affecting HDL-c. The reduction in TC, TG, and LDL can be related to the presence of Flavonoids, Saponins, Cumarins, Tannins, and Triterpens. Which decrease intestinal lipid absorption, decrease cholesterol synthesis and reduce cellular uptake of oxidized LDL-c.

Our finding regarding reduction of HDL-c, agrees with the finding of these authors [15-17]. The interpretation of decreased HDL-c can be attributed to the fact that the ingredients' of PJ have no effect on HDL-c, they act only on TC, TG, and LDL-c. Therefore the reduction of HDL-c can be attributed to intake of cholesterol formula and ghee which contains saturated fatty acids that lead to reduction of HDL-c and increase of TC, TG, and LDL-c.

For strong support of this study, it is recommended that the mechanism of action of PJ, in lowering blood lipids should be searched, in addition to studying the toxicity to ensure safety of its therapeutic use. Furthermore, it is recommended that this study can be conducted using a larger sample size, for greater convenience.

Conclusion

The present study concluded that oral administration of Punica granatum juice at two dosage levels for 14 days to Hypercholesterolemic rats lowers the elevated plasma levels of Total cholesterol and LDL-c (improves lipid profile). These effects are associated with decrease of plasma level HDL-c induced by lipid formula during experiment period. Therefore, fortification of food products with Punica granatum juice may be beneficial for patients who suffer from elevated Chronic Heart Diseases (CHD), Hypercholesterolemia, Arteriosclerosis or Oxidative stress.

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