

White Bean seeds and Pomegranate peel and fruit seeds as hypercholesterolemic and hypolipidemic agents in albino rats

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RESUMEN

Semillas de alubias blancas y piel y semillas de granadas como agentes hipercolesterolémicos e hipolipidémicos en ratas albinas

Se ha llevado a cabo un estudio para evaluar los efectos de las semillas de alubias blancas y de las de granadas así como de sus cáscaras secas y sus mezclas sobre los perfiles de lípidos de ratas que sufren de hiperlipidemia e hipercolesterolemia. La composición química de las alubias blancas y de las granadas (semillas y cáscaras) fue determinada en base a materia seca alcanzando una buena relación de proteínas, lípidos, fibra cruda y fenoles. Además, el contenido de fenoles de las semillas y las cáscaras de granada mostraron 16 compuestos que varían en cantidad entre ellos. Se observó que la catequina y los fenoles son los compuestos predominantes. Los resultados obtenidos mostraron un buen poder hipolipidémico de las semillas de alubias blancas y de granadas (semillas y las cáscaras), así como sus mezcla. Las semillas de alubias blancas en la dieta produjo una mejora general de la situación clínica, del perfil de lípidos en sangre (lípidos totales, triglicéridos, colesterol, HDL-C, LDL-C y VLDL-c), de la función hepática (ALT, AST y bilirrubina), de la función de los riñones (ácido úrico, urea y creatinina), de la peroxidación de lípidos en sangre y de las enzimas antioxidantes (SOD y CAT) por lo que se reduce la hiperlipidemia e hipercolesterolemia. La dieta mezcla presentó la mayor influencia sobre los estudios biológicos en relación con los otros tratamientos que utilizan solamente semillas de alubias blancas o granadas (semillas y cáscaras).

PALABRAS CLAVE: Alubia blanca (*Phaseolus vulgaris* L.) – Granada (*Punica granatum*) – Hipocolesterolemia – Hipolipidemia.

ABSTRACT

White Bean seeds and Pomegranate peel and fruit seeds as hypercholesterolemic and hypolipidemic agents in albino rats

This study was carried out to evaluate the effects of soaked white bean seeds, pomegranate seeds and dried peels and their mixtures on the lipid profiles of rats suffering from hyperlipidemia and hypercholesterolemia. The chemical compositions of dried soaked beans and pomegranate (seeds and peels) were determined on dry matter which amounted to good values for proteins, lipids, crude fiber and phenols. Also, the phenol contents of pomegranate seeds and peels showed 16 compounds varying in amount between them. It was noticed that catechin and phenol are the dominating compounds.

The obtained results showed a good hypolipidemic ability for soaked bean seeds and pomegranate (seeds, peels and their mixtures) as well as their mixtures. A 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 (ALT, AST and bilirubin), kidneys function (uric acid, urea and creatinine), blood lipid peroxidation and antioxidant enzymes (SOD and CAT) by which hyperlipidemia and hypercholesterolemia were reduced. The mixed diet had the best influence concerning biological studies than the other treatments which used bean seeds and pomegranate (seeds and peels) alone.

KEY-WORDS: Hypocholesterolemia – Hypolipidemia – Pomegranate (*Punica granatum*) – White bean (*Phaseolus vulgaris* L.).

1. INTRODUCTION

Lipid fractions are widely distributed in all cells of the body, but particularly in the nervous tissues. They play a major role in the functions of the mammalian body. They are required for the structural component of the plasma membrane of all cells and precursors for several factors of animal cells (hormones, bile salts and others). Hyperlipidemia and atherosclerosis induced by a dietary fatty diet with cholesterol has been reported in several studies in animals (Lovejoy *et al.*, 2002). The major contributors to cholesterol accumulation in arterial cells during the development of atherosclerosis include several factors such as a high level of plasma cholesterol (Murabito *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 increased oxidative stress (van Lieshout *et al.*, 2003). 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, which is of major importance. Atherosclerosis is a spectrum of arterial reactions that may result from many factors acting upon the vessel wall and producing their effect through different mechanisms and indifferent 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 an incidence of atherosclerosis and cardiovascular diseases. According to the World Health Reports (WHPs, 2002), more than 75% of cardiovascular diseases are the world's leading cause of premature death, resulting from high blood cholesterol and other factors such as smoking, drinking alcohol and high blood pressure. Therefore, the studies need to look for natural compounds that can help the patient to get rid of or reduce the health hazards of the disease as a care that they receive (Turnbull, 2005). Phenols are one of the major groups of non-essential dietary compounds appearing in fruits and vegetables. Phenols have traditionally been considered as antinutritive compounds due to the adverse effect of one of their main compounds, tannins and protein digestibility. However, currently 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 and scavenge free radicals (Martinez-Valverde *et al.*, 2000; Scalbert *et al.*, 2005; El-Beltagi and Mohamed, 2010; Afify *et al.*, 2011a). 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 hyperlipidemia, atherosclerosis and cardiovascular diseases (El-Beltagi *et al.*, 2008; Salama *et al.*, 2009; Shehab *et al.*, 2010; Afify *et al.*, 2011b,c; El-Beltagi, 2011; El-Beltagi *et al.*, 2011; Ibrahim *et al.*, 2011; Afify *et al.*, 2012a,b). Legume seeds utilized for human consumption have been of great interest to nutritionists and agricultural scientists for providing mankind with nutritional diets particularly when protein deficits occur. Bean seeds are not only a good choice for human nutrition but recently, the potential role of it in hyperlipidemia has received attention (Aly and El-Beltagi, 2010; Abdel-Rahim and El-Beltagi, 2011; Afify *et al.*, 2012c). Several compounds with hyperlipidemic or hypercholesterolemic activity are found in relatively high concentrations in bean seeds (Abdel-Rahim and El-Beltagi, 2010). The present studies were undertaken to investigate the effect of pomegranate seeds and peels as well as bean seeds and their biological and nutritional evaluation through an animal experiment as follows:

1. Chemical composition of pomegranate seeds and peels as well as raw bean seeds.
2. The total phenolic levels and their constitute 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 hypolipidemic agents on blood analysis, i.e., glucose, lipid profile, liver function, kidney function, lipid peroxidation and enzymatic antioxidant (superoxide dismutase (SOD) and catalase (CAT) activity).

2. MATERIALS AND METHODS

2.1. Preparation of samples

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

2.2. General chemical analysis

The determination of moisture, crude protein, total lipids, ash and crude fibers were made. A 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).

2.3. Determination of total phenols

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

2.4. HPLC analysis of pomegranate phenolic compounds

The phenolic compounds of pomegranate samples were extracted according to the method described by Duke *et al.* (2003) in which a known weight of dried sample was extracted by methanol. Each phenolic compound was identified and subjected to the JASCO HPLC using hypersil C₁₈ reversed phase column (250 × 4.6 mm) with 5 μ practical size. An injection by means of the Rheodyne injection valve with 50 PJ fixed loop was used. A constant flow rate of 1 mL min⁻¹ was used with two mobile phases: (A) 0.5% acetic acid in distilled water at PH 2,65; and solvent (B) over 35 min, using a UV detector set at wavelength 245 nm. The 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.

2.5. Experimental animals

Sprague – Dawley albino male rats weighing 65-75 g were used for the present study. The animals were obtained from the Agriculture

Research Center (ARC), Giza, Egypt. The animals were raised in the animal house and kept under normal laboratory conditions (temperature remain 25 ± 2 °C) for 48 h before the initiation of the experiment. During this period, animals were allowed free access to water and a basal diet. Food consumption and body weight were monitored daily for each animal.

2.6. Animal diet

The control diet is designed as reported by Lane – Peter and Pearson (1971), with 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture (Schneeman *et al.*, 1989), 1% vitamin mixture (Philip *et al.*, 1993) and starch 65%. The high fat diet was similar to the control diet but differed in more fat content which was 20% sheep fat, 2% cholesterol and 0.25% bile salts and starch 42.75%.

2.7. Experimental design

After a period of adaptation (one week), 48 of the male albino rats were divided into two groups as following:

Group I. Healthy, normal control group

The rats were fed a normal diet (8rats) as healthy, normal control animals.

Group II. Hyperlipidemic group

The rats were fed a high fat with cholesterol diet for 4 weeks (40 rats). At the end of the feeding period, blood samples were taken from the suborbital vein to test for blood cholesterol levels. A high level of serum cholesterol was considered an indication of hypercholesterolemia. The hyperlipidemic rats of the second group were subdivided into 5 subgroups (8 rats each).

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

Subgroup 2. Rats were fed the high fat/high cholesterol diet with 10% dried beans at the expense of starch (**Group 3:** Bean group).

Subgroup 3. Rats were fed the high fat/high cholesterol diet with 10% dried pomegranate seeds at the expense of starch (**Group 4:** Pomegranate seeds group).

Subgroup 4. Rats were fed the high fat/high cholesterol diet with 10% dried pomegranate peels at the expense of starch (**Group 5:** Pomegranate peels group).

Subgroup 5. Rats were fed the high fat/high cholesterol diet with 10% mixture of beans, dried pomegranate seeds and peels by a ratio of 1:1:1 at the expense of starch (**Group 6:** Mixture group).

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

2.8. Blood biochemical analysis

Determination of plasma glucose. Plasma glucose levels were determined enzymatic colorimetrically according to the method of Trinder (1969).

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

Kidney function. For kidney 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 method described by Faulkner and King (1976).

Plasma lipid and lipoprotein profile. For plasma lipid profile, total lipid, 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 the lipoprotein profile in plasma, HDL-c and LDL-c levels were determined according to Warnick *et al.*, (1983) and Bergmeyer (1985) respectively, but VLDL-c was calculated using the equation described by Fiedewald *et al.*, (1972).

Lipid peroxidation. Lipid peroxide was determined according to the method of Ohkawa *et al.*, (1979). Also, catalase (CAT) and superoxide dismutase (SOD) activities were determined according to the methods described by Aebi (1984) and Nishikimi *et al.* (1972) respectively.

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

3. RESULTS AND DISCUSSION

Legumes and fruits play important roles in human nutrition since they are rich sources of protein, soluble dietary fibers, calories, certain minerals, vitamins and antioxidants. Regarding the soaking of beans, the protein digestibility of beans was improved which may be attributed not only to the 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 -galactosidas activity to diminish oligosaccharides (Afify *et al.*, 2011d). This process not only improves the protein digestibility of beans

but also increases the bioavailability of nutrients (Shimelis and Rakshit, 2007).

Wheat beans and pomegranate are considered famous legumes and fruits 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 pomegranates (seeds and peels) are rich in protein, dietary fiber, elements and carbohydrates, but beans contain lower amounts of total phenols than dried pomegranate which were observed as 6-11 times the total phenols. The total phenols in dried pomegranate seeds and peels were 0.51 and 0.65% respectively, but for bean seeds were 0.11%. These values for pomegranate are comparable to the values reported by Gil *et al.* (2000), and showed that pomegranates contain an appreciable antioxidant power relative to bean

seeds. This means that the hypolipidemic effects of pomegranates (seeds and peels) were mainly related to the antioxidant agents, but related to protein for bean seeds. Recently, a special interest was given to legume protein and fruits antioxidants as good antilipidemic and anticholesterolemic agents. For this reason, the present studies analyzed the phenolic compounds of pomegranate methanol extract either for seeds or peels. The HPLC analysis of both fractions showed the presence of 12 compounds and 13 compounds in peel extracts varying in amount between both fractions (Table 2). The total known compounds in pomegranate seeds were 90.65% while, the total unknown compounds were 9.35%, also the total known compounds of pomegranate peels were 93.42% while, the total unknown compounds were 6.58%.

Table 1
Chemical composition of the white beans and pomegranate (seeds and peels) as (g 100g⁻¹) dry weight

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	55.0 ± 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

Values represent the mean ± SE of triplicate determinations.

Table 2
Phenolic compound profiles in the methanolic extract of pomegranate seeds and peels

No.	Compounds	pomegranate seeds		pomegranate peels	
		mg 100g ⁻¹ D.W.	%	mg 100g ⁻¹ D.W.	%
1	Catechin	406	71.99	850	71.73
2	Caffeic acid	–	–	9	0.76
3	Phenol	110	20	274	23.12
4	Daidzin	4	0.71	5	0.42
5	Salicylic acid	14	2.48	–	–
6	Ferulic acid	–	–	5	0.42
7	<i>p</i> -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	0.72	2	0.17
14	Chrysin	1	0.18	2	0.17
15	Galangin	1	0.18	2	0.17
16	Pinostrobin	7	1.24	–	–

It was noticed that compounds such as catechin, phenol, daidzin, genistein, cinnamic acid, kaempferol, eugenol, chrysin and galangin are detected in the extracts from pomegranate seeds and peels. On the other hand, more than the phenolic compounds salicylic acid, daidzein, and pinostrobin were detected in the seed extract, but in the peels, caffeic acid, ferulic, *p*-coumaric acid and quercetin were identified. Catechin is dominating which amounted to about 72% of the total phenolic compounds and phenol amounted to about 20% or more for seed and peel methanolic extracts respectively. Both fractions of pomegranate showed good values when total phenols were determined in terms of tannic acid. The pattern of the HPLC analysis seems to be in agreement with (Li *et al.*, 2006). It is clear that these phenolic compounds detected in pomegranate (seeds and peels) are responsible for the antioxidant effect and cholesterol lowering action. Pomegranate is one of these food items which are believed to possess strong antioxidant properties, 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 hypolipidemic effect may be due either to the retarding effect on lipid fraction absorption or the increase in the LDL receptor mediated cholesterol rein oval.

The studies are considered to be treatment investigations which were done on male albino rats weighing 70 ± 5 . The results of the evaluation of the three studies 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

were fed the high fat/cholesterol diet, the values amounted to 2.19, 2.80 and 2.49 fold respectively in comparison to that of the normal control. The results presented in Table 3 show the effect of the administration of 10% beans and pomegranate (seeds and peels) as well as their mixture on the lipid profile of hyperlipidemic animals. In the case of hypolipidemic animals, the feeding of the 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 effects of hyperlipidemia where the mixture and bean treatments were the most effective as hyperlipidemic and hypocholesterolemic agents. On the other hand, the mixture of pomegranate seeds and peels 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 of pomegranate seeds and peels produced the same alleviated effect on hyperlipidemia and hypocholesterolemia. The results are in agreement with those data of blood lipoprotein contents of the present study (table 3) except for the HDL-cholesterol (HDL-c). The effects of pomegranate (seeds and peels) and beans as well as their mixture treatments on hyperlipidemic animals were also different. Hyperlipidemia increased blood HDL-c, LDL-c, VLDL-c contents, but the increase in HDL-c was lower than the increases in LDL-c and VLDL-c. This drastic effect of hyperlipidemia was improved by the present treatments whereas, greater increases in the HDL-c content in the blood of hyperlipidemic animals were observed after treatments with feeding beans, pomegranate (seeds and peels) and their mixture.

Table 3
Plasma lipid fractions and lipoprotein profile (mg dL^{-1}) in experimental rats

Groups	mg dL^{-1}					
	Total lipid	Triglycerides	Total cholesterol	HDL-c	LDL-c	VLDL-c
Group 1 Normal control	270.0 ± 21.0^c	74.0 ± 4.21^c	65.0 ± 2.14^c	34.0 ± 2.00^c	26.0 ± 1.72^c	15.0 ± 0.99^c
Group 2 Hyperlipidemic control	590.0 ± 25.0^a	183.0 ± 8.92^a	182.0 ± 7.23^a	44.0 ± 2.74^b	86.0 ± 2.42^a	37.0 ± 2.00^a
Group 3 Raw beans	310.0 ± 20.0^c	81.0 ± 5.24^c	80.0 ± 3.23^b	46.0 ± 3.11^b	28.0 ± 1.83^c	16.0 ± 1.00^c
Group 4 Pomegranate seeds	351.0 ± 19.0^b	98.0 ± 4.49^b	87.0 ± 4.17^b	49.0 ± 2.19^a	31.0 ± 1.41^b	20.0 ± 1.21^b
Group 5 Pomegranate peels	349.0 ± 21.0^b	94.0 ± 5.31^b	86.0 ± 3.92^b	45.0 ± 3.01^b	32.0 ± 2.00^b	19.0 ± 1.02^b
Group 6 Mixture	300.0 ± 21.0^c	78.0 ± 4.11^c	86.0 ± 4.01^b	47.0 ± 2.02^{ab}	27.0 ± 1.46^c	16.0 ± 0.98^c
LSD 5%	29.42	9.12	4.35	3.26	2.53	1.85

Each value represents the mean of 8 rats (Means \pm SE).
The same letters in each column represent insignificant difference at $P < 0.05$.

In contrast, the LDL-c and VLDL-c contents in the blood of the hyperlipidemic rats were significantly alleviated by the present treatments which were reduced but the levels were still higher than that of the control animals. These effects are indicative of 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 is indicative of cholesterol accumulation in the reticular cell and the development of atherosclerosis (Singh *et al.*, 2001). The elevated HDL-c is indicated by the stimulation of paroxonase activity which is associated with HDL-c and protected it from oxidation (Aviram *et al.*, 2000). 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

The improvement in the lipid profile of blood could be attributed to several factors. The amino acids in protein, dietary fibers and antioxidants may play an important role in this action. The beneficial treatment of bean seeds and pomegranate (seeds and peels) showed that the dietary fibers have the potential to lower the levels of total cholesterol and LDL-c in blood. The absorption of bile salts by soluble dietary fibers (SDF) results in changes in cholesterol metabolism, loss in cholesterol and unavailability of bile salts in the intestine for micelle formation, which inhibits lipid fraction absorption; increased fecal bulk dilutes bile acids in the lower

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

The effects of the present antilipidemic agents on liver and kidney functions in hyperlipidemic rats were statistically analyzed and are illustrated in table (4). The results showed that hypolipidemia and hypocholesterolemia significantly stimulated ALT and AST activity as well as the plasma content of bilirubin. The same trend was observed for ALT and AST compared to the healthy control. These stimulations of AST activity indicated slight liver cell necrosis and the magnitude of increase correlated with the extent of necrosis (Murray *et al.*, 2006). All the treatments with beans and pomegranate (seeds and peels) as well as their mixture (as hypolipidemic agents) in diseased animals were characterized by an alleviation and normalization in the both transaminase activities (ALT and AST) and bilirubin content in plasma. These are conflicting reports on the changes in blood. Alterations in transaminases

Table 4
Liver (U L⁻¹) and kidney (mg L⁻¹) functions in experimental rats

Groups	U L ⁻¹		mg dL ⁻¹			
	ALT	AST	Bilirubin	Urea	Uric acid	Creatinine
Group 1 Normal control	141.0 ± 5.64 ^b	42.0 ± 3.23 ^b	0.42 ± 0.03 ^d	57.0 ± 3.15 ^b	3.55 ± 0.20 ^c	0.66 ± 0.04 ^c
Group 2 Hyperlipidemic control	176.0 ± 3.99 ^a	55.0 ± 3.74 ^a	0.71 ± 0.04 ^a	70.0 ± 4.54 ^a	5.01 ± 0.35 ^a	0.90 ± 0.05 ^a
Group 3 Raw beans	144.0 ± 5.86 ^b	46.0 ± 3.00 ^b	0.54 ± 0.03 ^c	60.0 ± 2.99 ^b	4.44 ± 0.30 ^b	0.69 ± 0.04 ^c
Group 4 Pomegranate seeds	153.0 ± 8.41 ^b	45.0 ± 2.79 ^b	0.60 ± 0.02 ^b	62.0 ± 3.16 ^b	4.30 ± 0.31 ^b	0.78 ± 0.02 ^b
Group 5 Pomegranate peels	157.0 ± 7.94 ^b	46.0 ± 3.24 ^b	0.53 ± 0.03 ^c	61.0 ± 3.00 ^b	4.25 ± 0.29 ^b	0.77 ± 0.05 ^b
Group 6 Mixture	143.0 ± 7.11 ^b	44.0 ± 2.77 ^b	0.50 ± 0.02 ^c	61.0 ± 2.78 ^b	4.01 ± 0.31 ^{bc}	0.75 ± 0.03 ^b
LSD 5%	8.85	3.95	0.05	4.43	0.34	0.04

Each value represents the mean of 8 rats (Means ± SE).
The same letters in each column represent insignificant difference at P < 0.05.

activity and bilirubin contents 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 the liver and RBCs (Chatterjea and Shinde, 2002).

The treatment with the present antilipidemic agents was characterized by normalization in both transaminase activity and bilirubin content in the plasma of diseased animals. The effects of the present treatments on the kidney function of hyperlipidemic animals were confirmed by the determination of blood uric acid, urea and creatinine which were statistically analyzed. The data in table (4) show that hyperlipidemia and hypercholesterolemia caused a significant increase in uric acid, urea and creatinine contents in 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 improvement in the three parameters of kidney function. The highest treatment effect on kidney function was detected by the mixture. The feeding of beans, 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 indicate the hyperlipidemia effects on blood glucose, lipid peroxidation and enzymatic antioxidant (SOD and CAT) activity in blood which were statistically analyzed. These results show that hyperlipidemia did not change the values of blood glucose levels which were around the normal value for all treatments compared to the healthy control animals. Also, the present data show that the activities of SOD and CAT were significantly inhibited by

lipidemia or cholesterolemia. In contrast, the data reveal that there is a significant increase in plasma lipid peroxide value in the diseased animals. Also, the results of Table 5 point out that the feeding of antioxidant diets (beans, pomegranate seeds and peels) and their mixture caused an improvement in blood peroxide values. Also, the present antioxidant diets alleviated the harmful effects of hyperlipidemia on SOD and CAT activities. These values were still far from that of normal healthy control animals.

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 the main activity responsible for the antilipidemic activity of protein and antioxidants, 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 of which produced a good alleviation and improvement in the lipidemic drastic effects as antilipidemic and anticholesterolemic agents influenced the blood lipid profiles. This may be due to the fact that the mixture consisted of lipotropic factors such as bean seed protein and antioxidative agents such as phenolic compounds of dried pomegranate (seeds and peels). This suggestion is confirmed by the observations of the present results, which show 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.

Table 5
Plasma glucose, lipid peroxidation (mg dL^{-1}) as well as SOD and CAT activities (U mL^{-1}) in experimental rats

Groups	mg dL^{-1}		U mL^{-1}	
	Glucose	Lipid peroxidation	SOD	CAT
Group 1 Normal control	100.0 \pm 4.42 ^b	2.00 \pm 0.15 ^c	490.0 \pm 18.0 ^a	10.0 \pm 0.52 ^a
Group 2 Hyperlipidemic control	119.0 \pm 5.52 ^a	5.01 \pm 0.29 ^a	125.0 \pm 10.0 ^e	2.01 \pm 0.14 ^d
Group 3 Raw beans	99.0 \pm 4.12 ^b	2.49 \pm 0.14 ^b	389.0 \pm 17.0 ^b	6.97 \pm 0.41 ^c
Group 4 Pomegranate seeds	107.0 \pm 4.97 ^b	2.51 \pm 0.16 ^b	269.0 \pm 14.0 ^d	6.66 \pm 0.40 ^c
Group 5 Pomegranate peels	104.0 \pm 3.91 ^b	2.36 \pm 0.17 ^b	221.0 \pm 15.0 ^d	6.02 \pm 0.37 ^c
Group 6 Mixture	104.0 \pm 3.23 ^b	2.22 \pm 0.12 ^c	300.0 \pm 19.0 ^c	8.71 \pm 0.51 ^b
LSD 5%	5.63	0.25	21.0	0.54

Each value represents the mean of 8 rats (Means \pm SE).

The same letters in each column represent insignificant difference at $P < 0.05$.

Thus the stimulation of transaminase activity and bilirubin content were used largely in the blood of the rats studied as indicators of liver function. Also, blood uric acid, urea and creatinine confirmed each other and previous findings.

From the present results it can be reported that the use of beans as therapy food against lipidemia and cholesterolemia, which had greatly improved effects on lipid profile, lipoprotein profile, lipid peroxidation, antioxidant enzyme activity, kidney functions and liver functions in the blood. Also, both dried pomegranate seeds and peel treatments improved and alleviated the harmful effects of hyperlipidemia and hypercholesterolemia. This rendered several studies to look for food rich in protein and antioxidants that can be preventive against atherosclerosis among exposed individuals. Therefore, further studies are required to evaluate the biochemical effects and mechanisms of the lipotropic factors studied and the antioxidant agents which may be used in food as recommended hypolipidemic food additives.

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