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Original research article

A STUDY OF ANTI-HYPERLIPIDEMIA, HYPOLIPEDIMIC AND ANTI-ATHEROGENIC ACTIVITY OF FRUIT OF EMBLICA OFFICINALIS (AMLA) IN HIGH FAT FED ALBINO RATS

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ABSTRACT

Background: Emblica Officinalis (Amla), belonging to the genus, Phyllanthus emblica is widely used for medicinal purpose. Its fruits have been used traditionally as a hypolipidemic. **Objectives:** The present study was aimed to evaluate hypolipedimic and anti-atherogenic activity of fruit of Emblica officinalis in high fat fed albino rats. **Materials and Methods**: For study of anti-hyperlipidemic, hypolipidemic, and anti-atherogenic activity. 5 groups of 6 animals in each received normal saline, E. Officinalis powder, high fat diet, High fat diet plus E. Officinalis powder both and Atorvastatin respectively for 8 weeks. Hyperlipidemia was induced by feeding animals with high fat diet per orally, consisting of coconut oil and vanaspati ghee, daily ad libitum. At the end of the study, blood samples of the animals were sent for the estimation of the lipid profile and effects of test drug studied by comparing levels of Total Cholesterol, Triglycerides, HDL, LDL, and Atherogenic index. The statistical significance between groups was analysed by using one way ANOVA, followed by Dunnet's multiple comparison test. **Results:** Fruit of Amla showed significant anti-hyperlipidemic, hypolipidemic, and anti-atherogenic effect. All these effects may contribute to its anti-atherogenic activity. **Conclusion:** Present study revealed the anti-hyperlipidemic, hypolipidemic, and anti-atherogenic effect of Amla fruit powder and can be safely used in the treatment of mild to moderate cases of hyperlipidemia considering its easy availability, cost effectiveness, and other beneficial effects.

Key Words: Emblica Officinalis, Hypolipidemic, Anti-Atherogenic, Atorvastatin, Atherogenic Index.

INTRODUCTION

Hyperlipidemia is one of the major culprits for various cardiovascular and central nervous system disorders. Both genetic disorders and diet enriched with saturated fats and cholesterol,

contribute to the elevated lipid levels in our population as well as in many other developed countries around the world [1].

Atherosclerosis is an age related disease. It is widely prevalent in industrialized countries, affecting primarily the intima of large and medium sized arteries and is characterized by fibrous-fatty plaques or atheroma [2]. The cause of atherosclerosis is not known, although several risk factors have been involved in the pathogenesis of atherosclerosis. Current experimental and epidemiological evidence suggests a strong relationship between atherosclerosis and elevated levels of plasma lipids. Recent work also incriminated folic acid deficiency leading to elevated plasma levels of homocysteine and chronic infection with chlamydia pneumonia in the pathogenesis [3]. Atherosclerosis, which was earlier thought to be always associated with hyper cholesterolemia, has now been proved as an inflammatory disease [4].

Over the last few years the changes in the lifestyle, particularly the westernization of the diet and a relatively sedentary lifestyle have led to an increased frequency of lifestyle related disorders such as hyperlipidemia, diabetes mellitus, and atherosclerosis [5]. The principle metabolic causes of atherosclerosis include hyperlipidemia, hypertension, obesity, insulin resistance, and diabetes mellitus [6]. Risk factors for the above are the following, Smoking, hypertension, serum cholesterol, genetic factors, physical activity, hormones, alcohol, thyroid disease, renal disease, and liver disease [7].

The current National Cholesterol Education Program (NCEP) for the management of patients with lipid disorder is of 2 types. One is population based approach, which is intended to lower blood cholesterol by dietary recommendations such as reduced total calories from fats to less than 305kCal and from saturated fats to less than 10%, consumption of less than 300mg of cholesterol per day and maintenance of desirable body weight. The second is a patient based approach described in the report 2001 report of NCEP.

Adult treatment panel-III (ATP-III) which continues to focus on lowering LDL-C levels as the primary goal of the therapy [8]. The ATP-III report recognizes four classes of drugs that may be used to achieve lipid goals. These include HMG-CoA reductase inhibitors (Statins), Bile Acid sequestrants, Niacin, and Fibric acid derivatives [9].

The history of natural products is as old as mankind. The importance of traditional systems of medicine and of certain traditional medical practices has now been recognized all over the world. Today, it is required to have an intelligent and pragmatic approach to evaluate selective drugs of herbal origin. Therefore, it should really matter for Pharmacologists to obtain information from traditional healers, about their remedies and to extract the active principles for development into drugs [10].

E. Officinalis or Phyllanthus emblica (Syn: Amla, Indian Gooseberry) is an evergreen tree which is highly prized in tropical Asia. The genus is natural to tropical Southeast Asia, particularly in Central and South India. It is commonly cultivated in gardens throughout India and grown commercially as a medicinal fruit [11]. It is among the most important medicinal plants in the Ayurveda Materia Medica and widely used in Indian medicines for the treatment of various ailments [12].

Apart from traditional uses, there are several reports in the pharmacological actions of Amla based on modern scientific investigations, especially anti-inflammatory action [13], antimicrobial action [14], anti-oxidant action [15], anti-carcinogenic action [16], anti-ulcerogenic action [17], anti-diabetic action [18], analgesic action [19], and hepato protective action [20].

The credit for initiating studies related to herbal extracts in our country goes to Sir Ram Nath Chopra and his team of dedicated workers, in Calcutta School of Tropical Medicine [21].

Keeping in view of the above ideas, the present study has been undertaken to evaluate the effect of E.officinalis powder on the serum lipids level, in albino rats fed with high fat diet comparing with standard hypolipidemic drug Atorvastatin.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Ethics Committee and Animal Ethics Committee of M.R Medical College, Gulbarga. The study was carried out in 30 healthy albino rats of Wister strain (Rattus norvegicus) weighing 150-200g either sex. Animals were maintained on a standard animal diet consisting of Bengal gram, Wheat, Maize, and Carrot in sufficient quantity for the entire period (8 weeks) of the study and Water *adlibitum*.

Drugs used in the study:

1. **Emblica officinalis**: The powder obtained from Phytopharma Ayurvedic firm from Kolhapur, Maharashtra. The dose in humans is 6g/day, which is equivalent to 540mg/kg in rats [22].
2. **Atorvastatin**: Atorvastatin powder was obtained from Biocon Pharmaceuticals, Bengaluru. Human dosages 80mg, which is 7.2mg/kg in rats [22].
3. **High Fat Diet**: Mixture of Coconut Oil (from Marico Industries Ltd., Mumbai) and Vanaspati Ghee procured from Ruchi Industries, Mumbai.
4. **Vehicle**: Gum Acacia: 4%, 2ml/kg procured from Nice chemicals, Kochi

Preparation of High Fat Diet for inducing Hyperlipidemia:

Edible Coconut oil and Vanaspati ghee mixed together in the ratio of 2:3 v/v as per the method of Shymala MP et al [23], at a dose of 10ml/kg body weight, was fed to the animals per oral daily in addition to a normal diet for 8 weeks.

Study Design

For the study, the animals were weighed, recorded, numbered, and randomly divided into 5 groups of 6 animals each for a period of 8 weeks according to CPCSEA (Committee for the purpose of control and supervision of experiments on animals) for laboratory animal facilities [24, 25].

Grouping and Treatment Schedules

Group 1: Normal Saline

Group 2: E. officinalis powder 540mg/kg/day along with normal diet.

Group 3: High Fat Diet (10ml/kg/day)

Group 4: High fat diet (10ml/kg/day) + E. officinalis powder (540mg/kg/day)

Group 5: High fat diet (10ml/kg/day) + Atorvastatin (7.2mg/kg/day)

All the animals used for the study were kept under observation for daily food intake. The drugs were administered to the animals for 8 weeks by an intra-gastric feeding tube. At the end of 8th week, all the group of animals was kept for overnight fasting, After overnight fasting 2ml of 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 retro-orbital plexus [26]. The blood was centrifuged; serum was collected and used for assessing the various biochemical parameters of the lipid profile.

Biochemical Estimation

Biochemical parameters were estimated in the Biochemistry Laboratory of Basaveshwara Teaching and General Hospital, attached to M. R. Medical College, Gulbarga.

The following parameters of Lipid Profile were measured:

1. Total Serum Cholesterol- It was estimated by using Erba Kit [27] manufactured by Transasia Bio-Medicals Ltd.
2. Serum Triglyceride- It was estimated by using a kit manufactured by AGAPPE Diagnostics [28].
3. High Density Lipoprotein Cholesterol (HDL) - It was estimated by using Erba Kit [27] manufactured by Transasia Bio-Medicals Ltd.

4. Low Density Lipoprotein Cholesterol (LDL) - It was estimated by using Erba Kit [27] manufactured by Transasia Bio-Medicals Ltd.

Atherogenic Index:

The Atherogenic index was calculated by using the formula [29].

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

Statistical Analysis

The statistical significance between groups was analysed by using one way ANOVA, followed by Dunnet's multiple comparison test. The significance was expressed by 'p' values, as mentioned in the table.

RESULTS

The results obtained are summarised in table 1. The values obtained were expressed in specific units of those parameters as mentioned in the table. The results of estimation were reported as Mean ± SEM (standard error of mean) of 6 animals at a time from each group. 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 Amla powder at a dose of 540mg/kg body weight along with high fat diet in the study animals, showed a significant decrease in all the lipid parameters ($p < 0.01$) i.e. hypolipidemic and antihyperlipidemic activity with a significant rise in the value of serum HDL ($p < 0.01$). Standard drug Atorvastatin at a dose of 7.2mg/kg administered along with a high fat diet, showed a significant decrease ($p < 0.01$) in all the lipid parameters, while there was a significant increase in serum HDL. The hypolipidemic activity of the test drug was found to be slightly less efficacious than that of the standard drug, in comparison to the control. (Figure 1)

Table: 1. Effects of fruit powder of E. officinalis on serum lipids at the end of 8th week of study.

Group	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL(mg/dl)	LDL(mg/dl)	Atherogenic Index ratio
Group 1	88.7 ± 5.5	66.76 ± 3.02	26.35 ± 1.68	48.98 ± 2.96	2.42 ± 0.20
Group 2	80.4 ± 5.75*	57.5 ± 1.39*	35.5 ± 1.28*	32.70 ± 1.21*	1.27 ± 0.04*
Group 3	267.0 ± 7.56*	218.48± 9.19*	16.0 ± 0.90*	207.25± 7.81*	15.92 ± 1.06*
Group 4	99.1 ± 1.47†	83.6 ± 1.88†	25.7 ± 1.5†	50.13 ± 3.92†	2.91 ± 0.23†
Group 5	77.5 ± 4.7†	57.03 ± 3.26†	37.05 ± 1.4†	29.0 ± 4.0†	1.11 ± 0.16†
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

***: p < 0.01, when compared with the normal control group;**

†: p < 0.01, when compared with the hyperlipidemic control group.

(One way ANOVA followed by Dunnet's multiple comparison test)

Data presented as Mean ± SEM

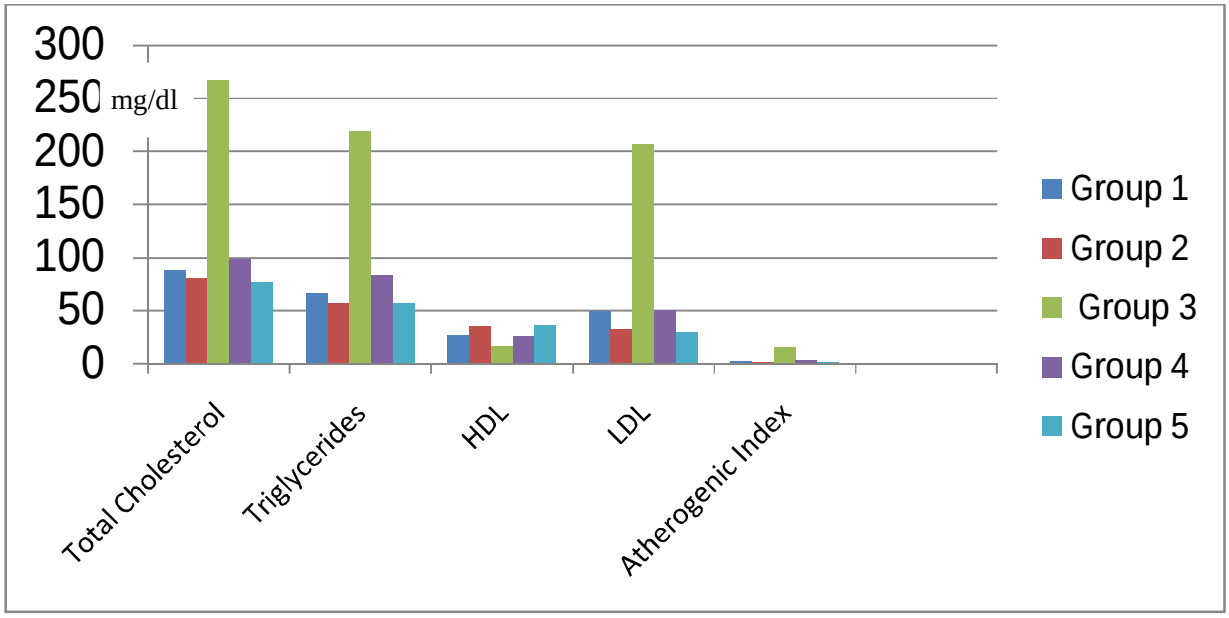

Figure-1: Graph showing mean serum lipid parameters in 5 groups at the end of 8th week.

DISCUSSION

Hyperlipidemia was induced by administering a high fat diet to the albino rats. Shyamala MP et al [23] stated that hyperlipidemia is a result of an oxidative abuse due to free radicals, formed by the interaction of high fat diet. They further stated that, an enhancement in the concentration of serum cholesterol and triglycerides of hyperlipidemia rats maybe result of lipid peroxidation evoked by high fat diet [30]. In the present study, Atorvastatin was used as a standard drug [31].

E. Officinalis powder, administered in hyperlipidemia rats can elicit a profound influence on the lipid metabolism. An enhancement in the concentration of total serum cholesterol, serum triglycerides, serum LDL, Atherogenic index of hyperlipidemia 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.[32] HDL concentration and HDL ratio would be useful in diseases like diabetes mellitus and coronary heart disease, because of their inverse relationship.[33] High LDL levels are usually associated with atherosclerosis[34]

Hyper triglyceridemia is also associated with metabolic consequences of hyper coagulability, hyperinsulinemia, insulin resistance, and glucose resistance, and is one of the risk factors in the coronary heart disease [35].

Hypolipidemic efficacy of E. officinalis powder is revealed by attainment of values below normal in the lipid profile of group 2 rats. The anti - hyperlipidemia activity of E. officinalis powder is established by the attainment of near normal values in lipid parameters of group 4 rats. The hypolipidemic effect of E. officinalis may have a protective mechanism against the development of atherosclerosis. Anti lipoperoxidative property of E.officinalis powder, maybe due to its rich flavonoids and poly phenol contents. It is well known that flavonoids and poly phenols are natural anti-oxidants [36, 37].

Recent epidemiological studies have revealed that the intake of flavonoids is inversely associated with the risk of coronary heart disease. E. Officinalis powder, rich in flavonoids and poly phenols may also be contributing towards its hypolipidemic effect, due to its ability to combat oxidative stress by quenching free radicals

generated in the body as a result of high fat diet. E. Officinalis powder may also act by triggering the secretion of anti-oxidant enzymes: Superoxide dismutase, Catalase, and Glutathione peroxidase in an enhanced level, which in turn stopped the oxidative damage due to hyperlipidemia. Dhuley JN et al [38] and Shyamala MP et al [23] have documented a similar observation with Cinnamonum verum bark and Amoma subulatum seeds and Syzygium aromaticum respectively in rats fed with high fat diet.

The present study which was done to evaluate the effect of E. officinalis on serum lipids and atherogenesis in albino rats is in agreement with other studies [39, 40]. As compared to the other studies, in the present study E. officinalis powder was used, which is easily available and inexpensive. In previous studies, E. officinalis aqueous and ethanolic extract has been used. The present study was carried out for a longer duration of time, i.e. 8 weeks as compared to the previous studies which were done for 4 week duration.

As atherosclerosis is closely associated with hyperlipidemia, it is beneficial to compare it with a standard drug such as Atorvastatin. Such a comparative study was not done before. Amla has shown to possess significant hypolipidemic and anti-atherogenic activity slightly lesser as compared to Atorvastatin. But if we compare Amla with Atorvastatin in terms of adverse effect profile, Atorvastatin can cause severe adverse effects like rhabdomyolysis to mention one of them [41].

CONCLUSION

Amla can be safely used in the treatment of mild to moderate cases of hyperlipidemia considering its easy availability, cost effectiveness, and other beneficial effects.

Not many studies have been undertaken to fully evaluate the molecular and biochemical basis of hypolipidemic action of Amla and further clinical studies are required to find out the hypolipidemic activity and molecular mechanism.

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