

Antihyperlipidaemic effects of *Eugenia Jambolana* fruit in diet induced hyperlipidaemic rats

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Abstract

Objective: To evaluate the antihyperlipidaemic effects of *Eugenia jambolana* fruit pulp in diet induced hyperlipidaemic rats and to compare them with Simvastatin.

Methods: An experimental randomised control study was conducted on seventy five male albino rats, 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 group D were given ethanolic extract of *Eugenia Jambolana* and Simvastatin respectively for eight weeks. Group E was given combination of both for same duration. Serum total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides were measured at zero, six and fourteen weeks.

Results: At fourteenth week significant reductions in total cholesterol, low density lipoprotein cholesterol and triglycerides and a rise in high density lipoprotein cholesterol 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 total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides of groups A, B, C, D & E; $p > 0.24$, $p > 0.37$, $p > 0.89$, $p > 0.2$, 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 ethanolic extract of *Eugenia Jambolana* fruit pulp was as effective as simvastatin in lowering serum total cholesterol, low density lipoprotein cholesterol and triglycerides and increasing high density lipoprotein cholesterol.

Keywords: Traditional medicine, rats, simvastatin (JPMA 61:433; 2011).

Introduction

Cardiovascular diseases are currently the leading cause of death in the West. Both genetic disorders and lifestyle (sedentary behaviour and diet high in calories, saturated fat and cholesterol) contribute to dyslipidaemias seen in developed countries around the world.¹

Dyslipidaemia is characterized by elevated low density lipoprotein cholesterol (LDL cholesterol), high triglycerides (TG) and low high density lipoprotein cholesterol (HDL cholesterol) level. Epidemiological, clinical, genetic and experimental studies indicate that high serum levels of TG, LDL cholesterol and low levels of HDL cholesterol are associated with atherosclerosis causing increased risk of vascular strokes and coronary heart disease.² 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.³

Traditional medical knowledge can serve as powerful search engine and offer a more holistic approach to drug

design and myriad possible targets for scientific analysis which will facilitate intentional, focused and safe natural product drug discovery and help to rediscover the drug discovery process.⁴ The use of natural products for treatment of various ailments has grown faster over the past few years which is undoubtedly driven by the belief that they are relatively safe, easily available and affordable to masses.⁵

In traditional medicine of the subcontinent, *Eugenia Jambolana* (*E. Jambolana*) commonly called as Jamun has been used for the treatment of Diabetes Mellitus and dyslipidaemias. In controlled experiments ethanolic and aqueous extracts of seeds administered orally to animals and human adults at various dose levels were found to have hypoglycaemic activity and lipid lowering effect.⁶ However, leaves of *E. jambolana* produced none of these effects.

No data is available on the antihyperlipidaemic effects of fruit extract of *E. Jambolana* in animals.

We designed this study to find out the hypolipidaemic effects of *E. jambolana* in experimental rats and also took this opportunity to compare it with simvastatin.

Material and Methods

This experimental randomised control study was started after taking approval from The Ethical 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 180-220gms were obtained from National Institute of Health, Islamabad. They were divided into 5 groups of 15 rats each by randomly generated computer numbers.⁷ 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 $22\pm 1^{\circ}\text{C}$ and humidity $50\pm 10\%$ was maintained throughout the experiment.⁸ 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 (normal control): Served as normal control, fed on regular rat diet till end of study.

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

Group C (experimental group): 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 (experimental group): This group was also fed on 2% cholesterol diet for initial six weeks, then was given Simvastatin for next eight weeks.

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

All the groups were fed water ad libitum. Simvastatin and Fruit extract of E. Jambolana was given orally as a single daily dose of 1.0mg/kg/day and 200mg/kg/day respectively.

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 rat diet and was given 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 Ethanol, Absolute, Merck (Germany) in a stopped conical

flask 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°C . The extract was then kept in freeze drier, LABCONCO, Frezone 2.5, at -40°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°C to be used throughout the experiment.⁹

Simvastatin: Simvastatin was purchased as Simvastatin pure 98%-101%, BIOCON Limited, India

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 3000rev/min for 15min and serum was separated.¹⁰ Serum total cholesterol, HDL cholesterol, LDL cholesterol and TG were measured using Randox laboratory kits in semi automatic clinical chemistry analyser.

Statistical analysis: We estimated that 15 rats had to be placed in every group to yield a statistical power of 80% with the p value of less than 0.05. These calculations assumed a standard deviation of 10mg for the mean lipid profile parameters values and calculations were performed with NQuery software. Results were expressed as Mean \pm S.E.M. The difference between groups were assessed by analysis of variance followed by Post hoc tukey test. Statistical significance was chosen as $p < 0.05$. Statistical analysis was performed using SPSS.

Results

Table-1 and 2 show the results of lipid profile of albino rats at the beginning of experiment and after giving them hyperlipidaemic diet respectively. Table 3 contains results after intervention with ethanolic extract of E.Jambolana, simvastatin 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-2).

Table-3 shows the effects of treatment with ethanolic extract of Eugenia jambolana fruit pulp on serum lipid profile

Table-1: Baseline Lipid profile parameters at beginning of experiment*.

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	58.50±3.90	93.30±3.10	19.90±1.30	19.94±4.58
B	57.45±1.45	94.80±5.60	21.50±2.00	16.99±4.57
C	68.60±3.20	90.00±1.60	27.15±2.95	23.45±6.47
D	61.30±2.30	93.25±5.75	23.40±3.80	19.25±0.35
E	68.20±6.2	106.50±3.50	25.00±1.00	21.90±5.9

*All values are expressed as ±S.E.M.

Table-2: Lipid profile parameters on first day of six week experiment*.

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	60.00±4.06	87.26±2.03	21.23±1.41	21.31±3.06
B	183.00±4.99	188.66±5.36	16.43±2.13	128.83±4.89
C	188.56±9.99	194.76±11.17	12.76±1.29	136.84±7.76
D	193.50±4.53	202.00±4.93	16.40±2.1	136.70±4.15
E	183.23±13.80	188.63±11.31	17.86±1.79	127.64 ± 13.23

*All values are expressed as ±S.E.M.

Table-3: Lipid profile parameters at the end of fourteen weeks of experiment*.

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	64.67±3.10	93.82±3.29	21.6±1.50	24.3±2.22
B	154.54±2.70	167.16±2.56	16.90±0.90	104.20±2.58
C	93.75±2.95	109.43±3.54	45.38±1.59	26.4±2.50
D	94.61±3.14	115.02±5.12	41.97±2.02	29.63±4.60
E	77.80±2.25	93.98±4.84	40.46±2.15	18.53±1.56

*All values are expressed as ±S.E.M.

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 fruit pulp, simvastatin 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 found to be significantly higher ($p<0.05$) in group B as compared to A. Intergroup comparison of C, D and E showed group E to possess significantly higher activity against serum total cholesterol 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 fruit pulp cause significant reductions in serum LDL cholesterol, TG, total cholesterol and increase in HDL cholesterol in 2% cholesterol fed

hyperlipidaemic rats. This study is in conformation with the earlier work done by S.B Sharma & A.Nasir on the ethanolic extract of seeds of Eugenia jambolana. They showed reduction in serum total cholesterol, LDL cholesterol and HMG-CoA reductase activity also with a significant fall in fasting blood glucose and glycosylated haemoglobin.¹¹ However this study is unique as it was carried out on fruit pulp of Eugenia jambolana which is the only edible portion of plant. In our knowledge, the antihyperlipidaemic pharmacological activity of this part of plant has not been studied before. This study is also unique because a comparison between antihyperlipidaemic effects of E.Jambolana and simvastatin was made. 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 simvastatin. Results in group E which were given a combination of simvastatin and E.Jambolana 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 simvastatin may be having different mechanism of action which needs to be explored further. We assume that antioxidant chemicals of E.Jambolana cause reduction in lipid profile. The fruit pulp of *Eugenia jambolana* is a rich source of anthocyanins and flavonoids. The flavonoids including Quercetin, Myrcetin and Kaempferol have 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 tendencies¹² 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.¹³ Flavonoids inhibit Hydroxy methyl glutaryl reductase (key enzyme involved in cholesterol biosynthesis) also it activates the enzyme 7 α -hydroxylase which accelerates cholesterol metabolism.¹⁴ Composition of water soluble vitamins of E.jambolana fruit (per 100gm of sample) is thiamine 0.12mg, niacin 0.272mg and ascorbic acid 30.0mg.¹⁵ Niacin in *Eugenia jambolana* fruit decreases the catabolism of HDL-*apo-A-1* apolipoprotein.¹⁶ The lipid lowering effect of this flavonoid is due to activation of cytochrome p450 dependant 7 α -hydroxylase which results in increased metabolism of cholesterol. Administration of flavonoids also results in suppression of oxidative modification of LDL and development of fatty streaks.⁸ However detailed analysis of chemical constituents of E.Jambolana needs to be carried out.

Treatment of hyperlipidaemia is a life long battle. The long term adverse effects of E.Jambolana were not addressed in the study. Simvastatin is known to cause hepatitis¹⁷ and myotoxicity.¹⁸ Idiosyncratic and immunoallergic mechanisms have been implicated in pathogenesis of rare cases of clinically significant liver injury caused by statins.¹⁹

Similar side effects are a possibility with E.Jambolana as well. In 2009 Rasheed S found the toxic effects of ethanolic extract of *Eugenia jambolana* seeds in liver of albino rats as shown by their significantly raised liver enzyme levels and disturbed liver histology.²⁰ Future studies are needed to evaluate these issues. Whether alteration in lipid profile parameters caused by E. Jambolana in experimental rats is reflected in the atherosclerotic process also needs to be addressed.

Changes in blood lipids have been associated with some herbs.²¹ Numerous clinical trials of diet, drug and surgical interventions have been established as lipid lowering agents. Dietary modifications and drug therapy has shown promising results to regulate HDL and LDL cholesterol level and to reduce the subsequent risk of

coronary artery disease associated pathological conditions.²² The possibility of retarding human atherosclerosis or even inducing its regression is one of the present therapeutic challenges.¹⁴ The use of herbal medicines in third world countries is quite high. In most Asian countries where the use of folk medicine is prevalent, the search for traditional cures is a common practice.¹⁰

In conclusion ethanolic extract of E.Jambolana fruit pulp is effective in ameliorating abnormalities in lipid profile in experimental rats. Data on the short and long term adverse effects of E.Jambolana fruit pulp ingestion needs to be collected. This *in vivo* study suggests a possible role of E.Jambolana as antihyperlipidaemic agent in humans.

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