Role of Elaeocarpus ganitrus Roxb. in Prevention of Coronary Artery Disease in Cholesterol fed Rabbits

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ABSTRACT
The present study was designed to investigate the antiatherosclerotic and antioxidant activity of 70% ethanolic crude extract of Elaeocarpus ganitrus seed in cholesterol fed rabbits. The E. ganitrus extract was administered at a dose level of 250 and 500 mg/kg/day (p.o) for 120 days to cholesterol fed rabbits. Lipid profile in serum and tissue biochemistry as well as antioxidant parameters in heart were determined. The histological change in coronary artery was examined. Statistical analysis was carried out by using One way ANOVA followed by Tukey’s multiple comparison tests using Graphpad PRISM software (version 5). Rabbits fed with cholesterole and E. ganitrus extract concurrently showed significant (P ≤ 0.01, ≤ 0.001) decrease in the levels of serum total cholesterol, triglycerides, LDL, VLDL and atherogenic index along with a considerable improvement in HDL ratio in a dose dependant manner. Further, a significant reduction was also recorded in the levels of total cholesterol, triglycerides, phospholipids in heart extract administered group animals. Lipid peroxidation levels decreases significantly as well as marked elevation in Glutathione, Catalase and SOD levels were observed in treated group rabbits. Histopathological analysis revealed significant increase in lumen size of coronary arteries when E. ganitrus extract supplemented concurrently to cholesterol fed animals. Our study exhibited that the phytoconstituents like phytosterols, fats, alkaloids, flavonoids, carbohydrates, proteins and tannins present in the E. ganitrus ethanolic extract may be attributed to the significant antiatherosclerotic as well as antioxidant activity, signifying the potential protective role in coronary artery disease.

Keywords: Atherosclerosis, cholesterol, Elaeocarpus ganitrus, ethanolic extract, hyperlipidaemic, phytoconstituents

INTRODUCTION
Regardless of the significant effort done by medical practitioner to treat coronary artery disease (CAD), heart disease is still the foremost cause of death in the US.1 Risk factors, such as hypercholesterolemia, hypertension, tobacco use and diabetes have all been found to cause vascular endothelial cell (VEC) injury and dysfunction.2 Coronary heart disease (CHD), also called coronary artery disease, occurs when plaque builds up in the coronary arteries. Oxygen-rich blood to heart is supplied by these arteries. CHD can lead to angina (chest pain) and heart attack. The leading cause of Coronary artery disease is atherosclerotic obstruction of the coronary arteries.

Atherosclerosis, which characterized by lipid accumulation, fibrosis, inflammation, development of focal plaques and arterial sclerosis is the foremost reason of morbidity and mortality in most developed countries.3,4 The disproportion of the lipid metabolites in the affected organism believed to a strong contributor towards atherosclerosis. Dyslipidemia is generally described by high levels of total cholesterol, triglycerides, low density lipoprotein cholesterol, and low levels of high density lipoprotein cholesterol.5 Further, the shift in equilibrium between oxidant/antioxidant in favor of oxidants is termed “oxidative stress” plays an important role in the development of atherosclerosis.6 An antioxidant which inhibits oxidation of LDL should be effective for suppressing atherosclerosis.7 Latest works advocates that managing the way of life that comprises an intake of mostly plants may help in preventing and reversing CAD.8 Recent literature emphasizes the potential therapeutic effects of phytoconstituates found in medicinal plants, indicating positive applications for controlling the pathogenesis of chronic cardiovascular disease driven by cardiovascular risk factors and oxidative stress.9

Elaeocarpus ganitrus Roxb. (Rudraksha) belongs to family Elaeocarpaceae is prevalent for its fascinating fruit stones and medicinal properties. It is a large evergreen broad-leaved tree whose seed is conventionally used for prayer beads in Hindu religion. The widespread investigation of literature exposed that Elaeocarpus ganitrus Roxb. is an imperative basis of various pharmacologically and medicinally significant chemicals, such as indispensable triterpenes, tannins like geraniin and 3, 4, 5 trimethoxy geraniin, indolizilidi alkaloids grandisines, rudrakine and flavnoids quercitin.10 Furthermore it is noted to have myriad pharmacological activities that involve anti-inflammatory,11 analgesic,12 hypoglycemic,13 antidepressant,14 antiasthmatic15 sedative,12 antihypertensive,16 hydrocholeric, smooth muscle relaxant,17 antiluercogenic,18 and antimicrobial.19 As per our literature survey, no scientific study has been conducted to evaluate antiatherosclerotic/cholesterol lowering activity of Elaeocarpus ganitrus Roxb. With this background information, the present study is undertaken to screen Elaeocarpus ganitrus Roxb. for its lipid lowering,
antiatherosclerotic as well as antioxidant activity in hypercholesterolic rabbits.

MATERIALS AND METHODS

Collection and extraction of plant material

Authentic seeds of *Elaeocarpus ganitrus* were obtained from the Jayoti Vidyapeeth Women’s University, Jaipur. Seeds were powdered and extracted with 70% ethanol for 36 to 48 hrs, by soxhlet extraction method. After this extraction solvent was removed under reduced pressure and controlled temperature (55-60°C) and dried to obtain a brown solid mass. This 70% ethanolic crude extract of the *E. ganitrus* was dissolved in distilled water and administered to the animals via oral gavage.

Animal model

New Zealand white male rabbits weighing 1.50-2.0 kg and age of 10-18 months were used for the conduction of experiments. The animals were acclimatized for 10 days before being used for the experiments. The animals were grouped (5 rabbits in each group) and housed in polypropylene cages at constant temperature and also maintained under a standard diet (Ashirwad Industrial Ltd., Punjab) and green leafy vegetables and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) and was executed according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Experimental design

The rabbits were divided into following groups of five animals in each:

**Group I:** Control –Placebo treated 120 days. (Vehicle treated)

**Group II:** Cholesterol feeding for 120 days

**Group III:** Cholesterol feeding + 250 mg/kg b.wt. /day *E.ganitrus* ethanolic extract treatment from day 1-120 (Concurrent feeding).

**Group IV** : Cholesterol feeding + 500 mg/kg b.wt. /day *E. ganitrus* ethanolic extract treatment from day 1-120 (Concurrent feeding).

Cholesterol feeding: 500 mg cholesterol/kg, b.wt./rabbit/day in 5ml coconut oil Animals were sacrificed after completion of treatment, blood and tissue were taken out for biological and histological examinations.

Induction of Hyperlipidaemia

Hyperlipidaemia was induced in New Zealand white male rabbits by daily oral administration of 500 mg cholesterol/kg, b.wt./rabbit/day in 5ml coconut oil.

Autopsy

Animals were autopsied under ether anesthesia after completion of 120 days of treatment. The blood was collected by cardiac puncture, serum was separated by centrifugation after 30 minutes and stored at -20°C for biochemical analysis.

Fixation

Heart (2–3 cm length) was quickly removed, cleared off the fat and connective tissue weighed on electronic balance. A small section of heart from each animal was soaked in a 10 % (v/v) form calcium solution for H & E staining. The heart was processed for normal histological section. The tissue samples were ultra sectioned (5–6 μm thickness), stained with haematoxylin and eosin (H&E) and examined under a light microscope for observation of structural abnormality.

Biochemical analysis

The serum total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low density lipoprotein (LDL) were measured in an autoanalyzer using special kits (Accurex, Biomedical Pvt. Ltd.) and very low density cholesterol was calculated by using formula VLDL: TG/5. Heart was analysed for Total cholesterol, Triglyceride, Phospholipid and antioxidant parameters i.e Lipid Peroxidation, Catalase, Glutathione and Super Oxide Dismutase (SOD). HDL Ratio was determined as

\[
\text{HDL Cholesterol X 100} \\
\text{Total Cholesterol - HDL Cholesterol}
\]

Atherogenic Index was derived using the formula-

\[
\text{LDL-Cholesterol + VLDL Cholesterol} \\
\text{HDL Cholesterol}
\]

Deviation Percent was calculated as

\[
\frac{\text{Final value – Initial value}}{\text{Initial Value}} \times 100
\]

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis was carried out by using One way ANOVA followed by Tukey’s multiple comparison tests using Graphpad PRISM software (version 5). P values <0.05 were considered as statistically significant.

RESULTS

Serum Biochemistry

As table 1 illustrated that cholesterol feeding for 120 days cause a significant increase in Total Cholesterol, Triglyceride, Phospholipids, LDL-C, VLDL-C in comparison with control group rabbits. Concurrent treatment groups showed the reduction in all these biochemical parameters when compared to cholesterol-fed rabbits in a dose dependent manner. In cholesterol fed rabbits, HDL cholesterol in comparison to total cholesterol was decreased, whereas it was again elevated by co-administration with various doses of *E. ganitrus* seed...
extract. (Table 1) Further, marked reduction was noticed in HDL ratio in rabbits after 120 days cholesterol feeding in comparison with controls which was improved considerably in a dose dependent manner after concurrent administration of *E. ganitrus* extract. A significant rise of 150.50% was observed in atherogenic index after 120 days of cholesterol feeding. An ameliorative action of plant extract on atherogenic index (i.e 55.44% at 250 mg and -67.94% at 500 mg dose level) of *E. ganitrus* crude extract was observed in concurrent group when compared with group II. (Table 1)

**Table- 1: Serum Biochemistry of Ethanolic *Elaeocarpus ganitrus* Extract Treated Rabbits**

<table>
<thead>
<tr>
<th>Identification</th>
<th>Group</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>VLDL Chol.</th>
<th>LDL Chol.</th>
<th>HDL Chol.</th>
<th>Chol/Phospho Ratio</th>
<th>HLD Ratio</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Placebo treated) from day 1-120</td>
<td>I</td>
<td>123.33 ± 7.75</td>
<td>77.05 ± 4.12</td>
<td>119.72 ±5.25</td>
<td>15.41 ±0.825</td>
<td>60.92 ±1.30</td>
<td>43.07 ±1.24</td>
<td>1.03</td>
<td>53.66</td>
<td>1.98</td>
</tr>
<tr>
<td>Cholesterol feeding* from day 1-120</td>
<td>II</td>
<td>% Deviation (I)</td>
<td>1041.66* ±27.82</td>
<td>475.66* ±9.02</td>
<td>611.00* ±8.50</td>
<td>95.13* ±1.80</td>
<td>644.67* ±10.33</td>
<td>148.89* ±3.58</td>
<td>1.70</td>
<td>16.67 ±150.50</td>
</tr>
<tr>
<td>Cholesterol feeding* + <em>Elaeocarpus ganitrus</em> ethanolic extract** from day 1-120 (Concurrent feeding)</td>
<td>III</td>
<td>% Deviation (II)</td>
<td>286.28b ±7.00</td>
<td>210.63b ±3.53</td>
<td>294.21b ±7.88</td>
<td>42.123b ±0.704</td>
<td>156.83b ±8.68</td>
<td>89.62b ±4.54</td>
<td>0.97</td>
<td>-42.94 ±173.36</td>
</tr>
<tr>
<td>Cholesterol feeding* + <em>Elaeocarpus ganitrus</em> ethanolic extract*** from day 1-120 (Concurrent feeding)</td>
<td>IV</td>
<td>% Deviation (II)</td>
<td>203.117b ±4.30</td>
<td>186.59b ±6.23</td>
<td>215.52b ±8.12</td>
<td>37.31b ±1.245</td>
<td>97.22b ±4.73</td>
<td>84.22b ±2.77</td>
<td>0.94</td>
<td>-44.70 ±224.89</td>
</tr>
</tbody>
</table>

VALUES ± 5 determination

a- P ≤ 0.001 Highly Significant Group II, compared with Group I
b- P ≤ 0.001 Highly Significant Group III, IV compared

d- P ≤ 0.001 Highly Significant Group II, compared with Group I

**Figure 1:** Effect of ethanolic extract of *E.ganitrus* on cholesterol, triglyceride and phospholipid in rabbits

Cholesterol feeding –500mg/ kg.b.wt in 5 ml coconut oil / day

**E. ganitrus-** 250 mg/ kg.b.wt. / day

*Cholesterol feeding –500mg/ kg.b.wt in 5 ml coconut oil / day

** E. ganitrus- 250 mg/ kg.b.wt. / day

**Group I

*** E. ganitrus 500mg/ kg.b.wt. / day with Group II

**Tissue Biochemistry

The heart showed significant (P ≤ 0.001) elevation in cholesterol, triglyceride and phospholipid content after cholesterol feeding for 120 days in comparison to the control group. In contrast, it was observed to be decreased significantly (P ≤ 0.001) by concurrent administration of *E. ganitrus* at different dose levels when compared to cholesterol fed rabbits (120 days). (Fig. 1)

**Antioxidant parameters**

For analyzing lipid peroxidation, MDA levels were measured in heart homogenates. A significant rise (P ≤
0.001) in MDA was observed in cholesterol fed rabbits for 120 days in comparison with the control. Moreover, lipid peroxidation was significantly reduced (P ≤ 0.01 and P ≤ 0.001) as depicted by the lower levels of MDA in heart when *E. ganitrus* supplemented concurrently with cholesterol feeding to rabbits in a dose-dependent manner as compared with cholesterol fed rabbits for 120 days. (Fig. 2a)

Cholesterol feeding to rabbits resulted in significant lowering of Glutathione, Catalase and Superoxide Dismutase concentration when compared to control ones in heart. In contrast a statistically significant elevation (P ≤ 0.01 and P ≤ 0.001) was observed in all these three parameters of heart when cholesterol fed rabbits concurrently supplemented with *E. ganitrus* extract in a dose-dependent manner. This indicates a positive effect of plant extract on enzymatic antioxidant defense in hyperlipidaemic rabbits. (Fig. 2b-2d)

**Histological Observations: Coronary Artery**

The histopathological examinations were also carried out in the coronary artery of control, cholesterol fed animals and cholesterol feeding accompanied with *E. ganitrus* extract group. The coronary artery of control group animals showed large and centrally placed lumen encircled by the arterial wall, which consisted of three distinct layers tunica intima, tunica media and tunica adventitia (Fig. 3a). The coronary arteries of cholesterol fed rabbits for 120 days showed atheroma, due to the presence of large number of lipid filled macrophages. The lumen size decreases considerably and fibrous tissues have replaced much of the muscles in media (Fig. 3b). As depicted in Fig. 3c and 3d, concurrent administration of *E. ganitrus* extract with cholesterol feeding, caused a significant increase in lumen size and exhibits almost normal histology with slight thickness in intima. However, plaque has restricted to grow in concurrent groups, but coronary artery of low dose level treated group showed few fatty changes.

**DISCUSSION**

Outcomes of the present study suggested that cholesterol feeding (500 mg/ kg.b.wt/day) accompanied with 70% ethanolic extract of *E. ganitrus* at a dose level of 250 and 500 mg/kg b.wt/day significantly attenuated the elevated total cholesterol, triglyceride, phospholipid, lipoprotein cholesterol (HDL, LDL and VLDL) concentrations in experimental animals.
A marked reduction was also observed in cholesterol/phospholipid ratio, atherogenic index and the HDL ratio improved significantly when cholesterol fed rabbits co-administered with seed extract at both the dose levels.

The overproduction of VLDL in the liver or delayed catabolism of VLDL or both results in elevated concentrations of VLDL remnants and eventually of LDL. These might be attributed to a significant elevation in serum cholesterol level after cholesterol feeding in rabbits. The cholesterol lowering effect of *E. ganitrus* extract were observed in cholesterol fed rabbits by the inhibition of HMG CoA reductase inhibition.

Cholesterol feeding rises serum triglyceride levels basically by preventing its uptake and clearance by inhibiting catabolising enzymes like lipoprotein lipase (LPL) and lecithin cholesterol acetyl transferase (LCAT). Apparently concurrent feeding of *E. ganitrus* along with cholesterol inhibits the elevation of serum triglyceride level suggesting its protective action against hyperlipidaemia. The decrease in serum triglyceride has been attributed to stimulation of the degradation of triglycerides through augmented expression and activity of lipoprotein lipase (LPL) and to reduced hepatic synthesis and secretion of triglycerides or may be due to inhibition of lipolysis so that fatty acid do not get converted into triglyceride. The reduction in phospholipids level possibly due to a higher level of phospholipase that metabolized the blood phospholipids in cholesterol fed animals which additionally approves the significant protecting influence of the plant extract against hyperlipidaemia.

Oxidative modification of LDL is one of the key steps in the development of atherosclerosis. The LDL-cholesterol lowering could result from a reduced LDL-synthesis and/or an increased LDL metabolism. Consumption of plant sterol and their esters has also been stated to not only reduce intestinal cholesterol absorption but lowered blood levels of the atherogenic LDL as well. The reduction might be due to an increased uptake by extra hepatic tissues as well as by increasing the fractional catabolic rate of LDL cholesterol and by increasing the liver LDL receptors activity. It is known that quercetin can reduce the activities and mRNA levels of several enzymes participated in the synthesis of fatty acids. Thereby decreasing the levels of cholesterol esters available to form very low density lipoproteins (VLDL), resulting in reduced VLDL secretion by the liver. The role of HDLs in Reverse Cholesterol Transport represents the major atheroprotective (prevention of the development of
atherosclerotic lesions in the vasculature) action of this class of lipoprotein. In the current study the ethanolic extract of the seeds of *E. ganitrus* is effective in increasing cholesterol induced decrease in HDL. The higher levels of cholesterol associated with HDL and the increase in the activity of plasma LCAT on feeding of *E. ganitrus* may result in a higher amount of cholesterol being removed from extra hepatic tissues which may contribute to the hypercholesterolemia observed in experimental animals.

The plant extract administration along with cholesterol feeding showed considerable improvement in the ratio of LDL-C to HDL-C indicating a protective role in cardiovascular disease occurrence. Further, the concurrent administration of ethanolic extract of *E. ganitrus* provides a favorable action on rabbit lipid metabolism with respect to a decline of AI. Again, the ethanolic extract of *E. ganitrus* consumption accompanied with cholesterol cause noticeable improvement in the HDL ratio showing the positive effect of this plant in preventing atherosclerosis incidence.

Cholesterol lowering effect of *E. ganitrus* might be due to inhibition of hepatic cholesterol biosynthesis, stimulation of receptor mediated catabolism of LDL-cholesterol, increased faecal bile acid excretion and enhanced uptake of LDL from blood by liver. The reduction in heart triacylglyceride has been ascribed to a stimulation of the degradation of triglycerides through enhanced expression of lipoprotein lipases and to a decrease of hepatic synthesis and secretion of triglycerides. Concurrent supplementation of ethanolic extract of *E. ganitrus* with cholesterol feeding, levels of phospholipid showed significant reduction as compared to hypercholesterolemia rabbits may also be due to the increased activity of phospholipases.

The medicinal plants are employed as an alternative source of medicine to mitigate the atherosclerosis associated with oxidative stress. The current study observed the defensive role of *E. ganitrus* extract in modifying oxidative stress markers due to its rich phytoconstituents, therefore, likely to be valuable as a prophylactic measure in managing consequences of atherosclerosis. *E. ganitrus* extract react with peroxy radicals including the inhibition of lipid peroxidation chain propagation. According to our data administration of *E. ganitrus* significantly buffered such impaired oxidant/antioxidant unbalance and decreased the content of MDA and suppressed the lipid peroxidation. Hence attenuated level of LPO in extract treated animals is suggestive of the antioxidant nature of this plant.

Significant decrease in the levels of total glutathione observed on cholesterol feeding might be due to reduced GSH biosynthesis and constant on slautgh of ONOOO formed by reactions of O₂⁻ and NO₁, both of which increased in hypercholesterolemia. A significant elevation in GSH content in *E. ganitrus* treated animals possibly due to lower extent of oxidative stress, resulting in to reduced GSH degradation or increase in the biosynthesis of GSH. Furthermore, it could be due to decreases in the free radicals by quenching and lowering oxidative stress.

*E. ganitrus* extract treated hyperlipidaemic rabbits had considerably elevated levels of SOD and CAT, reversing the ill effects of hyperlipidaemia. It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD and CAT activities. Total phenolic content in *E. ganitrus* was detected to be 56.79±1.6 mg gallic acid equivalents/g of dry material. Total flavonoids in *E. ganitrus* were detected to be 18.58± 0.3 mg rutin equivalents/g of dry material. These findings recommend 85% of the antioxidant capacity of *E. ganitrus* is by virtue of phenolics and flavonoid components. The currently noted elevated levels of both CAT and SOD levels could be due the influence of flavonoids and polyphenols of *E. ganitrus*. So experiments have shown that ethanolic extract (EE) is found to have 24.18 mg ascorbic acid equivalents at 500 µg/ml extract concentration proving antioxidant activity of extracts. So comparable with the findings in the literature for other extracts of plant products. Our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity.

*E. ganitrus* reduced the plaque of coronary arteries of hyperlipidemic rabbits in a dose dependent manner in both the treatment group animals. There is a marked restoration to the damaged coronary artery and also considerable decrease in coronary events. Our findings strongly suggest that the increase in the cholesterol concentration of blood of cholesterol fed animals is a major factor in the production of the significant dimensional and morphological changes in the arterial wall. Abdelhalim and Al-Ayed reported that softening of the arterial wall is due to the denaturation or fragmentation of collagen fibers and elastin, both of which are identified to play dominant roles in governing the mechanical properties of blood vessels. Further oxidation of cholesterol fractions (in particular LDL) has been accepted to play an important role in the atherosclerotic plaque formation process. The inhibition of atherosclerotic plaque formation by *E. ganitrus* might be mediated by its improvement of antioxidation status, lipid metabolism and anti-inflammation response. The active constituents like gallic acid, ellagic acid & quercetin, alkaloids like Elaeocarpidine, (+)-Elaeocarpine, (+)-Isoloelaeocarpin, phytosterols, fats, carbohydrates, proteins and tannins have been shown potent antioxidant and anti-inflammatory action.
CONCLUSION

In accordance with these findings, shifting to a plant-based diet may decrease CAD mortality by reversing or interrupting the progression of atherogenesis. A plant-based diet may down regulate the atherogenesis inflammatory initiation process and phytoconstituents protect the endothelium and inhibit atherogenesis. The study also implies that ethanolic extract of *E. ganitrus* seems to be a potential cardio protective candidate in rabbits by maintaining the membrane integrity and reestablishing the activities of antioxidant enzymes near to normal levels. The cardio protective, antiatherogenic and antioxidant effects of this plant are probably due to the presence of biologically active phytoconstituents such as alkaloids, flavanoids and tannins in the ethanolic extract, may demonstrates the multitarget, multicomponent features for regulating lipid metabolism. Further isolation and identification and processing of active principles in ethanolic fraction of *E. ganitrus* is required to establish the efficacy of this herb as a hypolipidaemic drug. Our study exhibited that the ethanol extract of *E. ganitrus* seed is a potent agent and contribute remarkably in evolving novel herbal remedies to recover the lives of patients suffering from cardiovascular diseases around the word.

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