



Research Article

**HYPOLIPIDAEMIC ACTIVITY OF *Punica granatum* FLOWERS ON HYDROGENATED
GROUNDNUT OIL INDUCED HYPERCHOLESTEROLEMIC RATS**

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Abstract: The present investigation was undertaken to evaluate the hypolipidemic activity of aqueous extract of *Punica granatum* flowers in hydrogenated groundnut oil induced hypercholesterolemic rats high fat diet produced a significant increase in total cholesterol, LDL, PL, Triglycerides and decrease in HDL. Reduction in the activity of lipo protein lipase was observed. High fat diet also decreased the levels of SOD, CAT and reduced glutathione with associated increase in lipid peroxidation. Treatment with *Punica granatum* flowers (100 mg./kg b.wt./day) altered the deranged metabolic profile and was effective in producing hypolipidemia.

Keywords: Lipid profile, antioxidant, *Punica granatum* flowers, high fat diet

INTRODUCTION

Coronary arterial diseases are responsible for more deaths than all other associated causes combined¹. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis associated conditions, such as Coronary Heart Disease (CHD), Ischemic cerebrovascular disease and peripheral vascular disease.² Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease.³ Reduction in serum cholesterol levels reduces risk for CHD¹. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease.⁴

Mortality rate due to cardiovascular disease has increased several folds in most developed and underdeveloped countries of the world. These cardiac ailments are directly related to hyperlipidemia⁵. Any abrupt change in blood lipid parameters such as total lipids, triglycerides and cholesterol and system may lead to atherosclerosis and arteriosclerosis. During the last two decades, both retrospective and prospective studies have shown strong correlation between levels of circulation lipids and mortality rates from coronary atherosclerotic heart disease.⁶

Cardiovascular diseases are the most common cause of death worldwide. Abnormalities in plasma lipoprotein and derangement in lipid metabolism rank as the most firmly established and best understood risk factor for atherosclerosis and cardiovascular complication.⁷ Approximately 10% of the global population is affected by dyslipidemia.⁸

Hyperlipidemia, is a major risk factor in the initiation and progression of atherosclerotic lesions,

conditions such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease. This leads to high mortality and morbidity rate in developed countries. This is mainly due to altered lipoprotein metabolism. Hyperlipidemia also has an indirect role by stimulating the production of oxygen free radicals from polymorphonuclear leukocytes and monocytes. It is considered as one of the five leading causes of the death in the world.⁹ High lipid content (hypolipidaemia) leads to many life threatening conditions such as atherosclerosis, myocardial infarction, ischemic heart disease, stroke and other vascular disease. Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia is a major risk factor for the development of cardiovascular disease. The search for new drugs able to reduce and/or to regulate serum cholesterol and triacylglycerol levels has gained importance over the years, resulting in numerous reports on significant activities of natural agents¹⁰

Punica granatum Linn. (Punicaceae) Shrub to small tree up to 6 m high leaves mostly opposite, short – petiolate, blades oblong – elliptical up to 8 cm long flower. Showy and up to 6 broad, bisexual, 5- 8 petals, reddish and up to 2.5 cm long. This flower was also used for the treatment of injuries from falls and grey hair of young man in the traditional Chinese medicine¹¹ Decoction of the flower is used in case of oral and throat inflammations. In the unani medicinal system, the flower parts serve as a remedy for diabetes mellitus either as a single drug or in polyherbal formulations¹² The ripe fruit is tonic, astringent, laxative, diuretic, used in brain diseases and chest troubles. It is useful in the treatment of dyspepsia and leprosy.¹³

MATERIALS AND METHODS
Preliminary Phytochemical Screening

In order to detect the various constituents present in the aqueous extract of *Punica granatum* flowers it was subjected to the tests as per standard method.

Animals

Male wistar rats weighing about 150-200 g were procured and maintained to laboratory condition. The animals were fed with standard pellet diet (Kamathenu Agencies, Bangalore, India) and clean water *ad libitum*, and routinely housed in controlled conditions with temperature of 25-26°C, relative humidity of 60-80% and 12-h light/dark cycle and animals were cared for in accordance with the principles and guidelines of Indian National Law on Animal care and use. The animals were acclimatized for 2 weeks before experimentation.

Experimental Design

Four groups of rats, six in each received the following treatment schedule.

- Group I** : Normal diet and water
Group II : Cholesterol 1% + Hydrogenated Ground nut oil (orally)
Group III : Cholesterol 1% + HGNO and aqueous extract of *punica granatum* flowers (100mg/kg Bw/day)
Group IV : Cholesterol 1% + HGNO and standard drug (Atorvastatin 3mg/kg Bw/day)

Collection of Blood

The experiment was continued for 30 days and the animals were sacrificed on the 31st day by cervical decapitation. The blood was collected and liver were removed quickly. The latter were washed with ice-cold saline and stored at 20°C for further analysis.

Liver was homogenized (10% W/V) in cold 100 mm phosphate buffer, pH 7.2 and used for the assay of lipoprotein lipase (LPL) activity.

Liver Lipid Extraction

Lipids were extracted from tissues by the method of Folch *et al.* using chloroform – methanol mixture (2:1 v/v). A known weight of tissue was homogenized in 7.0 ml of chloroform – methanol using potter Elvehjam homogenizer. The contents were filtered into a previously weighed side arm flask; residue on the filter paper was scrapped off and homogenized with 14 ml of chloroform – methanol mixture. This was again filtered into the side arm flask and the residue was successively homogenized in chloroform – methanol (2:1 v/v) and each time this extract was filtered.

Statistical Analysis

All the grouped data were evaluated statistically and significance of changes was determined using one-way analysis of variance followed by Duncan's multiple range test (Duncan 1957) using SPSS 11.0 for windows. Results are presented as mean \pm SD among values of 6 rats from each group statistical significance was set at P<0.05.

Biochemical Analysis

The serum and liver were assayed for total cholesterol, triglycerides, phospholipids, high density lipoprotein (HDL), low density lipoprotein (LDL), lipoprotein lipase (LPL). The serum cholesterol levels were determined by Zak's method. The triglyceride, phospholipids, HDL, LDL and LPL, LPO, SOD, CAT, GSH was calculated by using standard method.

RESULTS AND DISCUSSION

Phytochemicals are secondary metabolites is one more parts of the medicinal plants. These have the ability to produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, flavonoids, tannins, steroids, saponins, glycosides, carbohydrate, protein and amino acid.

Table 1 : Effect of *punica granatum* flowers on liver cholesterol, Triglycerides, phospholipids, LDL, HDL, LPL, SOD, CAT, GSH, LPO.

Groups	TC	TG	PL	LDL	HDL	LPL	SOD	CAT	GSH	LPO
	(mg/g)	(mg/g)	(mg/g)	(mg/dL)	(mg/dL)	(μ moles of free fatty acid released/h/mg protein)	(unit / min/mg protein)	(μ m of H ₂ O ₂ /min/mg protein)	(μ m of GSH/mg protein)	(μ m of MDA/mg protein)
	liver	liver	liver	Liver	Liver	liver	Liver	Liver	Liver	Liver
Group I	5.093 \pm 0.78 ^a	5.12 \pm 0.81 ^a	21.21 \pm 0.7 ^a	82.93 \pm 3.01 ^a	34.56 \pm 2.26 ^a	83.01 \pm 0.38 ^a	3.36 \pm 0.51 ^a	33.45 \pm 2.6 ^a	239.06 \pm 2.99 ^a	3.83 \pm 0.14 ^a
Group II	9.02 \pm 0.40 ^b	8.18 \pm 0.73 ^b	37.19 \pm 1.30 ^b	136.13 \pm 0.830 ^b	25.33 \pm 1.22 ^b	52.51 \pm 1.34 ^b	2.26 \pm 0.20 ^b	23.34 \pm 0.51 ^b	214.45 \pm 2.01 ^b	7.13 \pm 0.5 ^b
Group III	7.43 \pm 1.40 ^a	6.07 \pm 0.39 ^a	30.66 \pm 1.60 ^a	94.33 \pm 7.56 ^a	33.63 \pm 2.86 ^a	71.52 \pm 4.08 ^a	2.8 \pm 0.2 ^a	27.4 \pm 1.00 ^a	238.3 \pm 1.45 ^a	4.82 \pm 0.52 ^a
Group IV	7.1 \pm 1 ^a	5.1 \pm 0.36 ^a	26.1 \pm 0.76 ^a	87 \pm 1.24 ^a	34.3 \pm 1.80 ^a	73.18 \pm 4.49 ^a	2.7 \pm 0.1 ^a	30.5 \pm 0.21 ^a	244.9 \pm 2.24 ^a	4.28 \pm 0.33 ^a

Results were expressed as mean \pm S.E. The data were satisfically analysed by one way analysis of variance (ANOVA) and p values <0.05 were considered significant.

Phytochemical screening of fresh *punica granatum* flowers plant compounds flavonoids steroids, are reported to modulate lipid levels.¹⁴ The presence of flavonoids and steroids in *punica granatum* flowers might have contributed in lipid lowering effect to *punica granatum* flowers in similar manner. Tannins are reported to increase in activity of the endothelium bound lipoprotein lipase activity, which hydrolyzes triglycerides as reported.¹⁵ The presence of tannins in *punica granatum* flowers might be involved in triglyceride lowering activity but this need to be invested by further studies. Feeding HFD caused hyperlipidemia and altered the lipid component of plasma lipoproteins. Simultaneous *Punica granatum* flowers supplementation significantly lowered the serum levels of total cholesterol, LDL, TG, and PL and elevated HDL levels. Moreover *Punica granatum* flowers supplementation reduced the accumulation of total cholesterol, TG and PL in the liver and other peripheral tissues emphasizing its potent hypolipidemic properties. Simultaneous administration of animals with *Punica granatum* flowers and HFD caused increase in the level of LPL .

Punica granatum flowers also resulted in significant reduction the oxidative stress by lowering the serum and LPO. Elevated SOD, CAT and GSH activities, serum, levels on *Punica granatum* flowers supplementation may be due to elimination of the reactive toxic intermediates formed as a consequence of HFD induced oxidative stress. Similarly *Punica granatum* flowers supplementation significantly reduced the levels of LPO and elevated the activities of the antioxidant enzymes SOD, CAT, and GSH in liver.

The present study shows that *Punica granatum* flowers supplementation to HFD –fed rats has a profound effect on the levels of serum and liver, lipids and lipoproteins. Biological membranes are composed of PL and proteins. The lipid bilayer is highly impermeable to most polar molecules and ions but they are quite fluid in nature and interact with membrane proteins. The degree of fluidity of the plasma membrane was generally recognized to be important for appropriate functioning of cells.^{13,16} Abnormalities in membrane fluidity can give rise to pathological conditions. The dietary content of cholesterol, a component of cell membranes, generally parallels that of fat and could also influence the membrane fluidity and thereby, its function. Feeding HFD as in our study may also contribute to changes in membrane cholesterol composition and function. Moreover, intervention studies showed that dietary lipids are significant determinants of plasma cholesterol concentration.¹⁶ and evidence from animal and human studies have documented the hypercholesterolemia effect of dietary saturated fatty acids Thus, feeding a diet rich in highly saturated fatty acids and cholesterol is known to cause hyperlipidemia in most animal and human studies. The high concentration of plasma cholesterol observed in HFD – fed rats as compared to the control rats in the present study agree with our previous findings¹² also showed that increased intake of dietary cholesterol and hydrogenated groundnut oil significantly elevates serum cholesterol levels.

LPL plays an important role in the metabolism of plasma lipoproteins and thus, the transport of lipids to peripheral tissues. Absence or low LPL activity causes marked lipemia and triglyceridemia.¹⁷ The activities of both Serum LPL are significantly lowered in HFD–fed rats. These results correlate with previous findings, which showed significantly reduced LPL activity on feeding a high-fat, high –cholesterol diet.¹⁵ A number of studies reported that plasma LPL, directly or indirectly, may promote or protect against atherosclerosis.⁸ reported that increased LPL activity is antiatherogenic, and showed that a decrease in LPL activity is atherogenic. Significantly lowered LPL activity in HFD–fed rats can cause accumulation of cholesterol and VLDL. *Punica granatum* flowers administration resulted in the optimum activity of plasma LPL. Thus comparatively low levels of VLDL and LDL found in the *Punica granatum* flowers treated animals may be correlated with the optimal activity of plasma LPL observed in these animals.

Lipid peroxidation is a complex process that can occur in biological membrane the are made up of molecular oxygen – reactant polyunsaturated fatty acid, leading to the production of lipid hydroperoxides and their metabolites. The lipid peroxidation can occur in various pathological conditions including atherosclerosis, rheumatoid, arthritis, angina, cancer and irritable bowel diseases (IBA).

GSH is a multifunctional intracellular non-enzymic antioxidant which scavenge hydroxyl radical and singlet oxygen directly, and also detoxifies hydrogen peroxide and other lipid peroxide radicals by the catalytic action of offering protection to the leads from the deleterious effects of reactive oxygen species. Our results show that *Punica granatum* flowers has a definite and important role of play in preserving erythrocyte membrane structure and function by suppressing oxidative stress, enhancing antioxidant profile, and thus countering the hypercholesterolemia included alterations in cell functions.

CONCLUSION

The results from our study reveals the lipid lowering efficacy of *Punica granatum* flowers which act on a potential modulation of cellular lipid homeostasis and antioxidant status and may well form the basis for a model to develop an effective antihyperlipidemic drug that possess antioxidant capacity.

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