# EVALUATION OF BEAN SEEDS AND POMEGRANATE FRUIT FRACTIONS AS HYPOCHOLESTEROLEMIC AND HPOLIPIDEMIC AGENTS IN ALBINO RATS

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## ABSTRACT

The present study was undertaken to evaluate the influences of soaked white bean seeds and pomegranate fractions (seeds and peels) on lipid profiles of rats suffering from hyperlipidemia and hypercholesterolemia. The investigation was based on that legume and fruits as good hypolipidemic and hypocholesterolemic agents which contain protein and appreciable amount of phenols that are known to possess lipotropic and antioxidant characters. The lipotropic and antioxidant power of protein and phenols are supposed to protect blood lipid fractions from oxidation and precipitations on the walls of the blood vessel thus alleviated atherosclerosis.

The study was done on soaked bean seeds, pomegranate seeds and dried pomegranate peels and their mixture to evaluate them as hypolipidemic agents by rats feeding to clarify whether the effect is attributed to whole dried seeds and peels. The three present hypolipidemic agents individually and their mixture (1:1:1) amounted 10% of the basal diet. Chemical composition of dried soaked bean and pomegranate (seeds and peels) was determined on dry matter which amounted good values of protein, lipids, crude fiber and phenols. Also, HPLC analysis of pomegranate seeds and peels for the phenols content showed the present of about 16 compounds varying in amount between them. It was noticed that compounds such as catechin and phenol are dominating and daidzin, genistein, cinnamic acid, kaempferol, eugenol, chrysin and galangin were detected in the both

pomegranate fractions.

The present study showed that the treatments with bean seeds, pomegranate (seeds or peels) and specially their mixture can effect as hypolipidemic and hypocholesterolemic agents and improved the fractions of liver and kidneys as well as lipid peroxidation and the activity of antioxidative enzymes (SOD and catalase) but these treatments had insignificant effects on blood glucose of the hyperlipidemic and hypolipidemic rats. The obtained results showed a good hypolipidemic powerful for soaked bean seeds and pomegranate (seeds and peels) as well as their mixture. Bean seeds diet produced a general improvement in the clinical status of blood lipid profile (total lipids, triglycerides, cholesterol, HDL-c, LDL-c and VLDL-c), liver function (GOT, GPT and bilirubin), kidneys function (uric acid, urea and creatinine) and blood lipid peroxidation and antioxidant enzymes (SOD and catalase) by which hyperlipidemia and hypercholesterolemia were reduced. The mixture diet had the best influence concerning biological studies than the other treatments which used bean seeds and pomegranate (seeds or peels) alone. From the present results the anticholesterolemic and antilipidemic power of the different diet treatments can be arranged in the following from increasing to decreasing order:

Mixture ≥ Soaked bean seeds > Pomegranate seeds ≥ Pomegranate peels

Future studies are required to evaluate the interaction between lipotropic factor (protein) and antioxidative agent (phenols) which produced such synergetic effects.

**Key words:** pomegranate (*Punica granatum*) seeds or peels - white bean (*Phaseolus vulgaris* L.) - hypolipidemia - hypocholesterolemia

## INTRODUCTION

Lipid fractions are widely distributed in all cells of the body, but particularly in nervous tissue. They serve major functions in the mammalian body. It is required for structural component of the plasma membrane of all cells and precursors for several factors of animal cells (hormones, bile salts and others). Hyperlipimia and atherosclerosis induced by dietary fatty diet with cholesterol has been reported in several studies in animals (Lovejoy *et al.*, 2002).

Coronary atherosclerosis in primates not only resembles human lesions but can even produce myocardial infarction (McBride *et al.*, 1998). From the clinical view point, there is a strong relationship between plasma lipid levels and atherosclerosis, particularly of the coronary arteries that is major importance. Atherosclerosis is spectrum of arterial reaction that may result from many factors acting upon the vessel wall and producing their effect through different mechanisms in different subjects which may vary significantly from one patient to another. Among the health hazards is the deterioration in lipid parameters in the body which leads to incidence of atherosclerosis and cardiovascular diseases. According to the World Health Reports (WHPs, 2002), more than 75% of cardiovascular diseases, the world's leading cause of premature death, result from high blood cholesterol and other factors such as smoking, drinking alcohol and high blood pressure. For that, the studies need to look for natural compounds they can help the patient to get rid or reduce the health hazards of the disease as a care that they receiving (Turnbull, 2005). Phenols are one of the major group's of non essential dietary compounds appearing in fruits and vegetables food. Phenol have traditionally been considered as antinutritive compounds due to the adverse effect of one of their main compounds, tannins, on protein digestibility. However, actually there is an increased interest in these compounds because they have been associated with the inhibition of atherosclerosis and cancer. The bioactivity of phenolic compounds may be related to their antioxidant behavior which is attributed to their ability to chelate metals, inhibit lipoxygenase an scavenge free radical (Martínez-Valverde *et al*., 2000 and Scalbert *et al.*, 2005). Oxidative stress produces a modification of DNA, protein and lipids by reactive oxygen species (ROS) and other free radicals which played a role in aging and diseases, including hyperlipimia, atherosclerosis and cardiovascular diseases. Legume seeds utilized for human consumption has been of great interest to nutritionists and agricultural scientists in providing mankind with nutritional diets particularly when protein deficits occur. Bean seeds are not only a good choice for nutrition but recently, the potential role of it in hyperlipidimia has received attention. Several compounds with hyperlipidimic or hypercholesterolemic activity are found in relatively high concentrations in bean seeds.

The present studies were undertaken to investigate the effect of pomegranate seeds and peels as well as bean seeds on their biological

and nutritional evaluation through an animal experiment as following studies:

1- Chemical composition of pomegranate seeds and peels as well as raw bean.
2- The total phenolic levels and their constituent compounds of the pomegranate seeds and peels through analysis by HPLC technique.
3- The biological treatments of pomegranate seeds and peels as well as white bean seeds as hypolipidimic agents on blood analysis, i.e., glucose, lipid profile, liver function, kidneys function, lipid peroxidation and enzymatic antioxidant [superoxide dismutase (SOD) and catalase activity).

## MATERIALS AND METHODS

**Preparation of samples:**

Samples of the present study were purchased from the local market. White bean (*Phaseolus vulgaris* L.) was soaked in tap water (1:5 w/v) for 12 hr at room temperature. The soaked bean seeds were dried in a hot air oven maintained at 50$^{o}$C according to the method of Jood *et al.* (1988). For pomegranate (*Punica granatum*) the samples were cut and edible parts were separated from the peel, each of two parts (seeds and peels) were dried in an air oven at 50$^{o}$C till complete dryness, weighted to calculate its moisture content. The samples were ground to fine powder their analysis and used in animal experiments.

**General chemical analysis:**

The determination of moisture, crude protein, total lipids, ash and crude fibers were done, nitrogen free extract was calculated by difference, deducing the percentage of ash, crude protein, total lipids and crude fibers from 100 according to A.O.A.C. (2000).

**Determination of total phenols:**

The determination of total phenols was done according to the procedure described in the A.O.A.C. (2000) as tannic acid.

**HPLC analysis of pomegranate phenolic compounds:**

Phenolic compounds of pomegranate samples were extracted according to the method described by Duke *et al.* (2003) in which a known weight of dried samples was extracted by methanol. Each of

phenolic compounds was identified and performed on JASCO HPLC using hypersil $C_{18}$ reversed phase column (250 x 4.6 mm) with 5 µ particle size. Injection by means of Rheodyne injection value with 50 PJ fixed loop was used. A constant flow rate of 1 ml/min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at PH 2.65; and solvent (B) 0.5% acetic acid in pure (99.5%) acetonitrile, the elution gradient was linear starting with (A) and ending with (B) over 35 min, using an UV detector set at wavelength 254 nm. Phenolic compounds of the both samples were identified by comparing their retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements and then converted.

**Biological effects of investigated plants:**

**Experimental animals:**

The Spraque-Dawley albino male rats weighing 65-75g were used for the present study. The animals were obtained from Agriculture Research Centre (A.R.C), Giza, Egypt. The animals were raised in the animal house. The rats were kept under normal laboratory conditions (temperature remain 25± $2^0$C) for 48 hr before the initiation of experiment. During this period, the animals were allowed free access of water and basal diet. Food consumption and body weight were monitored daily for each animal.

**Animal diet:**

The control diet is composed of as reported by Lane-Peter and Pearson (1971) 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture (Schneeman *et al.*, 1989), 1% vitamins mixture (Philip *et al.*, 1993) and starch 65%. On the other hands, high fat diet was similar to the control diet but differ in fat content which was 20% sheep fat, 2% cholesterol and 0.25% bile salts and starch 42.75%.

**Experimental design:**

After a period of adaptation (48 hr), 48 adult rats weighing between 65-75g were divided into two groups:

**Group (1): (Healthy control group)**

Rats were given normal diet (8 rats) as health control (**group 1)**.

**Group (II): (Hyperlipidemic group)**

Rats were given high fat with cholesterol diet for 4 weeks (40 animals). At the end of the feeding period blood samples were taken from the suborbital vein to test for blood cholesterol level. A high level of serum cholesterol was considered as an indication to hypercholesterolemia. The hyperlipidimic rats of the second group were subdivided into 5 groups (8 rats/group):

**Subgroup 1:** Rats were fed on high fat/high cholesterol diet without any treatment (**Group 2:** hyperlipidemic control).

**Subgroup 2:** Rats were fed on high fat/high cholesterol diet with 10% beans (**Group 3:** bean group).

**Subgroup 3:** Rats were fed on high fat/high cholesterol diet with 10% dried pomegranate seeds (**Group 4:** pomegranate seeds group).

**Subgroup 4:** Rats were fed on high fat/high cholesterol diet with 10% dried pomegranate peels (**Group 5:** pomegranate peels group).

**Subgroup 5:** Rats were fed on high fat/high cholesterol diet with 10% mixture of bean, dried pomegranate seeds and dried pomegranate peels by ratio of 1:1:1 (**Group 6:** mixture group).

At the end of 10 weeks interval, rats were fasted overnight and then the animals were killed by decapitation and blood samples were collected from each rat and subjected to centrifugation tube at 3000 rpm to obtain the plasma which was kept in the deep freezer for the subsequent investigation.

**Blood biochemical analysis:**

**Determination of plasma glucose:**

Enzymatic determination of plasma glucose was carried out colorimetrically according to the method of Trinder (1969).

**Liver function:**

For liver function GOT (AST) and GPT (ALT) activities were determined colorimetrically according to the method of Reitman and Frankel (1957), also plasma total bilirubin were determined according to the method described by Tietz (1995).

**Kidneys function:**

For kidneys function uric acid and urea contents in plasma were determined colorimetrically according to the methods described by Caraway (1975) and the determination of plasma creatinine content was carried out colorimetrically according to the methods described by Faulkner and King (1976).

**Plasma lipid and lipoprotein profile:**

For plasma lipid profile, total lipids, total triglycerides and total cholesterol levels were determined colorimetrically according to the methods of Knight *et al.* (1972), Fossati and Prencipe (1982) and Allain *et al.* (1974) respectively. But for lipoprotein profile in plasma, HDL-cholesterol and LDL-cholesterol levels were determined according to Warnick *et al.* (1983) and Bergmenyer (1985) respectively. VLDL-cholesterol was calculated by using the equation which was described by Fiedewald *et al.* (1972).

**Lipid peroxidation:**

Lipid peroxide was determined according to the method of Ohkawa *et al*. (1979), Also catalase activity was determined according to the method Aebi (1984). The activity of superoxide dismutase (SOD) was determined according to the method described by Nishikimi *et al.* (1972).

**Statistical analysis:**

All data pooled through this study were proceeded by General Linear Model procedures (GLM) of the statistical analysis system described in SAS User's Guide (SAS Institute, 2000). The significance of the differences among treatment groups were tested using Waller-Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P<0.05$.

## RESULTS AND DISCUSSION

**Results**

White beans and pomegranate considered from famous legume and fruit in Egypt. The chemical composition of bean seeds as well as dried pomegranate seeds and peels are shown in table (1). It could be observed that beans and pomegranate (seeds and peels) are rich in protein, dietary fiber, elements and carbohydrates, but beans contain

low amount of total phenols than dried pomegranate which observed 6-11 fold of total phenols. Recently, a special interest was given to legumes protein and fruits antioxidants as good antilipidimic and anticholesterolemic agents. For that the present studies analyzed the phenolic compounds of pomegranate methanol extract either for seeds or peels. The HPLC analysis of the both fractions (seeds and peels) of pomegranate for their phenolic compounds (table 2) showed the presence 12 compounds and 13 compounds in peels extract varying in amount between the both fractions. Total known compounds of pomegranate seeds were 90.65% while, total unknown compounds were 9.35%, also total known compounds of pomegranate peels were 93.42% while, total unknown compounds were 6.58%.

**Table (1): Chemical composition of the beans and pomegranate (seed and peels)** as **(g/100g) dry weight basis**

| Samples | Moisture | Total Proteins | Total Lipids | Ash | Crude fiber | Nitrogen free extract | Total phenols |
|---|---|---|---|---|---|---|---|
| **Raw beans** | 8.09 ± 1.01 | 25.01 ± 2.22 | 1.08 ± 0.05 | 5.33 ± 0.16 | 7.01 ± 0.66 | 53.48 ± 4.12 | 0.11 ± 0.01 |
| **Pomegranate seeds** | 8.57 ± 1.02 | 13.15 ± 0.52 | 3.55 ± 0.21 | 9.72 ± 0.67 | 10.01 ± 0.91 | 55.00 ± 2.74 | 0.62 ± 0.03 |
| **Pomegranate peels** | 13.93 ± 1.00 | 14.00 ± 0.61 | 3.02 ± 0.22 | 15.01 ± 0.72 | 12.17 ± 0.92 | 41.87 ± 2.11 | 1.261 ± 0.03 |

* Moisture of fresh pomegranate (seeds and peels) were 79.00±3.21 and 77.00±3.0 respectively.

It was noticed that compounds such as catechin, phenol, daidzin, genistein, cinnamic acid, kaempferol, eugenol, chrysin and galangin are detected in seeds and peels pomegranate extracts. On the other hand more than those phenolic compounds salicylic acid, daidzein and pinostrobin were detected in seeds extract, but in peels caffiec acid, ferulic, p-coumaric acid and quercetin were identified. Catechin is dominating which amounted about 72% of total phenolic compounds and phenol amounted about 20% or more for seeds and peels methanolic extracts respectively. The both fraction of pomegranate showed a good value when total phenol was determined as tannic acid. The studies are considered to be a treatment investigation which were done on male albino rats weighing 70±5g. Results of the evaluation of three studied samples as hypocholesterolemic and hypolipidemic

agents in albino rats were statistically analyzed and summarized in table (3) which presented the lipid and lipoprotein profiles of six groups at the end of the experimental period (10 weeks). The data pointed out a significant increase in total lipids, cholesterol and triglycerides when rats fed on the high fat/cholesterol diet, the values amounted 2.19, 2.80 and 2.49 fold respectively relative to that of normal control. The results presented in table (3) show the effect of administration of 10% beans and pomegranate (seeds and peels) as well as their mixture on lipid profile of hyperlipidemic animals. In case of hypolipidemic animals, the feeding on different treatments (beans and pomegranate, seeds and peels, as well as their mixture) exhibited different effects on the blood lipid profiles and four treatments significantly alleviated the harmful of hypolipidemia where the mixture and beans treatments were the most effective as hypolipidemic and hypocholesterolemic agents than that of pomegranate seeds and peels alone. On the other hands, the mixture of seeds and peels pomegranate and beans treatment possessed remarkable hypolipidemic and hypocholesterolemic activity but the levels of total lipids, cholesterol and triglycerides in blood were still higher than that of the control. The feeding on pomegranate seeds and peels produced the same alleviated effect on hyperlipidemia and hypercholesterolemia. The results are in agreement of those data of blood lipoproteins content of the present study (table 3) except the HDL-cholesterol (HDL-c). The effects of pomegranate (seeds and peels) and beans as well as their mixture treatments on hyperlipidemic animals was also different. Hyperlipidemia increased blood HDL-c, LDL-c and VLDL-c contents, but the increase of HDL-c was lower than the increases of LDL-c and VLDL-c. This drastic effect of hyperlipidimia was improved by the present treatments whereas, more increases in HDL-c content of blood of hyperlipidemic animals observed after treatments by feeding on beans, pomegranate (seeds and peels) and their mixture. In contrast, LDL-c and VLDL-c contents in blood of hyperlipidemic rats were significantly alleviated by the present treatments which were reduced but the levels were still higher than that of control animals. From the present results the hypolipidemic and hypocholesterolemic effects of the different treatments can be arranged in the following increasing order:

Mixture ≥ Soaked bean seeds > Pomegranate seeds ≥ Pomegranate peels

**Table (2): HPLC analysis of phenolic compounds in methanolic extract of the pomegranate seeds and peels**

| No. | Components | pomegranate seeds | | pomegranate peels | |
|---|---|---|---|---|---|
| | | mg/100g | % | mg/100g | % |
| 1 | Catechin | 406 | 71.99 | 850 | 71.73 |
| 2 | Caffiec acid | - | - | 9 | 0.76 |
| 3 | Phenol | 109 | 19 | 274 | 23.12 |
| 4 | Daidzin | 4 | 0.71 | 5 | 0.42 |
| 5 | Salicylic acid | 14 | 2.48 | - | - |
| 6 | Ferulic | - | - | 5 | 0.42 |
| 7 | p-Coumaric acid | - | - | 14 | 1.18 |
| 8 | Daidzein | 1 | 0.18 | - | - |
| 9 | Quercetin | - | - | 5 | 0.42 |
| 10 | Genistein | 8 | 1.42 | 5 | 0.42 |
| 11 | Cinnamic acid | 4 | 0.71 | 2 | 0.17 |
| 12 | Kaempferol | 3 | 0.53 | 3 | 0.25 |
| 13 | Eugenol | 4 | 0.71 | 2 | 0.17 |
| 14 | Chrysin | 1 | 0.18 | 2 | 0.17 |
| 15 | Galangin | 1 | 0.18 | 2 | 0.17 |
| 16 | Pinostrobin | 7 | 1.24 | - | - |

**Table (3): Effect of administration of different samples to rats on serum lipid fractions and lipoprotein profile**

| Treatment | mg/dL | | | | | |
|---|---|---|---|---|---|---|
| | Total lipid | Triglycerides | Total cholesterol | HDL-cholesterol | LDL-cholesterol | VLDL-cholesterol |
| Group 1 Normal control | 270.00 [a] ± 21.0 | 74.00 [a] ± 4.21 | 65.00 [a] ± 2.14 | 34.00 [a] ± 2.00 | 26.00 [a] ± 1.72 | 15.00 [a] ± 0.99 |
| Group 2 Hyperlipidimic control | 590.00 [b] ± 39.00 | 183.00 [b] ± 8.92 | 182.00 [b] ± 7.23 | 44.00 [b] ± 2.74 | 86.00 [b] ± 3.42 | 37.00 [b] ± 2.00 |
| Group 3 Raw beans | 310.00 [a] ± 20.00 | 81.00 [a] ± 5.24 | 80.00 [c] ± 3.23 | 46.00 [c] ± 3.11 | 28.00 [a] ± 1.83 | 16.00 [a] ± 1.00 |
| Group 4 Pomegranate seed | 351.00 [c] ± 19.00 | 98.00 [c] ± 4.49 | 87.00 [c] ± 4.17 | 49.00 [b] ± 2.79 | 31.00 [c] ± 1.42 | 20.00 [c] ± 1.21 |
| Group 5 Pomegranate peels | 349.00 [c] ± 21.00 | 94.00 [c] ± 5.31 | 86.00 [c] ± 3.92 | 45.00 [b] ± 3.01 | 32.00 [c] ± 2.00 | 19.00 [c] ± 1.02 |
| Group 6 Mixture | 300.00 [a] ± 21.00 | 78.00 [a] ± 4.11 | 86.00 [c] ± 4.11 | 47.00 [cb] ± 2.42 | 27.00 [a] ± 1.46 | 16.00 [a] ± 0.99 |

- Each value represents the mean of 8 rats (Mean ± SE).
- Means in the same column followed by the same letter are not significantly different at ($P<0.05$).

The effects of the present antilipidemic agents on liver and kidney functions on hyperlipidemic rats were statistically analyzed and illustrated in table (4). The results showed that hyperlipidemia and hypercholesterolemia significantly stimulated GOT and GPT activity as well as the plasma content of bilirubin. The same trend was observed either for GOT or GPT relative to healthy control. These stimulations of GPT activity indicated slight liver cell necrosis and the magnitude of increase correlated with the extent necrosis (Murray *et al.*, 2006). The all treatments with beans and pomegranate (seeds and peels) as well as their mixture (as hypolipidemic agents) into diseased animals were characterized by an alleviation and normalization in the both transaminases activity (GOT and GPT) and bilirubin content of plasma. These are conflicting report on the change in the blood changes. Alterations in transaminases activity and bilirubin content in plasma have been thought to be significant in the pathogenesis of

lipidemia and cholesterolemia. The increases in plasma bilirubin and stimulation in transaminases activity is unlikely to be due to damage in liver and RBCs (Chatterjea and Shinde, 2002).

**Table (4): Effect of administration of different samples to rats on liver and kidney functions**

| Treatment | U/L | | mg/dL | | | |
|---|---|---|---|---|---|---|
| | GOT | GPT | Bilirubin | Urea | Uric acid | Creatinine |
| **Group 1 Normal control** | 141.00 [a] ± 8.64 | 42.00 [a] ± 3.12 | 0.42 [a] ± 0.03 | 57.00 [a] ± 3.15 | 3.55 [a] ± 0.20 | 0.66 [a] ± 0.04 |
| **Group 2 Hyperlipidimic control** | 176.00 [b] ± 9.99 | 55.00 [b] ± 3.74 | 0.71 [b] ± 0.04 | 70.00 [b] ± 4.54 | 5.01 [b] ± 0.35 | 0.90 [b] ± 0.05 |
| **Group 3 Raw beans** | 144.00 [a] ± 7.86 | 46.00 [a] ± 3.00 | 0.54 [c] ± 0.03 | 60.00 [a] ± 2.99 | 4.44 [c] ± 0.30 | 0.69 [a] ± 0.04 |
| **Group 4 Pomegranate seed** | 153.00 [a] ± 8.42 | 45.00 [a] ± 2.79 | 0.60 [d] ± 0.02 | 62.00 [a] ± 3.16 | 4.30 [c] ± 0.31 | 0.78 [c] ± 0.04 |
| **Group 5 Pomegranate peels** | 157.00 [a] ± 7.94 | 46.00 [a] ± 3.24 | 0.53 [d] ± 0.03 | 61.00 [a] ± 3.00 | 4.25 [c] ± 0.29 | 0.77 [c] ± 0.05 |
| **Group 6 Mixture** | 143.00 [a] ± 7.11 | 44.00 [a] ± 2.77 | 0.50 [c] ± 0.02 | 61.00 [a] ± 2.78 | 4.01 [ac] ± 0.31 | 0.75 [c] ± 0.05 |

- Each value represents the mean of 8 rats (Mean ± SE).
- Means in the same column followed by the same letter are not significantly different at ($P<0.05$).

The treatment with the present antilipidemic agents was characterized by normalization in both transaminases activity and bilirubin content of plasma in diseased animals. The effects of present treatments on kidneys function of hyperlipidemic animals was done by determination of blood uric acid, urea and creatinine which were statistically analyzed. The data in table (4) showed that hyperlipidemia and hypercholesterolemia caused a significant increase at control in uric acid, urea and creatinine contents of the diseased animal blood. The treatments with beans, pomegranate (seeds and peels) and their mixture as lipotropic factors for present diseased rats produced a significant improvements in the three parameters of kidneys function. The highest treatment effect on kidneys function was detected by the

mixture. The feeding of beans and pomegranate (seeds and peels) produced lower improved effects in the same respect. These effects were similar to those of the liver function.

The results presented in table (5) indicated that the hyperlipidemia effects on blood glucose, lipid peroxidation and enzymatic antioxidant (SOD and Catalase activity) in blood which were statistically analyzed. These results showed that hyperlipidemia did not change the values of blood glucose level which were around the normal value for all treatments relative to healthy control animals. Also, the present data showed that the activities of SOD and catalase were significantly inhibited by lipidemia or cholesterolemia. In contract the data observed that there occurred a significant increase in plasma lipid peroxide value in the diseased animals. Also, the results of table (5) pointed out that the feeding antioxidant diets (beans, pomegranate seeds and peels) and their mixture caused an improvement in the lipid peroxide values. Also, the present antioxidant diets alleviated the harmful effects of hyperlipidemia on SOD and catalase activities. These values were still far from that of normal healthy control animals.

**Table (5): Effect of administration of different samples to rats on serum glucose and lipid peroxidation as well as SOD and Catalase activity**

| Treatment | mg/dl | | U/mL | |
|---|---|---|---|---|
| | Glucose | Lipid peroxides | SOD | Catalase |
| **Group 1 Normal control** | 100.00 [a] ± 8.41 | 2.00 [a] ± 0.15 | 490.00 [a] ± 31.00 | 10.00 [a] ± 0.72 |
| **Group 2 Hyperlipidimic control** | 119.00 [b] ± 9.52 | 5.01 [b] ± 0.29 | 125.00 [b] ± 10.00 | 2.01 [b] ± 0.14 |
| **Group 3 Raw beans** | 99.00 [a] ± 8.12 | 2.49 [c] ± 0.14 | 389.00 [c] ± 17.00 | 6.97 [c] ± 0.41 |
| **Group 4 Pomegranate seed** | 107.00 [a] ± 8.97 | 2.51 [c] ± 0.16 | 269.00 [c] ± 14.00 | 6.66 [c] ± 0.40 |
| **Group 5 Pomegranate peels** | 104.00 [a] ± 9.91 | 2.36 [c] ± 0.17 | 221.00 [d] ± 15.00 | 6.02 [c] ± 0.37 |
| **Group 6 Mixture** | 104.00 [a] ± 8.23 | 2.22 [a] ± 0.12 | 300.00 [c] ± 19.00 | 8.71 [d] ± 0.51 |

- Each value represents the mean of 8 rats (Mean ± SE).
- Means in the same column followed by the same letter are not significantly different at ($P<0.05$).

## Discussion

Legumes and fruits play important role in human nutrition since they are rich source of protein, soluble dietary fibers, calories, certain minerals, vitamins and antioxidants. Regarding soaking of beans, protein digestibility of beans was improved which may be attributed not only to removal/reduction of antinutrients but also to the structural disintegration of the native protein, including enzyme inhibitors and lectins, differential solubility of the individual oligosaccharides and their diffusion rates. Phytase activity to break down phytic acid in seeds and the development of endogenous α-galactosidase activity to diminish oligosaccharides. This process not only improves pal at ability of beans but also increases the bioavailability of nutrients (Shimelis and Rakshit, 2007). From the present results it can be reported the use of beans as therapy food against lipidemia and cholesterolemia which had a large improved effects on lipid profile, lipoprotein profile, lipid peroxidation, antioxidant enzymes activity, kidneys function and liver function of blood. Also, either dried pomegranate seeds or peels treatments improved and alleviated the harmful effects of hyperlipidemia and hypercholesterolemia. The major contributors to cholesterol accumulation in arterial cell during development of atherosclerosis include several factors such as high level of plasma cholesterol (Joanne *et al.*, 2005), inhibited blood paraoxonase (Li *et al.*, 2006), enhanced macrophage cholesterol esterification rate (Aviram *et al*., 2000). In connection to the most effective factor which is the increased oxidative stress (van Lieshout *et al.*, 2003). This rendered several studies to look for feeding on food rich in protein and antioxidant that can be preventive to atherosclerosis among exposed individuals.

The improvement in lipid profile of blood could be referred to a multi factors beside on the role of amino acids of protein, dietary fibers and antioxidants may play a good part in this action. Beneficial treatment of bean seeds and pomegranate (seeds and peels) showed that the dietary fibers are having the potential to lower the levels of total cholesterol and LDL-c in blood. Absorption of bile salts by soluble dietary fiber (SDF) results in changes in cholesterol metabolism, loss of cholesterol, unavailability of bile salts in the intestine for micelle formation, which inhibits lipid fractions absorption, increased fecal bulk dilutes bile acids in the lower

intestinal tract, and short chain fatty acids produced especially the propionate, which has been proposed to inhibit hepatic cholesterol synthesis (Tharanathan and Mahadevamma, 2003). Also, amino acids of the present dietary food used as precursors of lipoprotein, including HDL-c which has 50% of its structure protein (Elliott and Elliott, 2001), that via protein biosynthesis and also the three hypophseal peptides that simulate fatty acids release from adipose tissue for their biodegradation (Campbell, 1995). In addition, these amino acids are considered as lipotropic factors used in the protein biosynthesis of β-oxidation enzymes, glucose oxidase, antioxidant enzymes (SOD and catalase) and other protein factors. These enzymes stimulate fat oxidation rate, and antioxidative power, suggesting that dietary protein exerts its hypolipidemic effects by stimulation of β-oxidation fatty acids at the expense of fatty acids esterification (Wang and Jones, 2004).

Pomegranate is one of these food items that is believed to possess strong antioxidant property, including its capability to scavenge or prevent several reactive oxygen species and inhibit lipid peroxidation (Kaplan *et al.*, 2001 and Li *et al.*, 2006). The total phenols in dried pomegranate seeds and peels 0.51 and 0.65% respectively, but for bean seeds was 0.11%. These values of pomegranate are comparable with similar reported values by Gil *et al.* (2000) and showed that pomegranate contain an appreciable antioxidant power relative to bean seeds. It means that, the hypolipidemic effects of pomegranate (seeds and peels) were mainly related to the antioxidant agents, but related to protein for bean seeds. The pattern of HPLC analysis (table 2) seems to be in agreement with of Li *et al.* (2006). It is clear that these phenolic compounds detect in the pomegranate (seeds and peels) are responsible for the antioxidative effect and cholesterol lowering action. The hypolipidemic effect may be either due to retarding effect on lipid fraction absorption or increase in LDL receptor mediated cholesterol rein oval.

Hyperlipidemia showed a significant increased in the value of blood total lipids, cholesterol, triglycerides, LDL-c, VLDL-c, lipid peroxidation and slightly increased HDL-c, but activity of SOD or catalase was inhibited. The treatments with bean seeds or pomegranate and their mixture observed that the lipid fractions started to change toward normalization and alleviated the harmful effects on liver and

kidney functions produced by lipidemia and cholesterolemia. The present results confirmed each other in which the roles of pomegranate antioxidants and bean seeds protein to correct that derangement in either lipid fractions or oxidative stress. Consequently, the reduction in blood lipid fractions (triglycerides and cholesterol) produced due to treatments of either bean seeds or pomegranate (seeds and peels) as well as their mixture, a significant reduction was observed in lipid peroxidation and stimulation in SOD and catalase activities. These effects are indicative to a lower rate of lipid oxidation and show that the treated diets could exert antioxidant influences on lipid compartments of the lipidemic animals. The potent antioxidant activity of pomegranate was reported by Aviram *et al*. (2000) and Murthy *et al.* (2002) and may also contribute to the reducing effect of pomegranate on the high blood cholesterol accumulation, which indicative to cholesterol accumulation in the retrial cell and development of atherosclerosis (Singh *et al.*, 2001). The elevated HDL-c is indicated to stimulation of paraoxonase activity which associated to HDL-c and protected it from oxidation (Aviram *et al*., 1998). The antioxidative action of pomegranate is suggested to be due to the capacity of phenols to transfer electron anion free radicals, chelate metal catalyst, active antioxidative enzymes as reported in the present studies and inhibit oxidases (Spencer *et al.*, 2001).

Therefore, the peripheral mechanism of action of the present treatments especially the mixture of dried pomegranate (seeds and peels) and bean seeds may be main activity responsible for antilipidemic activity of protein and antioxidant, although other target organs (liver and kidneys) cannot be discarded. The more pronounced effect of the mixture than the others alone may be due to the synergetic effect of the three diets each other which produced a good alleviation and improvement in the lipidemic drastic effects as antilipidemic and anticholesterolemic agents influenced on blood lipid profiles. These may be due to that the mixture was consisted of lipotropic factor such as bean seeds protein and antioxidative agents such as the phenolic compounds of pomegranate (seeds and peels). This suggestion is confirmed by the observations of the present results, which showed that the biological activity has been attributed to two main factors (lipotropic and antioxidant factors) either alone or synergistically. The present results are in harmony with each other. Thus the stimulation of transaminases activity and bilirubin content in

blood of the studied rats, which largely was used as indicator of liver function. Also, blood uric acid, urea and creatinine confirmed each other and also the previous findings. Therefore, further of studies are required to evaluate the biochemical effects and mechanism of the studied lipotropic factors and antioxidant agents as antilipidemic and anticholesterolemic agents which may use in addible food to recommend their use as hypolipidemic food additives.

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# تقييم بذور الفاصوليا ومكونات ثمار الرمان كعوامل خافضة للكولسترول وليبيدات الدم في الفئران البيضاء

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تم إجراء هذه الدراسة لتقييم التأثيرات الناتجة من معاملة الفئران البيضاء التي تعاني من زيادة في ليبيدات الدم hyperlipidemia وزيادة في الكولسترول hypercholesterolemia ببذور الفاصوليا البيضاء ومكونات ثمار الرمان (البذور و القشر)، وقد اعتمدت الدراسة على أن البقوليات والفواكه تعتبر عوامل خافضة لليبيدات الدم والكولسترول hypolipidemic and hypocholesterolemic agents حيث تحتوي علي بروتين والمعروفة بقدرته على ربط الليبيدات وكمية من الفينولات والمعروفة كذلك كمضادات للأكسدة، الأمر الذي يساعد على حماية مكونات ليبيدات الدم من الأكسدة أو الترسيب على جدر الأوعية الدموية وبالتالي التخفيف من أمراض تصلب الشرايين. أجريت الدراسة على بذور الفاصوليا البيضاء وبذور الرمان و قشر الرمان المجففة وكذلك خليط من الثلاثة بنسبة (1:1:1) لتقييم تأثيرهم كعوامل خافضة للدهون عن طريق التغذية للفئران البيضاء لتوضيح ما إذا كان التأثير راجع إلى البذور أم القشر المجفف ، وقد تم إضافة بذور الفاصوليا البيضاء وبذور الرمان و قشر الرمان المجفف بجانب الخليط المكون منهم بنسبة (1:1:1) إلى العليقة الغذائية الأساسية بنسبة 10%.

تم التعرف على التركيب الكيميائي لبذور الفاصوليا وكذا ثمار الرمان (بذور وقشر) في المادة الجافة وقد أظهرت النتائج قيم جيدة في محتوى البروتين والليبيدات والألياف والفينولات، كما تم إجراء تحليل للمركبات الفينولية من بذور وقشر الرمان باستخدام جهاز التفريد الكروماتوجرافى السائل عالي الكفاءة (HPLC) وقد أظهرت النتائج فصل 16 مركب يختلف كل منهم في نسبته، وقد لوحظ أن مركبا الكاتشين و الفينول تمثل النسبة الأعلى مقارنة بباقي المركبات الفينولية ، كما تم التعرف على العديد من المركبات الفينولية الأخرى منها دايدزين ، جنستين ، حمض السينامك ، كامفيرول ، إيجنول ، شيرسن ، جالانجين.

أظهرت الدراسة أن المعاملة ببذور الفاصوليا البيضاء وبذور الرمان و قشر الرمان المجفف وكذلك الخليط لها تأثيرات كعوامل خافضة للدهون والكولسترول وكذلك المحافظة على وظائف الكبد والكلى ، كذلك حماية ليبيدات الدم من الأكسدة والمحافظة على نشاط الإنزيمات المضادة للأكسدة (الكتاليز و SOD)، فيما أظهرت هذه المعاملات تأثيرات خافضة معنوية على جلوكوز الدم في الفئران التي تعاني من زيادة ليبيدات الدم. أيضا أوضحت النتائج المتحصل عليها أن المعاملة ببذور الفاصوليا أدت لتحسن عام في الحالات المرضية المختلفة سواء خفض مكونات ليبيدات الدم (الدهون الكلية ، و الجلسريدات الثلاثية ، والكولسترول ، والكوليستيرول فى الدهون البروتينية ذات الكثافة المرتفعة HDL-C وكذلك ذات الكثافة المنخفضة LDL-C و ذات الكثافة المنخفضة جدا VLDL-C) ، وتحسين وظائف الكبد (GOT, GPT and bilirubin)، وتحسين وظائف الكلى ( حمض اليوريك ، واليوريا ، والكرياتينين) ، وكذلك خفض في أكسدة الدهون وزيادة في نشاط الإنزيمات المضادة للأكسدة (كتاليز و SOD) سواء في حالة الفئران المصابة بارتفاع ليبيدات الدم والكولسترول، كما أظهرت المعاملة بمخلوط بذور الفاصوليا البيضاء وبذور الرمان و قشر الرمان (1:1:1) أفضل تأثر في الدراسة البيولوجية مقارنة بباقي المعاملات.

عموما من النتائج المتحصل عليها يمكن ترتيب المعاملات الغذائية المختلفة تحت الدراسة من الأعلى للأقل من حيث قوة التأثير كعوامل مضادة للزيادة في ليبيدات الدم والكولسترول كالتالي:

المخلوط ≥ بذور الفاصوليا > بذور الرمان ≥ قشر الرمان

وبصفة عامة هناك احتياج لدراسات مستقبلية لتقييم التداخل الحادث بين العامل الرابط للدهون (البروتين) والعامل المضاد للأكسدة (الفينولات) لإحداث التأثيرات المشتركة.