COMPARISON OF ANTIHYPERLIPIDAEMIC ACTIVITY OF EUGENIA JAMBOLANA FRUIT WITH PUNICA GRANATUM FRUIT IN DIET INDUCED HYPERLIPIDAEMIC RATS

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ABSTRACT

Objective: To compare the antihyperlipidemic effects of Eugenia Jambolana fruit pulp with Punica Granatum fruit in diet induced hyperlipidaemic rats at the same dose level.

Methods: An experimental randomized control study was conducted on seventy five male albino rats over a period of 14 weeks in University of Health Sciences Lahore. They were divided into five groups labelled A, B, C, D and E with fifteen rats in each group. Group A was kept as normal control, groups B, C, D and E were given hyperlipidaemic diet for six weeks. In group B no further intervention was done, group C and D were given ethanolic extract of Eugenia Jambolana and Punica Granatum respectively for eight weeks. Group E was given combination of both for same duration. Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), lowdensity lipoprotein cholesterol (LDL-c) and triglycerides (TG) were measured at zero, six and fourteen weeks.

Results: At fourteenth week significant reductions in TC, LDL-c and TG and a rise in HDL-c was observed in interventional groups C, D and E as compared to experimental hyperlipidaemic control group B (p <0.05). There was no significant difference at baseline (zero weeks) serum TC, HDL-c, LDL-c and TG of groups A, B, C, D and E; $p > 0.57$, $p > 0.22$, $p > 0.56$, $p > 0.76$, respectively. On sixth week, there was no significant difference between groups B, C, D and E ($p > 0.05$). However, 15 rats of group A had significant lower levels of cholesterol, high density lipoproteins, low density lipoproteins and triglycerides when compared to 60 rats of groups B, C, D and E (p<0.05).

Conclusion: In male albino rats combination of ethanolic extracts of Eugenia Jambolana and Punica Granatum fruit pulps was most effective in lowering serum total cholesterol and triglycerides while decrease in low density lipoprotein cholesterol and rise in high density lipoprotein cholesterol was same as the extracts given alone.

Keywords: Eugenia jambolana, Punica granatum, Rats, Traditional medicine.

INTRODUCTION

Natural products are the source of synthetic and traditional herbal medicine[1]. The therapeutic efficacy of many indigenous plants has been described by traditional herbal medicinal practitioners for various ailments[2]. Consumption of plant foods is also associated with lower risk of CVD and hypertension[3]. Hyperlipidemia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases[4]. Fifty percent of mortality in developed countries and twenty five percent deaths in the developing world are due to the diseases related to atherosclerosis with dyslipidaemias being its root cause[5]. Flavanoids extracted from gingko, soybean, and some other plants have been reported as the antioxidants and could be beneficial to hyperlipidemic patients[4].

The pomegranate, Punica granatum, an ancient, mystical, and highly distinctive fruit, is the predominant member of Punicaceae family. The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease and diabetes[1]. The extract of flower of Punica Granatum has shown to decrease cardiac triglycerides content along with reduction in plasma total cholesterol and triglycerides[6].

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Eugenia Jambolana (E.Jambolana) commonly called as Jamun has been used for the treatment of diabetes mellitus and dyslipidaemias[7]. In our previous study we have observed the comparison between antihyperlipidaemic effects of flavonoids rich fruits E jambolana with widely used antihyperlipidemic drug Simvastatin in experimental hyperlipidemic rats and the fruit extract was found to be as effective as the HMG CoA reductase inhibitor simvastatin[8].

We designed this study to observe the comparison between hypolipidaemic effects of flavanoids rich fruits E jambolana and Punica Granatum in experimental hyperlipidemic rats and also took this opportunity to observe their combined effect at the same dose level.

MATERIAL AND METHODS

This experimental randomized control study was started after taking approval from the Ethic committee on animal experiments, University of Health Sciences (UHS) Lahore. A total of 75 male albino rats of Wistar strain, 4-5 months old and weighing 180-220 gms were obtained from National Institute of Health, Islamabad. They were divided into 5 groups of 15 rats each by randomly generated computer numbers[9]. The cages were labelled as A, B, C, D and E and were kept in experimental animal house of UHS Lahore. The conventional light regimen with light and dark cycles for 12 hours at room temperature of 22 ± 10C and humidity 50 ± 10% was maintained throughout the experiment[10]. For initial one week, all rats were fed on standard rat diet. Body weight of animals was recorded twice weekly, to calculate the dose of drugs.

Experiment:

Group A (NC): Served as normal control, fed on regular rat diet till end of study.

Group B (EC): This group served as experimental control and was treated for initial six weeks with 2% cholesterol diet. After baseline lipid profile at the end of six weeks, this group was fed on regular rat diet till the end of study.

Group C (EG): This group was also fed on 2% cholesterol diet for initial six weeks, then was given E.Jambolana fruit pulp extract, once daily.

Group D (EG): This group was also fed on 2% cholesterol diet for initial six weeks, then was given Punica Granatum fruit extract for next eight weeks.

Group E (EG): This group was also fed on 2% cholesterol diet for initial six weeks, then was given both E Jambolana fruit pulp extract and Punica Granatum fruit extract for eight weeks.

All the groups were fed water ad libitum. Fruit extract of E Jambolana and Punica Granatum was given orally as a single daily dose of 200 mg/kg/day each[8].

Preparation of 2% cholesterol diet: Two grams cholesterol, Extra pure, Scharlau (Spain) and 500 milligram Cholic acid, minimum 98%, Sigma-Aldrich (Germany) was thoroughly mixed and mashed with 97.5 grams of commercial standard rat chow (18% protein; Global 2018, Harlan Teklad, Madison, WI)[11] and was given in the form of pellets.

Preparation of Ethanolic Extract of Eugenia jambolana Fruit: Two kg of E Jambolana fruit was purchased from local fruit market of Lahore.The identity was established with the help of a qualified botanist of Hagler Bailley Pakistan (Pvt.) Ltd. using taxonomic rules. The pulp of fruit was separated from seeds and was dipped in 1 litre of Absolute Ethanol, Merck (Germany) in a stopped conicalflask for 48 hours at room temperature with occasional stirring. The dark purple solvent obtained was then filtered with the help of a filter funnel. The ethanol infiltrate was evaporated by putting it in a rotary vacuum evaporator, Heidolph, Laborota 4002, at 45^0C. The extract was then kept in freeze drier, LABCONCO, Frezone 2.5, at -40^0C temperature under vacuum for 6 hours so that the moisture was completely removed. The extract obtained was weighed and found to be 100 grams from 1 kg of fruit pulp. It was kept in a tightly closed bottle, protected from light in the refrigerator at 2 to 8^0C to be used throughout the experiment[12].

Preparation of ethanolic extract of Punica Granatum fruit: Punica Granatum fruit was purchased from local fruit market of Lahore. The identity was established with the help of a qualified botanist of Hagler Bailley Pakistan (Pvt.) Ltd. using taxonomic rules. One kg grains of fruit were separated from the peel, air-dried away from direct sunlight, powdered with an electrical grinder and then soaked in 1 litre of absolute ethanol, Merck (Germany) in a stopped conical flask for 48 hours at room temperature with occasional stirring. The dark red solvent obtained was then filtered with the help of a filter funnel. The ethanol infiltrate was evaporated by putting it in a rotary vacuum evaporator, Heidolph, Laborota 4002, at 45^{0}C. The extract was then kept in freeze drier, LABCONCO, Frezone 2.5, at -40^{0}C temperature under vacuum for 6 hours so that the moisture was completely removed. The extract obtained was weighed and found to be 100 grams from 1 kg of fruit pulp. It was kept in a tightly closed bottle, protected from light in the refrigerator at 2 to 8^{0}C to be used throughout the experiment[12].

Collection of blood sample: Blood sampling was performed through cardiac puncture. On day 1 of week one, two rats were randomly picked from all groups and were sacrificed for baseline readings.

On day 1 of week six three rats were randomly picked from all groups and sacrificed to confirm hyperlipidaemia in groups B, C, D and E. Rats of group A served as normal control. On last day of week fourteen at the end of study all the remaining rats were sacrificed to observe the effects of drugs given for 8 weeks. Blood was centrifuged at 3000 rev/min for 15 min and serum was separated[13]. Serum total cholesterol, HDL cholesterol, LDL cholesterol and TG were measured using Randox laboratory kits in semi automatic clinical chemistry analyser.

Table-1: Effect of Eugenia Jambolana and Punica Granatum on lipid profile of rats at zero, 6 and 14 weeks of experiment*.

Groups	Weeks	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
A	0	58.50 ± 1.90	93.30 ± 3.10	19.90 ± 1.30	19.94 ± 4.58
	6	60.00 ± 4.06	87.26 ± 2.03	21.23 ± 1.41	21.31 ± 3.06
	14	65.95 ± 1.51	94.94 ± 1.47	21.95 ± 0.73	24.5 ± 1.54
B	0	57.45 ± 1.45	94.80 ± 5.60	21.50 ± 2.00	16.99 ± 4.57
	6	183.0 ± 4.99	188.66 ± 5.36	16.43 ± 2.13	128.83 ±
	14	153.2 ± 1.48	165.58 ± 1.54	17.15 ± 0.5	4.89
					102.98 ± 1.3
C	0	68.60 ± 3.20	90.00 + 1.60	27.15 ± 2.95	23.45 ± 6.47
	6	188.56 ± 9.99	194.76 ± 11.17	12.76 ± 1.29	136.8 ± 7.78
	14	93.38 ± 2.6	108.5 ± 3.52	45.25 ± 1.4	26.4 ± 2.24
D	0	61.30 ± 2.30	93.25 ± 5.75	23.40 ± 3.80	19.25 ± 0.35
	6	193.50 ± 4.53	202.00 ± 4.93	16.40 ± 0.51	136.70 ±
	14	92.54 ± 3.5	115.2 ± 4.12	40.86 ± 2.01	4.15
					28.6 ± 8.87
E	0	68.20 ± 6.2	106.50 ± 3.50	25.00 ± 1.00	21.90 ± 5.9
	6	183.23 ± 13.80	188.63 ±11.31	17.86 ± 1.79	127.63 ±

RESULTS

Table-1 shows the results of lipid profile of albino rats at the beginning of experiment and after giving them hyperlipidaemic diet respectively. Table contains results after intervention with ethanolic extract of E. Jambolana, Punica Granatum and combination of

both. Results in table 1 show that there was no significant difference in lipid profile parameters among groups A, B, C, D and E (p>0.05). Animals in group B, C, D and E fed on hyperlipidaemic diet had significantly higher lipid profile parameters as compared to normal control group A (p<0.05) (Table-1).

Table-1 shows the effects of treatment with ethanolic extract of Eugenia jambolana and Punica Granatum fruit pulps on serum lipid profile in albino rats. In hyperlipidaemic animals after administration of 2% cholesterol diet given over a period of six weeks there was an overall increase in serum lipids, except HDL cholesterol which was decreased as compared to normal control. After eight weeks of administration of ethanolic extract of Eugenia Jambolana, Punica Granatum and combination of both to groups C, D and E respectively, lipid profile parameters were found to be significantly lowered (p<0.05) in groups C, D and E as compared to group B. However, in comparison with group A, values in these groups were found to be near normal, showing reduction in total cholesterol, LDL cholesterol and TG (p<0.05) and increase in HDL cholesterol (p<0.05). Lipid profile values were

Table-2: Comparison of groups for mean change in lipid profile.

Multiple comparisons

Tukey HSD

Dependent Variable	(I) Time (Weeks)	(J) Time (Weeks)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total cholesterol (mg/dl)	0	6	-98.8500*	14.3602	.000	-133.216	-64.484
		14	-33.7360*	12.1851	.019	-62.896	-4.576
	6	0	98.8500*	14.3602	.000	64.484	133.216
		14	65.1140*	10.3553	.000	40.332	89.896
	14	0	33.7360*	12.1851	.019	4.576	62.896
		6	-65.1140*	10.3553	.000	-89.896	-40.332
Triglycerides (mg/dl)	0	6	-76.6967*	12.7275	.000	-107.155	-46.238
		14	-19.0740	10.7996	.188	-44.919	6.771
	6	0	76.6967*	12.7275	.000	46.238	107.155
		14	57.6227*	9.1779	.000	35.659	79.587
	14	0	19.0740	10.7996	.188	-6.771	44.919
		6	-57.6227*	9.1779	.000	-79.587	-35.659
HDL-c (mg/dl)	0	6	6.4500	4.1958	.280	-3.591	16.491
		14	-9.9800*	3.5602	.018	-18.500	-1.460
	6	0	-6.4500	4.1958	.280	-16.491	3.591
		14	-16.4300*	3.0256	.000	-23.671	-9.189
	14	0	9.9800*	3.5602	.018	1.460	18.500
		6	16.4300*	3.0256	.000	9.189	23.671
LDL-c (mg/dl)	0	6	-89.95667*	13.95734	.000	-123.3583	-56.5550
		14	-19.85080	11.84320	.221	-48.1930	8.4914
	6	0	89.95667*	13.95734	.000	56.5550	123.3583
		14	70.10587*	10.06478	.000	46.0196	94.1921
	14	0	19.85080	11.84320	.221	-8.4914	48.1930
		6	-70.10587*	10.06478	.000	-94.1921	-46.0196

* The mean difference is significant at the 0.05 level.

56 Post-hoc comparison at zero, six and fourteen weeks

found to be significantly higher (p<0.05) in group B as compared to A. Inter group comparison of C, D and E showed group E to possess significantly higher activity against serum total cholesterol

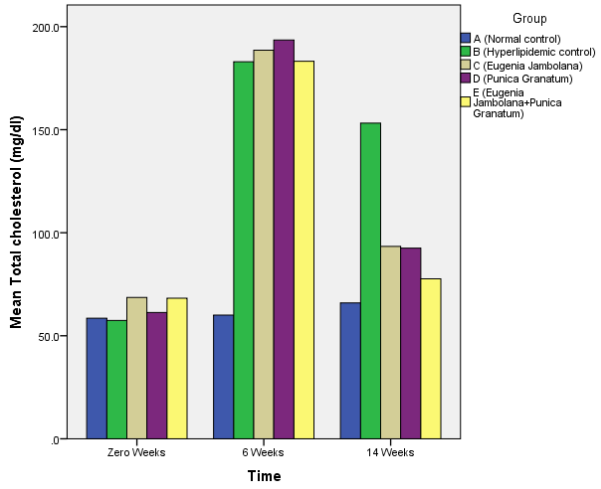

Figure-1: Comparison of mean total cholesterol level at zero, six and fourteen weeks.

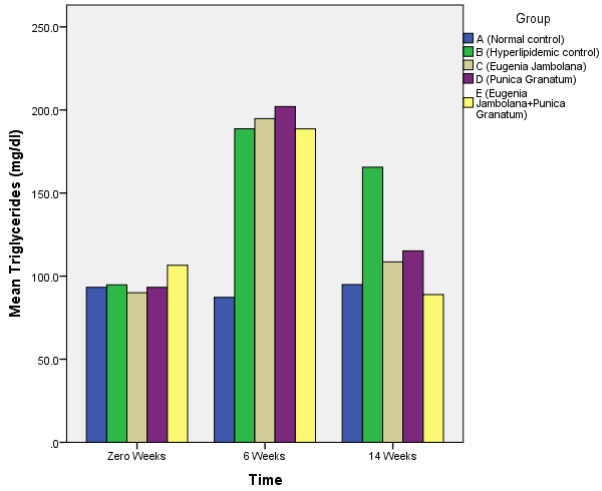

Figure-2: Comparison of mean Triglycerides at zero, six and fourteen weeks.

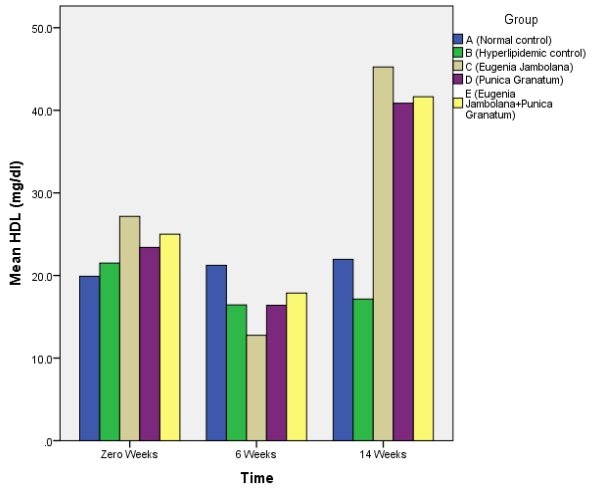

Figure-3: Comparison of mean HDL-c at zero, six and fourteen weeks.

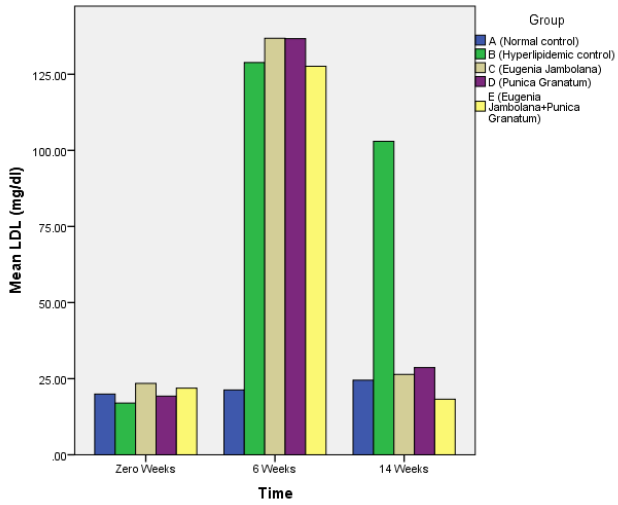

Figure-4: Comparison of mean LDL-c at zero, six and fourteen weeks.

and TG when compared with groups C and D (p<0.05) while effects on serum HDL cholesterol and LDL cholesterol were found to be same in groups C, D and E (p>0.05).

DISCUSSION

The results of this experimental study show that ethanolic extracts of E Jambolana and Punica Granatum fruit pulps cause significant reductions in serum LDL cholesterol, TG, total cholesterol and increase in HDL cholesterol in 2% cholesterol fed hyperlipidaemic rats. According to a recent study prolonged exposure to reduce LDL-c beginning early in life is associated with a substantially greater reduction in the risk of coronary heart disease (CHD) than the current practice of lowering LDL-c beginning later in life[14]. Another study elucidates the facts that phytotherapy is safe and less toxic[15]. So, for the management of the diseases which require life long therapy fruit extracts can be a good option as in most Asian countries where the use of folk

medicine is prevalent, the search for traditional cures is a common practice[13].

The antihyperlipidemic activity of Eugenia Jambolana has been studied and was found to be as effective as Simvastatins (HMGCoA reductase inhibitors) in experimental rats[8]. This study is unique because a comparison is made between the antihyperlipidemic activity of Eugenia Jambolana and Punica Granatum which is also a flavonoid rich fruit. The purpose was to compare their effects at the same dose level. We divided the experimental animals into five groups feeding 2% cholesterol rich diet to groups B, C, D and E. This converted them into hyperlipidaemic rats. Cessation of hyperlipidaemic diet after six weeks showed that group B remained hyperlipidaemic till the end of the study. This provided an opportunity to do pharmacological interventions in group C, D and E. Interestingly groups C and D showed equal response to E. Jambolana and Punica Granatum. Results in group E which were given a combination of E. Tambolana and Punica Granatum were intriguing. They showed a better response against serum total cholesterol and TG. However, fall in LDL cholesterol and rise in HDL cholesterol were same as in group C and D. This finding suggests that the fruit extract of E Jambolana and Punica Granatum may be having different mechanism of action which needs to be explored further. We assume that antioxidant chemicals of E.Jambolanaand Punica Granatum cause reduction in lipid profile.

Eugenia Jambolana is a rich source of anthocyanins and flavonoids. The flavonoids include Quercetin, Myrcetin and Kaempherol[16]. Punica Granatum fruit extract contains the components of both peel and seeds, it was found to contain the highest number of bioactive compounds. The fruit extract is a rich source of flavonoids, tannins, vitamin C and glycosides. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds have been found to be present in the Punica Granatum extract, it might be responsible for the potent antioxidant capacity of this fruit[17].

Flavonids have also been shown to exhibit a series of biological effects among which stand out the inhibition of lipid peroxidation and platelet aggregation which contributes to reduced thrombotic tendencies[16] and also cholesterol lowering effects by alteration in cholesterol absorption, triglycerides assembly and processing of lipoproteins in plasma. Multiple functions of dietary polyphenols help in reduction of coronary heart disease risk by improving plasma lipid profile[17]. Flavonoids

ANOVA

		Sum of squares	df	Mean square	F	Sig. (*p*-value)
Total cholesterol (mg/dl)	Between groups	69528.426	2	34764.213	28.097	.000
	Within groups	89085.389	72	1237.297		
	Total	158613.815	74			
Triglycerides (mg/dl)	Between groups	47393.924	2	23696.962	24.381	.000
	Within groups	69979.098	72	971.932		
	Total	117373.022	74			
HDL-c (mg/dl)	Between groups	3446.657	2	1723.328	16.315	.000
	Within groups	7605.150	72	105.627		
	Total	11051.807	74			
LDL-c (mg/dl)	Between groups	67959.758	2	33979.879	29.071	.000
	Within groups	84156.763	72	1168.844		
	Total	152116.521	74			

*Value of $p<0.05$ is significant

inhibit hydroxy methyl glutryl reductase (key enzyme involved in cholesterol biosynthesis) also it activates the enzyme 7 a-hydroxylase which accelerates cholesterol metabolism[18].

The aim was to evaluate which of the two fruit extracts has a better effect on lipid profile at the same dose level. We also had an opportunity to study the effect of combination therapy of Eugenia Jambolana and Punica Granatum fruit extracts to observe if there is an increase or decrease in response. As shown by our results the combination therapy had significantly more total cholesterol and triglycerides lowering effects than the fruit extracts alone rather the TC and TG levels were brought to near normal in group E as compared to groups C and D.

CONCLUSION

Medicinal plants have low cost, they are less toxic and free from side effects[19] therefore, can be started in early years of life to prevent the development of hyperlipidemias, atherosclerosis and CHD. For future studies we can perform a bioassay guided isolation of bioactive flavonoids from both fruit extracts[20]. Their structures can be elucidated and antihyperlipidemic evaluation can be performed.

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