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ANTI-HYPERLIPIDEMIC ACTIVITY OF THE METHANOLIC EXTRACT OF EUGENIA JAMBOLANA BARK IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RAT

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ABSTRACT

Eugenia jambolana is therapeutically evaluated for anti-hyperlipidemic activity. Significant anti-hyperlipidemic effects were obtained as evident from the restoration of biochemical parameters altered by cholesterol towards the normal. Almost normal histological appearance of liver was observed in treated groups. Among the three doses, 400 mg/kg showed better activity. However, the activity was found to be less than the standard atorvastatin given at 1.2 mg/kg dose. The results showed that the methanolic extract of *Eugenia jambolana* has potential hypolipidemic effect along with recovery of liver functions.

INTRODUCTION

The use of natural products for treatment of various ailments has grown faster over the past few years which are undoubtedly driven by the belief that they are relativity safe, easily available and affordable to masses [1, 2]**.** In traditional medicine of the subcontinent, *Eugenia Jambolana* commonly called as Jamun has been used for the treatment of Diabetes Mellitus and dyslipidaemias [3]. In controlled experiments ethanolic and aqueous extracts of seeds administered orally to animals and human adults at various dose levels were found to have hypoglycemic activity and lipid lowering effect. No data is available on the Antihyperlipidaemic effects of bark extract of *E. Jambolana* in animals. So 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 atorvastatin [4]**.**

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The *Eugenia jambolana* is well known for its medicinal properties in indigenous medicine in India. Earlier studies carried out have proved its anti-inflammatory, anti-diabetes, diuretic, and antibacterial activities of the bark. Except these studies, so far no other biological and phytochemical investigations have been reported on the bark. Hence a systematic pharmacognostical and pharmacological investigation of ethanol extract of stem bark of *Eugenia jambolana* is carried out. Based on the traditional uses as well as earlier work on other species of *Eugenia jambolana* anti hyperlipidemic study is selected for pharmacological screening of the extract using animal models. The toxicity of the extract was also assed in animal models [5-7].

MATERIALS AND METHODS

Plant material

The bark of *Eugenia jambolana* was collected from Vangapally, Nalgonda District, Andhra Pradesh, India and authenticated by Dr. S. Srinivas Rao, Swami Degree College, Bhongir, Nalgonda District, where a

voucher specimen has been deposited for further reference. It was shade dried and powdered.

Extraction and Phytochemical Analysis

The plant powder (50 g) was extracted with 250 ml of methanol in a soxhlet apparatus for 72 hrs. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50 ºC) in a rotavapor.

Animals

Male Wistar Albino rats (200-220 g) were used for anti-hyperlipidemic activity. The animals were fed on a standard pellet diet and water *ad libitum*. Environmental conditions were standardized (temp 23 ± 2 ºC, humidity 55 – 60% with a 12 h light and dark cycle). Animals were fasted, but allowed water 12 h prior to the experiments. Animal studies were performed according to the prescribed guidelines of CPCSEA, Government of India.

Acute toxicity studies

Acute oral toxicity study in rats was carried out as per OECD-425 guidelines. Graded doses (200, 500 and 2000 mg/kg bw) of methanolic extract of *Eugenia jambolana* was administered orally to various groups of rats containing ten in each group. The animals were observed for mortality, clinical signs and body weight changes for a period of 0,1,2,4 and 24 h [8].

Anti - Hyperlipidemic activity

Preparation of 2 % cholesterol diet

2 g of cholesterol (Extra pure, Molychem, Mumbai, India) and 0.5 g of Cholic acid, Rolex chemical industries, Mumbai, India was thoroughly mixed and mashed with 97.5 g of standard rat diet and was given in the form of pellets.

Animals were divided into six groups of six animals each. Group I was served as normal control and received fed on regular standard rat diet till end of study. Groups II animals served as disease control and was treated for initial four weeks with high fat diet. After baseline lipid profile at end of four weeks, this group was fed on regular rat diet till end of study. Group III, IV and V received fed on 2 % cholesterol diet for initial six weeks and then was given methanolic extract of *Eugenia jambolana* 100, 200 and 400 mg/kg, respectively. Group VI received fed on 2 % cholesterol diet for initial four weeks and then was given with Atorvastatin at the dose 1.2 mg/kg orally 10 days. All these treatments were given orally once daily for 10 days.

Induction of hyperlipidemia

The high fat diet was prepared by mixing 2 % (w/w) cholesterol and 1% (w/w) cholic acid in standard animal chow and administered for 4 weeks. All the rats except the normal control were fed with high fat diet. The normal control group was fed with standard chow only. At the end of 4th week, total cholesterol level in serum was estimated and the animal with greater than 250 mg/dl level was selected and considered as hyperlipidemia rats.

At the end of the study, blood was collected by retro-orbital plexus puncture under mild ether anesthesia from overnight fasted rats and serum was separated and analyzed for total cholesterol, HDL, LDL and TG by autoanalyzer using Ecoline Kits.

After the collection of blood samples, the liver was excised and rinsed in ice-cold normal saline. A portion of the tissues were fixed in 10% formalin, cut into 5 µm thick sections and stained using heamatoxylin-eosin and histopathological observations were made [5]**.**

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett Multiple Comparison Test. P values < 0.05 were considered as significant [9].

RESULTS

Anti - hyperlipidemic activity of the methanolic extract of *Eugenia jambolana* bark

Acute toxicity results showed no clinical signs of toxicity and mortality of the animals. Therefore, an LD_{50} > 3000 mg/kg bw may be assumed. Hyperlipidemic induced rats showed significant increase in the levels of total cholesterol, LDL, TGL and VLDL and a significant reduction in the level of HDL when compared to normal animals (Table 1). Treatment with methanolic extract of *Eugenia jambolana* at all the three doses and atorvastatin caused a significant reversal of all these changes towards the normal. Among the three doses, methanolic extract of *Eugenia jambolana* at 400 mg/kg dose was found to be more active. However, the standard atorvastatin exhibited better activity than *Eugenia jambolana* in all the above parameters.

In the histopathological study, normal animals liver tissue showed sinusoidal cards of hepatocyte with central vein and portal tracts. Hyperlipidemic animals liver section, the degrees of hepatic steatosis was examined. The treatment with methanolic extract of *Eugenia jambolana* at all the doses and standard atorvastatin decreased the hepatic steatosis remarkably when compared to hyperlipidemic animals.

DISCUSSION

The bark of *Eugenia jambolana* is a rich source of flavonoids. The flavonoids including Quercetin, Myrcetin and Kaempherol 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 tendencies 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 [10]**.** Flavonoids inhibit Hydroxy methyl glutryl reductase (key enzyme involved in cholesterol biosynthesis) also it activates the enzyme 7 a-hydroxylase which accelerates cholesterol metabolism [11]**.** The lipid lowering effect of this flavonoid is due to activation of cytochrome p450 dependant 7a-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 [12]**.** However detailed analysis of phytochemical constituents of *Eugenia jambolana* needs to be carried out. Treatment of hyperlipidaemia is a lifelong battle. The long term adverse effects of Eugenia jambolana were not addressed in the study [13]**.** Atorvastatin is known to cause hepatitis and myotoxicity. Idiosyncratic and immunoallergic mechanisms have been implicated in pathogenesis of rare cases of clinically significant liver injury caused by statins [14-15]**.**

Similar side effects are a possibility with *Eugenia jambolana* as well. In 2009 Rasheed S has 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 and this finding has been confirmed with our histopathological observations [16]**.** Future studies are needed to evaluate these issues. Whether alteration in lipid profile parameters caused by *Eugenia jambolana* in experimental rats is reflected in the atherosclerotic process also needs to be addressed.

Table 1. Effect of the methanolic extract of *Eugenia jambolana* bark on cholesterol levels in hyperlipidemic rats

Groups	**CHO. T** mg/dl	**HDL** mg/dl	**LDL** mg/dl	**TGL** mg/dl	**VLDL** mg/dl	AI
NORMAL	110.00±14.14	13.00±02.83	100.00±14.14	127.50±10.61	11.00±1.41	1.00±0.06
HFD	300.00±28.28	09.00±01.41	130.00±09.60	187.50±03.54	16.70±3.54	1.32±0.08
ATOR (1.2 mg/kg)	195.00±07.07	13.50±02.12	095.00±07.07	094.50±06.36	12.50±3.54	0.85±0.04
EJ BARK (100 mg/kg)	280.00±14.14	10.50±00.71	115.00±09.08	105.00±07.07	15.00±1.41	1.00±0.01
EJ BARK (200 mg/kg)	185.00±21.21	12.50±00.71	105.00±05.10	110.00±14.14	13.76±1.48	0.94±0.03
EJ BARK (400 mg/kg)	130.00±14.14	12.00±02.83	085.00±07.07	087.50±10.61	13.50±2.12	0.87±0.16

CONCLUSION

The bark has a potential to be used in the treatment of hyperlipidemia as this is the risk factor for cardiovascular disease. Data on the short and long term adverse effects of *Eugenia jambolana* bark ingestion needs to be collected. Further pharmacological investigations are in progress to support that *Eugenia jambolana* bark is a kind of promising extract and can be developed to a new drug hopefully.

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