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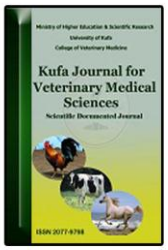

Effect of Powder of Pomegranate (*Punica granatum*) Peels on Lipid Profile in Hypercholesterolemic Rats

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Abstract:

A present study was carried out to investigate the effects of Pomegranate Peel Powder (PPP) on the lipid profile and body weight gain ratio on hypercholestrolemic male rats. PPP was added to hypercholesterolemic diet by (10%, 15% and 20%) as dietary fiber supplement. Forty adult male albino rats were randomly divided into five groups (8/group). First group (A) kept as control negative, second group (B) control positive given hypercholesterolemic diet, while group (C, D and E) Given hypercholestrolemic diet plus (10%, 15% and 20%) of PPP respectively. Food consumption was recorded daily. Blood samples collected at the end of the experiment (28 days) for measuring the following parameters: serum total cholesterol, triglycerides, HDL, LDL and VLDL. At the end of the experiment animals were scarified to measuring the kidney to body weight ratio, liver to body weight ratio. Results indicated that all (B, C, D and E) groups showed significant ($P<0.05$) changes in all tested lipids profile comparing with group A. On the other hand, the groups (C, D and E) revealed significant ($P<0.05$) decrease in all tested lipids profile comparing with group B except HDL in group E. Furthermore, all (C, D and E) groups had significant ($P<0.05$) decrease in body weight gain ratio and food consumption when compared with group B. Moreover, kidney and liver weights to body weight ratio had highly significant ($P<0.05$) decrease for all (C, D and E) groups comparing with group B.

Key words: Pomegranate**,** Pomegranate Peel Powder**,** lipid profile**,** HDL, LDL and VLDL.

تأثير مسحوق لب الرمان (*Punica granatum*) على معايير الدهون في الجرذان المعاملة بالكولسترول

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الخلاصة:

صممت هذه الدراسة لمعرفة تاثير مسحوق لب الرمان على معايير الدهون ونسبة زيادة وزن الجسم في ذكور الجرذان المعاملة بالكولسترول تم اضافة مسحوق لب الرمان بواقع (10%، 15% و20%) الى الغذاء العالي الكولسترول كمحسن غذائي ليفي. تم استخدام اربعون من ذكور الجرذان البيضاء البالغة حيث قسمت عشوائياً الى خمسة مجاميع (8/مجموعة). المجموعة الاولى (أ) اعتبرت مجموعة سيطرة سلبية، المجموعة الثانية (ب) مجموعة السيطرة الايجابية حيث اعطيت غذاء عالي الكولسترول، بينما المجموعات (ج، د، ه) اعطيت غذاء عالي الكولسترول اضافة الى (10%، 15% و20%) من مسحوق لب الرمان على التوالي. تم تسجيل معدل استهلاك الغذاء يومياً. جمعت عينات الدم في نهاية التجربة (28 يوم)

وذالك لقياس المعايير التالية: الكولسترول الكلي في مصل الدم، الدهون الثلاثية، البروتينات الدهنية عالية الكثافة، البروتينات الدهنية واطئة الكثافة، البروتينات الدهنية الواطئة الكثافة جداً. تم في نهاية فترة التجربة تم قتل الحيوانات لقياس نسبة وزن الكلية الى وزن الجسم، ونسبة وزن الكبد الى وزن الجسم. بينت النتائج بأن المجاميع (ب، ج، د، ه) اظهرت تغيرات معنوية ($P<0.05$) في كل معايير الدهون المقاسة بالمقارنة مع المجموعة (أ). من جانب اخر، اظهرت نتائج المجاميع (ج، د، ه) حصول انخفاض معنوي ($P<0.05$) في كل معايير الدهون المقاسة بالمقارنة مع مجموعة (ب) عدا البروتينات الدهنية عالية الكثافة في المجموعة (ه). بالاضافة الى ان كل المجاميع (ج، د، ه) اظهرت انخفاض معنوي ($P<0.05$) في نسبة زيادة وزن الجسم و معدل استهلاك الغذاء بالمقارنة مع المجموعة (ب). علاوة على ذلك، ان نسبة وزن الكلية والكبد الى وزن الجسم اضهرت انخفاض معنوي($P<0.05$) في كل من مجموعة (ج، د، ه) بالمقارنة مع مجموعة (ب).

الكلمات المفتاحية: الرمان، مسحوق لب الرمان، معايير الدهون، HDL ، LDL ، VLDL

Introduction:

It is said that the fruit shared by Adam and Eve in the Garden of Eden was the pomegranate, not an apple. King Tut was buried with pomegranates he believed that he would have a happy rebirth. The pomegranate was favorite fruit of the prophet Mohammed. The goddess Persephone's taste for pomegranate led her to spend half of each year in Hades and half in the light of the world. Pomegranates are symbols of sexuality, rebirth and abundance (1).

Pomegranate (*Punica granatum* L.) fruits are widely consumed fresh and in beverage forms as juice and wines (2). Commercial pomegranate juice shows potent antioxidant and anti-atherosclerotic properties attributed to its high content of polyphenols (3). Pomegranate juice is an important source of phenolic compounds (4). Phenolic compounds are important components of many fruits, vegetables, and beverages contributing to their color and sensory properties, epidemiological studies have demonstrated that the composition of phenol-rich food retards the progression of arteriosclerosis and reduces the incidence of heart diseases by preventing the oxidative stress, that is, lipid peroxidation in arterial macrophages and in lipoproteins (5, 6). Pomegranate juice has been demonstrated to be high in antioxidant activity and is effective in the prevention of atherosclerosis (7). Major risk factors for atherosclerosis include high plasma LDL concentration and LDL modifications such as its retention, oxidation and aggregation (8, 9, 10). Oxidative modification of LDL is thought to play a key role during early atherogenesis. Oxidized LDL is taken up by macrophages at an enhanced rate via their scavenger receptors (11), leading to the formation of lipid-laden foam cells, the hallmark of the early atherosclerosis (12).

There is little information about the effect of Pomegranate Peel Powder on lipid profile. Therefore, this experiment designed to demonstrate this effect on the hypercholestrolemic rats.

Materials and Methods:

Pomegranate fruit was brought from my garden which is lies in Al-Mukdhadia-Diyala-Iraq. The middle part of pomegranate between seed and husk were collected, dried and grinded with electrical grinder. Cholesterol was obtained from local market.

Animals: Forty albino Wister male rats were used, the age of these animals ranged between (10-12) weeks and weight was about (180-220) gm. Rats were kept under suitable environment condition and maintained in temperature and humidity control with 12 h light/dark cycle daily and housed in plastic cage 50*35*15 cm (length, width and height respectively), the food was given as pellets of freshly prepared ration, all rats were allowed to free access of drinking water and basal diet. The animals were kept for seven days for adaptation before beginning the experiment.

Experimental design: Rats divided randomly in to five groups (8) rats for each group and handled as follows: (I) Control negative (group A): Animals of this group fed on basal diet (pellet) and ordinary tap water. (II) Control positive (group B): Animals of this group fed on hypercholesterolenmic diet composed of pellet + 1% cholesterol + 10% saturated fat according to (13). (III) Group C: Rats fed on hypercholestrlemic diet + Pomegranate Peel Powder 10%. (VI) Group D: Rats fed on hypercholesterolemic diet + Pomegranate Peel Powder 15%. (V) Group E: Rats fed on hypercholesterolemic diet + Pomegranate Peel Powder 20%.

Food consumption was recorded every day and body weight was recorded weekly. At the end of the experiment (28 days) fasting blood samples were collected via cardiac puncture after anesthetized animals by (ketamine 90mg/kg B.W. and xylazine 40mg/kg B.W.). Blood samples were centrifuged at (3000 rpm). Organs was removed then washed in saline and weighted after dried with filter paper.

The estimation of serum total cholesterol, triglycerides, high density lipoprotein HDL, low density lipoprotein LDL and very low density lipoprotein VLDL was performed by using biochemistry analyzer (Nuve, Japan).

Statistical analysis of data was performed on the basis of variance (ANOVA) Group difference were determined using less significant difference (LSD) test at $P<0.05$ (14).

Result and Discussion:

Table I: Effect of (PPP) on lipids profile (Total serum cholesterol, HDL, LDL, VLDL, Triglycerides) in hypercholesterolemic male rats.

No.	Groups	Total choles-terol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Triglycerides (mg/dl)
1-	Group A	70.26 ± 3.03 **c**	32.36 ± 2.87**c**	21.92 ± 1.92**c**	15.98 ± 0.64**c**	64.21 ± 4.04 **c**
2-	Group B	146.05 ± 3.91**a**	40.60 ± 2.55**a**	84.34 ± 2.31**a**	21.11 ± 0.84**a**	131.24 ± 4.33**a**
3-	Group C	89.54 ± 4.12 **b**	35.89 ± 2.16**b**	40.75 ± 1.87**b**	12.90 ± 0.95**b**	70.03 ± 3.97 **b**
4-	Group D	92.03 ± 3.64 **b**	35.87 ± 1.97**b**	44.25 ± 2.04**b**	11.91 ± 0.51**b**	68.81 ± 4.20 **b**
5-	Group E	95.32 ± 3.37 **b**	39.64 ± 2.73**b**	44.72 ± 2.22**b**	10.96 ± 0.92**b**	68.27 ± 4.17 **b**

-LSD of (total cholesterol = 6.77, HDL = 2.28, LDL = 4.01, VLDL = 2.52, triglycerides = 3.61).

- Values are presented as means ± SE (n= 8 rat/ group), different small letter denote significant differences among groups ($P<0.05$).

-Group A = control negative, group B = control positive given hypercholesterolemic diet, Group C: Given hypercholestrolemic diet plus 10% of PPP, Group D: Given hypercholestrolemic diet plus 15% of PPP, Group E: Given hypercholestrolemic diet plus 20% of PPP.

When we looking for the data deeply in the (table :I) it could be observed that the hypercholesterolemic rats of (B, C, D and E) groups showed significant (P<0.05) increase in total cholesterol, triglycerides, high density lipoprotein HDL, low density lipoprotein LDL and very low density lipoprotein comparing with rats of control negative group (group A). As well as, all tested lipid parameters (except HDL of group E) had highly significant (P<0.05) decrease for all hypercholesterolemic rats administrated with different levels of Pomegranate Peel Powder PPP (10, 15 and 20%) comparing with control positive group (group B). Pomegranates are a rich source of polyphenols and other antioxidants (15), pomegranate peel extract appeared to have more potential as a health supplement rich in natural antioxidants than pulp extract so the effects of PPP on the lipid profile may be attributed to its antioxidant activity (7). The result came compatible with (16) who reported that consumption of concentrated pomegranate juice for type ll diabetic patient with hyperlipidemia caused significant reduction were seen in serum total cholesterol and low density lipoprotein LDL and had slight changes in serum HDL. On the other hand, dietary supplementation with nutrients rich in polyphenols and antioxidants is associated with inhibition of atherogenic modifications to LDL and atherosclerosis (17). Furthermore, polyphenols of pomegranate may accelerate and promote cholesterol metabolism by reversing cholesterol transport via HDL (18, 19).

Perhaps the greatest mechanism of PPP (which include ellagic acid that combating cholesterol) is associated with an enzyme known as paraoxonase (PON), paraoxonase is an HDL-associated enzyme whose activity is related to cholesterol and atherosclerosis, decreased activity of PON is associated with increase cholesterol and increase risk for atherosclerosis, hypocholesterolemic properties of PON may be related to its ability to protect against lipid peroxidation (17, 20).

Polyphenolic compounds was showed to improve the serum lipid profile this is might be due to it posses a powerful antioxidant activity and it is able to reduce lipid peroxidation, ellagic acid, tannic acid and other polyphenolic compounds can remove free radicals and can prevent lipid peroxidation (21, 22).

Table II: Effect of (PPP) on food consumption, body weight gain ratio, kidney to body weight and liver to body weight in hypercholesterolemic male rats.

No.	Groups	Food consumption (gm/ day)	Body weight gain ratio	Kidney to body weight ratio	Liver to body weight ratio
1-	Group A	27.17 ± 2.21 **c**	31.20 ± 2.93 **c**	1.12 ± 0.14 **b**	4.22 ± 0.41 **b**
2-	Group B	36.16 ± 2.33 **a**	65.49 ± 2.81 **a**	1.63 ± 0.09 **a**	5.91 ± 0.37 **a**
3-	Group C	29.93 ± 1.85 **b**	43.37 ± 2.77 **b**	1.09 ± 0.10 **b**	4.43 ± 0.51 **b**
4-	Group D	30.66 ± 2.70 **b**	30.94 ± 2.63 **c**	1.12 ± 0.12 **b**	4.26 ± 0.40 **b**
5-	Group E	30.48 ± 1.97 **b**	30.44 ± 2.86 **c**	1.07 ± 0.14 **b**	4.41 ± 0.28 **b**

-LSD of (Food consumption = 2.1, Body weight gain ratio = 3.63, Kidney to body weight ratio = 0.21, Liver to body weight ratio = 1.06).

- Values are presented as means ± SE (n= 8 rat/ group), different small letter denote significant differences among groups (P<0.05).

-Group A = control negative, group B = control positive given hypercholesterolemic diet, Group C: Given hypercholestrolemic diet plus 10% of PPP, Group D: Given

hypercholestrolemic diet plus 15% of PPP, Group E: Given hypercholestrolemic diet plus 20% of PPP.

From the data in (Table: II) it could be observed that the result in control positive group (group B) showed significant ($P<0.05$) increase in food consumption and body weight gain ratio, kidney and liver weight to body weight ratio comparing with control negative group (group A). Moreover, all PPP administrated rats (10, 15 and 20) % had significant ($P<0.05$) decrease in food consumption and body weight gain ratio, liver and kidney weight to body weight ratio comparing with control positive group (group B). The increased fiber intake might de associated with improves body weight and reduction in other cardiovascular risk factors (23). The enhancement of body weight may be due to the biological function of pomegranate polyphenols including ellagic and tannic acids which increases total serum protein and protein synthesis in the body (24).

The liver of different groups support the protective effects probably exhibited by the extract of pomegranate peel (25). On the other hand, pomegranate polyphenols are apotent antioxidant that has antioxidative activity (15), so it might inhibit the protein catabolism by the combating of free radicals. Moreover, PPP has an important role in improvement of body immunity by increasing total protein, immunoglobulins and improves body health (26).

We can be concluded that Pomegranate Peel Powder is capable to improve lipid profile and it is able reduce the probability of incidence of atherosclerosis in hypercholesterolemic rats .The potent antioxidative capacity of PPP may be the central link for the antiatherogenic effects on lipoproteins. Moreover, it suggested that consumption of PPP may modify the risk of hypercholesterolemia and it have more potential as a health supplement rich in natural antioxidants.

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