

Original Research Article

Antihyperlipidemic effect of pomegranate peel and Iranian fenugreek extracts on cholesterol-rich diet-induced hypercholesterolemia in guinea pigs

Nabil A. Hasona^{1,2*}, Mohammed Q. Ahmed¹, Turki A. Alghassab¹, Muath A. Alghassab¹ and Abdullah A. Alghabban¹

Abstract:

¹College of Medicine, Ha'il University, Ha'il, KSA

²Beni-Suef University, Faculty of Science, Beni-Suef, Egypt

*Corresponding Author's Email: drnabil80@yahoo.com
Tel.: 00966-552707142

Composition of the diet played an important role in the management of lipid and lipoprotein concentrations in blood. Therefore, the aim of this study was to evaluate the effects of pomegranate peels and Iranian fenugreek extracts on the liver, cardiac and antioxidant status of the serum and tissues from diet-induced hypercholesterolemic guinea pigs. The guinea pigs were divided into six groups (10 per group). The first group served as normal control. The second and third groups were treated with Iranian fenugreek and pomegranate extract alone, respectively. The fourth group was fed with hypercholesterolemic diet only for 30 days; whereas the fifth and sixth groups were treated with Iranian fenugreek extract or with pomegranate extract respectively and fed with hypercholesterolemic diet also for 30 days. Blood and tissue were removed for further biochemical analysis. Oral treatment with extracts of pomegranate peel and Iranian fenugreek demonstrated significant protective effects on all the biochemical parameters studied including lipid profile, antioxidant status and liver functions tests. These results suggest that pomegranate and Iranian fenugreek extended protection against various biochemical changes in hypercholesterolemic guinea pigs. Thus, methanolic extract of pomegranate and Iranian fenugreek are recommended to prevent the development of hypercholesterolemic and its complications.

Key Words: Antioxidant, Fenugreek, Guinea Pigs, Hypercholesterolemia, Lipid, Liver, Pomegranate

INTRODUCTION

The management of the blood cholesterol and triacylglycerides are necessary for cardiovascular health. Cholesterol is an essential part of every cell in the body and principal building blocks of the plasma membrane, also; used in biosynthesis of some hormone; bile acids and Vitamin D. High level of blood cholesterol are a contributory factor in atherosclerosis and many lipids associated ailments like obesity, heart attacks and stroke and kidney failure (Basulaiman et al., 2014).

Saudi Arabia, considered a rapidly developing country, faces progressive urbanization and the fast adoption of a western lifestyle, factors which contribute to the rising

burden of cardiovascular disease (Soofi and Youssef, 2015). Hyperlipidemia is a widely known key risk factor for cardiovascular diseases (Huang et al., 2015).

High cholesterol diet is regarded as a crucial factor in the development of hypercholesterolemia, atherosclerosis and ischemic heart disease. In the majority of people with hypercholesterolemia among the general public, the condition is attributable to a high-fat diet. The elevation of serum total cholesterol and low-density lipoprotein cholesterol as well as alteration of other lipid parameters has been implicated as a primary risk factor for cardiovascular diseases. Diet and lifestyle factors

continue to be the cornerstone of health and prevention of chronic disease. Fruit and vegetable consumption is associated with the primary prevention of cardiovascular disease. Many of the health benefits associated with diets high in fruits and vegetables are attributed to their phytochemical content (Esmailzadeh et al., 2006).

Punica granatum, commonly known as pomegranate (PMG), is a fruit that grows in tropical and subtropical areas (Sen et al., 2014). The pomegranate (*Punica granatum L.*) originated in the Middle East and was used extensively in ancient cultures for medicinal purposes for many centuries (Mertens-Talcott et al., 2006). Pomegranate fruit is a rich source of polyphenolic compounds such as anthocyanins and hydrolysable tannins. In addition to, punicalagin, punicalin, ellagic acid and gallic acid (Kamal et al., 2015). Pomegranate consumption has become increasingly popular due to its reported benefits on human health (Herber et al., 2007).

Fenugreek (*Trigonella foenum-graecum L.*) is one of the oldest medicinal plants from Fabaceae family originated in central Asia ~4000 BC [9]. It is being commercially grown in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, Middle East and Argentina (Altuntas et al., 2005). Fenugreek is one of the oldest medicinal plants with exceptional medicinal and nutritional profile. Fenugreek contains a fairly high amount of flavonoids, alkaloids, saponins and other antioxidants (Ahmad et al., 2016).

Thus, the present study was designed to evaluate the possible beneficial effect of the pomegranate peels and Iranian fenugreek extracts on serum lipid parameters and hepatic oxidative stress parameters in guinea pigs fed with cholesterol-rich diet.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Scientific and Research Ethics Committees college of Medicine, Hail University, KSA.

Chemicals

Cholesterol was purchased from Sigma – Aldrich. All other chemicals and reagents used were of analytical grade.

Preparation of pomegranate peel extract

Fresh mature *Punica granatum L.* (*Punicaceae*) fruits were used and collected from private market in the city. For extraction purpose, a peel is removed and dried in shade for 10 days before grinding. The grounded material (50 gm) is shaken in 500 ml of absolute methanol for 24 hours at room temperature, followed by

filtration. The filtrate is centrifuged at 8000 rpm for 15 min, the clear supernatant is collected, and then the methanol is evaporated in a rotary evaporator at 45 °C under reduced pressure. The crude extract (23.5%, w/w) is kept at 20 °C till further use. Pomegranate peel extract (500 mg/kg) is administered orally to the guinea pigs in aqueous solution once per day (Ahmed et al., 2014).

Preparation of Iranian fenugreek extract

Seeds of Iranian fenugreek were collected from the private market. The whole seeds were cleaned and crushed into a fine powder using a grinding machine. The grounded material (50 gm) is shaken in 500 ml of absolute methanol for 24 h at room temperature, followed by filtration. The filtrate is centrifuged at 8000 rpm for 15 min, the clear supernatant is collected, and then the methanol is evaporated in a rotary evaporator at 45 °C under reduced pressure. Crude extract is kept at 20 °C until further use. Fenugreek extract (500 mg/kg) is administered orally to the guinea pigs in aqueous solution once per day (Parveen et al., 2014).

Phytochemical screening

The extracts were subjected to preliminary phytochemical analysis to test the presence of various phytochemical constituents in the extracts such as flavonoids, Phlobatannins, Terpenoids, tannins, alkaloids, glycosides, saponins, Coumarins, Anthraquinones and proteins as described by (Khandelwal et al., 1996).

Experimental Animals

A total of 60 male guinea pigs (Weighing between 490 and 550 g.) were used in the study. Animals were individually housed in stainless steel cages. They were fed with the standard commercial diet (Al-Karawan Company, KSA), and provided with tap water for 7 days to be acclimatized with the hold.

Induction of hypercholesterolemia

Hypercholesterolaemia was induced in animals by feeding powdered fodder containing 2% cholesterol for 30 days (Ahmad-RausRaha et al., 2001).

Experimental Design for animal study

After one week acclimation period, the guinea pigs were grouped into 6 groups, comprising of 10 guinea pigs in

each group as follows:

Group I: Experimental animals fed with a standard diet and served as normal control.

Group II: Control guinea pigs treated with Iranian fenugreek extract only (500 mg/kg) in aqueous solution orally for 30 days.

Group III: Control guinea pigs treated with pomegranate extract only (500 mg/kg) in aqueous solution orally for 30 days.

Group IV: Negative control fed with a high cholesterol diet [pure cholesterol (2% w/w)].

Group V: Fed with high cholesterol diet and also supplemented with extract of *Iranian fenugreek* orally (500 mg/kg) once daily for 30 days.

Group VI: Fed with high cholesterol diet and also supplemented with extract of *pomegranate peels* orally (500 mg/kg) once daily for 30 days.

By the end of the 4th week, body weight gain was calculated from the difference between the initial weight at the beginning and the final weight at the end of the experiment. Blood samples and tissues were collected from all experimental guinea pigs after overnight fasting by decapitation after diethyl ether inhalation. Serum was separated for biochemical and antioxidants measurement.

Serum cholesterol, triglycerides (TG), HDL-C, LDL-C and glucose levels and alanine transaminase (ALT), aspartate transaminase (AST) and creatine kinase (CK) activities were measured using commercial kits purchased from United Diagnostics, KSA.

The animals were quickly dissected and the liver removed. The liver was cleaned of blood and later blotted with a clean tissue paper, weighed and homogenized. The homogenate was later transferred to specimen bottles and kept frozen for 24 hours at -4°C before analyzes.

Hepatic cholesterol content was determined according to enzymatic method using kits purchased from United Diagnostics, KSA.

The hepatic catalase (CAT) activity was assayed as described by Cohen et al. (1970).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS for WINDOWS, version 18.0; SPSS Inc, Chicago) was used for the statistical analyses. Comparative analyzes were conducted by using the general linear models procedure (SPSS Inc). Results are expressed as mean \pm standard error and values of $P > 0.05$ were considered statistically insignificant, while values of $P < 0.05$ were considered statistically significant, values of $P < 0.01$ was considered statistically highly significant and $P < 0.001$ were considered statistically very highly significant.

RESULTS

Phytochemical screening

The crude extracts of pomegranate and fenugreek were tested for the most common phytochemical constituents of medicinal plants for which have hypolipidemic and hepatoprotective activity of other plants has been attributed. These included; flavonoids, alkaloids, tannins, Phlobatannins, terpenoids, carbohydrates, coumarins, anthraquinones, saponins and proteins. The results showed that the most abundant phytochemicals were flavonoids, tannins, alkaloids, coumarins and terpenoids as shown in table (1):

The body weight of hypercholesterolemic guinea pigs was increased whereas it increased throughout the 4-weeks experimental period in control animals. Treatment of normal guinea pigs with fenugreek extract or pomegranate extract showed a significant increase in body weight gain as compared to normal control group. While the hypercholesterolemic control guinea pigs showed a very highly significant increase in body weight gain. Treatments of hypercholesterolemic guinea pigs with fenugreek extract or pomegranate extract were fairly beneficial since they elicited some weight gain (Table 2).

Hypercholesterolemic untreated guinea pigs showed a significant increase ($P < 0.01$) in the serum cholesterol and triglycerides when compared with normal control group. Administration of fenugreek and pomegranate showed a very significant decrease ($P < 0.01$) in serum triglyceride and cholesterol levels when compared with the hypercholesterolemic control group as shown in the table (3).

The results were showed the serum ALT, AST and CK activities significantly increased ($P < 0.001$) in hypercholesterolemic untreated guinea pigs as compared to normal control group. Treatment of hypercholesterolemic guinea pigs with fenugreek and pomegranate extracts showed significantly decrease ($P < 0.001$) when compared with the hypercholesterolemic control group as shown in the table (4). Fasting serum glucose concentration of the hypercholesterolemic control group was significantly higher than that of the normal control group and treatment of hypercholesterolemic guinea pigs with fenugreek and pomegranate showed a decrease in glucose level as compared to hypercholesterolemic control one as shown in the table (5).

Table (6) showed that the change in hepatic cholesterol content and catalase significantly increase induced by high cholesterol diet feeding in guinea pigs compared to the normal control group. Administration of fenugreek and pomegranate showed a significant decrease ($P < 0.001$) in hepatic cholesterol content and catalase activity when compared to hypercholesterolemic control group.

Table 1. Phytochemical screening of ethanolic extracts of pomegranate peel and fenugreek.

Components	Ethanolic pomegranate	Ethanolic fenugreek
Flavonoids	+++ve	+ve
Alkaloids	+ve	+ve
Tannis	+++ve	+ve
Phlobatannins	+ve	+ve
Terpenoids	+ve	+ve
Carbohydrates	+ve	+ve
Coumarins	+ve	+ve
Anthraquinones	+ve	-ve
Saponins	+ve	-ve
Proteins	-ve	-ve

+ ve present; - ve absent

Table 2. Effects of fenugreek and pomegranate extract on body weight gain and liver weight in normal and hypercholesterolemic guinea pigs.

Groups	Body weight gain(gm.)	Liver weight (gm.)
Group I	10.75 ± 3.8	16.75 ± 0.32
Group II	60.50*** ± 8.5	18.29** ± 0.23
Group III	61.87*** ± 9.09	19.46*** ± 0.40
Group IV	99.38*** ± 2.37	22.06 ± 0.200
Group V	73.00** ± 6.83	22.57 ± 0.15
Group VI	50.63*** ± 4.41	21.00 ⁺ ± 0.32

Values are expressed as means ± SE; n = 10 for each treatment group. **P<0.01 as compared to normal control group. ***P<0.001 as compared to normal control group. ⁺P<0.05 as compared to hypercholesterolemic control group. **P<0.01 as compared to hypercholesterolemic control group. ***P<0.001 as compared to hypercholesterolemic control group

Table 3. Effects of fenugreek and pomegranate extract on serum levels cholesterol and triglycerides in normal and hypercholesterolemic guinea pigs.

Groups	Serum Cholesterol (mmol/l)	Serum Triglycerides (mmol/l)
Group I	0.78±0.02	0.701±0.022
Group II	0.72±0.14	0.701±0.018
Group III	0.58**±0.30	0.623**±0.012
Group IV	3.32***±0.12	1.53***±0.071
Group V	2.128***±0.035	1.022***±0.02
Group VI	1.67***±0.103	0.835***±0.034

Values are expressed as means ± SE; n = 10 for each treatment group. **P<0.01 as compared to normal control group. ***P<0.001 as compared to normal control group. +++ P<0.001 as compared to hypercholesterolemic control group

Table 4. Effects of fenugreek extract and pomegranate extract on serum levels of HDL-C and LDL-C in normal and hypercholesterolemic guinea pigs.

Groups	Serum HDL-C (mmol/l)	Serum LDL-C (mmol/l)
Group I	0.585 ± 0.02	1.09 ± 0.02
Group II	0.573 ± 0.03	1.02* ± 0.02
Group III	0.620 ± 0.02	0.92*** ± 0.02
Group IV	0.23*** ± 0.01	2.74*** ± 0.04
Group V	0.33*** ± 0.01	1.98*** ± 0.04
Group VI	0.48*** ± 0.03	1.59*** ± 0.05

Values are expressed as means ± SE; n = 10 for each treatment group. **P<0.01 as compared to normal control group. ***P<0.001 as compared to normal control group. +++ P<0.001 as compared to hypercholesterolemic control group.

Table 5. Effects of fenugreek and pomegranate extract on serum glucose level and activities of ALT, AST and CK in normal and hypercholesterolemic guinea pigs.

Groups	Serum glucose(mg/dl)	Serum ALT(U/L)	Serum AST(U/L)	Serum CK (U/L)
Group I	78.75 ± 3.03	56.88 ± 0.64	70.88 ± 1.52	1012.00 ± 11.01
Group II	74.38 ± 1.99	53.25* ± 1.13	61.88** ± 2.13	995.90 ± 20.96
Group III	68.88** ± 2.94	53.37* ± 1.15	53.00*** ± 1.15	974.10 ± 15.00
Group IV	160.75*** ± 1.58	101.87*** ± 1.87	198.63*** ± 1.93	2317.10*** ± 16.45
Group V	133.63*** ± 1.61	79.13*** ± 1.04	160.62*** ± 2.09	1450.60*** ± 21.40
Group VI	121.37*** ± 1.51	68.88*** ± 1.08	131.50*** ± 1.64	1086.70*** ± 24.56

Values are expressed as means ± SE; n = 10 for each treatment group. **P<0.01 as compared to normal control group. ***P<0.001 as compared to normal control group. +++ P<0.001 as compared to hypercholesterolemic control group.

Table 6. Effects of fenugreek and pomegranate extract on hepatic cholesterol content and activity of catalase in normal and hypercholesterolemic guinea pigs.

Groups	Hepatic CAT(U/g protein)	Hepatic cholesterol content (mg/g liver tissue)
Group I	6.93±0.15	6.14±0.11
Group II	7.50*±0.21	5.90±0.09
Group III	7.69***±0.09	5.00***±0.10
Group IV	4.58***±0.14	8.92***±0.09
Group V	5.99***±0.122	7.37***±0.11
Group VI	6.92***±0.10	6.53***±0.34

Values are expressed as means ± SE; n = 10 for each treatment group. **P<0.01 as compared to normal control group. ***P<0.001 as compared to normal control group. +++ P<0.001 as compared to hypercholesterolemic control group.

DISCUSSION

The aim of this study was to investigate the effect of cholesterol feeding on the liver and cardiac functions tests also to investigate the hypolipidemic, cardioprotective and hepatoprotective effects of Iranian fenugreek and pomegranate peel extracts.

We choose guinea pig as a model for diet-induced hypercholesterolemia because, in addition to the possible advantages in cost, physiologically, guinea pigs present

the closest lipid metabolism to humans and they carry the majority of their serum cholesterol in LDL-like humans (Fernandez and Volek, 2006).

In the current study, Phytochemical screening of Iranian fenugreek and pomegranate peel extracts showed the presence of alkaloids, Phlobatannins, flavonoids, carbohydrates, tannins, coumarins and terpenoids and absence of anthraquinones in fenugreek extract only.

In this study, the high cholesterol diet is used which

consists of a normal diet supplemented with 2% cholesterol for induction of hypercholesterolemia according to (Ahmad-RausRaha et al., 2001).

Concerning body weight gain, significant increases observed in hypercholesterolemic guinea pigs when compared to the normal control rats. These findings were in agreement with those obtained by (Otunola et al., 2010; Amin et al., 2011). The increase in body weight of hypercholesterolemic guinea pigs might be due to the increase of feed and caloric intake by guinea pigs (Manal et al., 2013).

With regard to the effects of Pomegranate and fenugreek when orally given to hypercholesterolemic guinea pigs for 30 days on body weight gain, the results revealed that Pomegranate juice significantly reduction in body weight gain when compared to the hypercholesterolemic control group. These findings might be due to decreased appetite of guinea pigs and/or reduction of intestinal fat absorption or due to an inhibition of pancreatic lipase activity (McFarlin Brian et al., 2009).

In relation to the relative weight of liver, there was a significant increase in relative weight in the liver of hypercholesterolemic guinea pigs as compared to the normal group. These results might be due to the accumulation of fat in the liver cells leading to an increase in their weight. Our findings were in accordance with those obtained by (Matos et al., 2005).

Concerning relative weights of the liver of Pomegranate and fenugreek-treated hypercholesterolemic guinea pigs; the results showed that weight of liver decreased when compared to the hypercholesterolemic group. Our results were in agreement with those of (Chidambara et al., 2002).

Guinea pigs fed with high cholesterol diet showed increased levels of serum cholesterol, triglycerides and LDL-C levels compared to normal control groups. While, the serum level of the HDL-C decreased significantly as compared to normal control group. These results run parallel to those reported by (Sumathi and venkatakrishnanKamesh, 2012; Frantz et al., 2012) who demonstrated that lipid metabolism in rats fed high-fat diet (HFD) presented disorders and levels of serum TC and TG increased significantly, compared with the normal control group.

The high cholesterol level in serum may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids (Saikrishna et al., 2010). The metabolism of free and ester cholesterol are impaired in the liver and the rate of turnover was specifically decreased in liver tissues of hyperlipidemic guinea pigs. Increase in LDL levels is increase the risk of cardiovascular diseases (Saikrishna et al., 2010).

Oral administration of fenugreek and pomegranate extracts significantly decreases the levels of serum TC,

TG and LDL-C when compared to hypercholesterolemic control group. These results run parallel to those reported by (Fatma and Ahmed, 2009; Aviram et al., 2002; Noda et al., 2002) who concluded that Pomegranate is rich in polyphenols and demonstrates high capability in scavenging free radicals, reduced cholesterol accumulation and inhibiting LDL-c oxidation *in vitro* and *in vivo*. Also, these results run parallel to those reported by (Parveen et al., 2014; HamzaNawel et al., 2012) who concluded that fenugreek seed extract inhibits fat accumulation and ameliorates dyslipidemia in high fat diet-induced obese rats.

In view of liver function tests, hypercholesterolemia endangers vital organs like the liver. High-fat diets increase the retention of lipid in the liver, followed by hepatic steatosis and reduce hepatic functions (Suanarunsawat et al., 2011). This study results showed that a high cholesterol diet suppressed hepatic functions which were expressed as augmentation of serum levels of AST and ALT as compared to the normal control group. These data are consistent with (Hiren et al., 2014; Prasad, 2010; Saki et al., 2011) who showed that a high cholesterol diet moderately elevated serum levels of ALT and AST enzymes in rats.

Results of the present study showed that there were significant decreases in serum levels of AST and ALT enzymes in hypercholesterolemic guinea pigs orally given Pomegranate and fenugreek, compared to the hypercholesterolemic control group. The present results partially agreed with the results obtained by (Osman et al., 2012) who examined the antioxidant effect of Pomegranate peel and juice on diabetes mellitus induced by alloxan in Female Rats.

Hypercholesterolemic untreated guinea pigs showed a significant elevation in the serum glucose level as compared to normal control one. Our result in accordance with (Parveen et al., 2014; Hamza Nawel et al., 2012).

Hypercholesterolemia and obesity leads to the development of insulin resistance which in turn causes hyperglycemia (Guo et al., 2009).

Our result showed that the treatment of hypercholesterolemic guinea pigs with fenugreek extract made decreasing in serum glucose level. This is in agreement with the results of (Hamza Nawel et al., 2012).

In view of oxidative stress, the decrease in the activity of hepatic catalase enzyme in the hypercholesterolemic control group could be attributed to the excessive utilization of the enzyme in inactivating the free radicals generated due to the high cholesterol diet or insufficient availability of GSH. Our results are in agreement (Chtourou et al., 2015; Thiruchenduran et al., 2011) who studied the effect of high-fat diet on the liver and antioxidant enzyme systems.

Decreased CAT is accompanied with an enhanced risk of chronic diseases that induced by oxidative stress include neurodegenerative diseases, diabetes,

atherosclerosis, and postmenopausal osteoporosis (Oh et al., 2007).

Treatment of hypercholesterolemic guinea pigs with fenugreek and pomegranate peel extracts attenuated oxidative stress and improved guinea pigs antioxidant defense system in the liver. These results are in agreement with (Mansouri et al., 2014; Al-Moraie et al., 2013; Spencer et al., 2001) who revealed that the significant hydroxyl radical scavenging activity of the pomegranate and fenugreek is due to phenolic compounds present in its extracts.

CONCLUSIONS

In our study, the presence of phytoconstituents in pomegranate and fenugreek inhibit fat accumulation and ameliorate dyslipidemia in hypercholesterolemic guinea pigs, which is due to prevention of impaired lipid digestion and absorption, in addition to improvement in glucose and lipid metabolism, and increased antioxidant defense.

Conflicts of Interest

The authors declare no conflict of interest.

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