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Effect of Aqueous Extracts of *Emblica officinalis* Fruit and *Terminalia arjuna* Bark on Serum Lipid Profile in Male Albino Wistar Rats Fed with High Fat Diet

Vijayalakshmi Korlam[1]* and Rajendran K.[2]

[1]Department of Anatomy, NRI Institute of Medical Sciences, Sangivalsa, Visakhapatnam, India
[2] Department of General Medicine, Saveetha Institute of Medical and Technological Sciences, Thandalam, Chennai, India

**Corresponding Author*

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Abstract: The present study was aimed to investigate the effects of aqueous extracts of *Emblica officinalis* (Amla) fruit and *Terminalia arjuna* bark on serum lipid profile in relation to the atherogenesis in male albino Wistar rats fed with high fat diet. Hyperlipidaemia was induced by feeding the rats with vanaspathi and edible oil in the ratio of 3:2. Hyperlipidaemia was evidenced by the elevated levels of the serum lipid profile. At the end of the study, blood samples of the animals were estimated for 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 turkey's post hoc test. The aqueous extract of *E.offiinalis* and *T. arjuna* showed significant hypolipidemic effect by change in the serum lipid profile levels compared to that of hyperlipidemic rats at dose of 400 mg /kg body wt and 200 mg/kg body wt of the extracts. The findings of this study reveals that the aqueous extract of *E. officinalis* fruit of 400 mg showed marked reduction in the serum lipid levels than *T. arjuna* bark extract. Present study revealed the antihyperlipidemic and anti-atherogenic effect of Amla fruit extract and *Terminalia arjuna* bark extract 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.

Keywords: *Emblica officinalis*, Hyperlipidaemia, Lipid profile, *Terminalia arjuna*, Atorvastatin, Vitamin C

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Introduction

Coronary heart disease is the main cause of death in developed and developing countries which is the contributing factor all over the world. There are many causes for the heart failure like hypertension, high fat diet, sedentary life which lead to hyperlipedemia that is responsible for the

myocardial infarction and its complications. The known hypolipidemic drugs such as statins and fibrates have many side effects like hyperuricemia, diarrhoea, nausea, gastric irritation, flushing and dry skin; in order to avoid such side effects the natural products have been chosen which are easily available within our resources (Gaire and Subedi, 2014).

Herbal medicines are being used by nearly 80% of the world's population primarily in the developing countries for primary health care. The chemical constituents present in them are a part of the physiological functions of living flora and hence, they are believed to have better compatibility with the human body. It is used both as a medicine and as a tonic to build up lost vitality and vigour (Srivsuki, 2012). Amla is highly nutritious and is one of the richest sources of vitamin C, amino-acids, minerals containing mainly the gallic acid and ellagic acid (Dasangi, 2014). The fruit juice of amla contains the highest concentration of vitamin C than in the oranges, tangerines and lemons. The fruit of Amla possesses antioxidant, anti-diabetic, hypolipidemic, antibacterial and hepato-protective properties (Patel *et al*., 2013).

E. officinalis, widely known as Indian gooseberry (amla), used as a rejuvenating herb in Ayurveda since ancient times. The plant is widely distributed throughout tropical and sub-tropical countries. *E. officinalis* extracts contain various antioxidants such as emblicanin A, emblicanin B, gallic acid, ascorbic acid and ellagic acid which are effective in a range of ailments. Presence of various active phytocompounds bestows *Emblica officinalis* fruit extract with various therapeutic benefits consequential of radioprotective, anti-atherosclerotic, antidiabetic, antiaging, gastro-protective, cytoprotective and immunomodulatory properties. Vitamin C is considered the effective therapeutic approach in lowering total serum cholesterol level (Dwivedi and Chopra, 2014).

Efforts are being directed towards discovering safe and effective agents that will be beneficial in correcting the lipid metabolism and thus preventing cardiac diseases. Recently, there has been a renewed interest in medicinal plants attributed with therapeutic virtues. India has a rich heritage of medicinal plants of wide diversity, which are used by the local population and traditional healers for the treatment of several diseases including lipid disorders (Santoshkumar *et al*., 2013)

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.

The current National Cholesterol Education Program (NCEP) for the management of patients with lipid disorder is of two 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 305 kCal and from saturated fats to less than 10%, consumption of less than 300 mg of cholesterol per day and maintenance of desirable body weight. The second is a patient based approach (Lama and Sikia, 2011).

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 (David *et al.*, 2012).

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. 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 (Bheemshetty, 2015).

Terminalia arjuna, commonly known as Arjuna, belongs to the family Combretaceae. In the Indian subcontinent its bark decotation is used for angina pain, hypertension, congestive heart failure, and dyslipidemia. As most of the studies were done on the ethanolic, methanolic, and hydroalcoholic extracts of *E.officinalis* fruit and *T.arjuna* bark, the present study was taken to understand the nutritional value and to evaluate the biochemical parameters for the hypolipidemic activity of the aqueous extracts of *E.officinalis* fruit and *T.arjuna* bark (Sadeghi *et al.*, 2014).

Materials and Methods

Chemicals and Reagents:

The chemicals and reagents used in the current study were analytical grades. The biochemical estimation kits used in the study were purchased from Transasia Bio-Medicals Ltd., India. Edible vegetable oil and vanaspati for high fat diet procured from local market.The standard drug Atorvastatin 20 mg was purchased from a local medical shop.

Plant Extract:

Aqueous extracts of *E. officinalis* fruit (AEEOF) and *T. arjuna* bark (AETAB) were obtained from Bhagavathi herbals, Gujarat, India.

Phytochemical analysis:

The collected extracts were analyzed (Table 1) to explore extracts' chemical profile using standard phytochemical tests qualitatively.

Selection of animals:

Healthy albino Wistar rats (150-200 g) of 60-90 days age were procured from M/S Mahaveer Enterprizes, Hyderabad, India to study the toxicity and anti-hyperlipedemic activity of *Emblica officinalis and Terminalia arjuna* extracts. The animals were maintained with controlled conditions (12 h light/12 h dark; 24±2°C; 40-70% relative humidity) by providing food and water. The experimental protocol and the animal studies were approved by Institutional Animal Ethical Committee (IAEC) of Maharajah's Institute of Medical Sciences (M.I.M.S), Vizianagaram, Andhra Pradesh, India with CPCSEA No. 753/03/C/CPCSEA.

The animals were acclimatized for 7 days before the study. The animals were grouped into experimental and control groups. They were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h before experimental protocol to minimize non-specific stress. All experimental protocols were followed as per the Ethics Committee on Research in Animals. Care was taken for animals during entire experiment process (Chidambaram *et al.*, 2007).

Induction of hyperlipidemia:

High fat diet (HFD) was prepared by mixing vanaspati and palm oil in the ratio of 3:2. It was administered for a period of 6 weeks to all the groups except for normal control group which were fed with the standard rat pellets and provided with sufficient water (Dove *et al*., 2005).

Acute toxicity study:

The acute toxicity of *E. officinalis* fruit and *T.arjuna* bark aqueous extracts was determined. Rats were fasted overnight and randomly divided into six rats per group. Extract doses were given as per Organization for Economic Co-operation and Development (OECD) guidelines with 5, 50, 300, 1000 and 2000 mg/kg b wt. The doses were administered separately to the rats of each group using bulbed steel needle and animals were observed at regular intervals for any physiological and psychological changes and finally mortality. All rats were then allowed free access to food and water and observed over a period of 48 hr for signs of acute toxicity. The number of deaths occur in this period were recorded (Mouhssen, 2007).

Table 1: Qualitative phytochemical analysis of aqueous extracts of *E. officinalis* fruit (AEEOF) and *T. arjuna* Bark (AETAB)

S. No.	Phytochemicals	AEEOF	AETAB
1	Alkaloids	-	+
2	Tannins	+	+
3	Proteins	-	-
4	Saponins	+	+
5	Coumarins	-	-
6	Flavanoids	-	+
7	Phenols	+	+
8	Carbohydrates	+	+
9	Glycosides	+	+
10	Sterols	-	+

+ Present; - Absent

Biochemical Estimation:

Biochemical parameters were estimated for serum lipid profile in the Biochemistry Laboratory of Maharaja's Institute of Medical Sciences (MIMS), Vizianagaram.

The following parameters of Lipid Profile were measured:

Total Serum Cholesterol: It was estimated by using Erba Kit 27 manufactured by Transasia Bio-Medicals Ltd.

Serum Triglyceride: It was estimated by using a kit manufactured by AGAPPE Diagnostics.

High Density Lipoprotein (HDL): It was estimated by using Erba Kit 27 manufactured by Transasia Bio-Medicals Ltd.

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

Atherogenic index: It was calculated by using the formula:

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

Experimental Design:

Experimental animal groups: The animals were divided into 7 groups (six animals in each group) and treated as follow:

Group I: Normal rats Diet - Control

Group II: High Fat Diet (HFD)

Group III: HFD + Atorvastatin (20 mg) - Standard

Group IV: HFD + Aqueous extract of *E.officinalis* fruit (200 mg/kg b wt)

Group V: HFD + Aqueous extract of *E.officinalis* fruit (400 mg/kg b wt)

Group VI: HFD + Aqueous extract of *T.arjuna* bark (200 mg/kg b wt)

Group VII: HFD + Aqueous extract of *T.arjuna* bark (400 mg/kg b wt)

The drugs were administered to the animals through intragastric feeding tube for 6 weeks daily except for the normal control group. At the end of the 6th week all the rats were sacrificed after overnight fasting and the blood was collected.

Collection of Samples:

The blood samples were collected on 6th week through the retro-orbital path into the sterile containers, after the separation of the serum the biochemical parameters was done.

Determination of serum lipid profile:

Blood was withdrawn from the retro-orbital plexus of rats under mild ether anesthesia on day 0 (before starting the induction of hyperlipidemia), day 10 (end of the induction period) and day 25 (end of the drug intervention period) of the study. The animals were fasted for 18 h prior to blood withdrawal. The blood was collected and centrifuged to separate serum for estimation of lipid profile (Mukherjee *et al*., 2007).

The serum total cholesterol, triglyceride and HDL, LDL, VLDL levels were analyzed by enzymatic colorimetric method using commercially kits.

Estimation of fecal cholesterol excretion:

Feces were collected for three consecutive days, i.e, 23 to 25th day of the study and dried for 1 h at 60°C. The dried fecal matter was powdered and extracted with chloroform: methanol (2:1). The fecal cholesterol content in the extract was estimated by enzymatic method using kits (Yang *et al.*, 2006).

Estimation of hepatic hydroxymethyl glutaryl coenzyme (HMGCoA) reductase activity:

Animals were sacrificed at the end of the drug intervention period. The hepatic HMGCoA reductase enzyme activity was indirectly measured based on the HMGCoA/ mevalonate ratio. The ratio between HMGCoA and mevalonate is inversely proportional to HMGCoA reductase activity, i.e., an increase in ratio indicates decreased activity.

Histopathological Study:

The formalin-fixed Peripheral adipose tissues were processed and embedded in paraffin blocks using standard histological techniques. A rotary microtome was used to cut the slices, which were then stained with Ehlrich's hematoxylin and eosin (H and E). To analyse histopathological alterations generated by AEEOF, AETAB, histological sections were inspected and photos were acquired using an Olympus light microscope (Olympus, Japan, magnification 40 x) (Yokozawa *et al.*, 2003).

Statistical Analysis:

The statistical significance between groups was analysed by using one way ANOVA, followed by Tukey's post hoc test. The significance was expressed by 'p' values, data are expressed as Mean±SEM.

Results and Discussion

Table 2 shows the effect of oral administration of aqueous extracts of *Emblica officnalis* fruit and *Terminalia arjuna* bark for 6 weeks. In the HFD group, there was an elevated level in the serum total cholesterol, triglycerides, VLDL, LDL, VLDL, AI and decrease in the level of HDL for 6 weeks (Fig. 1). Groups IV and VI shows decrease in Total cholesterol levels for 6 weeks compared to group III and V. *E. officinalis* (400 mg) and *T. arjuna* (400 mg) shows reduced levels of triglycerides than 200 mg of both extracts. Group IV (*E. officinalis* 400 mg) shows decreased levels of VLDL, LDL and Atherogenic Index than Group VI (*T. arjuna* 400 mg). The higher and improved values of HDL were noticed in the group IV compared to group III, V, and VI for 6 weeks. In group IV the good value for HDL was observed for 6 weeks. Dose of 400 mg aqueous extract of *E. officinalis* showed much reduced levels in the TC, TG, VLDL, LDL, and atherogenic index (AI) with a rise in the HDL level for 6 weeks compared to similar dose of *T. arjuna* and 200 mg of both extracts. In the earlier study of *E.officinalis* at a dose of 540 mg for 8 weeks showed the decreased values of TC, TG, VLDL, LDL (Santoshkumar *et al*., 2013) whereas in the present study it was observed for 6 weeks at a dose of 400 mg. A high-fat diet may cause elevated levels of cholesterol and decreased level of HDL which ultimately leads to obesity. Whereas in the present study the HDL values were elevated with the aqueous extracts of *E.officinalis* and *T.arjuna* which indicates anti-hyperlipedemic activity. The importance of medicinal plants in the treatment of hyperlipidaemia was experimentally studied in recent years where oxidative stress induced apoptosis in adipose tissue was noticed (Ghosh, 2008). Medicinal plants have been used to control the lipid level to improve the oxidative stress. Increase in LDL level causes deposition of cholesterol in the arteries and aorta and hence, it leads to coronary heart disease (CHD), whereas LDL transports cholesterol from the liver to the periphery. After treatment with *E. officinalis* fruit extract the VLDL level reduced significantly in the hyperlipidaemic group (CPCSEA, 2003).

Effect of 6 weeks treatment period with aqueous extract of *E. officinalis* 200 and 400 mg/kg b wt and *T. arjuna* at a dose of 400

Table 2: Effect of aqueous extracts of *T. arjuna* bark and *E. officinalis* fruit on serum lipid profile after 6 weeks

	TCH	TGL	VLDL	HDL	LDL	AI
Control	187.24±2.23	105.22±1.07	25.52±1.08	37.4±1.53	41.44±3.15	4.028±0.16
HFD	262.04±3.10##	173.72±6.96##	59.68±1.65##	27.2±1.59##	108.2±1.31##	8.81±0.82##
EO (200 mg)	236.44±6.36**	130.02±1.56**	47.84±2.04**	33.6±1.69NS	59.08±1.74**	6.14±0.56**
EO (400 mg)	203.94±2.94**	118.62±0.42**	35.44±1.70**	41.62±1.36**	51.04±1.11**	3.91±0.16**
TA (200 mg)	246.44±5.32**	135.62±3.36**	49.28±1.34**	28.6±1.16**	65.08±2.72**	7.65±0.31 NS
TA (400 mg)	228.02±3.00**	127.7±3.55**	42.64±3.34**	37.82±2.16NS	57.68±2.84**	5.1±0.33**
AV	192.76±1.86**	110.82±1.20**	31.48±1.83**	47±2.58**	46.44±1.69**	3.14±0.22**

Values are expressed as Mean ± SEM (n=6); one way ANOVA followed by Tukeys post hoc test. ## p<0.01 compared to normal control group, **p<0.01 Compared to HFD treated group, NS= not significant, EA=*Emblica officinalis*, TA= *Terminalia arjuna*

mg/kg b wt significantly showed reduced levels of serum cholesterol, triglycerides, VLDL, LDL levels and AI with an increase in the HDL levels. So, the aqueous extract of *E. officinalis* drug is more preferable than *T. arjuna*. The analysis found that taking a vitamin C supplement of 500 mg/day significantly reduced LDL (bad) cholesterol by approximately 7.9 mg/dl and blood triglycerides by 20.1 mg/dl. A minimum of 500 mg/d of vitamin C supplementation for at least four weeks can result in a significant decrease in serum LDL cholesterol and triglyceride concentrations with a non-significant increase in the levels of HDL cholesterol in the serum (Akhtar *et al.*, 2011).

The oral administration of aqueous extract for 6 weeks was found to reduce the cholesterol absorption from the intestine which was evident by significant ($p < 0.05$) increase in fecal cholesterol excretion demonstrated by aqueous extract at four doses employed. However, at the high dose of 400 mg kg^{-1} the aqueous extract was also found to decrease the cholesterogenesis in liver by significantly ($p < 0.05$) increasing the HMGCoA/mevalonate ratio. HMGCoA/mevalonate ratio has an inverse relationship to the activity of HMGCoA reductase, a key enzyme in cholesterol biosynthesis (Mishra *et al.*, 1981).

The other mechanistic pathway responsible for the cholesterol lowering effect could be via inhibition of acyl-CoA: cholesterol acyltransferase (ACAT) enzyme, a primary enzyme responsible for the intracellular esterification of free cholesterol to cholesterol ester. The esterification of free cholesterol is essential for its intestinal absorption and the assembly of very low density lipoprotein (VLDL) (Singh *et al.*, 2015). Thus, inhibition of ACAT may exhibit cholesterol lowering and anti-atherosclerotic activities by blocking intestinal absorption of dietary cholesterol, inhibiting hepatic secretion of VLDL and preventing formation of foam cells in the arterial wall, thus inhibiting the growth of atherosclerotic lesions (Yokozawa *et al.*, 2007). In our study we tried to

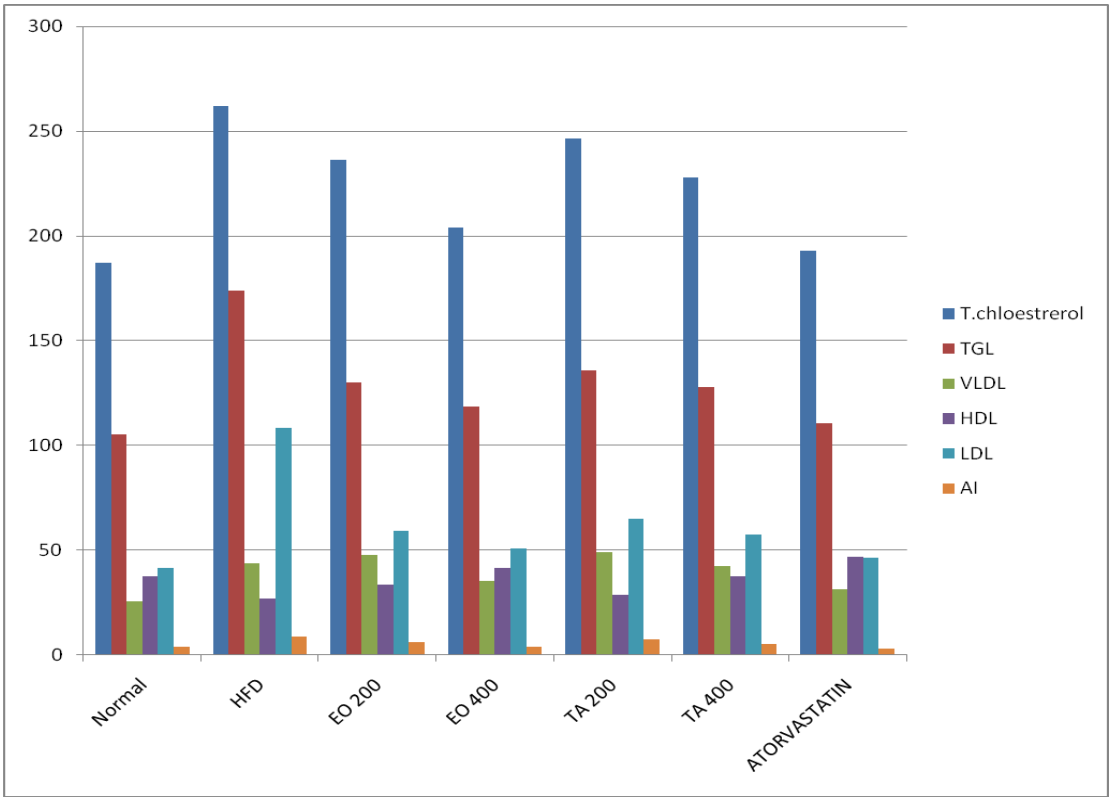

Fig. 1: Effect of aqueous extracts of *T. arjuna* bark (TA) and *E. officinalis* fruit (EO) on serum lipid profile after 6 weeks.

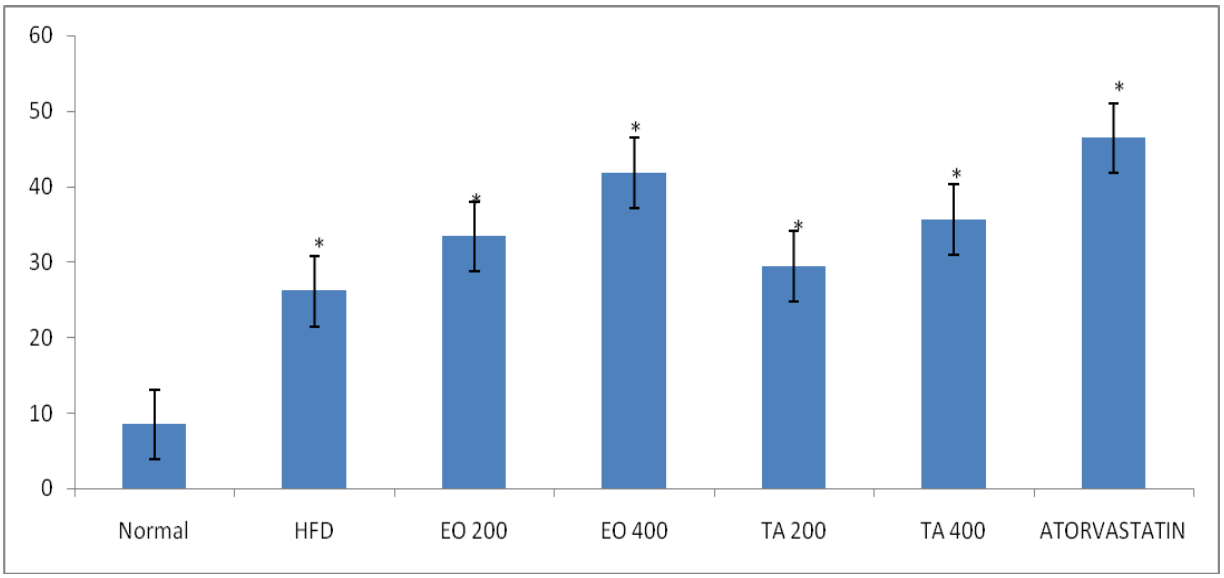

Fig. 2: Effects of aqueous fruit extracts of and *E. officinalis* and *T. arjuna* on faecal cholesterol after 6 weeks. Values are expressed as Mean ± SEM (n=6); one way ANOVA followed by Tukeys post hoc test. *p<0.01 compared to normal control group, HFD – High Fat Diet, EO - *Emblica officinalis*, TA- *Terminalia arjuna.*

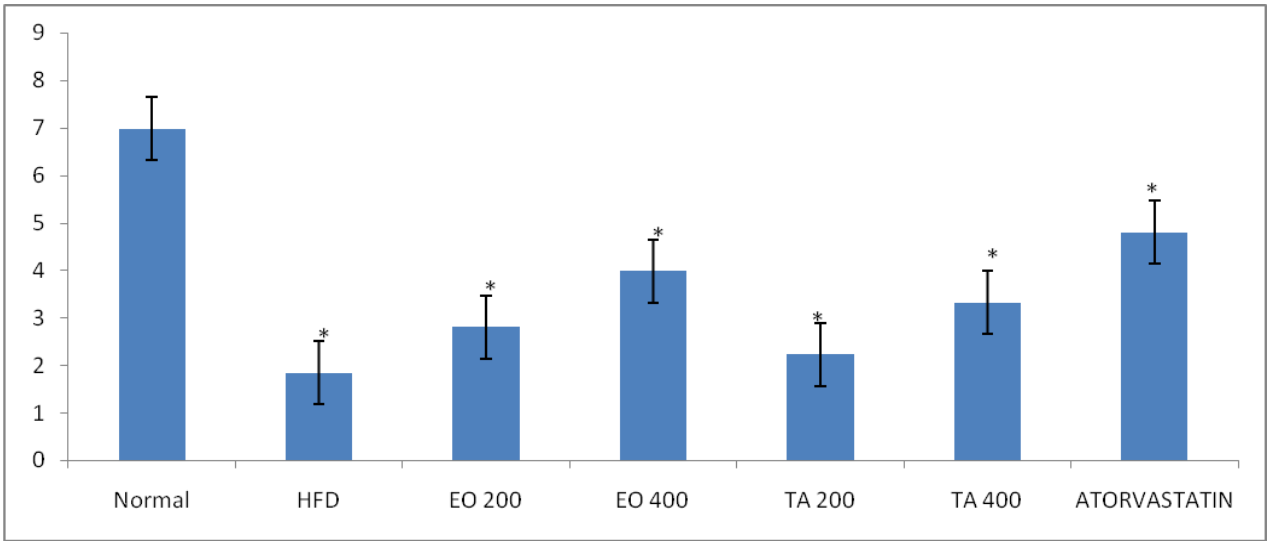

Fig. 3: Effect of aqueous fruit extracts of and *E.officinalis* and *T.arjuna* on hepatic HMGCoA enzyme activity after 6 weeks. Values are expressed as Mean ± SEM (n=6); one way ANOVA followed by Tukeys post hoc test. *p<0.01 compared to normal control group, HFD – High Fat Diet, EO - *Emblica officinalis*, TA- *Terminalia arjuna.*

investigate positive influence of aqueous extracts of *Emblica officinalis* fruit *and Terminalia arjuna* bark on Adipocytes of hyperlipidemic albino Wistar rats and observed *Embilica officinalis* was useful in regulating hyperlipidemia than *Terminalia arjuna* in albino Wistar rats fed with hyperlipidemic diet.

Hyperlipidemic animal models express clinical manifestations of fatty liver and other cardiovascular risk factors. The hyperlipidemic effects of the diet demonstrated in the increased body weight and lipid profile in albino Wistar rats. There was a significant decrease in % body weight gain in rats treated with *Embilica officinalis* compared to hyperlipidemic rats and control after 6 weeks of treatment. There was no significant difference observed between hyperlipidemic rats treated with *Embilica officinalis* and rats treated with *Terminalia arjuna* after 6 weeks of treatment, this could be due to excessive breakdown of tissue proteins and catabolism of fats and proteins

Effect of aqueous extract on fecal cholesterol excretion:

At the end of the drug intervention period the aqueous extract of *Emblica offcinalis* and *Terminalia arjuna* was found to significantly ($p < 0.05$) increase the fecal cholesterol excretion at doses of 400 mg kg^{-1} b wt of both extracts with respect to the high fat diet control group (Fig. 2).

Effect of aqueous extract on HMGCoA reductase activity:

The HMGCoA/mevalonate ratio was significantly ($p < 0.05$) increased by the standard atorvastatin and aqueous extract of *Emblica officinalis* and *Terminalia arjuna* at the dose of 400 mg kg^{-1} as compared to the high fat diet control group (Fig. 3).

Histopathological study:

In Group II the adipocytes appeared ring shaped studded with the lymphocytes at the edges and in between them are filled with inflammatory cells.Few Ring shaped adipocytes with edges of adipocytes studded with lymphocytes were observed in Group IV (Fig. 4). Adipocytes appeared normal and no abnormalities were observed in Group V.

Conclusion

The aqueous extract of *Emblica officinalis* fruit revealed maximum hypolipedemic effect at a dose of 400 mg/kg wt for a period of 6 weeks than 200

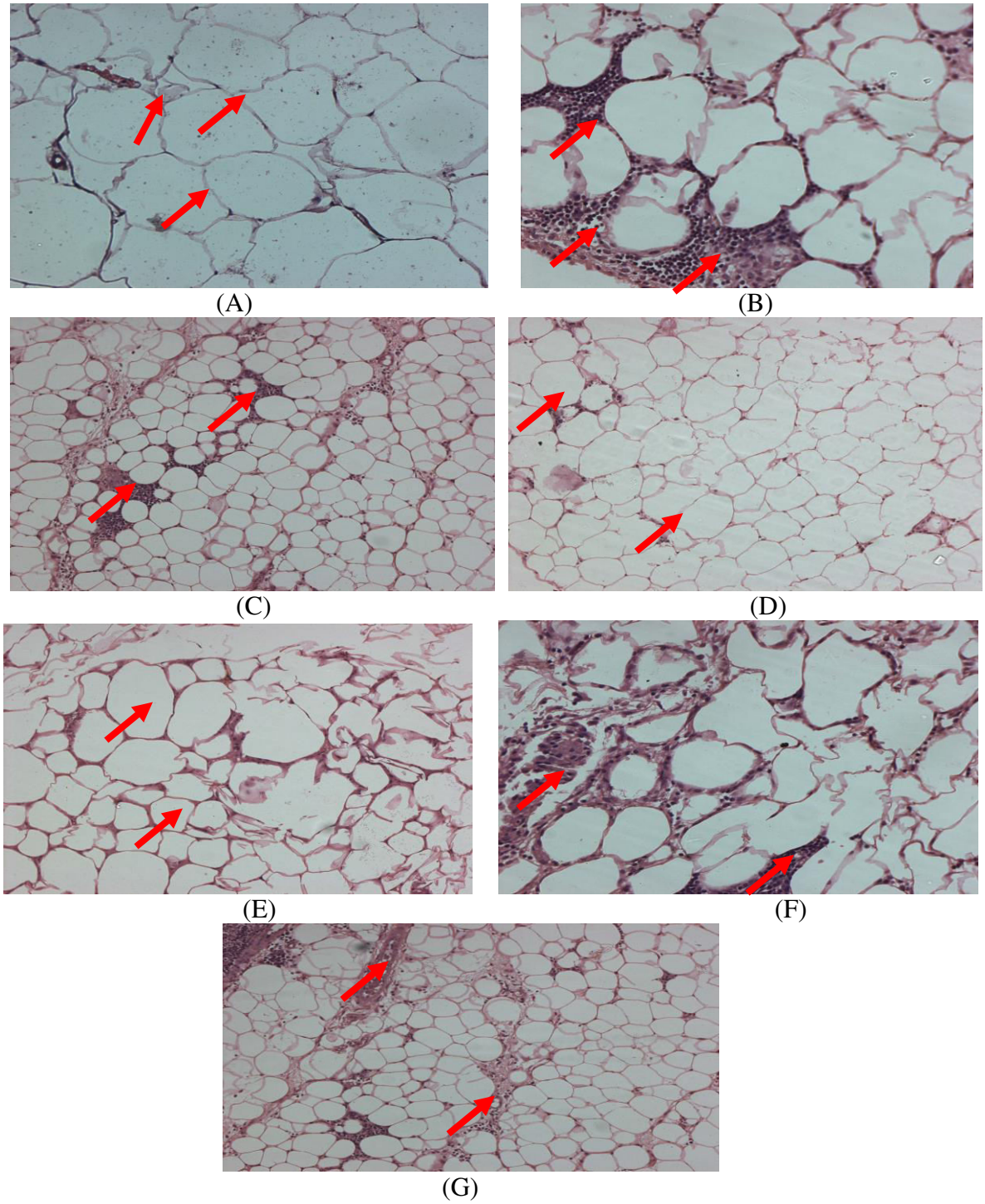

Fig. 4: Histopathological studies of *Emblica officinalis, Terminalia arjuna* extracts at different doses and Atorvostatin on High fat induced diet on Adipose tissue. (A) Adipocytes appear clearly in control group (Group-I); (B) In the toxic group (Group-II) the adipocytes appeared ring shaped studded with the lymphocytes at the edges and in between them are filled with inflammatory cells; (C) Few foci of inflammatory cells studded with adipocytes and remaining adipocytes are appeared normal in Standard group (Atorvastatin) Group III; (D) Few Ring shaped adipocytes with edges of adipocytes studded with lymphocytes were observed in AEEOF 200 mg/kg b wt treated group (Group IV); (E) Adipocytes appeared normal and no abnormalities were observed in AEEOF 400mg/kg b wt treated group (Group V); (F) Ring shaped adipocytes with edges of adipocytes studded with lymphocytes observed in AETAB 200 mg/kg b wt treated group (Group VI); (G) Few foci of adipocytes are studded with inflammatory cells observed in AETAB 400 mg/kg b wt treated group (Group VII).

mg/kg and the aqueous extracts of *T. arjuna* bark both doses. This study was taken in order to know the functionality and to utilise the naturally available drug resources which is easily available for the poor economy people. Further studies should be confirmed with the molecular action of the drug on the organs and the changes in the aortic structure.

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