ABSTRACT
In the present study 70% ethanolic crude extract of *Elaeocarpus ganitrus* seed has been screened for its anti-atherosclerotic and antioxidant activity in liver of liver of cholesterol induced atherogenic rabbits. Lipid profile, lipid peroxidation level as well as histological changes in liver were investigated. The statistical analysis were carried out by using one way ANOVA followed by Tukey’s multiple comparison test. Cholesterol feeding produced a significant increase in total cholesterol, phospholipids and triglycerides in liver. It also increases lipid peroxidation as well as showed severe fatty changes in liver. *E. ganitrus* extract was administered at a dose level of 250 mg/kg and 500 mg/kg (p.o) daily for 60 days to cholesterol-fed rabbits rectified the disturbed lipid profile significantly and a considerable decrease in lipid peroxidation (P ≤ 0.01, ≤ 0.001) was observed. Normal histology of liver regained in cholesterol fed animals given *E. ganitrus* extract compared to the cholesterol fed animals. The phytochemical analysis of ethanolic extract of *E. ganitrus* indicated strong presence of alkaloids, flavanoids, tannins, phenols, saponins and fatty acids that may be responsible for the significant antiatherosclerotic as well as antioxidant activity. Our study exhibited that the ethanol extract of *E. ganitrus* seed is a potent agent and contribute remarkably in developing novel herbal medicines to improve the lives of patients suffering from cardiovascular diseases around the world.

KEYWORDS: Atherosclerosis, Hyperlipidaemic, Phytoconstituents, Cholesterol.

INTRODUCTION
Diseases of the heart and circulatory system (or CVD) were the second most common cause of death in the United Kingdom in 2014, with a total of around 155,000 deaths. In 2014, CVD caused 27% of all deaths and cancers caused 29%. Abnormalities of lipid metabolism are associated with cardiac diseases, obesity and their associated disorders. Thus regulation of dyslipidemia is crucial for the prevention and treatment of cardiovascular events.

It is generally established that increased generation of free radicals/ reactive oxygen species (ROS) contributes considerably in the manifestation and development of atherogenesis. The formation of enormous number of reactive oxygen species can devastate the intracellular antioxidant defense, causing activation, DNA breaks and protein modification. Antioxidants have the ability to protect the body from oxidative damage by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes.

Due to the growing concern of consumers regarding the better compatibility, improved patient tolerance of long-term pharmaceutical drug usage, the demand for nutraceutical compounds keeps on rising. Scientific researchers worldwide study alternative sources of pharmaceutical remedies one of these possible alternative sources would be medicinal plants. Natural plants have a dramatic cholesterol lowering properties without any side effects which are normally associated currently available hypolipidemic drugs. The consumption of synthetic hypolipidemic drugs having adverse effects like hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. Medicinal plants play a major role in mitigating the atherosclerosis associated with oxidative stress and suggest that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine.

*Elaeocarpus ganitrus* Roxb. (Syn. *E. sphaericus* Gaertn; family Elaeocarpaceae) is commonly known as Rudraksha is a large evergreen broad-leaved tree, prevalent for its fascinating fruit stones and medicinal properties. The widespread investigation of literature exposed that *E. 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, indolizilidine alkaloids grandisines, rudrakine and flavonoids quercitin. Furthermore it is noted to have myriad pharmacological activities that involve anti-inflammatory, analgesic and sedative hypoglycemic, antidepressant, antiasthmatic and antiulcerogenic, antihypertensive, anticonvulsant and antimicrobial.

As there is no indication of publications regarding the anti hyperlipidemic and anti-atherosclerotic activity of the plant. The present study is undertaken to screen 70% ethanolic extract of E. ganitrus at two dose levels (250 and 500 mg/kg, b.wt./day/rabbit) for its ability to decrease lipid levels and oxidative stress as well as proved effective in regaining normal histology of liver in cholesterol fed rabbits.

**MATERIALS AND METHODS**

**Collection of plant material**

The seeds of E. ganitrus were procured from Jayoti Vidyapeeth Women’s University, Jaipur and authenticated by authority of Department of Botany, University of Rajasthan, Jaipur. A voucher specimen number (RUBL21180) was submitted at University herbarium department for future reference.

**Extraction of plant material**

The seeds were coarsely powdered with mechanical grinder and extracted with 70% ethanol for 48 hrs by soxhlet extraction method. The ethanol was filtered and then separated under reduced pressure and controlled temperature (55-60°C) to obtain solid mass in a rotary evaporator and this was stored in a desiccator. This 70% ethanolic crude extract of E. ganitrus seed 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 in the study. 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 for 120 days.
- **Group II:** Cholesterol feeding for 120 days
- **Group III:** Cholesterol feeding for 60 days then treated with 250 mg/Kg ethanolic extract of E. ganitrus for next 60 days.
- **Group IV:** Cholesterol feeding for 60 days then treated with 500mg/Kg ethanolic extract of E. ganitrus for next 60 days.

Cholesterol feeding for 60 days then treated with 500 mg cholesterol/kg b.wt./rabbit/day in 5ml coconut oil.

**Biochemical analysis**

Total cholesterol, triglycerides and phospholipids were estimated in liver. Liver was also analysed for antioxidant parameter i.e lipid Peroxidation.

**Histological analysis**

The liver was fixed in Bouin’s solution for 24 hrs and then washed with water and stored in 70% alcohol. The tissue was dehydrated successively through upgrading xylene and embedded in paraffin wax for the preparation of wax impregnated blocks. From these blocks, tissue was ultrasectioned (5–6 μm thickness) on a rotary microtome and stained with haematoxyline and eosin (H & E) and examined under a light microscope for observation of structural abnormality.

**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.

Deviation Percent was calculated as:

\[
\text{Deviation Percent} = \frac{\text{Final value} - \text{Initial value}}{\text{Initial value}} \times 100
\]

**RESULTS**

**Antihyperlipidaemic parameters**

As illustrated in table 1, the total cholesterol, triglyceride and phospholipid level of liver were elevated significantly by 196.26%, 140.90% and 86.01% respectively in the hyperlipidaemic rabbits of 120 days in comparison with the control group. In contrast, treatment with E. ganitrus seed extract carried about a significant (P ≤ 0.01 and P ≤ 0.001) dose dependent decline of -44.50% and -50.77% in total cholesterol -39.26% and -44.56% in triglycerides level, -29.00% and -38.42% in phospholipids level at various doses i.e
Lipid Peroxidation (LPO) (n mol MDA/mg)

For analyzing lipid peroxidation, MDA levels were measured in liver homogenates. Lipid peroxidation value in liver tissue was raised significantly (P ≤ 0.001) by 279.31% in rabbits fed with cholesterol for 120 days as compared to control animals. However, administration of E. ganitrus extract to hyperlipidaemic rabbits prevented the rise in lipid peroxides by -31.81% and -71.87% according to the dose level when compared to cholesterol fed rabbits (120 days). (Table 1).

<table>
<thead>
<tr>
<th>Identification</th>
<th>Group</th>
<th>Cholesterol (mg/gm)</th>
<th>Triglycerides (mg/gm)</th>
<th>Phospholipids (mg/gm)</th>
<th>Lipid peroxidation (n mole MDA/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Placebo treated) from day 1-120</td>
<td>I</td>
<td>7.22±0.59</td>
<td>4.62±0.38</td>
<td>7.08±0.11</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Chol. feeding* from day 1-120</td>
<td>II</td>
<td>21.39±0.82</td>
<td>11.13±0.40</td>
<td>13.17±0.41</td>
<td>1.10±0.04</td>
</tr>
<tr>
<td>Chol. feeding* from day 1-60 +Elaeocarpus ganitrus ethanolic extract** from day 61-120</td>
<td>III</td>
<td>11.87±0.25</td>
<td>6.76±0.35</td>
<td>9.35±0.40</td>
<td>0.75±0.04</td>
</tr>
<tr>
<td>Chol. feeding* from day 1-60 +Elaeocarpus ganitrus ethanolic extract*** from day 61-120</td>
<td>IV</td>
<td>10.53±0.30</td>
<td>6.17±0.26</td>
<td>8.11±0.41</td>
<td>0.64±0.06</td>
</tr>
</tbody>
</table>

*a-Cholesterol feeding –500mg/kg.b.wt in 5 ml coconut oil / day
** E. ganitrus - 250 mg/kg.b.wt / day
*** E. ganitrus 500mg/kg.b.wt / day
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 with Group II
c-P≤0.01 Significant ns- non significant

Histology of Liver

Control group animals showed normal histology of liver, having large polygonal hepatocytes with binucleated sinusoids. In the center of each lobule a central or intralobular vein is present. Sinusoids converge radially in to the central vein. In cholesterol fed group, the hepatocytes and sinusoids became enlarged, congested and few seemed to be enucleated. Liver showed granular cytoplasm with foamy vacuolization of most of the cells. In some cells pyknotic and karyolitic changes were observed. After treatment with various doses of E. ganitrus extract to hyperlipidaemic animals, histology of damaged liver recovered along with normal shape of hepatocytes and binucleated sinusoids. However few foamy cytoplasm containing gloli and fatty material was present. The degree of vascularization was also reduced as compare to hyperlipidaemic group. Effect of Elaeocarpus ganitrus ethanolic extract on histopathological changes in liver tissue, (Hematoxylin and eosin, 100x) in cholesterol fed rabbits depicted in (Fig 1-4).
Fig. 1: Liver of control rabbit

Fig. 2: Liver of rabbit after cholesterol feeding for 120 days
Fig. 3: Liver of rabbit- Cholesterol feeding from day 1-60 + *Elaeocarpus ganitrus* ethanolic extract (250 mg/kg, b.wt./day) from day 61-120

Fig. 4: Liver of rabbit- Cholesterol feeding from day 1-60 + *Elaeocarpus ganitrus* ethanolic extract (500 mg/kg, b.wt./day) from day 61-120
DISCUSSION

The threat of cardiovascular disease development directly powers by the level of cholesterol, higher levels of cholesterol then more will be the risk of cardiovascular diseases. [22,23] Normally hepatocyte initiate synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver (e.g., after the fatty meal or in the situation of increased lipolysis) but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte cholesterol concentration by increasing the catabolic conversion of cholesterol to bile acids in liver. [24]

Total cholesterol content in the liver showed a significant elevation in hyperlipidaemic rabbits after 120 days of cholesterol feeding. Liver plays an imperative role in regulation of cholesterol metabolism with the aid of two key enzymes HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase (ACAT). E. ganitrus extract administration significantly lowered the cholesterol level in liver might be due to inhibition of ACAT. [25] Further, Cholesterol lowering effect of E. ganitrus may be speculated 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. [26]

Present investigation showed a significant increase in triglyceride (TG) contents of liver in cholesterol fed rabbits for 120 days. This increase could be due to increased secretion of triglyceride from the liver or from decreased triglyceride removal from the blood. Prolonged consumption of high-fat diet increases synthesis of TG and inhibit β-oxidation of fatty acids which consequently leads to the accumulation of excess TG in the liver. [27, 28] The accumulation of the TG causes an increase in liver weight and adipose tissues. [29] After E. ganitrus administration, a dose dependent reduction in the concentration of triglycerides in liver was observed. The reduction has been credited to a stimulation of the degradation of triglycerides through increased expression and activity of lipoprotein lipases and to a decrease of hepatic synthesis and secretion of triglycerides. [29]

An increased storage of phospholipid in the liver were seen after 120 days of cholesterol feeding when compared to control rabbits. This may be due to decreased phospholipase activity. [30] Liver showed highest phospholipid concentration, may be due to mobilization of the lipid from the heart and aorta where the turnover is slow to the liver which has a higher turnover. [31] After supplementation with ethanolic extract of E. ganitrus, levels of phospholipid showed significant reduction as compared to hyperlipidaemic rabbits may also be due to the enhanced activity of phospholipases. [32]

The shift in balance between oxidant/antioxidant in favor of oxidants is termed “oxidative stress” plays an important role in the development of atherosclerosis. [33] In present study, the content of serum MDA in cholesterol fed rabbits was elevated significantly compared with the control rabbits, suggesting that hyperlipidaemia might enhance the process of lipid peroxidation. It could be explained by the finding that excess cholesterol in platelets, polymorphonuclear cells, leukocytes and endothelial cells could lead to the generation of reactive oxygen species (ROS) and speeding up the course of lipid peroxidation, leading to tissue damage. [34,35] Study further revealed ethanolic extracts of E. ganitrus showed preventive function against atherogenesis as it reduced the lipid peroxidative markers, MDA levels in the tissues. This indicates that E. ganitrus extract react with peroxyl radicals including the inhibition of lipid peroxidation chain propagation. [36] Hence Attenuated level of LPO in extract treated animals is suggestive of the antioxidant nature of this plant.

In the histopathological examination of cholesterol fed rabbits showed progression of hepatic steatosis, inflammation, and fibrosis. [37,38] Administration with ethanolic extract of E. ganitrus resulted in less fatty cytoplasmic vacuolated cells in liver parenchyma as well as liver cell necrosis was prevented as compared to hyperlipidaemic rabbits.

Elaeocarpus ganitrus are reported to possess promising antioxidant capacity. Phytochemical analysis has revealed that different extracts contain constituents like flavonoids, polyphenols, biflavones, tannins and phenolic compounds etc. 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. Reducing power of a compound also reflects its potential of antioxidant capacity. Reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. Reducing power of EE ranged from 1.112 to 1.973 Abs (Arbitrary units) for 100 mg/ml and 200 mg/ml concentration.

Total phenolic compounds of E. ganitrus are 56.79 mg gallic acid equivalent/g of dry material. Total flavonoids present are 18.58 mg equivalent/g of dry material. [39] So comparable with the findings in the literature for other extracts of plant products. [40] Our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity. Thakur et al., 2001 [41] and Borraidaile et al., 2002 [42] studies suggest that antihypercholesterolemic effect of flavonoids is related to decrease of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) and decrease in apo B secretion in hepatocytes.

Regarding observed outcomes, the possible mechanism could be the presence of biologically active phytoconstituents such as phytosteres, fats, alkaloids, flavonoids, phenols, terpenoids, carbohydrates, proteins and tannins in the ethanolic extract of E. ganitrus that may demonstrate the multitarget, multicomponent
features for regulating lipid metabolism.\textsuperscript{43} Thus the isolation of the pure secondary metabolites responsible for the extracts activity and their molecular mechanism as well as expression studies related to lipid metabolism will be a good addition to the \textit{Elaeocarpus} literature.

CONCLUSIONS

In accordance with these results, it may be confirmed that consumption of \textit{E. ganitrus} extract could prevent or be helpful in reducing the complications of dyslipidemia associated with oxidative stress in hyperlipidaemic rabbits. Antiatherosclerotic and lipid lowering activities of this plant are probably due to its phytochemicals. These health promoting effects can range from providing dietary antioxidant effects to modifying signal transduction in the biological systems through alterations in gene expression. As a concluding remark, ethanolic extract of \textit{E. ganitrus} seems to be a potential cardioprotective candidate in rabbits. Advance studies need to be done in order to establish a detailed assessment of metabolic effects, anti-oxidant actions and the efficacy of this plant extract as a hypolipidaemic drug.

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DECLARATION OF INTEREST

The authors report no declarations of interest.

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