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## Effect of Pomegranate Peels and Juice on Hypercholesterolemic Rats

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### Abstract:

The aim of the current study to evaluate five products of cake were prepared by adding levels from pomegranate (peel and juice), moreover evaluate the effect of pomegranate on hypercholesterolemic rats. Total of 49 adult male albino rats were used, divided into main groups: The first main group (7 rats) fed on basal diet (control negative), while the other 6 groups were fed on high fat diet for two weeks, to induce hypercholesterolemia. After that, they were divided into six subgroups each group contain (7) rats. Subgroup1: fed on high fat diet contents (positive control group), Subgroup2: fed on high food in fat containing 15% pomegranate peel replacement, Subgroup 3: fed on high food in fat containing 20% pomegranate peel replacement, Subgroup 4: fed on high food in fat containing 15% pomegranate juice replacement of water, Subgroup5: fed on high food in fat containing 20% pomegranate juice replacement of water, Subgroup6: fed on high food in fat containing 10% pomegranate peel + 10% pomegranate juice replacement respectively for six weeks. The results of the sensory evaluation showed that adding pomegranate peel and juice to the diet of rats suffering from hypercholesterolemia led to decrease in acquired weight, feed efficiency, total cholesterol, triglyceride, low density lipoprotein cholesterol, liver enzymes (ALT, AST and ALP) and food intake. A slight recovery of fatty liver was observed in group which treated with 20% fruit juice and 10% fruit peel and a mixture of (10% pomegranate peel + 10% pomegranate juice).

*Key words: cholesterol, blood lipid profile, liver enzymes, sensory evaluation, cake*

### Introduction:

Cholesterol is a lipid that serves a variety of purposes. It is critical for the structure and function of cell membranes in vertebrates. Hypercholesterolemia is a major risk factor for heart disease and stroke. It is caused by a discrepancy between cholesterol secretions into the blood and uptake. Cholesterol is necessary, but too much of it causes deposition in the blood vessels, which constricts them, resulting in blockage and, eventually, heart

stroke <sup>(1)</sup> It's a serious metabolic disorder. It is usually caused by an imbalance of energy consumption versus energy expenditure, as well as a lack of nutritional knowledge, and is characterized by the accumulation of excess fat in adipose tissue, which is linked to a number of chronic diseases, including type2 diabetes, hypertension, coronary heart disease, hyperlipidemia, and cancer. <sup>(2)</sup>

The pomegranate (*Punica granatum L.*) is an ancient edible fruit that is widely grown in many tropical and subtropical countries <sup>(3)</sup>. Pomegranate is a juicy fruit that provides about 16 percent of an adult's vitamin C requirement per 100 ml of juice, as well as a good source of vitamin B5, potassium, and polyphenols like tannins and flavonoids <sup>(4)</sup>. Sugars, vitamins, polysaccharides, polyphenols, and minerals abound in pomegranate (*Punica granatum L.*) seeds. They contain little oil but are high in polyunsaturated fatty acids <sup>(5)</sup>. Pomegranates of various types have long been used in folk medicine for a variety of therapeutic purposes <sup>(6)</sup>. Pomegranate fruit peel also has antioxidant <sup>(7)</sup>, cytotoxic <sup>(8)</sup>, hepatoprotective <sup>(9)</sup>, and hypoglycemic <sup>(9)</sup> pharmacological functions <sup>(10)</sup>. Pomegranate is a rich source of bioactive compounds and has been used in traditional medicine for centuries. Pomegranate juice has been shown to have a high level of antioxidant activity and to help prevent atherosclerosis. According to the FRAP (ferric reducing antioxidant power) assay, pomegranate peel had the highest antioxidant activity among the peel, pulp, and seed fractions of 28 different fruits commonly consumed in China <sup>(11)</sup>. In terms of scavenging or preventing superoxide anion, pomegranate peel extract had significantly higher antioxidant capacity than pulp extract.

CuSO<sub>4</sub>-induced LDL is inhibited by hydroxyl and peroxy radicals, as well as by hydroxyl and peroxy radical's oxidation. Total phenolics, flavonoids, and proanthocyanidins were measured. Peel extract is also more concentrated than pulp extract. The high concentration of phenolics

It's possible that the antioxidant properties of peel extract are the cause of its potent antioxidant properties <sup>(11)</sup>.

Dietary supplementation with antioxidant-rich foods has been linked to a reduction in atherogenic LDL modifications, macrophage foam cell formation, and atherosclerosis. Pomegranates are high in polyphenols and other antioxidants. Antioxidants are beneficial to the body <sup>(12)</sup>.

The aim of this study is to realize if pomegranate peel and juice can be used in food products, as well as to see how they affect hypercholesterolemia in rats.

## **Material and methods:**

### **Materials:**

1-Fresh pomegranate (*Punica granatum L.*) was obtained from Damietta city, Egypt. in autumn (2019).

- 2- Food products (flour, sugar, sun flower oil, milk, eggs, Baking powder, vanilla obtained local market, from Damietta city, Egypt.
- 3- Forty-nine males albino rats (Sprague Dawley rats) weighting between (150-200 gm each) from Faculty of Medicine, Al-Mansoura University, Egypt.
- 4-Danni fats from local market from Damietta city.
- 5- Kits were used to determine Total Cholesterol(TC), Triglycerides(TG), High density lipoprotein(HDL-c), Low density lipoprotein (LDL-c), Alanine Amino Transferase (ALT), Amino Transferase (AST), Alkaline Phosphatase (ALP), were obtained from EL-Gomhoriya Company for Trading, Chemicals and Medical instruments, Cairo, Egypt.
- 6- Bile Salts were obtained from EL-Gomhoriya Company for Trading, Chemicals and Medical instruments, Cairo, Egypt
- 7- Minerals, vitamins, choline chloride, cellulose, casein, corn oil, corn starch, all chemicals and diagnostic kits were purchased from EL-Gomhoriya Company for Trading, Chemicals and Medical instruments, Cairo, Egypt.

#### **Methods:**

##### **Preparation of pomegranate peel:**

In addition to the inner yellow peels, the exterior fresh pomegranate peels are cleaned with water, filtered, and microwave dried. After drying the peels, they are ground into a powder in a food processor, making them ready to use and store in a glass or plastic container<sup>(13)</sup>.

##### **Preparation of pomegranate juice:**

The pomegranate is split in half and then squeezed with an electric juicer to extract the juice.<sup>(14)</sup>

**1- Control:** Control cake was made from 220gm flour ,200gm sugar ,55gm sun flower oil, 200 gm milk, 4 eggs, 10gm baking powder and 5gm vanilla.

##### **2-Different Formulas:**

**A- Treatment:** Were made from adding 33 gm pomegranate peel on wheat flours per 187 gm.

**B- Treatment:** Were made from adding 44 gm pomegranate peels on wheat flours per 176 gm.

**C- Treatment:** Were made from adding 18 mg pomegranate juice on milk per 182 mg.

**D- Treatment:** Were made from adding 24mg pomegranate juice on milk per 176mg.

**E- Treatment:** Were created by combining 22 gm of pomegranate peel and 12gm of pomegranate juice with 198gm of wheat flour and 188 gm of milk.

Cakes are made according to the method used by<sup>(15)</sup>

##### **Specific Gravity of Batter and Measurements of Cakes (physical properties)**

Volume (cm<sup>3</sup>) and weight (gm) of three cake samples of each treatment were recorded. Specific volume (gm/ cm<sup>3</sup>) was calculated by dividing of the volume to weight according to the method described in <sup>(16)</sup>

#### **Color determination method**

Changes in Hunter color parameter (L, a and b) of different cakes were followed up using Tristimulus Color Analyzer (Hunter, Lab Scan XE, Reston, Virginia) with standard white tile. (Tests were carried out in Cairo National Research Centre)

#### **Experimental design:**

The biological investigation was carried out in compliance with the Laboratory Animal Welfare Ethics Act's rules. Rats were kept in standard settings for one week before to the experimental investigation to allow them to adjust. During this time, rats were fed a basal meal according to the protocol <sup>(17)</sup>. Following a one-week adaption period on a basal diet only, the rats were separated into two groups:

The first group (7 rats) was fed a standard diet (as a control negative group).

The second main group (42 rats) was fed a high fat-content diet.

(20% Danni fat and 0.255% bile salt) <sup>(18)</sup>

After high cholesterol were determined in second main group to insure the induction. The rats in the second main group were divided into six subgroups: each group contain (7) rats.

**Subgroup1:** fed on high food in fat content as positive control group.

**Subgroup2:** fed on high food in fat containing 15% pomegranate peel (Pp 15%).

**Subgroup 3:** fed on high food in fat containing 20% pomegranate peel (Pp 20%).

**Subgroup4:** fed on high food in fat containing 15% pomegranate juice (Pj 15%). replacement of water.

**Subgroup5:** fed on high food in fat containing 20% pomegranate juice (Pj 20%) replacement of water.

**Subgroup6:** fed on high food in fat containing 10% pomegranate peel + 10% pomegranate juice (Pp 10%+Pj 10%) replacement of water.

#### **Sensory evaluation:**

Cake products were evaluated for, smell, taste, texture, color, overall acceptability and total score by 15 people specialized arbitrators from Home Economics Department. Faculty of specific Education, Damietta university. The evaluation was carried out according to the method of Sammak <sup>(19)</sup>.

#### **Biological determination:**

Every day during the experiment period (56 days), the amounts of diet ingested and/or discarded were recorded. In addition, the rat's weight was monitored on a weekly basis to calculate food intake and percent body weight gain according to Chapman <sup>(20)</sup>.

**Body weight gain% was determined using the following equation:**

$$\text{Body weight gain} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100$$

**Feed efficiency ratio was determined using the following equation:**

$$\text{Feed Efficiency Ratio} = \text{Body weight gain (g)} / \text{Food intake (g)}$$

**Animal:**

Forty-nine adult male Sprague Dawley rats weighing 150-200 gm. Rats were obtained from the Medical Experimental Research Center, Faculty of Medicine, Mansoura University. Rats were housed under standard conditions (12 h. light – dark cycles, 8 rats per 1500 cm<sup>2</sup> cage in 22±3 C°) for one week to acclimate before experimental study, during this period, rats were feed on standard rat diet with freely access to food and water. The basal diet consists of 14% casein (protein 80%), soya oil 10%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.25% and the remainder is corn starch (17)

**Blood sampling:**

After 56 days, rats were sacrificed using an over-dose of thiopental sodium (ip 75 mg/kg) (21) The blood withdrawn from the heart of rats using 5 ml syringe and collected in a dry test tube for serum preparation. Blood samples allowed to clot for 2 hours at room temperature and then centrifuged at 10000 xg for 15 minutes and then the serum is separated and collected into three tubes and stored at – 20°C for assay of Serum cholesterol, triglycerides, HDL, LDL, ALT, AST, ALP.

**Biochemical analysis of serum:**

total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL)

Serum total cholesterol (TC) was determined according to the method described by Allain *et al.*, (22). Serum triglycerides (TG) were determined according to the method described by Fossati and Principe (23). Serum high density lipoprotein (HDL-c) was determining according to the method described by Burstein *et al.*, (24). Serum low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) was determined according to the method described by Fried *et al.*, (25). As for liver enzyme, ALT was determined according to the method described by (26). Determination of AST in serum according to the method described by Bergmeyer and Wahlefeld (27). Alkaline phosphatase activity was determined according to the method described by Belfield and Goldberg (28).

**Histopathological Examination:**

Saline was irrigated into the liver and heart multiple times via a syringe inserted into the thoracic aorta to cleanse blood. The liver and heart were removed and placed in a 10%

formalin solution before being used to create 6 mm thick paraffin embedded slices for histological analysis <sup>(29)</sup>.

#### **Statistical analysis:**

The data were performed with SPSS version 10.0 for windows® (SPSS Inc., USA), obtained were statistically analyzed by using computer. The results were expressed as mean t standard deviation "SD" and tested for significance ( $P \leq 0.05$ ) using one way analysis of variance "ANOVA" test, according to Duncan <sup>(30)</sup>.

#### **Result And Discussion**

The effect of Pp (15% & 20%), Pj (15% & 20%) and (Pp 10% + Pj 10%) on the body weight gain (BWG %), food intake and feed efficiency of hypercholesterolemic rats are summarized in table (1). Data in table (1) showed significant decrease ( $p \leq 0.05$ ) in body weight gain (%) between the positive control group and groups pomegranate peel and juice (Pp 20%, Pj 20% and Pp 10% + Pj 10%), and also this data showed significant increase ( $p \leq 0.05$ ) in food intake between the positive control group and groups pomegranate peel and juice (Pp 20%, Pj 20% and (Pp 10% + Pj 10%),  $19 \pm 3.17$ ,  $27 \pm 4.50$ ,  $29 \pm 4.83$  and  $32 \pm 5.33$  g/day/rat respectively.

As well as, feed efficiency also declared significant decrease ( $p \leq 0.05$ ) between the positive control group and treatment groups ( $6.38 \pm 1.06$ ,  $3.87 \pm 0.65$ ,  $2.94 \pm 0.49$ ,  $4.04 \pm 0.67$ ,  $2.78 \pm 0.46$  and  $2.58 \pm 0.43$ ) respectively.

Meanwhile, another hypercholesterolemic study <sup>(31)</sup> showed that, oral administration of pomegranate juice in doses of 1, 3, and 5 ml/kg b.wt. significantly ( $P \leq 0.05$ ) decreased BWG percent (positive control group) These results could be due to a decrease in intestinal fat absorption or a decrease in pancreatic lipase activity. It is well known that dietary fat is not directly absorbed from the intestine unless it has been treated with pancreatic lipase, and that the extract reduces obesity and hyperlipidemia.

The effect of Pp (15% & 20%), Pj (15% & 20%) and Pp 10% + Pj 10% on TC, TG, HDL-c and LDL-c of hypercholesterolemic rats show in the table (2).

Data in table (2) showed significant decrease ( $P \leq 0.05$ ) of TC and LDL-c in treatment groups Pp 15%, Pp 20%, Pj 15%, Pj 20% and (Pp 10% + Pj 10%) when compared with positive control group.

treating hypercholesterolemic group on diet containing levels of (Pp 10% + Pj 10%) recorded the highest decrease in TC and LDL-c, as compared to other treated groups. Furthermore, the same table showed significant decrease ( $P \leq 0.05$ ) between the positive control group and groups 20%Pp and 10%Pp + 10%Pj in TG, the highest reduction in TG recorded for the group fed on diet containing the Pp 20% as compared to other treated group.

**Table (1) The effect of treatments on body weight gain (%), food intake and feed efficiency of hypercholesterolemic rats.**

Parameters Groups	BWG (%)	Food intake g/day/rat	Feed efficiency
NC (-)	35.30±6.84 <sup>d</sup>	33±5.50 <sup>a</sup>	1.07±0.18 <sup>d</sup>
PC (+)	121.15±10.05 <sup>a</sup>	19±3.17 <sup>c</sup>	6.38±1.06 <sup>a</sup>
15% Pp	92.94±5.36 <sup>ab</sup>	24±4.00 <sup>bc</sup>	3.87±0.65 <sup>bc</sup>
20% Pp	79.47±6.08 <sup>c</sup>	27±4.50 <sup>ab</sup>	2.94±0.49 <sup>c</sup>
15% Pj	93.00±7.32 <sup>ab</sup>	23±3.83 <sup>bc</sup>	4.04±0.67 <sup>b</sup>
20% Pj	80.56±8.84 <sup>bc</sup>	29±4.83 <sup>ab</sup>	2.78±0.46 <sup>c</sup>
10%Pp +10%Pj	82.57±8.73 <sup>bc</sup>	32±5.33 <sup>a</sup>	2.58±0.43 <sup>c</sup>

NC= Control (-), Pc= Control (+), Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $p \leq 0.05$ ).

In contrast, data showed non- significant decrease ( $P \geq 0.05$ ) between the positive control group and group 15%Pp, 15%Pj and 20%Pj. Meanwhile, there was significant increase ( $P \leq 0.05$ ) between the positive control group and groups Pp 15%, Pp 20%, Pj 15% and (Pp 10% + Pj 10%) in HDL-c, nevertheless data showed non- significant increase between Pj 20% and positive control group in HDL-c.

In another study<sup>(32)</sup> when compared to the control positive group, oral administration of Pomegranate juice to hypercholesterolemia rats for 28 days significantly reduced serum levels of TC, TG, and low density lipoprotein cholesterol (LDL-c).

VLDL-C, LDL-C, total cholesterol, and triglycerides were all reduced when the amount of pomegranate peel in the cupcake was increased; however, HDL-C was significantly increased<sup>(33)</sup>.

In another study showed that giving rats with high cholesterol three different doses of pomegranate peel (5, 10, and 15%) and pomegranate juice extract (1,2, and 3%) reduced lipid levels and increased high-density lipoprotein levels<sup>(34)</sup>.

Pomegranate juice, given at doses of 3 or 5 mL kg<sup>-1</sup>, reduced total cholesterol, triglyceride, and LDL-C levels in hypercholesterolemic rats fed a high-cholesterol diet<sup>(35)</sup>.

In patients with hypercholesterolemia, consumption of a concentrated pomegranate juice containing 875 mg per 100 g total polyphenols significantly reduced plasma levels of total cholesterol and LDL-C<sup>(36)</sup>.

**Table (2): The effect of treatments on Total cholesterol, Triglycerides, HDL and LDL of hypercholesterolemic rats.**

Parameters Groups	TC (mg/dl)	TG (mg/dl)	HDL(mg/dl)	LDL(mg/dl)
NC	61.86±4.10 <sup>d</sup>	64.89±1.89	55.17±2.91 <sup>a</sup>	33.33±2.74 <sup>d</sup>
PC	119.19±13.13 <sup>a</sup>	97.09±6.39 <sup>a</sup>	34.66±3.99 <sup>c</sup>	57.19±5.16 <sup>a</sup>

<b>15% Pp</b>	89.03±9.99 <sup>bc</sup>	88.66±3.05 <sup>ab</sup>	50.90±3.92 <sup>a</sup>	43.33±1.29 <sup>bc</sup>
<b>20% Pp</b>	84.04±9.40 <sup>c</sup>	73.27±5.41 <sup>c</sup>	55.03±3.32 <sup>a</sup>	41.39±2.22 <sup>c</sup>
<b>15% Pj</b>	97.89±6.32 <sup>b</sup>	95.46±11.72 <sup>a</sup>	42.99±2.77 <sup>b</sup>	47.14±2.88 <sup>b</sup>
<b>20% Pj</b>	97.67±4.97 <sup>b</sup>	87.79±6.86 <sup>ab</sup>	45.66±4.31 <sup>bc</sup>	47.20±1.72 <sup>b</sup>
<b>10%Pp +10%Pj</b>	83.46±5.18 <sup>c</sup>	81.33±6.42 <sup>bc</sup>	52.07±3.51 <sup>a</sup>	43.01±2.78 <sup>bc</sup>

NC= Control (-), Pc= Control (+), Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $p \leq 0.05$ ).

The effect of Pp (15% & 20%), Pj (15% & 20%) and (Pp 10% + Pj 10%) on serum ALT, AST and ALP of rats fed a high-cholesterol diet are given in table (3).

Data in table (3) showed significant decrease ( $p \leq 0.05$ ) between the positive control group and groups Pp 15%, Pp 20%, Pj 15%, Pj 20% and (Pp 10% + Pj 10%) in AST and ALP, also showed significant decrease ( $p \leq 0.05$ ) between the positive control group and Pp 20%, Pj 15%, Pj 20% and (Pp 10% + Pj 10%) in ALT.

Pomegranate peel powder, which is high in phenolic compounds, can be used as a nutraceutical food ingredient for people with liver diseases because it protects against oxidative stress<sup>(37)</sup>.

Ellagic and gallic acids are powerful free radical scavengers found in pomegranate peel, restoring the activity of hepatic enzymes (catalase, peroxidase, and superoxide dismutase) and inhibiting the lipid peroxidation process<sup>(38)</sup>.

Data presented in Photo (A1, A2, A3, A4, A5, A6, A7) illustrate Effect of Pp (15%, 20%), Pj (15%, 20%) and (Pp10% + Pj10%) on histopathological examination of liver.

Photo (A1) Histological section of liver tissues of normal control group shows no fatty changes with normal tissue architecture and regular morphology of hepatic cell. Photo (A2) Histological section of liver tissues of positive control groups shows fatty changes presented in fat droplets. Photo (A3, A4) Photomicrograph of liver of Pp 15% and Pj 15% shows fatty changes presented in fat droplets. Photo (A5, A6) Photomicrograph of liver of Pp 20% and Pj 20% shows fatty changes presented in fat droplets. Photo (A7) Photomicrograph of liver of Pp + Pj group shows significant enhancement in hepatic tissue presented in reduced fat droplets.

**Table (3): The effect of treatments on liver enzymes (ALT, AST and ALP) of hypercholesterolemic rats.**

Parameters Groups	ALT(U/dl)	AST(U/dl)	ALP(U/l)
NC	17.93±2.08 <sup>d</sup>	70.67±3.50 <sup>d</sup>	200.33±14.1 <sup>d</sup>
PC	39.99±2.51 <sup>a</sup>	104.39±7.12 <sup>a</sup>	373.41±28.4 <sup>a</sup>
<b>15% Pp</b>	37.11±2.86 <sup>ab</sup>	78.44±7.05 <sup>cd</sup>	281.13±37.7 <sup>bc</sup>

<b>20% Pp</b>	32.16±1.99 <sup>c</sup>	72.47±3.83 <sup>d</sup>	261.17±28.2 <sup>c</sup>
<b>15% Pj</b>	35.34±1.78 <sup>bc</sup>	92.75±6.73 <sup>b</sup>	310.96±23.3 <sup>b</sup>
<b>20% Pj</b>	35.49±2.13 <sup>bc</sup>	84.47±8.69 <sup>bc</sup>	254.76±11.42 <sup>c</sup>
<b>10%Pp +10%Pj</b>	32.11±3.36 <sup>c</sup>	76.51±2.81 <sup>cd</sup>	274.46±22.3 <sup>bc</sup>

NC= Control (-), Pc= Control (+), Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $p \leq 0.05$ ).

A histopathological study proved that Pomegranate protected Wistar albino rats from carbon tetrachloride toxicity by restoring normal hepatic architecture, according to a histopathological study<sup>(38)</sup>.

In biochemical and histopathological studies of liver,<sup>(39)</sup> proved methanolic pomegranate's therapeutic potential against oxidative damage and fibrosis caused by biliary obstruction with its antioxidant and antifibrotic properties, pomegranate administration reduced liver oxidative injury and improved hepatic structure and function.

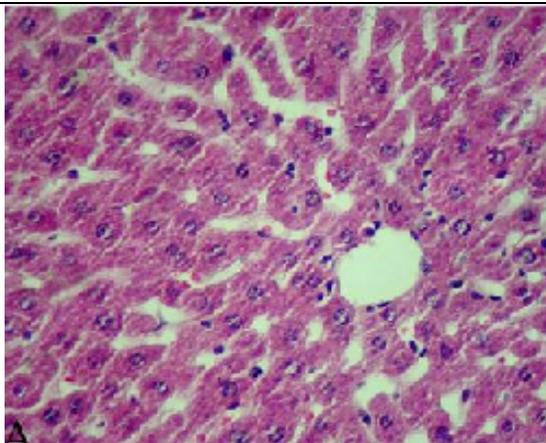


Photo (A1) Histological section of liver tissues. 40 X stained with H&E of normal control group shows no fatty changes with normal tissue architecture and regular morphology of hepatic cell.

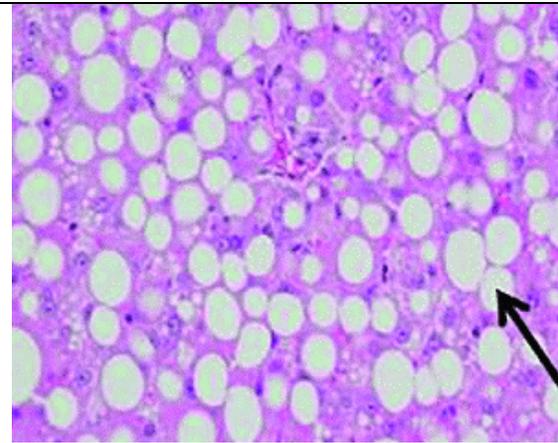


Photo (A2) Histological section of liver tissues. 40 X stained with H&E of HFD group shows fatty changes presented in fat droplets.

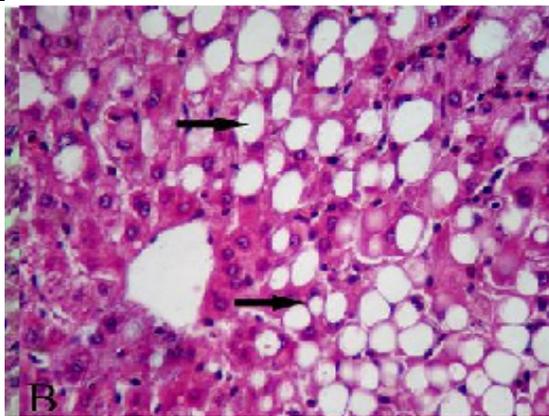


Photo (A3) Histological section of liver tissues. 40 X stained with H&E of peel 15 group shows fatty changes presented in fat droplets.

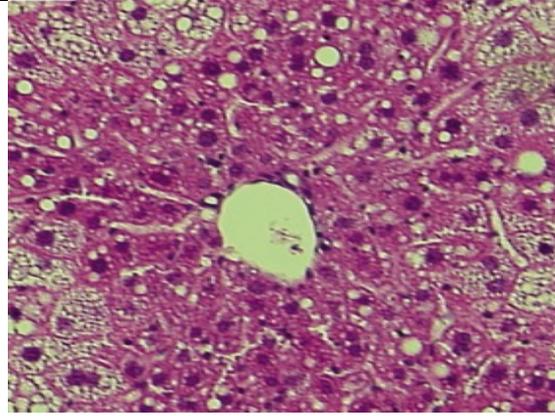


Photo (A4) Histological section of liver tissues. 40 X stained with H&E of peel 20 group shows fatty changes presented in fat droplets with slight improvement

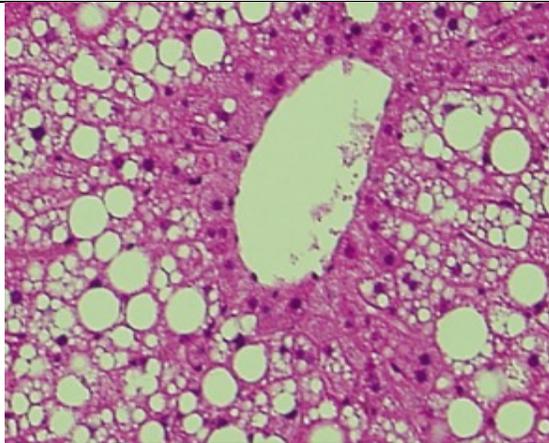


Photo (A5) Histological section of liver tissues. 40 X stained with H&E of juice 15 group shows fatty changes presented in fat droplets.

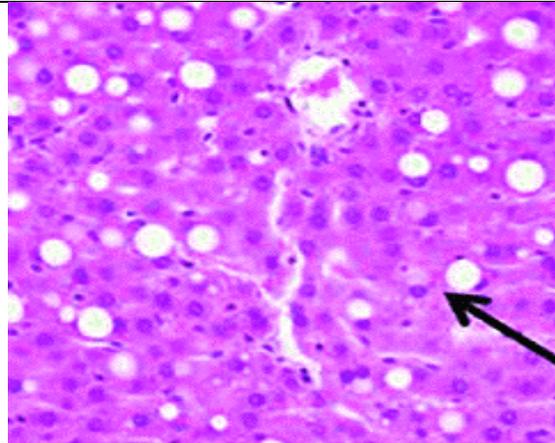


Photo (A6) Histological section of liver tissues. 40 X stained with H&E of juice 20 group shows fatty changes presented in fat droplets with slight improvement.

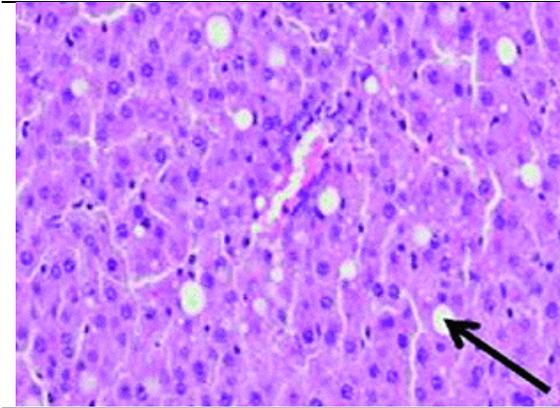
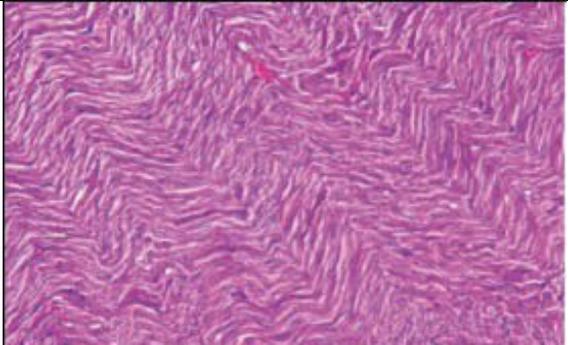
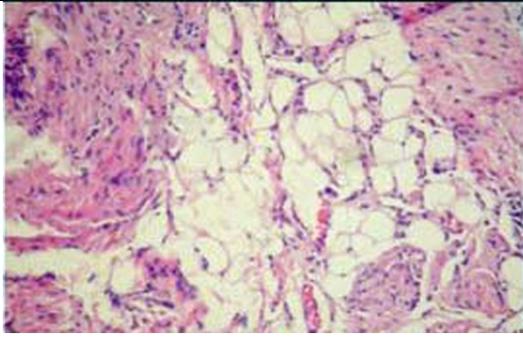
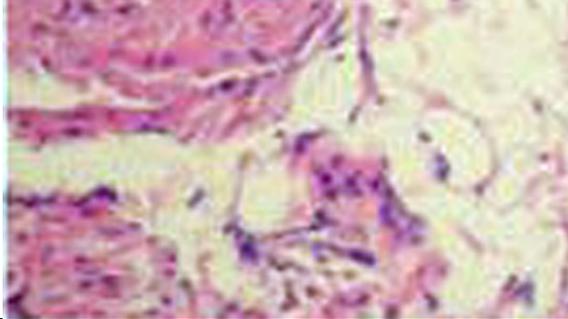
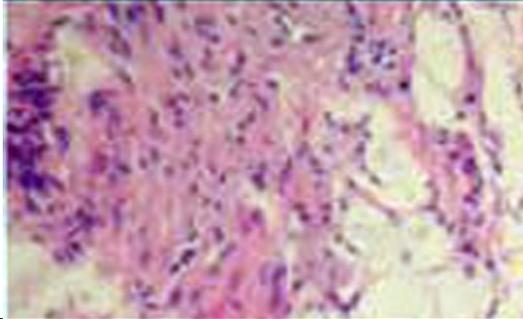


Photo (A7) Histological section of liver tissues. 40 X stained with H&E of juice 10+10 peel group shows significant enhancement in hepatic tissue presented in reduced fat droplets.

Data presented in Photo (B1, B2, B3, B4, B5, B6, B7) illustrate effect of Pp (15%, 20%), Pj (15%, 20%) and pomegranate peel and juice (10% + 10%) on histopathological examination of heart. Histological section of heart tissues. 40 X stained with H&E Photo (B1) Photomicrograph of heart of normal control group shows no fatty changes with normal tissue architecture and regular morphology of myocardial cell membrane. Picture (B2) Photomicrograph of heart of positive control groups shows focal fatty infiltration in myocardial cells with high degree of infiltration Photo (B3, B4, B5, B6) Photomicrograph of heart of Pp 15%, Pp 20%, Pj 15% and Pj 20% groups shows focal fatty infiltration in myocardial cells with different degrees, no significant enhancement was observed. Photo (B7) Photomicrograph of heart of Pp + Pj group shows significant enhancement in myocardium presented in reduced fatty infiltration.

In this respect, the present results agreed with the study <sup>(40)</sup> reported that pomegranate juice's antioxidant and free radical scavenging properties appear to protect the myocardium from oxidative damage in heart tissue.

	
<p>Photo (B1) Histological section of heart tissues, 40 X stained with H&amp;E of normal control group shows no fatty changes with normal tissue architecture and regular morphology of myocardial cell membrane.</p>	<p>Photo (B2) Histological section of heart tissues, 40 X stained with H&amp;E of HFD group shows focal fatty infiltration in myocardial cells with high degree of infiltration.</p>
	
<p>Photo (B3) Histological section of heart tissues, 40 X stained with H&amp;E of peel 15 group shows focal fatty infiltration in myocardial cells with high degree of infiltration.</p>	<p>Photo (B4) Histological section of heart tissues, 40 X stained with H&amp;E of peel 20 group shows focal fatty infiltration in myocardial cells with high degree of infiltration.</p>

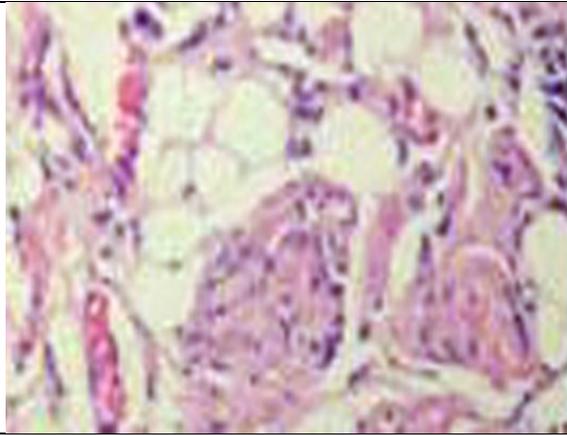


Photo (B5) Histological section of heart tissues, 40 X stained with H&E of juice 15 group shows focal fatty infiltration in myocardial cells with high degree of infiltration.

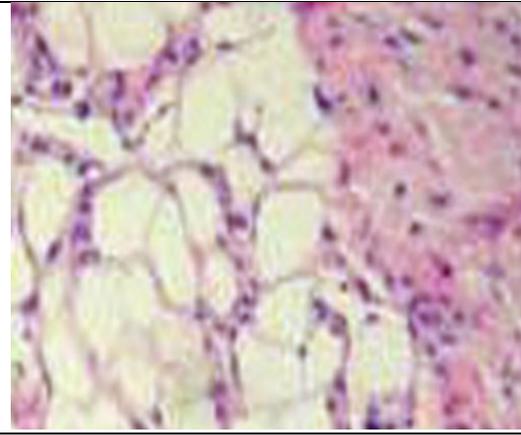
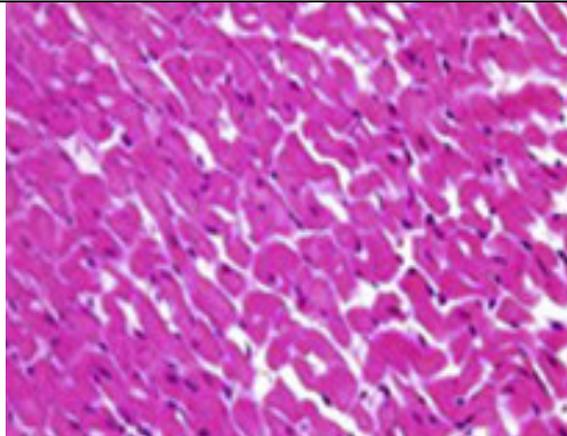


Photo (B6) Histological section of heart tissues, 40 X stained with H&E of juice 20 group shows focal fatty infiltration in myocardial cells with high degree of infiltration.



Photo(B7) Histological section of heart tissues, 40 X stained with H&E of 10 peel +10 juice group shows significant enhancement in myocardium presented in reduced fatty infiltration.

Table (4) showed that, the mean values + SD in the color, odor, texture, taste, over all acceptability and total were 10.00±00, 10.00±00, 20.00±00, 40.00±00, 20.00±00,

100.00±00 of cake fortified with different levels of Pp (15%, 20%), Pj (15%, 20%), and (Pp 10% + Pj 10%) respectively.

The results obtained from the sensory tests indicated that, sensory evaluation was decreased gradually with increasing the levels of Pp and Pj. Generally, sensory evaluation showed an acceptance of cake fortified with (Pp 15%, 20%, Pj 15%, 20% and (Pp 10% + Pj 10%).

The highest record of color was the cake fortified with Pp 20% and Pj 20%, the highest record of odor was the cake fortified with (Pp 10% + Pj 10%), the highest record of texture was the cake fortified

with Pp 15% and (Pp 10% + Pj 10%), the highest record of taste was the cake fortified with (Pp 10% + Pj 10%), the highest record of overall acceptability was the cake fortified with Pj 20%.

**Table (4) Sensory evaluation of the cake fortified with (Pp 15%, 20%, Pj 15%, 20%, and (Pp 10% + Pj 10%).**

Groups	Color	Odor	Texture	Taste	Over all acceptability	Total
<b>control</b>	9.87±0.35 <sup>a</sup>	9.65±0.48 <sup>a</sup>	19.97±0.13 <sub>a</sub>	39.73±0.59 <sub>a</sub>	19.70±0.46 <sub>a</sub>	98.92±1.27 <sub>a</sub>
<b>Pp 15%</b>	9.57±0.62 <sup>a</sup> <sub>b</sub>	9.23±0.56 <sup>b</sup>	19.77±0.56 <sub>ab</sub>	39.10±0.71 <sub>c</sub>	19.30±0.80 <sub>a</sub>	96.97±2.03 <sub>b</sub>
<b>Pp 20%</b>	9.70±0.59 <sup>a</sup> <sub>b</sub>	9.37±0.61 <sup>a</sup> <sub>b</sub>	19.50±0.73 <sub>b</sub>	38.70±1.07 <sub>bc</sub>	19.37±0.97 <sub>a</sub>	96.63±2.80 <sub>b</sub>
<b>Pj 15%</b>	9.27±0.88 <sup>b</sup>	9.25±0.69 <sup>a</sup> <sub>b</sub>	19.60±0.63 <sub>ab</sub>	38.83±0.84 <sub>c</sub>	19.33±0.72 <sub>a</sub>	96.29±2.32 <sub>b</sub>
<b>Pj 20%</b>	9.70±0.46 <sup>a</sup> <sub>b</sub>	9.40±0.54 <sup>a</sup> <sub>b</sub>	19.70±0.65 <sub>ab</sub>	39.20±0.86 <sub>abc</sub>	19.50±0.68 <sub>a</sub>	97.50±2.19 <sub>ab</sub>
<b>Pp 10% + pj 10%</b>	9.50±0.82 <sup>a</sup> <sub>b</sub>	9.53±0.52 <sup>a</sup> <sub>b</sub>	19.77±0.56 <sup>a</sup> <sub>b</sub>	39.36±0.89 <sub>ac</sub>	19.37±0.72 <sub>a</sub>	97.53±2.30 <sub>ab</sub>

Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $P \leq 0.05$ ).

Baking quality of cakes are presented in Table (5). Cake weight produced from Wheat Flour (WF) and Pp were highest compared with other samples, while cake volume produced from WF and Pj were lowest compared with other samples. This effect may be due to high fiber contents in pomegranate peel. Fiber are characterized by their high water holding capacity. From the same table, specific volume of cake produced from WF had higher values compared with that of other samples. On the other hand, the processing of cake from WF and Pj specific volume was lower compared to those of the other samples.

**Table (5): Baking quality of cakes**

Samples	Weight (g)	Volume (cm <sup>3</sup> )	Specific volume (cm <sup>3</sup> /g)
Control	395.0±7.07 <sup>a</sup>	845±7.07 <sup>a</sup>	2.14±0.01 <sup>a</sup>
15% Pp	391.0±4.24 <sup>ab</sup>	785±7.07 <sup>ab</sup>	2.01±0.01 <sup>a</sup>
20% Pp	394.0±2.82 <sup>ab</sup>	805±21.21 <sup>a</sup>	2.04±0.03 <sup>a</sup>
15% Pj	351.5±2.12 <sup>c</sup>	585±21.21 <sup>c</sup>	1.66±0.07 <sup>b</sup>
20% Pj	376.0±1.41 <sup>b</sup>	710±14.14 <sup>b</sup>	1.89±0.02 <sup>ab</sup>
10% Pp+10% Pj	385.0±7.07 <sup>ab</sup>	805±35.35 <sup>a</sup>	2.09±0.12 <sup>a</sup>

Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $P \leq 0.05$ ).

The color parameters of crust and crumb in table (6) of cake samples were evaluated using a Hunter laboratory colorimeter. The L scale ranges from 0 black to 100 white; the a scale extends from a negative value (green hue) to a positive value (red hue) and the b scale ranges from negative blue to positive yellow. Cake from WF and Pp was darker than other samples, where lightness ( $L^*$ ) and redness values ( $b^*$ ) decreased as pomegranate peel used in cake processing increased. The same trend was observed in case of yellowness ( $a^*$ ) of cake samples, where their values were getting higher in cake samples compared with cake control. This result could be attributed to the darkness of raw materials (Pp, Pj and mixed from tem) than WF so, darkness increased as a result of the presence of Pp, Pj and mixed from tem in cakes. Such findings are in-agreement with <sup>(41, 42, 43)</sup>.

**Table (6): Hunter colour parameter of crust cakes produced**

Samples	Crust color values		
	l	a	b
Control	65.68±0.77 <sup>a</sup>	11.10±0.16 <sup>c</sup>	26.91±0.44 <sup>a</sup>
Pp 15%	40.63±0.84 <sup>c</sup>	19.99±0.45 <sup>b</sup>	15.81±0.48 <sup>c</sup>
Pp 20%	31.24±0.59 <sup>f</sup>	22.78±0.89 <sup>a</sup>	13.96±0.13 <sup>f</sup>
Pj 15%	53.63±0.64 <sup>c</sup>	13.20±0.07 <sup>dc</sup>	23.23±0.11 <sup>b</sup>
Pj 20%	57.66±0.36 <sup>b</sup>	13.95±0.79 <sup>cd</sup>	20.74±0.12 <sup>c</sup>
Pp 10% + Pj 10%	49.19±1.37 <sup>d</sup>	15.73±0.72 <sup>c</sup>	18.90±0.17 <sup>d</sup>

Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $P \leq 0.05$ ).

**Table (7): Hunter colour parameter of Crumb cakes produced**

Samples	Crumb color values		
	l	a	b
Control	75.63±0.39 <sup>a</sup>	0.88±0.04 <sup>f</sup>	16.54±0.45 <sup>a</sup>
Pp 15%	49.10±0.60 <sup>e</sup>	7.97±0.10 <sup>b</sup>	7.19±0.13 <sup>d</sup>
Pp 20%	38.24±0.32 <sup>f</sup>	10.08±0.13 <sup>a</sup>	6.47±0.28 <sup>d</sup>
Pj 15%	66.79±0.59 <sup>b</sup>	1.30±0.03 <sup>e</sup>	10.21±0.13 <sup>b</sup>
Pj 20%	61.15±1.08 <sup>c</sup>	4.18±0.06 <sup>d</sup>	9.13±0.17 <sup>c</sup>
Pp 10% + Pj 10%	53.91±0.32 <sup>d</sup>	6.06±0.07 <sup>c</sup>	8.34±0.06 <sup>c</sup>

*Pp = pomegranate peel, Pj = pomegranate juice. Means in the same column with different superscript letters are statistically significant at ( $P \leq 0.05$ ).*

### Conclusion:

This study indicated that pomegranate peel and juice play an important role in controlling and treating hypercholesterolemia, the results obtained from the sensory tests indicated that, sensory evaluation showed an acceptance of supplemented food products (cake) with Pp (15% & 20%), Pj (15% & 20%) and (Pp 10% + Pj 10%), in this study to management or treatment hypercholesterolemia disease and its complications. Pomegranate juice and peel is a polyphenol-rich with high antioxidant capacity, it has a high nutritional value and beneficial effects on human health, these properties have been attributed to the phenolic fraction, which has high antioxidant and antibacterial activities, it is a good source for vitamin C, vitamin B 5, potassium and polyphenols, such as tannins and flavonoids. The study recommended adding pomegranate peels and juice to the diet of hypercholesterolemic patient.

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## تأثير قشور وعصير الرومان على الفئران المصابة بارتفاع الكوليسترول

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### الملخص العربي

تهدف الدراسة إلى إمكانية استخدام قشور وعصير الرومان في إعداد منتجات غذائية، وتأثيرها على الفئران المصابة بارتفاع الكوليسترول، وتم إعداد خمس منتجات غذائية من الكيك باستخدام مسحوق قشور وعصير الرومان بنسب 15%، 20%، وخليطهما 10% قشور رمان + 10% عصير رمان، وتم اخضاعها للتقييم الحسي. واستخدم 49 فأر من ذكور الألبينو تم تقسيمهم إلى مجموعتين المجموعة الأولى 7 فئران تم تغذيتها علي الغذاء الأساسي كمجموعة ضابطة سالبة المجموعة الثانية 42 فأر تم تغذيتها علي غذاء عالي الدهن لمدة أسبوعين لإحداث الإصابة بارتفاع الكوليسترول، وتم تقسيم المجموعة الثانية إلى ست مجموعات كل مجموعة 7 فئران، المجموعة الأولى تم تغذيتهم علي غذاء عالي الدهن كمجموعة ضابطة موجبة، المجموعة الثانية تم تغذيتهم علي غذاء عالي الدهن مع 15% قشور الرومان، المجموعة الثالثة تم تغذيتهم علي غذاء عالي الدهن مع 20% قشور الرومان، المجموعة الرابعة تم تغذيتهم علي غذاء عالي الدهن + 15% عصير رمان، المجموعة الخامسة تم تغذيتهم علي غذاء عالي الدهن + 20% عصير رمان، المجموعة السادسة تم تغذيتهم علي غذاء عالي الدهن + 10% قشور الرومان + 10% عصير الرومان لمدة 6 أسابيع وظهرت نتائج الاختبارات الحسية قبول حسي مرتفع لهذه المنتجات كما اوضحت النتائج ان تغذية الفئران المصابة بارتفاع الكوليسترول على قشور وعصير الرومان مقارنة بالمجموعة الضابطة الموجبة ادت إلي انخفاض في الوزن المكتسب، كفاءة الغذاء، TC، TG، LDL، انزيمات الكبد ALT,AST and ALP وزيادة المآخوذ من الغذاء والبروتينات الدهنية عالية الكثافة وأظهر الفحص الهستوبولوجي وجود تغيرات دهنية في خلايا كبد فئران المجموعة الضابطة الموجبة وحدوث تحسن لخلايا الكبد الدهني للمجموعة التي تغذت علي 20% قشور رمان وخليط من 10% قشور + 10% عصير.. وتوصي الدراسة بإضافة قشور وعصير الرومان الي النظام الغذائي للأفراد المصابين بارتفاع الكوليسترول

الكلمات المفتاحية: الكوليسترول، دهون الدم، انزيمات الكبد، التقييم الحسي، الكيك