



ANTI HYPERLIPIDEMIC EFFECT OF ETHANOLIC EXTRACT OF *PUNICA GRANATUM* L. MESOCARP IN HYPERLIPIDEMIC RATS

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Abstract

This study Characterize and identify phytochemical compound of *Punica granatum* L. mesocarp extract and study their an anti-hyperlipidemic activity. The Thirty *Rattus rattus* male rats were divided into 2 main group A [normal control group (6 rats)] and B [induced hyperlipidemic group (24 rats)] group B [high cholesterol diet induced HL (24 rats)]. Each sub group were further divided into (4) sub- groups 1 2 3 and 4. According to the concentration of administrated extract (400 300 200) mg/kg body weight respectively. Some biochemical and physiological tests parameters were estimated which were body weight triglyceride cholesterol high density lipoprotein (HDL) low density lipoprotein (LDL) very low-density lipoprotein (VLDL). for induction hyperlipidemia in rats were administrated high diet cholesterol feeding and high fructose feeding. And the results revealed significantly rising ($p \leq 0.02$) in the body weight mean of both high diet feeding group in compare with negative control group and the high diet cholesterol group mean of body weight was significantly higher than high fructose feeding group. In addition to that The results showed significant elevation in all lipid profile compare with negative control group. And the levels of lipid profile was higher significantly ($p \leq 0.05$) compared with the levels of lipid profile of high fructose feeding group.

Hydro-ethanolic extract was used as anti- hyperlipidemia which administrated in three different doses (400 300 200) mg/kg body weight for 30 day. And the results revealed that the administration of mesocarp pomegranate extract after one month of induction causes decreased in body weight for all extract doses and this decrease become more significant as the dose of extract increase. and this activity was higher significantly ($p \leq 0.000$) in dose (400 mg) compared with other doses. This activity represented in significantly decreasing in all lipid profile compared with positive control.

Key words : Antihyperlipidemic - *Punica granatum* L. Mesocarp.

Introduction

Medicinal plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. Plants play as important role in our life. Plants not only provide us nutrition but also they have medicinal values (Albrecht *et al.*, 2004). Hyperlipidemia is a secondary metabolic deregulation associated with increased risk factors development of diabetes. Beside the cause effect relationship with diabetes elevated serum level of triglycerides cholesterol and LDL are major risk factors for the premature development of cardiovascular diseases such as

atherosclerosis hypertension and coronary heart disease (Ansarullah *et al.*, 2009). Increased plasma lipid levels mainly total cholesterol, triglycerides and LDL along with decrease in HDL are known to cause hyperlipidemia which is the reason for initiation and progression of atherosclerosis impasse (Ghule *et al.*, 2006). Hyperlipidemia with increased concentration of cholesterol triglycerides carrying lipoproteins is considered to be the cause of arteriosclerosis with its dual sequel of thrombosis and infraction. Hyperlipidemia is caused by over-ingestion of alcohol or foods (Ansarullah *et al.*, 2009). Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis therefore two ways are feasible to reduce

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hyperlipidemia, to block endogenous synthesis or to decrease absorption (Sikarwar and Mrityunjaya 2014). Pomegranate (*Punica granatum* L.) has been used in traditional medicine for treating a wide variety of illness. Pomegranate fruits have purported use for expelling parasites (Dell'Agli *et al.*,2009). Seeds and fruit peels for treating diarrhea flowers for managing diabetes tree barks and roots for stopping bleeding and healing ulcers and leaves for controlling inflammation and treating digestive system disorders (Qnais *et al.*,2007). The pomegranate grease (mesocarp) means that it is the white matter that surrounds the pomegranate seeds. The importance of pomegranate grease is due to containing a substance called tannic that has many benefits on the digestive system, it is a medicine for the stomach and its problems so that it helps to get rid of stomach ulcers and duodenal ulcers. Fragility is a disease and also protects against cancers because it contains antioxidants (Muhammad *et al.*, 2017, Kim *et al.*,2002).

Materials and Methods

Plant Collection

Punica granatum L. was collected at duration November to December 2019 from one of Kufa farm then the plant was identified by Dr. Nedaa Adnan (Plant herbarium/ department of biology/ college of science/ university of Babylon). The mesocarp (grease) of the collected plant was taken and then dried in shade at room temperature for 10 days. Dried mesocarp (grease) was milled by using electric miller.

Preparation of *Punica granatum* L. Mesocarp Extract

The method published by Liu *et al.*, (2013) was used for extraction with some modifications. Three different solvents were used for extraction of secondary metabolites from *Punica granatum* L. Mesocarp which included the following solvents, hot water cold water and ethanol (50%). One gram of dried powdered of pomegranate mesocarp was soaked in 10 ml of cold distilled water at temperature of 22 °C and another 1 g was soaked in 10 ml hot distilled water at temperature of 45 °C. The ethanolic extract was prepared by dissolving 1 g of dried powdered of pomegranate mesocarp in 10 ml of ethanol (50%). All three extracts were homogenized in ultrasonic incubator for overnight at room temperature. The extracts were filtered through filter paper. Then the filtrate was dried at 45 °C for 24 hours by using oven and then the dried matter was collected in sealed glass container and stored in the refrigerator for use at a later time.

Body Weight Estimation

The body weight of each rat was assessed weekly by using balance during study periods (Ekpenyong *et al.*, 2012).

Blood Samples and Biochemical Tests

After 30 days of administration rats were fasted overnight and nearly 5 ml of blood was collected from 6 rats of each group with drawn by collected by cardiac puncture under chlorophorm anesthesia and put in tubes without EDTA at room temperature for clotting. Serum was separated by centrifugation at 2500 rpm for 15 min. for serum lipid profile analyzes total cholesterol triglycerides HDL LDL and VLDL

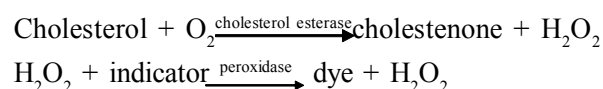
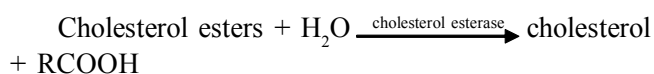
Measurement of Lipid Profile

One of the most important parameters should be measured was the serum parameters were estimated which were triglyceride cholesterol high density lipoprotein (HDL) low density lipoprotein (LDL) very low-density lipoprotein (VLDL)

Measurement of Total Serum Cholesterol (Thomas 2000)

Test Principle

The sample flows to the reaction zone after it was applied to the strip. The reaction scheme is as follow:



A blue stain is yielded from the reaction which is proportional to the concentration of cholesterol in the sample. The concentration of cholesterol is measured at 37°C in mg/dL or mmol/L.

Reagent Component

Cholesterol esterase cholesterol oxidase peroxidase (POD) tetramethylbenzidine buffer.

Storage and Stability

Store at 2-30°C

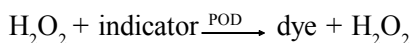
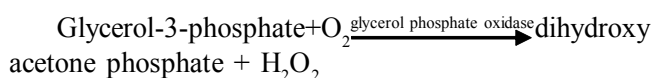
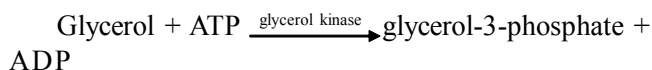
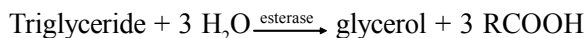
Procedure

- For each test one strip was used provided by Roche and Reflotron instrument.
- Thirty µL of the sample was applied on the center of the red application zone.
- The sliding cover of Reflotron instrument was opened the test strip was placed and pushed horizontally forward till it locks into place.
- The result is shown on the display.

Calculation

- The concentration of cholesterol is automatically calculated from the measurement taken. The concentration is displayed either in mg/dL or mmol/L depending on the apparatus has been situated to exhibit either conversional or SI units.

Conversion factor: mg/dL x 0.026 = mmol/L

Measurement of Serum Triglyceride Concentration (Thomas 2000)**Test principle**

The triglyceride concentration is proportional to the color formed and is measured in mg/dL or mmol/L.

Reagent components

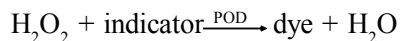
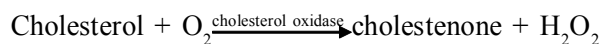
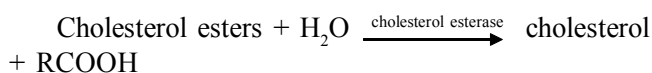
Esterase glycerol phosphate oxidase glycerol kinase peroxidase (POD) adenosine triphosphate (ATP) 4-(4-dimethylaminophenyl)-5-methyl-2-(35-dimethoxy-4-hydroxyphenyl)-imidazole dihydrochloride buffer.

Procedure

- For each test one strip was used provided by Roche and Reflotron instrument.
- 30 μL of the sample was applied on the centre of the red application zone.
- The sliding cover of Reflotron instrument was opened the test strip was placed and pushed horizontally forward till it locks into place.
- The result is shown on the display.

Calculation

- The concentration of triglyceride is automatically calculated from the measurement taken. The concentration is displayed either in mg/dL or mmol/L appropriation on whether the apparatus has been situated to exhibit either conversional or SI units.
- Conversion factor: mg/dL x 0.026 = mmol/L.

Measurement of Serum HDL-C Concentration (National Cholesterol Education Program (NCEP) Expert Panel 2002)**Test principle**

The dye formed at a temperature of 37°C is measured and the HDL-C concentration is displayed in mg/dL or mmol/L.

Reagent Components

Magnesium acetate dextran sulphate cholesterol esterase ascorbate oxidase cholesterol oxidase 4-(4-dimethylaminophenyl)-5-methyl-2-(35-dimethoxy-4-hydroxyphenyl)-imidazole dihydrochloride buffer.

Procedure

- For each test one strip was used provided by Roche and Reflotron instrument.
- 30 μL of the sample was applied on the center of the red application zone.
- The sliding cover of Reflotron instrument was opened the test strip was placed and pushed horizontally forward till it locks into place.
- The result is shown on the display.

Calculation

- The concentration of HDL-C is automatically calculated from the measurement taken. The concentration is displayed either in mg/dL or mmol/L appropriation on whether the apparatus has been situated to exhibit either conversional or SI units.
- Conversion factor: mg/dL x 0.026 = mmol/L.

Calculation of LDL VLDL (De- Cordova & De Cordova 2013)

$$\text{VLDL} = \text{serum triglyceride} / 5$$

$$\text{LDL} = \text{total cholesterol} - \text{HDL-Cholesterol} - \text{TG} / 2.2$$

Results and Discussion**1. Effect of pomegranate extract on hyperlipidemic rat**

The differences in the body weight levels of experimental rats groups with high diet cholesterol and high fructose compare with negative control the data showed that the body weight show significant elevation at fourth week as shown in (Table 1).

Concerning increase in body weight of experimental rat after intake feed there was an effect of high cholesterol supplemented diet on feed intake than fructose during experiment period with significant rising in body weight comparing with initial body weight. AL-Morai (2013) observed increase in the final body weight of

Table 1: The body weight mean (g) of different induced groups after 30 day.

| Group | Body weight (g) (Mean ± SE) | | | |
|-----------------------------|-----------------------------|---------------|---------------|---------------|
| | 1st week | 2nd week | 3rd week | 4th week |
| Negative control | 184.66±3.040b | 191.16±4.206b | 205.5±3.403b | 217.33±3.272c |
| High fat diet induced group | 200.16±4.292a | 222.33±3.800a | 239.16±8.711a | 262.16±2.676a |
| Fructose dietinduced group | 188.5±5.175ab | 200.16±5.016b | 215.16±5.362b | 231.33±3.602b |
| <i>P</i> value (0.05) | 0.053 | 0.000 | 0.005 | 0.000 |

Table 2:The Lipid profile levels of different induced rats groups after 30 day of induction.

| Test Group | Cholesterol (Mean ± SE) | Triglyceride (Mean ± SE) | HDL (Mean ± SE) | LDL (Mean ± SE) | VLDL (Mean ± SE) |
|-----------------------------|-------------------------|--------------------------|-----------------|-----------------|------------------|
| Negative control | 0.85±0.15b | 0.51±0.14b | 0.9±0.11b | 0.14±0.02b | 0.06±0.01b |
| High fat diet induced group | 2.39±0.18a | 1.05±0.14a | 2.1±0.16a | 0.57±0.06a | 0.2±0.06a |
| Fructose dietinduced group | 1.21±0.15b | 0.63±0.10ab | 1.23±0.18b | 0.28±0.05b | 0.08±0.01ab |
| <i>P</i> value (0.05) | 0.001 | 0.062 | 0.004 | 0.003 | 0.079 |

Different letter means (refer to significant differences).

HDL means(High density lipoprotein) LDL means (Low density lipoprotein)V LDL means(Very low-density lipoprotein).

P value (0.05) for the difference between (Fructose diet induced group andHigh fat diet induced group compare with Negative control).

Table 3: Mean differences of body weight at 4 weeks of animal feeding.

| Group | Body weight (g) (Mean ± SE) | | | | |
|-----------------------|-----------------------------|----------------------|----------------------|----------------------|-----------------------|
| | 1 st week | 2 nd week | 3 rd week | 4 th week | <i>P</i> value (0.05) |
| Negative control | 226.25±2.92Eb | 238.25±3.59Db | 252.75±5.63DC a | 263.25±5.34Ca | 0.000 |
| Positive control | 271.75±1.37Ad | 277.50±1.70Ac | 283.75±1.37Ab | 290.50±1.55Aa | 0.000 |
| HL+Ex (200 mg) | 260.75±3.03Bc | 265.75±4.21Bcb | 274.75±4.32BAba | 286.25±3.83BAa | 0.003 |
| HL+Ex (300 mg) | 249.00±3.80Cc | 255.25±3.59Ccb | 265.00±5.61CB ba | 276.25±3.17Ba | 0.002 |
| HL+Ex (400 mg) | 237.50±3.79Dc | 243.75±3.03Dcb | 250.75±3.47Dba | 260.25±3.83Ca | 0.004 |
| <i>P</i> value (0.05) | 0.000 | 0.000 | 0.000 | 0.000 | |

Different letter means (refer to significant differences).

Capital letters mean (refer to differences between groups) Small letters means (refer to differences among time period in same group) HL + Ex means(High fat diet induced group + extract).

P value (0.05) for the difference between (1st2nd3rd 4thweek).

P value (0.05) for the difference between(HL + Ex (400 mg) HL + Ex (300 mg)HL + Ex (200 mg) compare withPositive control and Negative control.

Table 4: The lipid profile levels of rats groups after the administration of different doses of extract for 30 day.

| Test Group | Cholesterol (Mean ± SE) | Triglyceride (Mean ± SE) | HDL (Mean ± SE) | LDL (Mean ± SE) | VLDL (Mean ± SE) |
|-----------------------|-------------------------|--------------------------|-----------------|-----------------|------------------|
| Negative control | 1.11±0.130cd | 0.59±0.132bc | 1.03±0.118bc | 0.2±0.049b | 0.09±0.020bc |
| Positive control | 2.55±0.211a | 1.17±0.130a | 2.16±0.150a | 0.68±0.050a | 0.27±0.072a |
| HL+Ex (200 mg) | 1.93±0.191b | 1.05±0.175ab | 1.54±0.208b | 0.58±0.118a | 0.23±0.060ab |
| HL+Ex (300 mg) | 1.63±0.125bc | 0.91±0.128abc | 1.29±0.68bc | 0.21±0.057b | 0.10±0.028bc |
| HL+Ex (400 mg) | 1.04±0.159d | 0.46±0.158c | 0.91±0.117c | 0.17±0.049b | 0.07±0.026c |
| <i>P</i> value (0.05) | 0.000 | 0.029 | 0.002 | 0.001 | 0.039 |

Different letter means (refer to significant differences).

HDL means (High density lipoprotein) LDL means (Low density lipoprotein)V LDL means (Very low-density lipoprotein).

P value (0.05) for the difference between(HL + Ex (400 mg) HL + Ex (300 mg) HL + Ex (200 mg) compare with Positive control and Negative control.

hypercholesterolemic rats (positive control group) compared to the normal rats (negative control group). Harnafi *et al.*, (2009) show that there were no significant changes in weight between healthy and hypercholesterolemic rats. Nwozo *et al.*, (2011) revealed incidence of significant increases in body weight through feed intake of hypercholesterolemic rats compared to the negative control rats.

Results of our study revealed that feeding of rats on high - cholesterol diet resulted in significant increases than high - fructose diet in cholesterol (0.001) TG (p 0.062) HDL (P 0.004) and LDL (P 0.003) while in VEDL it decreased in non-significant differences (p 0.079) compare with negative control.

These results could be explained on the basis that feeding of rats on high cholesterol diet leads to raise in cholesterol absorption and hence serum cholesterol and other parameter of lipid profile increment. Frantz *et al.*, (2012) who demonstrated that lipid metabolism in rats fed high fat - diet presented disorders and levels of serum TC and TG increased significantly compared with the negative control group.

While AL-Morai (2013) observed in significant increases in serum levels of TC TG LDL-c and VLDL accompanied with a significant decrease in HDL level as compared to the negative control group.

With regard to the effects of Pomegranate mesocarp extract when orally given to hypercholesterolemic rats at different concentration for 4 weeks on feed intake and body weight gain. Our results study revealed that body weight decreased with an increased feeding concentration of pomegranate mesocarp extract (400mg) during period of feeding when compare with positive control of rat fed high cholesterol diet as shown in (Table 3).

Our result agreed with Al-Morai (2013) reported that oral administration of Pomegranate juice significantly (P < 0.05) decreased the body weight when compared to positive control group (hypercholesterolemic rats). These findings might be due to decreased appetite (anorexia) of rats and/or reduction of intestinal fat absorption or due to an inhibition of pancreatic lipase activity. It is well known that dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase (Lei *et al.*, 2007).

Results in table 4 illustrate the effect of of hydroethanolic extract from *P.granatum* mesocarp in different concentration on serum levels of lipoprotein fractions in hypercholesterolemic rats showed marked antihyperlipidemic effects in high cholesterol diet fed rats.

These results of Pomegranate extract indicated significant differences in lipid profile level in experimental rat with significant decrease (p 0.00) in cholesterol TG LDL and VLDL with HL + Ex (400 mg) while HDL show increment with non-significant differences (p 0.002) when compare with positive control.

Previous studies like Al-Fartosi *et al.*, (2015) show that significant reduced (P< 0.01) in the total cholesterol LDL level and in VLDL level of rat which treated with high cholesterol diet and (0.2 mL/animal) of pomegranate juice compared with rat which treated with high cholesterol diet (positive control). While there results indicated there was a significant increase observed in (P< 0.01) in TG level in group of rat which treated with high cholesterol diet and pomegranate juice compared with positive control.

Sadeghipour *et al.*, (2014) demonstrated that treated with pomegranate peel extract significantly decreased serum triglycerides cholesterol LDL while increasing serum HDL level in high lipid diet fed rats compared with group of rat that fed on 10% lipid diet and given saline 0.5mL/rat.

Al-Muslehi (2013) observed that the effective of Pomegranate peel extract on the lipid profile and body weight gain ratio on hypercholesterolemic male rats that was increase in weigh body of rat compare with initial weight and indicated significant (P<0.05) decrease in cholesterol triglycerides LDL and VLDL. While increase in HDL level for all hypercholesterolemic rats administrated with different concentration of pomegranate peel powder comparing with positive control group (animal fed on hypercholesterolemic diet composed of pellet + 1% cholesterol + 10% saturated fat).

Mustafa and Abdelrahman (2015) show that the levels of cholesterol triglycerides LDL were a significantly decreased (P. value < 0.05) while significant reduction (P. value < 0.05) in HDL-C after treatment at days 14 and 21 with pomegranate peels powder extract to obese hypercholesterolemic rat due to feeding with high cholesterol diet that cause increase in weight body.

Conclusions

The 400 mg /kg body weight of hydro-ethanolic extract of pomegranate grease was effective as an antihyperlipidemic treatment by significantly decreasing both of body weight and the levels of lipid profile in hyperlipidemic rats compare with positive control.

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