

ANTI-ATHEROSCLEROTIC ACTIVITY OF *ELAEOCARPUS GANITRUS* ROXB. IN CHOLESTEROL-FED RABBITS

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ABSTRACT

Objectives: Atherosclerosis was experimentally induced in New Zealand white male rabbits by cholesterol feeding for 120 days for investigating effects of 70% ethanolic *Elaeocarpus ganitrus* seed extract (EEGS) against atherosclerosis.

Methods: Anti-atherosclerotic activity of *E. ganitrus* extract was evaluated at a dose level of 250 and 500 mg/kg of body weight (p.o). On 121st day, the animals were sacrificed to collect the aorta for determining tissue lipid profile, antioxidant parameters, and histopathological changes. One-way ANOVA followed by Tukey's multiple comparison test was used for the statistical analysis.

Results: Aorta of cholesterol-fed rabbits for 120 days showed marked elevation in total cholesterol, triglycerides, and phospholipids. EEGS extract treatment significantly ($p \leq 0.01$, ≤ 0.001) corrected disturbed lipid profile in cholesterol-fed rabbits in a dose-dependent manner during the 60 days of the treatment period. A significant reduction in lipid peroxidation and a considerable raise in glutathione, catalase, and superoxide dismutase levels ($p \leq 0.01$, ≤ 0.001) were accompanied by post-treatment with EEGS extract to cholesterol-fed rabbits. The histopathological modifications such as accumulation of foam cells, atheromatous plaque formation, and lumen size reduction supported the successful induction of atherosclerosis in rabbits. The treated rabbits showed significant protective effect by lowering the deposition of cholesterol and increasing the lumen size compared to cholesterol-fed group.

Conclusions: Phytoconstituents such as alkaloids, tannins, flavonoids, steroids, triterpenoids, and carbohydrates in the ethanolic extract may be attributed to effective anti-atherosclerotic and antioxidant activity of plant extract. Our study exhibited that EEGS extract could be a potent herbal therapeutic agent for the treatment of atherosclerosis and hypercholesterolemia-related diseases.

Keywords: *Elaeocarpus ganitrus*, Atherosclerosis, Hyperlipidemic, Phytoconstituents, Cholesterol, Ethanolic extract.

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INTRODUCTION

Hypercholesterolemia contributes significantly to the occurrence and rigorosity of atherosclerosis and cardiovascular disease that could eventually affect the majority of the adult population of developed countries [1]. The disproportion of the lipid metabolites in the affected organism is mainly accountable for these conditions. Atherosclerosis is the major lipid disorder that is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall [2]. Further, increased oxidative stress due to overproduction of oxygen free radicals resulting in disorders of lipid metabolism. There is evidence to support an association between lipid oxidation and atherosclerosis development [3]. An antioxidant, which inhibits processes that lead to oxidative stress should be effective for overwhelming atherosclerosis [4].

Several studies have established that obesity and hyperlipidemia can be induced by high-fat or high-calorie diets in the normal rodent model [5,6]. Herbal drugs, derivative of plant extracts, are abundantly utilized to treat a wide range of clinical diseases. *Elaeocarpus ganitrus* Roxb. (Syn. *E. sphaericus* Gaertn; family *Elaeocarpaceae*) which is commonly known as Rudraksha is a large evergreen broad-leaved tree. It is ethnomedicinally important plant, and different parts (seeds, leaves, and outer shell of beads) of it exhibit diverse pharmacological activities. The extensive investigation of literature exposed that *E. ganitrus* Roxb. is a vital source of many pharmacologically and therapeutically significant chemicals such as triterpenes, flavonoids like quercetin, tannins which include geraniin, and 3, 4, 5-trimethoxy

geraniin and indolizidine alkaloids, grandisines, and rudrakine [7]. In the experimental studies, extracts of *E. ganitrus* showed large range of pharmacological properties such as anti-inflammatory [8], analgesic, sedative [9], hypoglycemic [10], antidepressant [11], antiasthmatic [12], antihypertensive [13], and antimicrobial [14].

Therefore, the present study is carried out to elucidate the effects of *E. ganitrus* on histopathology of the aorta using a cholesterol-induced hypercholesterolemic rabbit model. The outcomes of this study provide evidence to support the viability of developing *E. ganitrus* as a potential natural-based medicine for the treatment of atherosclerosis as well as other diseases related with hypercholesterolemia.

METHODS

Collection and extraction of plant material

Authentic seeds of *E. ganitrus* were obtained from Jayoti Vidyapeeth Women's University, Jaipur, and authenticated by authority of the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen number (RUBL21180) was submitted at University Herbarium Department for future reference. The seeds were coarsely powdered in a cutter and grinding mill. Powdered seeds of *E. ganitrus* were extracted with 70% ethanol for 48 hrs, by Soxhlet extraction method. Then, ethanol was filtered and evaporated to dryness at 55-60°C under reduced pressure in a rotary evaporator. The extract was stored in a desiccator. This 70% ethanolic crude extract of the *E. ganitrus* seed extract (EEGS) was dissolved in distilled water and administered to the animals through 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 and housed in polypropylene cages at controlled temperature ($23\pm 2^\circ\text{C}$). The animals were maintained under a standard diet pellet (Ashirwad Industrial Ltd., Punjab), green leafy vegetables, and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee and was executed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India.

Experimental design

The rabbits were divided into following groups:

- 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 EEGS extract for the next 60 days
- Group IV: Cholesterol feeding for 60 days then treated with 500 mg/kg EEGS extract for the next 60 days.

Cholesterol feeding: 500 mg cholesterol//kg. b.wt./day/rabbit/day in 5 ml coconut oil.

At the end of the treatment, all the rabbits were sacrificed; blood and aorta were collected for biochemical and histopathological examinations.

Induction of hyperlipidemia

New Zealand white male rabbits were made hyperlipidemic by daily oral administration of 500 mg cholesterol/kg.b.wt./rabbit/day in 5 ml coconut oil.

Autopsy and fixation

Animals were autopsied under ether anesthesia after completion of treatment. Aorta was quickly removed, cleared off the fat, and connective tissue weighed on electronic balance. A small section of the aorta of each animal was soaked in a 10 % (v/v) formocalcium solution for hematoxylin and eosin (H and E) staining. The aorta sections were processed for normal histological section. The tissue samples were ultrasectioned (5-6 μm thickness), stained with H and E, and analyzed under a light microscope for observation of structural abnormality.

Biochemical analysis

Aorta was analyzed for total cholesterol [15], triglyceride [16], phospholipid [17], and antioxidant parameters, i.e., lipid peroxidation [18], catalase (CAT) [19], glutathione (GSH) [20], and superoxide dismutase (SOD) [21].

Statistical analysis

All values were expressed as mean \pm standard error of the mean. The data obtained were subjected to statistical analysis using one-way ANOVA followed by Tukey's multiple comparison tests by GraphPad Prism software (version 5). $p < 0.05$ was considered to be statistically significant.

RESULTS

Cholesterol, phospholipid, and triglyceride level of the aorta after cholesterol feeding in Group II showed elevation in comparison to the control group. However, it was found to be decreased significantly ($p \leq 0.001$) in both the treated groups in comparison to hyperlipidemic rabbits in dose-dependent manner (Fig. 1).

Further, we found that cholesterol feeding to rabbits for 120 days produced a significant decrease in the activity of CAT, SOD, and GSH levels whereas an increase in thiobarbituric acid reactive substances (marker of lipid peroxidation) activity of aorta was observed. A dose dependent Reduction in lipid peroxidation levels, whereas an elevation in GSH content, catalase as well as SOD activity was observed after the Treatment with EEGS extract (Table 1).

The histopathological analysis of control group rabbits showed characteristic three layers of arteries without any lesion in intima or media (Fig. 2). Cholesterol-fed rabbits for 120 days depicted well-developed atheromatous intimal plaque. The plaque consists of lipid-laden foam cells, collagenous fibers, and smooth muscle cells. The lumen size was reduced to a great extent as plaque is protruding into the lumen of the aorta when compared to control aorta. Few foam cells were also presenting in media (Fig. 3). Microphotographs of the

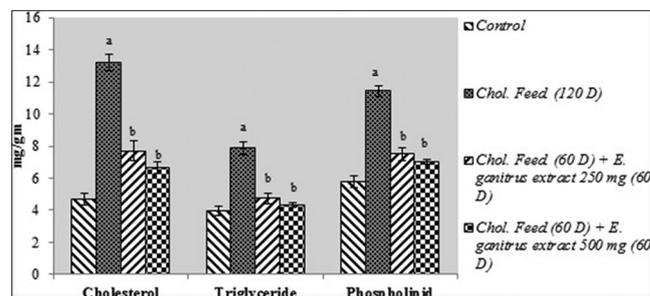


Fig. 1: Effect of ethanol extract of *Elaeocarpus ganitrus* on tissue biochemistry of cholesterol-fed rabbits. *Cholesterol feeding: 500 mg/kg body weight (b.wt.) in 5 ml coconut oil/day, *E. ganitrus*: 250 mg/kg. b.wt./day, ****E. ganitrus* 500 mg/kg. b.wt./day, values \pm 5 determination, ^a $p \leq 0.001$ highly significant, ^b $p \leq 0.001$ highly significant Group II compared with Group I, Group III, IV compared with Group II**

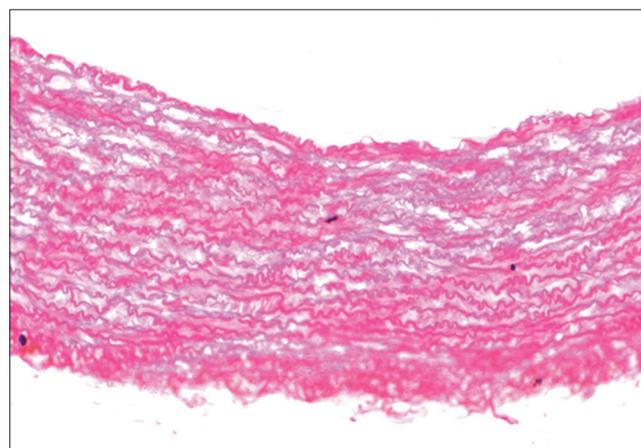


Fig. 2: Ascending aorta of control rabbit

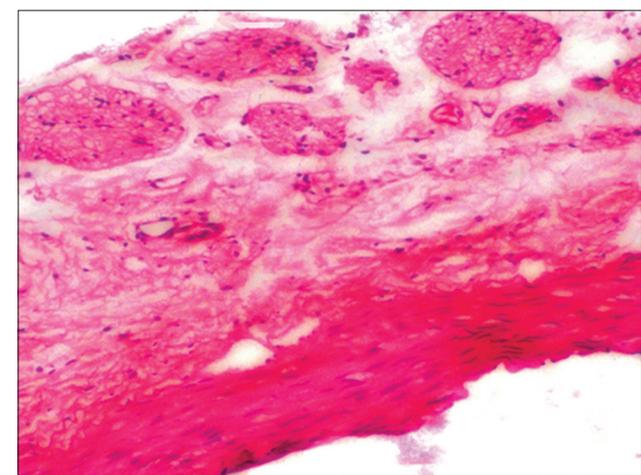


Fig. 3: Ascending aorta of rabbit after cholesterol feeding for 120 days

Table 1: Effect of ethanolic extract of *E. ganitrus* on antioxidant parameters

Identification	Group	Lipid peroxidation (n mole MDA/mg)	GSH (n mole/gm)	CAT (nmole of H ₂ O ₂ consumed/minute/mg protein)	SOD (units/mg of protein)
Control (placebo treated) from day 1 to 120	I	0.11±0.02	20.59±1.11	69.95±2.07	17.87±0.64
Cholesterol feeding from day 1 to 120	II	0.79 ^a ±0.05	9.74 ^a ±1.19	43.94 ^a ±1.43	6.04 ^a ±1.10
Cholesterol feeding* from day 1 to 60+ <i>E. ganitrus</i> ethanolic extract** from day 61 to 120	III	0.52 ^c ±0.02	16.63 ^c ±0.63	55.55 ^c ±1.27	13.76 ^c ±1.15
Cholesterol feeding from day 1 to 60+ <i>E. ganitrus</i> ethanolic extract*** from day 61 to 120	IV	0.45 ^b ±0.02	19.55 ^b ±0.52	66.01 ^b ±1.916	15.34 ^b ±1.21

E. ganitrus: *Elaeocarpus ganitrus*, *cholesterol feeding: 500 mg/kg.b.wt. in 5 ml coconut oil/day, ***E. ganitrus* 250 mg/kg.b.wt./day, ****E. ganitrus* 500 mg/kg.b.wt./day. Values±5 determination, ^ap≤0.001 highly significant Group II compared with Group I, ^bp≤0.01 significant, ^cp≤0.001 highly significant Group III, IV compared with Group II, ^pp≤0.01 significant, MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase, b.wt: Body weight, SOD: Superoxide dismutase

animals after administration of different doses of EEGS extract showed regressed intimal plaque, and three distinct layers of intima, media, and adventitia are reverting to their normal position. However, few lipid-laden cells and fibrous tissue were present in the middle layer in comparison to Group II rabbits (Figs. 4 and 5).

DISCUSSION

Plant sterols of *E. ganitrus* may be attributed to cholesterol lowering effect by interference in the absorption of dietary fat and cholesterol as well as improved endogenous cholesterol excretion [22]. Previous studies have shown that a high-fat diet in rats increases the long-chain acyl-CoA content in liver and red muscles. Our study further confirmed that cholesterol feeding for 120 days would increase the acyl-CoA pool, resulting into increased triglyceride storage [23]. Phytoconstituents of EEGS extract may credit to decreased level by a stimulation of the degradation of triglycerides through better expression and activity of lipoprotein lipases and to a reduction of hepatic production and excretion of triglycerides [24]. Post-treatment with EEGS extract showed considerable reduction in phospholipid levels as compared to hypercholesterolemic rabbits. This may be attributed to the improved activity of phospholipases [25].

High cholesterol, particularly low-density lipoprotein (LDL) is the major Reason attributed to cardiovascular diseases. The lipid peroxidation is aggravated by the imbalance in the lipid metabolism [26,27]. The previous study demonstrated that a decline in lipid peroxidation result in the reduction of hypercholesterolemia-induced atherosclerosis [28]. In the current study, the malondialdehyde (MDA) level was raised significantly in cholesterol-fed rabbits in comparison with the control rabbits. Experimental outcomes suggested that surplus cholesterol result in the generation of reactive oxygen species and speedy progression of lipid peroxidation, leading to tissue damage [29,30]. Furthermore, EEGS extract revealed preventive function against atherogenesis as it decreased the lipid peroxidative markers, MDA levels in the tissues. This could be due to the reaction of EEGS extract with peroxy radicals including the inhibition of lipid peroxidation chain propagation [31].

A significant decrease in the levels of total GSH observed on cholesterol feeding might be due to reduced GSH biosynthesis which increased in hypercholesterolemia [32]. Cholesterol feeding followed by administration of plant extract to hypercholesterolemic animals showed a significantly elevated level of GSH. It is possible that extract might have reduced the extent of oxidative stress, leading to lesser GSH degradation or increase in the biosynthesis of GSH [33]. Furthermore, it could be due to decreases in the free radicals by quenching and lowering oxidative stress [34].

The decrease in the SOD and the catalase activities in the cholesterol-fed rabbits are recognized to increased oxidative stress on cholesterol feeding in these animals [35]. EEGS extract-treated hyperlipidemic rabbits had noticeably elevated levels of SOD and CAT, reversing the ill effects of hyperlipidemia. It is well established that polyphenols and

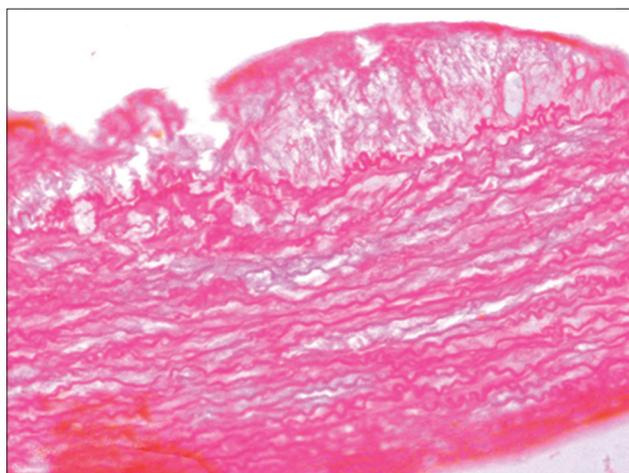


Fig. 4: Ascending aorta of rabbit - cholesterol feeding from day 1 to 60+*Elaeocarpus ganitrus* seed extract extract (250 mg/kg. body weight/day) from day 61 to 120

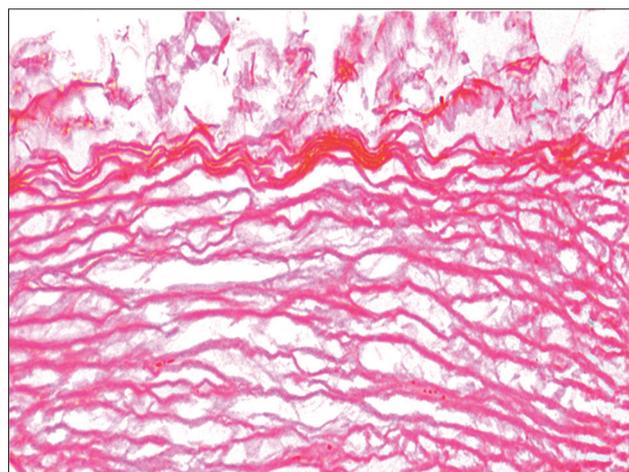


Fig. 5: Ascending aorta of rabbit - cholesterol feeding from day 1 to 60+*Elaeocarpus ganitrus* seed extract (500 mg/kg. body weight/day) from day 61 to 120

flavonoids are natural antioxidants and have also been reported to considerably increase SOD and CAT levels [36,37]. The 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 [38]. These findings recommend 85% of the antioxidant capacity of *E. ganitrus* is by virtue of phenolics and flavonoid components. The presently illustrated raised levels of both SOD and CAT could be

explained as the influence of flavonoids and polyphenols of *E. ganitrus*. This is in agreement with the findings of other studies in the literature for extracts of plant products [39].

Aorta is the main artery originating from the left ventricle of the heart and distributes oxygenated blood to all the body parts. The control rabbit aorta showed all the three layers, i.e., tunica intima, tunica media, and the outer most tunica adventitia. In the present study, cholesterol feeding for 120 days demonstrated atheromatous plaque when compared to control group rabbits. In hyperlipidemic rabbits, cholesterol deposits were concentrated in the media tunica and intima tunica regions, mainly in the endothelial layer. The focal thickening of the intima is due to a large increase in smooth muscle cells, formation of new connective tissue matrix by these smooth muscle cells, and in hyperlipidemic individuals, the accumulation of intracellular and extracellular lipid.

Preclinical interpretations have shown that hypercholesterolemia induces the buildup of oxLDL in the arterial wall, stimulating endothelial cell dysfunction and atherosclerosis development [40]. These substances are noxious to endothelial cells, leading in lesions that promote monocytes and macrophages to grow into foam cells, eventually resulting into atheroma [41]. A marked decrease in plaques was observed in the EEGS extract-treated groups as compared to the cholesterol-fed group. The improvement might be associated with the free radical scavenging activity of plant extract which prevents LDL oxidation. In addition, it could be due to the strong presence of sterols which stimulate bile fluid secretion as well as biliary cholesterol secretion and improve excretion of bile acids in feces [42]. Furthermore, it is well known that polyphenols have various pharmacological properties, including hypolipidemic and antiatherogenic activity [43]. The tannins and flavones may perform in free radical scavenging mechanism and may check atherogenesis in rabbit aorta [44,45].

CONCLUSION

Considering all the above findings, it can be concluded that consumption of *E. ganitrus* extract might check atherogenesis. Biologically active components such as phytosterols, flavonoids, alkaloids, tannins, fats, carbohydrates, and proteins could be credited to anti-atherosclerotic property of the plant. There is a requirement of advance studies to be done for isolating the pure secondary metabolites and to study the exact underlying vascular protective mechanisms of EEGS extract. Our study demonstrated that the seed of *E. ganitrus* has strong cardiovascular protective effects and could be a potential candidate for the development as a herbal therapeutic for preventing atherosclerosis progression triggered by unusual lipid metabolism and oxidative stress.

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