Original Article

Comparison of simvastatin with Eugenia Jambolana fruit pulp in their effects on Alanine Transferase, Aspartate Aminotransferase and Creatinine Phosphokinase levels of hyperlipidaemic rats

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Abstract

Objectives: To compare the effects of Eugenia Jambolana fruit extract with simvastatin on liver enzymes, aspartate aminotransferase (AST), alanine transferase (ALT) and muscle enzyme creatinine phosphokinase (CPK) in diet induced hyperlipidaemic rats.

Methods: An experimental randomized 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 (TC), Low density lipoprotein (LDL), High density lipoprotein (HDL), Triglycerides (TG), ALT, AST and CPK were measured at zero, six and fourteen weeks.

Results: At fourteenth week significant reductions in serum ALT , AST and CPK levels were observed in hyperlipidaemic group C as compared to other hyperlipidaemic groups B, D and E ($p<0.05$). Serum ALT level which is considered to be the most important parameter of hepatotoxicity returned to normal after 8 weeks in group C fed on Eugenia Jambolana fruit pulp only and the values were equal to control group A. There was no significant difference at baseline (zero weeks) serum TC, LDL, HDL, TG, ALT, AST and CPK of groups A, B, C, D and E; $p>0.24$, $p>0.37$, $p>0.89$, respectively. On sixth week, serum ALT, AST and CPK levels of hyperlipidaemic groups B,C,D and E were found to be significantly higher as compared to group A ($p<0.05$).

Conclusion: Ethanolic extract of Eugenia Jambolana fruit caused a reduction in serum ALT, AST and CPK level in male albino rats when compared with simvastatin.

Keywords: Traditional medicine, Rats, Simvastatin, Eugenia Jambolana (JPMA 61: 1190; 2011).

Introduction

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 the root cause.[1]

Drugs used in hyperlipidaemia are 3-hydroxy-3methyl glutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors, niacin, fibric acid derivatives and bile acid binding resins.[2] The statins are the most effective and best tolerated agents for treating dyslipidaemias. These drugs are competitive inhibitors of HMG-CoA reductase, which catalyzes an early rate limiting step in cholesterol biosynthesis.[3]

Since hypercholesterolaemia is a life long condition, the long term safety and tolerability of these agents is an important issue.[4] Although well tolerated by most patients, statins have also been associated with few hazardous adverse events like myotoxicity, hepatotoxicity, increased risk of carcinogenesis and drug interactions.[5] Lipid lowering drugs cause hepatotoxicity in three percent of population.[6]

Serum levels of AST, ALT and CPK are the most frequently utilized surrogatae markers of hepatocellular injury.[7] The serum CPK level of statin treated patients having skeletal myopathy is usually ten times the upper limit of normal.[5]

In traditional medicine of the subcontinent Eugenia 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.[8] However, leaves of Eugenia Jambolana produced none of these effects. The lipid lowering effects of ethanolic extract of pulp of Eugenia Jambolana have already been demonstrated and they were found equal to simvastatin.[9]

The hepatoprotective activity of Eugenia Jambolana pulp in Paracetamol induced hepatotoxicity has been undertaken in albino rats[10] but no data is available on the comparison of hepatotoxic and myopathic effecs of Eugenia Jambolana fruit pulp and simvastatin in hyperlipidaemic rats.

We designed this study to find out hepatic and myopathic effects of Eugenia Jambolana in hyperlipidaemic 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.[11] 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 ± 1ºC and humidity 50%±10% was maintained throughout the experiment.[12] 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 Eugenia 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 Eugenia Jambolana fruit pulp extract and Simvastatin for eight weeks.

All the groups were fed water ad libitum. Simvastatin and fruit extract of Eugenia Jambolana was given orally as a single daily dose of 1.0mg/kg/day and 200mg/kg/day respectively.[9]

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 Eugenia 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 filter funnel. The ethanol in filtrate was evaporated by putting it in rotary vacuum evaporator, Heidolph, Laborota 4002, at 45ºC. The extract was then kept in freeze drier, LABCONCO, Frezone 2.5, at -40ºC 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 tightly closed bottle, protected from light in refrigerator at 2 to 8ºC to be used throughout the experiment.[13]

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

Collection of blood sample: Rats were sacrificed by stunning and pithing and 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.[14] Serum TC, HDL , LDL, TG, ALT, AST and CPK 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 and 10 U/L for ALT, AST and CPK values and calculations were performed with NQuery software. Results were expressed as Mean ± S.D. 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 serum ALT, AST and CPK levels 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 Eugenia Jambolana, simvastatin and combination of both. Results in Table-1 show that there was no significant difference in ALT, AST and CPK levels among groups A,B,C,D and E ($p>0.05$) at the beginning of experiment. Animals in group B,C,D and E fed on hyperlipidaemic diet had significantly higher ALT, AST and CPK levels 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, Simvastatin and combination of both on serum ALT, AST and CPK levels in albino rats.

In hyperlipidaemic animals due to administration of 2% cholesterol diet given over a period of six weeks there was an overall increase in serum ALT, AST and CPK levels 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, serum ALT, AST and CPK levels were found to be significantly lowered ($p= <0.005$) in groups C as compared to group B, D and E. However, in comparison with group A , values in this group were found to be near normal for ALT and AST, showing significant reduction ($p= <0.005$) . Serum CPK value although not brought to near normal in group A but was significantly lower than groups B, D and E. Intergroup comparison of B, C, D and E showed group C to be showing significantly lower values of ALT, AST and CPK after 8 weeks of treatment with Eugenia Jambolana fruit pulp extract ($p=<0.005$). The effects of Eugenia Jambolana fruit

Table-1: Baseline ALT, AST & CPK levels at beginning of experiment.*

Groups	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Creatinine Phosphokinase (U/L)
A	49.80±1.9	67.2±2.8	110.9±13.3
B	57.85±3.6	78.80±4.2	178.25±32.8
C	60.80±2.2	71.00±15.5	142.20±8.2
D	57.25±10.9	69.4±8.4	152.75±66.1
E	62.00±1.4	69.5±14.5	137.75±16.6
Value of P (Between groups)	0.3000	0.8000	0.4000

*All values are expressed as ±S.D.
*Value of P<0.05 is significant.

Table-2: ALT, AST & CPK levels on first day of six week experiment.*

Groups	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Creatinine Phosphokinase (U/L)
A	55.33±8.7	68.00±2.6	122.6±11.5
B	72.56±2.7	95.5±5.6	220.0±25.2
C	71.80±7.3	97.5±14.9	265.33±60.9
D	87.20±6.6	97.8±3.4	292.66±22.4
E	81.73±5.1	100.3±11.2	308.00±11.5
Value of P (Between groups)	0.001	0.007	<0.0001

*All values are expressed as ±S.D.
*Value of P<0.05 is significant.

Table-3: ALT, AST & CPK levels at the end of fourteen weeks of experiment.*

Groups	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Creatinine Phosphokinase (U/L)
A	52.85±3.7	64.3±3.9	152.32±5.1
B	76.6±4.1	93.9±6.8	345.16±19.3
C	60.66±8.0	76.36±6.1	296.33±20.5
D	93.31±7.1	116.00±12.5	401.05±34.1
E	87.9±7.09	102.2±8.1	367.81±24.3
Value of P (Between groups)	<0.001	<0.001	<0.0001

*All values are expressed as ±S.D.
*Value of P<0.05 is significant.

extract on lipid levels has already been reported.[9]

Discussion

The results of this experimental study showed that ethanolic extract of Eugenia Jambolana fruit pulp caused significant reductions in serum ALT, AST and CPK levels in 2% cholesterol fed hyperlipidaemic rats. This study is in conformation with the earlier work done by Das S and Sharma G who evaluated hepatoprotective effects of ethanolic extract of Eugenia Jambolana pulp on paracetamol induced hepatotoxicity in rats.[10] However this study is unique as it was carried out on diet induced Hyperlipidaemic rats who were treated with Simvastatin, Eugenia Jambolana fruit pulp extract and the combination of both. The purpose of the research was to study the adverse effects of the aforementioned drugs on liver and skeletal muscle by taking into consideration serum ALT, AST and CPK levels. In our knowledge the effect of this part of the plant on liver and skeletal muscle has never been studied in hyperlipidaemic rats with deranged LFTs and CPK. Also this study was unique because a comparison was made between simvastatin, a drug known to have hepatotoxic and myopathic effects and ethanolic extract of Eugenia Jambolana whose action on liver and muscle enzymes are not known.

The study proved that Eugenia Jambolana significantly lowered ALT, AST and CPK levels in hyperlipidaemic rats.

Interestingly serum ALT, AST and CPK level in group D and E which were given simvastatin or simvastatin with Eugenia Jambolana further deteriorated showing that continued use of Simvastatin can worsen the liver and skeletal muscle pathology. This is in consistence with the findings of Arora R who studied statin induced myopathy and observed an increase in serum CPK level around 10 times above the baseline.[5]

The major mechanism behind the skeletal muscle and hepatoprotective activity is probably the diminution of the intensity of oxidative stress, which is brought about by the antioxidant activity of Eugenia Jambolana fruit pulp extract. In 2008 Bhawna Sharma performed chemical analysis of Eugenia Jambolana seed extract by using HPLC and demonstrated the presence of different flavanoids and their derivatives which are known for their antioxidant potential.[16]

Since the phytoconstituents of the seed kernel and the pulp of Eugenia Jambolana are almost the same, the pulp may also be presumed to have similar effects on antioxidant enzymes. The flavonoids, gallic acid and anthrocyanins present in the pulp of Eugenia Jambolana, are natural antioxidants.[17] The seed kernel of Eugenia Jambolana has been reported to increase the hepatocellular reduced glutathione content and also to increase the activities of the antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase in the liver of experimental animals.[10] Earlier investigators have screened the hepatoprotective activity of several flavonoid compounds found in plants.[18] Thus it is postulated that Eugenia Jambolana fruit extract, because of the presence of natural antioxidants like flavonoids in it might have exerted protective action against hyperlipidaemia-induced liver damage. The mechanism probably increases the activity of the antioxidant enzymes like superoxide dismutase causing scavenging of freely generated toxic radicals. This possibly ameliorates the extent of oxidative stress mediated cellular damage caused by a hyperlipidaemic diet. The flavonoids including Quercetin, Myrcetin and Kaempherol have been shown to exhibit a series of biological effects among which the inhibition of lipid peroxidation and platelet aggregation are more prominent as they contribute to reduced thrombotic tendencies[19] and also cholesterol lowering effects by alteration in cholesterol absorption, triglycerides assembly and processing of lipoproteins in plasma. Flavonoids inhibit HMG CoA Reductase which is the key enzyme involved in biosynthesis of cholesterol.The lipid lowering effect of this flavonoid is due to activation of cytochrome p450 dependant 7a-hydroxylase which results in increased metabolism of cholesterol.[20]

Treatment of hyperlipidaemia is a life long battle. The long term adverse effects of all antihyperlipidaemic drugs are needed to be elucidated. Simvastatin is known to cause hepatitis[5] and myotoxicity.[21]

Contrary to our findings, in 2009, Rasheed et al reported the toxic effects of ethanolic extract of Eugenia Jambolana seeds in liver of albino rats with paracetamol induced hepatotoxicity. They demonstrated significantly raised liver enzyme levels and disturbed liver histology.[22]

Future studies are needed to evaluate the effect of Eugenia Jambolana fruit extract in different forms of hepatitis like viral, autoimmune,alcoholic and metabolic. These studies should also help in further elucidating the protective effect of this fruit in hyperlipidaemia induced liver and muscle injury. The long term beneficial and harmful effect of this flavanoid rich fruit in humans should also be studied.

Conclusion

The study concluded that ethanolic extract of Eugenia Jambolana fruit pulp is effective in ameliorating abnormalities in liver and skeletal muscle serum enzymes of hyperlipidaemic rats. It has no adverse effects on liver and muscle enzymes when compared to simvastatin in hyperlipidaemic rats. This fruit has a potential to be used in the treatment of hyperlipidaemias.

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