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Research Article

The effects of *Emblica officinalis* on serum lipids and atherogenesis, in albino rats fed with high fat diet.

DR. ANJU LAMA *, DR. HITESWAR SAIKIA
Department of Pharmacology,Melmaruvathur Adhiparasakthi Institute of Medical Science & Research.
Melmaruvathur- 603319,Telephone- 09789693382

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Abstract

The present work is aimed to evaluate the effects of Emblica officinalis (Amla) extract on serum lipids and atherogenesis, in albino rats fed with high fat diet. Healthy albino rats of Wistar strain (150-200 gm each) were randomized into five groups of six animals each- Group A (received normal diet), Group B (received normal diet + Emblica officinalis extract 1 gm/kg BW), Group C (received high fat diet consisting of vanaspati ghee and coconut oil at a ratio of 3:2, at a dose of 10 ml/kg/day), Group D (received high fat diet + Emblica officinalis extract 1 gm/kg BW) and Group E (received high fat diet + simvastatin 1.8 mg/kg BW). Treatment period was 8 weeks. At the end of 8 weeks, lipid profile was evaluated by estimating total cholesterol, serum triglyceride, serum LDL, serum HDL and atherogenic index. Ethanolic extract of Emblica officinalis showed significant antihyperlipidaemic activity ($P < 0.01$) with significant improvement in atherogenic index ($P<0.01$). The results suggest that Emblica officinalis extract at a dose of 1 gm/kg BW exerts antihyperlipidaemic effect comparable to that of simvastatin. It also possesses hypolipidaemic activity.

Keywords: - Emblica officinalis, Phyllanthus emblica, Amla, Indian Gooseberry, High fat diet, Atherogenesis, Simvastatin, Antihyperlipidaemic, Hypolipidaemic.

Introduction

Hyperlipidaemia and its associated consequences are undisputed risk factors in the development of atherosclerosis. Moreover, a World Health Organization (WHO) survey reveals that India is predicted to have a large number of mortalities due to coronary artery disease by the year 2015. Atherosclerosis is a disease that involves the interplay of several factors. There are three main issues to be addressed in atherosclerosis, *viz*., hyperlipidemia, clotting factors and oxidation of lipoproteins. These factors collectively contribute to the development and rupture of the atherosclerotic plaque [1]. The coronary and the cerebral circulations are common sites of atherosclerosis. The cause of atherosclerosis is not known although several factors have been blamed in the pathogenesis of atherosclerosis. A lot of experimental and epidemiological evidence suggests a relationship between atherosclerosis and elevated levels of plasma lipids [2].

Emblica officinalis or *Phyllanthus emblica* (Family euphorbiaceae) (***syn:*** Amla, Indian Gooseberry) is an evergreen tree which is highly prized in tropical Asia. The genus is nature to tropical southeastern Asia, particularly in central and southern India, Pakistan, Bangladesh, Sri Lanka, Malaysia, Southern China and Mascarene Islands. It is commonly cultivated in gardens throughout India and grown commercially as a medicinal fruit [3]. It is among the most important medicinal plants in the Ayurvedic Materia Medica and widely used in Indian Medicine for the treatment of various diseases [4]. *Emblica officinalis* primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C(478.56 mg/100 ml). Compounds isolated from *Emblica officinalis* were gallic acid, ellagic acid, 1- O-galloyl-beta-D-glucose,3,6-di-O-

Correspondence Address:
DR. ANJU LAMA
Assistant Professor,Department of Pharmacology
Melmaruvathur Adhiparasakthi Institute of Medical Science & Research,Melmaruvathur- 603319
Email: ritetoanju@gmail.com
Phone:91-9789693382

galloyl-Dglucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O - galloyl beta D glucose, 3 Ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and isostrictiniin. It also contains flavonoids, kaempferol 3 O alpha L (6" methyl) rhamnopyranoside and kaempferol 3 O alpha L (6"ethyl) rhamnopyranoside. A new acylated apigenin glucoside (apigenin 7 O (6" butyryl beta glucopyranoside) was isolated from the methanolic extract of the leaves of Phyllanthus emblica together with the known compounds; gallic acid, methyl gallate, 1,2,3,4,6-penta-O-galloylglucose and luteolin-4'-Oneohesperiodoside were also reported [5].

Each part of the plant has a different therapeutic value –the root, the bark and the ripe fruit are astringent, the flowers are cooling and aperients, the unripe fruit is cooling, diuretic and laxative. The exudation from the incisions on the fruit is used as an external application in inflammation of the eye. Every part of the plant is equally useful in the antidotal treatment of snake –bite and scorpion sting [3]. Apart from the traditional uses, there are several reports on the pharmacological actions of Amla based on modern scientific investigations specially anti–inflammatory action [6], antimicrobial action [7], antioxidant action [8], anticarcinogenic action[9], antiulcerogenic action [10], anti– diabetic action [11],analgesic action [12],and hepatoprotective action [13].Keeping in view the above ideas, the present study has been undertaken to evaluate the effect of *Emblica officinalis* on serum lipids and atherogenesis in albino rats fed with high fat diet.

MATERIALS AND METHODS:

Drugs used in the study:

Alcoholic extracts of *Emblica officinalis* (**EEO**), Simvastatin, High Fat Diet, Normal saline (0.9 %) as vehicle.

Method of Preparation of Alcoholic Extract of *Emblica Officinalis* (EEO):

Fresh, mature, healthy and good quality fruits of *Emblica officinalis* were procured from the local market, during the months of November–December 2005 and identified and authenticated. Five (5) kgs of fresh fruits were washed thoroughly and shade dried. The dried fruits were then powdered by electrical grinder and kept in a tight container.500 gms of powdered fruits were soaked in sufficient quantity of 70% ethanol.and allowed to stand for minutes. Then it was transferred to a percolator and firmly packed. Enough of the solvent was poured on it for saturation and top of the percolator was closed. The drug was allowed to macerate for 24 hours at room temperature. Percolation was allowed slowly after 24 hours of maceration at a rate not exceeding 1 ml/minute. This procedure was repeated twice after full percolation by adding fresh solvent to the previously used drug powder. The extract obtained from percolation was collected in a flask. The extract was flask evaporated by using controlled temperature (bath temperature 40–50°C) until the solvent part was evaporated. A brownish, gummy extract was obtained. The extract was collected in glass Petri dishes, kept in a vacuum dessicator and used in the experiment. The yield at the end of extraction was 40 gms (8% of the dry powder) **[**14**].**

Method of preparation of the test drug:

Stock solution of **EEO** was prepared by dissolving 30 gms of extract in 30 ml of normal saline and 1 ml of stock solution provided 1 gm of drug. The dose of **EEO** for this experiment (1 gm/kg body weight) was selected based on previous studies done by *Mishra M et al* **[**15**],** and *Gulati RK et al* **[**16**]**.

Method of Preparation of Simvastatin Suspension:

The stock solution was prepared by dissolving 20 mg of simvastatin in 70 ml of normal saline and used as a standard drug in a dose of 1.8 mg/kg body weight for the respective group. The daily dose of simvastatin for albino rats was calculated by extrapolation from the human dose (20 mg/day) **[**17**]**.

Method of Preparation of High Fat Diet:

Edible coconut oil and vanaspathi ghee were procured from the market and a mixture of the two was prepared in a ratio of 2: 3 respectively v/v **[**18**]**. This high fat diet, at a dose of 10 ml/kg body weight, was fed to the animals, per orally, daily in addition to normal diet for 8 weeks to produce Hyperlipidaemia.

Experimental Design:

The experiment was carried out for a period of 8 weeks. For this purpose, 30 numbers of healthy albino rats (Wistar strain) of both sex and weighing approximately 150–200 gm were collected from the Central Animal House of Assam Medical College, Dibrugarh. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for one week and they were provided with a standard diet consisting of Bengal gram, wheat, maize and carrot. Water was given *add libitum* during the entire period of the experiment. For the experiment, the animals were weighed, recorded, numbered and randomly divided into five

groups of 6 animals each.All the animals were taken care of under ethical consideration and the experimental protocol was duly approved by institutional ethic committee.

Group–A: (Normal Control Group): Received vehicle normal saline 10 ml/kg body weight/day.

Group–B: (Test Drug Group): Received **EEO** 1 gm/kg body weight/day.

Group–C: (Hyperlipidaemic Control Group): High fat diet (10 ml/kg body weight/day).

Group–D: (Hyperlipidaemic Test Group): High fat diet + **EEO** (1gm/kg/day).

Group–E: (Hyperlipidaemic Standard Drug Group):High fat diet + Simvastatin(1.8 mg/ kg/day)

All the animals used for the experiment were kept under observation for daily food intake. The drugs were administered to the animals in the doses given above, for 8 weeks, by means of an intragastric feeding tube. At the end of the 8th week, all the animals were taken group wise and blood collected from each of them for assessing the various parameters of lipid profile.

Method of Collection of Blood:

Blood was collected from the orbital sinus with the help of a capillary tube by pressing the thumb behind the angle of the jaw resulting in the engorgement of the retro-orbital plexus **[**17**]**. The serum of each animal was estimated for different biochemical parameters.

Biochemical Estimation:

After separation of serum from blood, the various biochemical parameters were estimated in the Department of Pathology, Assam Medical College by using standard kits of Randox, Mumbai. The parameters of lipid profile which were observed were Total Serum Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein Cholesterol (LDL), High Density Lipoprotein Cholesterol (HDL) and Atherogenic Index (AI). Serum LDL Cholesterol was estimated by calculation based on *Friedewalds equation*: **[**19**]**.

[LDL Cholesterol mg/dl = Total cholesterol – HDL cholesterol- TGL/5]. The Atherogenic Index was calculated by using the formula of Kleopatra Schu pis et al[20]

$$\text{Atherogenic Index} = \frac{\text{Total Cholesterol} - \text{HDL}}{\text{HDL}}$$

RESULTS & OBSERVATIONS

The values obtained were expressed in specific units for those parameters as mentioned in the table.

TABLE–1

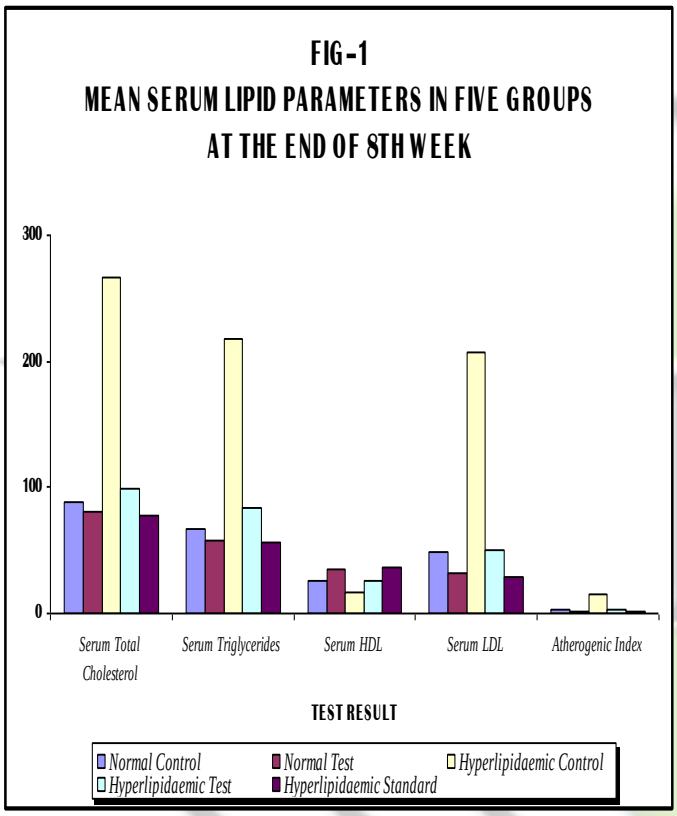
EFFECTS OF ETHANOLIC EXTRACT OF *EMBLICA OFFICINALIS* ON SERUM LIPIDS AT THE END OF 8TH WEEK OF EXPERIMENT

GROUP	DRUG	DOSE, ORAL, SINGLE DOSE DAILY	TEST RESULT (MEAN ± SEM) (in mg/dl)				TEST RESULT (MEAN ± SEM) (in ratio)
			Serum Total Cholesterol	Serum Triglycerides	Serum High Density Lipoprotein	Serum Low Density Lipoprotein	Atherogenic Index
Group-A (Normal Control)	Normal Saline	10ml/kg	88.7 ± 5.52	66.76 ± 3.02	26.35 ± 1.68	48.98 ± 2.96	2.42 ± 0.20
Group-B (Test Control)	Ethanolic extract of *Emblica officinalis*	1 gm/kg	80.4 ± 5.75[a]	57.5 ± 1.39[a]	35.3 ± 1.28[a]	32.70 ± 1.21[a]	1.27 ± 0.04[a]
Group-C (Hyper-lipidaemic Control)	High Fat Diet	10 ml/kg	267 ± 7.56[a]	218.48 ± 9.19[a]	16.0 ± 0.90[a]	207.25 ± 7.81[a]	15.92 ± 1.06[a]
Group-D (Experimental Control)	High Fat Diet + Ethanolic extract of *Emblica officinalis*	10 ml/kg + 1gm/kg respectively	99.1 ± 1.47[b]	83.6 ± 1.88[b]	25.7 ± 1.5[b]	50.13 ± 3.92[b]	2.91 ± 0.23[b]
Group-E (Standard)	High Fat Diet + Simvastatin	10 ml/kg + 1.8 mg/kg respectively	77.5 ± 4.7[b]	57.03 ± 3.26[b]	37.05 ± 1.4[b]	29.0 ± 4.0[b]	1.11 ± 0.16[b]
One way ANOVA		F	330.01	219.80	44.28	312.84	149.97
		df	25,4	25,4	25,4	25,4	25,4
		p	<0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a] : $p < 0.01$ when compared with Normal Control Group;
[b] : $p < 0.01$ when compared with Hyperlipidaemic Control Group
(ANOVA followed by Dunnet's multiple comparison test)

Results of estimation were reported as Mean ± SEM (standard error of mean) of 6 animals at a time from each group. The statistical significance between groups was analyzed using one–way ANOVA, followed by Dunnet's multiple comparison tests. The significance was expressed by '*p*' values, as mentioned in the table. '*p*'value of < 0.01 was considered significant.Table–1 has shown the different serum lipid parameters at the end of the 8th week of the experiment. It was seen that there was a significant increase in all the lipid parameters ($p < 0.01$) except HDL following administration of high fat diet.It was also seen that concomitant administration of the **EEO** at a dose of 1 gm/kg body weight along with high fat diet in the experiment animals, showed a significant decrease in all the lipid parameters ($p < 0.01$) with a significant rise in the value of

HDL ($p < 0.01$). Standard drug at a dose of 1.8 mg/kg administered along with high fat diet, showed a significant decrease ($p < 0.01$) in all the lipid parameters while there was a significant increase in HDL. The Hypolipidaemic activity of the test drug was found to be less efficacious than that of the standard drug, in comparison to the control (Fig–1).



DISCUSSION

In the present study hypolipidaemic activity of **EEO** was tested on albino rats by the method of *Mishra M et al* **[**15**],** with slight modification. In this study, experimental animals used were albino rats, whereas rabbits were used in the study of *Mishra M et al*.

Group–B, which was treated with **EEO**, when compared with the normal control group, showed a significant decrease in the levels of the TC [80.4 ± 5.75 ($p < 0.01$)], TG [57.5 ± 1.39 ($p < 0.01$)], LDL [32.70 ± 1.21 ($p < 0.01$)], while there was a significant increase in the level of HDL [35.3 ± 1.28 ($p < 0.01$)] when compared to the normal control group.

The high fat diet treated group (Group–C) showed a significant rise in the level of TC [267 ± 7.56 ($p < 0.01$)], TG [218.48 ± 9.19 ($p < 0.01$)], LDL [207.25 ± 7.81 ($p < 0.01$))], as well as a significant decrease in HDL [16.0 ± 0.90 ($p < 0.01$)], when compared to the normal control group. At the end of the 8th week of the experiment, such changes in lipid parameters by production of Hyperlipidaemia by high fat diet were also reported by *Shyamala MP et al* [18].

Group–D, which was treated with high fat diet + **EEO**, when compared with the hyperlipidaemic control group (Group–C), showed a significant decrease in the levels of TC [99.1 ± 1.47 ($p < 0.01$)], TG [83.6±1.88 ($p<0.01$)], LDL [50.13 ± 3.92 ($p < 0.01$)] while there was a significant increase in HDL [25.7 ± 1.5 ($p <0.01$)] at the end of the experimental period. The reduction in serum lipids show significant fall ($p<0.01$) which confirms the findings of other workers [15].

Mishra M et al [15] studied the effects of fruits of *Emblica officinalis* on healthy rabbits for 4 weeks. In that study the animals were divided into 3 groups of 4 animals each and fed with high fat diet. The group receiving high fat diet and *Emblica officinalis* simultaneously (at a dose of 1 gm/kg BW), showed significantly lower mean serum cholesterol levels at the end of 4 weeks than their counterparts in the other two groups.

Gulati RK et al [16] have documented similar findings after evaluating the effects of 50% ethanolic extract of *Emblica officinalis* on alcohol and high fat diet-fed albino rats for 3 weeks. Results showed a mean decrease in serum cholesterol 83.75 ± 4.44 ($p<0.05$) and serum triglycerides 27.93 ± 5.27 ($p<0.05$) in the group fed with alcohol and high fat diet and *Emblica officinalis* extract simultaneously at a dose of 1 gm/kg body weight, when compared to the hyperlipidaemic control group, which showed serum cholesterol 102.3 ± 7.94 ($p<0.05$) and serum triglyceride 31.75 ± 3.36 ($p<0.05$).

The above studies reported by *Mishra M et al* [15] and *Gulati RK et al* [16] suggest that fruits of *Emblica officinalis* has significant hypolipidaemic activity. The present study done to evaluate the effects of *Emblica officinalis* on serum lipids in albino rats, is in agreement with these studies. In this study, the group (Group–E) receiving high fat diet along with simvastatin simultaneously showed a significant decrease in the levels of TC [77.5 ± 4.7 ($p < 0.01$)], TG [57.03 ± 3.26 ($p < 0.01$)], LDL [29.0 ± 4.53 ($p < 0.01$)] while there was a significant increase in HDL [37.05 ± 1.40 ($p < 0.01$)]. Group–B which was treated with **EOE**, showed a significant decrease in the level of **AI** [1.27 ± 0.04 ($p < 0.01$)] as compared to the normal control group [2.42± 0.20]. The high fat diet treated group (Group–C) showed a significant rise of **AI** [15.92 ± 1.06($p< 0.01$)].

The Group–D (high fat diet + **EOE**)**,** when compared with

Group–C (hyperlipidaemic control group), showed a significant decrease in **AI** [2.91 ± 0.23 (p< 0.01)].

The Group–E (high fat diet + simvastatin), showed a significant decrease in the level of **AI** [1.11 ± 0.16 (p <0.01)]. A similar change in the value of **AI** at the 3rd week of study was reported by *Mukherjee B et al* **[**21**]**. In that study, the baseline **AI** was 1.2 ± 0.34. In the hyperlipidaemic group, there was a significant increase in the value of **AI** [6.1 ± 1.51], while the group receiving *C. roseus* methanolic extract along with high fat diet showed a significant decrease in **AI,** comparable to the normal control group 1.4 ±0.88. A decrease in the **AI** is believed to be beneficial, since the HDL level is inversely related with coronary heart disease and its elevation is considered as an antiatherosclerotic factor **[**21**]**.

EOE administration in hyperlipidaemic rats can elicit a profound influence on lipid metabolism. An enhancement in concentration of TC, TG, LDL and AI of hyperlipidaemic rats was observed which was probably due to lipid peroxidation evoked by high fat diet. Lipid peroxidation is a free radical mediated process which has been implicated in a variety of disease states. HDL concentration and HDL ratio would be useful in diseases like diabetes mellitus and coronary heart disease because of their inverse relationship. High LDL levels are usually associated with atherosclerosis. Hypertriglyceridemia is also associated in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose resistance and is one of the risk factors in coronary heart disease **[**22**]**. Hypolipidaemic efficacy of **EOE** is revealed by the attainment of values below normal in lipid profile of Group–B rats. Antihyperlipidaemic activity of **EOE** is revealed by the attainment of near normal values in lipid parameters of Group–D rats. The hypolipidaemic effect of **EOE** may have a protective mechanism against the development of atherosclerosis. The antilipoperoxidative property of **EOE** may be due to its rich flavonoids and polyphenol contents. It is well known that flavonoids and polyphenols are natural antioxidants **[**23**]**.

Recent epidemiological studies have revealed that the intake of flavonoids is inversely associated with the risk of coronary heart disease **[**24**]**. **EOE** is rich in flavonoids and polyphenols may also be contributing towards its hypolipidaemic effects due to its ability to combat oxidative stress by quenching free radicals, generated in the body as a result of high fat diet. **EOE** may also act by triggering the secretion of antioxidant enzymes Superoxide dismutase, Catalase and Glutathione peroxidase in enhanced levels which in turn stopped the oxidative damage due to Hyperlipidaemia. *Dhuley JN* [25] and *Shyamala MP et al* [18] have documented similar observations with Cinnamonum verum bark and Amoma subula-tum seeds and Syzygium aromaticum Linn respectively in rats fed with high fat diet .

CONCLUSION

The hypolipidaemic activity of *Emblica officinalis* extract was evaluated at the end of the experimental period of 8 weeks, by estimation and comparison of the different serum lipid parameters and atherogenic index between each group. Considering the entire results from the study it was observed that the **EEO** significantly inhibited high fat diet induced hyperlipidaemia in albino rats. On comparison, the hypolipidaemic activity of simvastatin was found to be more efficacious than that of **EEO**. *Emblica officinalis* (Amla) is the most commonly used traditional, household plant, which is safe, cost–effective and easily available. It can be utilized for providing dietary management in the prevention of atherosclerosis in hyperlipidaemic patients. *Emblica officinalis*, a commonly used natural product, deserves further evaluation from the stand point of its hypolipidaemic effect in therapy. Today there is widespread interest in drugs derived from plants. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapautic agents in medicinal plants. So emphasis should be laid upon development of more purified products from Amla for the control of various diseases.

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