

## Antihyperlipidemic potential of *Eugenia caryophyllus* extract in Wistar rats

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### Abstract

**Objective:** The present study was aimed to evaluate the antihyperlipidemic activity of the aqueous ethanolic extract of *Eugenia caryophyllus* in triton induced hyperlipidemia.

**Methods:** Antihyperlipidemic activity was evaluated using Triton X 100 induced hyperlipidemia in rats as an experimental model. Hyperlipidemia was induced in rats by a single intraperitoneal (ip) injection of Triton X 100 (400 mg/kg b.w.) The aqueous ethanolic extract of *Eugenia caryophyllus* extract was administered at two different doses of 200mg/kg and 400mg/kg for 7 days to hyperlipidemic rats. Atorvastatin was used as a reference standard. Serum triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C, atherogenic index and glucose were determined to assess the antihyperlipidemic activity.

**Result:** It was found that the aqueous ethanolic extract of *Eugenia caryophyllus* at the doses of 200mg/kg and 400mg/kg showed a significant decrease in the levels of serum total cholesterol, triglycerides, LDL-C, VLDL-C, glucose and significant increase in the level of serum HDL-C.

**Conclusion:** The current study provided a strong evidence that the *Eugenia caryophyllus* extracts at both the doses (200mg/kg and 400mg/kg) possess significant antihyperlipidemic activity. The dose of 400mg/kg had a higher beneficial effect and its efficacy was similar to that of Atorvastatin in treating hyperlipidemia. However, further study is needed to understand the precise mechanism.

**Keywords:** Triton, hyperlipidemia, Atorvastatin, *Eugenia caryophyllus*

### Introduction

Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low density lipoprotein cholesterol and reduced high density lipoproteins but increased triglycerides in blood. It is a primary risk factor for atherosclerosis which is also referred as a "silent killer" as being responsible for coronary artery disease and cerebral vascular diseases. Atherosclerosis is one of the leading causes of death in the developed countries and is on the rise in developing countries like India [1]. World Health Organization (WHO) survey has reported that India is predicted to have a large no. of mortalities due to coronary artery disease by the year 2015 [2]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischaemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [3]. Currently available hypolipidaemic drugs have been associated with a number of side effects [4,5]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [6]. Therefore it is a need of the day to search other materials from natural sources that are less toxic, less expensive, which can provide better safety and efficacy on a long term usage. Natural products from plants are a rich source used for centuries to cure various ailments.

*Eugenia caryophyllus* is a small ever-green tree belongs to the botanical family *Myrtaceae* (subfamily: *Myrtoideae* and tribe:

*Syzygiae*) and scientifically known as *Syzygium aromaticum* (L.) Merr. & L. M. Perry. Dried flower buds of *Eugenia caryophyllus* are used as a spice. The essential oil obtained from the buds of *Eugenia caryophyllus* L. is widely known for its medicinal properties. Chemical analysis has identified the major constituents as eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone [7]. Its oil has anti-oxidant properties and in dentistry used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavouring industries. The essential oil has shown to have anti-microbial activity [8,9] and anti-fungal properties against dermatophytes [10,11]. In addition, it is found to have antimutagenic [12], anti-inflammatory [13], antiulcerogenic [14,15], antithrombotic [16] and antiparasitic [17] and an anti-convulsant [18] activity. The anti-oxidant effect of eugenol has been reported on carbon tetrachloride induced erythrocyte damage in rats [19]. Pharmacological studies with *Eugenia caryophyllus* extract have also demonstrated anti-stress activity [20], analgesic activities [21,22] and found to be more potent than aspirin in inhibiting platelet aggregation [23]. However there were no documented reports on the antihyperlipidemic activity of this plant so the present study was undertaken to investigate antihyperlipidemic activity of the aqueous ethanolic extract of flower buds of *Eugenia caryophyllus*.

### Materials and Methods



## Chemicals

Triton X-100 was obtained from Sigma-Aldrich, USA. Atorvastatin was obtained from Alkem Research Centre, Navi Mumbai. All other chemicals were of analytical grade.

## Plant material

The flower buds of *Eugenia caryophyllus* were collected from an authorized vendor Global Herbs and authenticated by Professor Mohammed Ali, Department of Pharmacognosy, Faculty of Pharmacy, Jamia Hamdard, New Delhi. A voucher specimen coded PRL/JH/11/01 was deposited in the Jamia Hamdard for future reference.

## Extraction procedure

The flower buds of *Eugenia caryophyllus* were dried at room temperature and reduced to coarse powder. The powdered material was subjected to qualitative tests [24,25] for the identification of various phytoconstituents like alkaloids, flavanoids, phenols, phytosterols etc. The dried powder was subjected to cold maceration with 70% ethanol for 7 days at room temperature with in between stirring & shaking. After 7 days, it was filtered and the filtrate was concentrated on water bath to obtain a dark brownish residue. The aqueous ethanolic extract of *Eugenia caryophyllus* will be called as EEC [26].

## Preliminary Phytochemical analysis

The aqueous ethanolic extract of the flower buds of *Eugenia caryophyllus* was subjected to preliminary phytochemical screening [27]

## Experimental Animals

The present study was approved by Institutional Animal Ethical Committee (IAEC) of KIET School of Pharmacy, Ghaziabad constituted under CPCSEA. (IAEC ref no. IAEC/KSOP/02/2013-2014 dtd 18.10.13).

Wistar albino adult rats weighing 175-250g of either sex were obtained from the animal house, KIET School of Pharmacy, Ghaziabad, India. The animals were grouped and housed in polyacrylic cages (38x 23 x 10 cm) with not more than five animals per cage and maintained under standard laboratory conditions (temperature 25±2°C), relative humidity (50% ± 5%) and 12 h light and dark cycle. They were allowed free access to standard pellet rat chow diet (Amrut feeds, Pranav Agro Industries Ltd., New Delhi, India) and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

## Acute toxicity studies

Albino mice weighing 22-25 g selected by random sampling technique, were used in the study. Acute oral toxicity was

performed as per OECD- 423 guidelines [28]. The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight p.o. and the groups were observed for 14 days. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

## Antihyperlipidemic studies

### Induction of hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats by a single intraperitoneal injection of freshly prepared solution of Triton X-100 (400mg/kg) after overnight fasting for 18hrs. The animals were divided into five groups of 6 rats each. The first group was given standard pellet diet and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 400mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. The third and fourth group was administered with 200mg/kg and 400 mg/kg suspended in 5%CMC respectively of aqueous ethanolic extract of *Eugenia caryophyllus* p.o., for daily 7 days, after inducing hyperlipidemia. Fifth group was administered with the standard drug Atorvastatin (10mg/kg) p.o. for 7 days [29].

### Collection of blood

On the 8<sup>th</sup> day, the blood was collected by retro orbital sinus puncture, under mild anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments.

### Biochemical analysis

The serum was assayed for total cholesterol, triglycerides, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL) using standard protocol methods. Atherogenic index was calculated as  $A.I. = \frac{\text{Total Cholesterol}}{\text{HDL-C}}$ . The serum was also assayed for glucose to assess its effect on liver and pancreas.

### Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis was carried out by using ANOVA followed by Dunnett's multiple comparison tests using Graph pad PRISM software version 4.03. P values < 0.05 were considered as statistically significant.

## Results & Discussion

*Eugenia caryophyllus* was found to be non-toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. On basis of the acute toxicity studies, the doses 1/10<sup>th</sup> i.e. 200mg and 1/20<sup>th</sup> i.e. 400mg/kg were selected.



The phytochemical tests with the aqueous ethanolic extract of *Eugenia caryophyllus* indicated the presence of flavanoids, carbohydrates, alkaloids, phytosterols, saponins.

Hyperlipidemia has been associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values have been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The present study found that there was an extremely significant ( $P<0.05$ ) increase in serum total cholesterol, triglycerides, low density lipoprotein (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and atherogenic index in triton treated group as compared to normal control group. All the treated groups showed an extremely significant ( $P<0.05$ ) decrease in serum total cholesterol, triglycerides, LDL-C, VLDL-C and atherogenic index.

In comparison to 200mg/kg, the dose 400mg/kg possessed better antihyperlipidemic property.

There was an extremely significant ( $P<0.05$ ) decrease in serum HDL cholesterol in triton treated group as compared to normal control.

Atorvastatin 10mg/kg, the extract at doses 200mg/kg and 400 mg/kg showed an extremely significant increase in HDL cholesterol as compared to hyperlipidemic control group. Among all 400 mg/kg was found to be most protective group.

There was an extremely significant ( $P<0.05$ ) increase in serum glucose level of hyperlipidemic control group as compared to normal control group. Atorvastatin 10mg/kg, 200mg/kg and extract at 400 mg/kg showed an extremely significant decrease in serum blood glucose level as compared to hyperlipidemic control group. Among all 400 mg/kg was found to be most protective group.

**Table 1: Effect of ethanolic extract of *Eugenia caryophyllus* (EEC) on total cholesterol, HDL-C, LDL-C, VLDL-C in serum.**

Groups	Total-Chol	HDL-C	LDL-C	VLDL-C
Group-I Normal Control	112.08±0.95	27.59±1.43	60.03±2.25	24.46±0.42
GroupII :Triton treated (Hyperlipidemic control)	229.59±2.13 <sup>a</sup>	13.12±0.94 <sup>a</sup>	176.27±2.22 <sup>a</sup>	40.21±0.33 <sup>a</sup>
Group III : Triton+EEC 200mg/kg	136.41±1.95 <sup>b</sup>	21.58±0.98 <sup>b</sup>	86.50±2.39 <sup>b</sup>	28.32±0.32 <sup>b</sup>
Group IV: Triton+EEC 400mg/kg	122.78±1.68 <sup>b,c</sup>	26.78±0.81 <sup>b,c</sup>	71.21±1.90 <sup>b,c</sup>	24.79±0.23 <sup>b,c</sup>
Group V: Triton + Atorvastatin 10mg/kg	123.99±1.57 <sup>b</sup>	26.23±1.19 <sup>b</sup>	72.26±2.51 <sup>b</sup>	25.50±0.30 <sup>b</sup>

Values are mean ±SEM(n=6). <sup>a</sup> $P<0.05$  vs Group I and <sup>b</sup> $P<0.05$  vs Group II, <sup>c</sup> $P<0.05$  vs Group III using one way ANOVA followed by Dunnet's test.

**Table 2: Effect of ethanolic extract of *Eugenia caryophyllus* on Triglycerides, atherogenic index and glucose in serum.**

Groups	TG's	Atherogenic Index	Glucose
Group-1 Normal Control	122.31±2.08	4.13±0.26	82.64±2.77
GroupII :Triton treated (Hyperlipidemic Control)	201.07±1.65 <sup>a</sup>	17.96±1.28 <sup>a</sup>	150.27±2.84 <sup>a</sup>
GroupIII Triton+EC 200mg/kg	141.59±1.62 <sup>b</sup>	6.39±0.35 <sup>b</sup>	117.89±1.39 <sup>b</sup>
Group IV Triton +EC 400mg/kg	123.95±1.16 <sup>b,c</sup>	4.61±0.17 <sup>b,c</sup>	89.60±1.88 <sup>b,c</sup>
Group V Triton + Atorvastatin 10mg/kg	127.48±1.50 <sup>b</sup>	4.78±0.26 <sup>b</sup>	114.17±1.96 <sup>b</sup>

Values are mean ±SEM(n=6). <sup>a</sup> $P<0.001$  vs Group I and <sup>b</sup> $P<0.001$  vs Group II, <sup>c</sup> $P<0.05$  vs Group III using one way ANOVA followed by Dunnet's test.

The aim of the present study was to elucidate the role of *Eugenia caryophyllus* extract during hyperlipidemia induced by Triton X100 in rats. Raised level of LDL-C is a major risk factor for coronary

artery disease and HDL-C works as a cardioprotective protein. It is well known that Triton X 100 (a non-ionic detergent) blocks the clearance of triglyceride-rich lipoproteins to induce active



hyperlipidemia. Triton acts as a surfactant and suppresses the action of lipoprotein lipases to block the uptake of lipoproteins from the circulation by the extrahepatic tissues resulting into increased blood lipid concentration [30]. With the aid of lipoprotein lipase present on the endothelial walls of the vessels, VLDL is converted into LDL where the cholesterol can be either used as a substrate for steroid synthesis or for bile acids. Triton induced hyperlipidemia model has been used as a screening method for hyperlipidemic agents and also used for elucidating lipid metabolism[31].

Treatment with the aqueous ethanolic extract of *Eugenia caryophyllus* at the doses of 200mg/kg and 400mg/kg reduced the levels of serum total cholesterol, triglycerides, VLDL-C, LDL-C and raised good cholesterol carrier HDL as compared to Triton treated group. The reduction in the triglycerides level may be due to an increase in the activity of endothelial bound lipoprotein lipase that hydrolyzes the triglycerides into fatty acids or could be inhibition of lipolysis so that fatty acids do not get converted into triglycerides. The cholesterol lowering activity of the extract could be due to rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids. Furthermore the components like flavanoids and polyphenols have exhibited a variety of pharmacological activities including anti-atherogenic effect.

## Conclusion

The findings of the present study revealed that *Eugenia caryophyllus* in both low (200mg/kg) and high (400mg/kg) doses showed antihyperlipidemic activity against Triton X-100 induced hyperlipidaemia. Among all the treated groups, *Eugenia caryophyllus* extract at the dose of 400mg/kg had shown better protection. The anti-hyperlipidemic activity of the plant could be attributed to the presence of flavanoids in them so further research is needed to carry out to find out the exact mechanism of action.

## Author's Contributions

Dr. K. Nagarajan has given guidance in designing the overall protocol of my research work. Dr. Vinay Kumar has helped me in writing and editing the complete manuscript. Mrs. Parul Grover has given her full co-operation during the extraction and phytochemical screening work.

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