

**STUDY OF THE ANTIHYPERLIPIDEMIC, ANTIOXIDATIVE AND ANTIATHEROGENIC ACTIVITY OF *Aegle marmelos* LINN. IN RABBIT RECEIVING HIGH FAT DIET**

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**ABSTRACT**

*Aegle marmelos* Linn. has been traditionally used as antihyperlipidemic. So we want to prove it scientifically. Twenty rabbits of either sex were taken and divided into four groups five in each as - Normal Control- received normal diet, Experimental Control- received high fat diet, Test drug - received high fat diet plus ethanolic extract of *Aegle marmelos* Linn.500 mg/Kg /day orally and Standard Drug- received high fat diet plus Atorvastatin 2.1 mg/Kg/day orally for 12 weeks and then animals were sacrificed. Blood sample collected and lipid profile, Catalase, Superoxide Dismutase and Malondialdehyde levels were measured. The antiatherogenic effect was assessed by atherogenic index and histopathology of aorta. Data were statistically analysed by one way ANOVA followed by multiple Dunnett's test. *Aegle marmelos* Linn. significantly decreased serum lipids towards normal levels. It also increased catalase and SOD activity and decreased Malondialdehyde activity. *Aegle marmelos* Linn. has antihyperlipidemic, antioxidant and antiatherogenic effects.

**Keywords:** antihyperlipidemic, anti-oxidative, antiatherogenesis, high fat diet, atorvastatin, lipid profile.

**INTRODUCTION**

Dys-lipidemia is a major contributor towards many chronic non-infectious diseases like atherosclerosis, diabetes, MI, angina, stroke etc. Dys-lipidemia are mainly related to some genetic variations in lipid metabolism or dietary food habits or both which are highly prevalent in Indian sub-continent<sup>1</sup>.

The main cause of atherogenesis is dys-lipidemia, in human typically occurs over a period of many years, usually decades. Generally after a prolonged "silent" period atherogenesis may become clinically significant. The fatty streak and thickening of intima in blood vessels represent the initial lesion of atherosclerosis<sup>2</sup>.

*Aegle marmelos* Linn. also known as Bilwa in Hindi and Bel in Bengali and Assamese. It belongs to the family Rutaceae and grows wild in dry forest, outer Himalayas and Shivaliks. It is a medium to large sized deciduous glabrous, armed tree with the axillary and 2.5 cm long alternate trifoliate leaves, short flower and globular fruits. This plant has shown various activities including anti-diabetic, anti-inflammatory, anti-hyperlipidemic, anti-cancer and anti-viral properties<sup>3</sup>.

*Aegle marmelos* leaves contains sitosterol, aegelin, lupcol, rutin, marmesinin, eugenol,  $\beta$ - sitosterol, flavon, glycoside, montanine, o-isopentenyl halfordiol marmelin and phenylethyl cinnamamides<sup>4</sup>.

In modern practice, there are many drugs like statins and fibrates which are in use as hypolipidemic agent but the therapy is not cost-effective and as such these drugs do not fulfill the WHO guidelines of essential drugs.

So, herbal drugs proved a boon here. As antihyperlipidemic activity of *Aegle marmelos* had not yet been elucidated in an exclusive hyperlipidemic model, the present study has been designed to evaluate lipid controlling, anti-atherosclerotic and ant-oxidant activity of this plant.

**MATERIAL AND METHOD****Plant**

The leaves of *Aegle marmelos* Linn. were collected in the month of May from Assam Medical College and Hospital campus (AMCH), Dibrugarh and authenticated by Dr. M. Islam, Professor, Department of Life Science, Dibrugarh University. A voucher specimen (No. DU/LS/211) was deposited at Dibrugarh University.

**Preparation of plant extract**

The leaves were washed, air dried, powdered and then kept in percolator with 90% ethanol for 72 hours. The extract obtained

from percolation was collected in a flask, and then evaporated by using controlled temperature until the solvent part was evaporated<sup>5</sup>.

**Drug**

Atorvastatin was obtained from Lupin LTD., Kartholi, Jammu.

**Chemicals**

HDL-Cholesterol Kit, Total Cholesterol Kit and Triglyceride Kit were obtained from CREST BIOSYSTEMS, Goa, India. Potassium Phosphate Buffer, Hydrogen Peroxide Solution and Tricarboxylic acid were obtained from Sigma Pvt. Limited, Bangalore, India. Thiobarbituric acid was obtained from HiMedia Laboratories Pvt. Limited, Mumbai, India. Malondialdehyde bis was obtained from Merk Schuchardt OHG, Hohenbrunn, Germany.

**High fat diet**

This was prepared by mixing coconut oil and vanaspati ghee in a ratio of 2 : 3 (v/v) . It was given to the rabbits at a dose of 10 ml/Kg body weight per day mixed with food<sup>6</sup>.

**Animal**

Healthy New Zealand white rabbit (*Oryctolagus cuniculus*) weighing from 1.5-2.5 kg of either sex were taken from Central Animal House, Assam Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages and maintained under normal room temperature. The rabbits were fed with normal diet, high fat diet according to their group and water *ad libitum*. Before commencing the work permission from the Institutional Animal Ethical Committee was taken.

**Acute oral toxicity studies**

Acute oral toxicity test was done according to the OECD guidelines 425<sup>7</sup>. Albino rats of either sex were used. A total of five animals were used. After overnight fasting they received a single oral-dose (2000 mg/kg body weight) of ethanolic extract of leaves of *Aegle marmelos*. Then food was withheld for further 3-4 hrs. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. At the end of the study the animals were observed for general toxic signs, morphological behaviour and mortality.

**Experimental Design**

Twenty numbers of rabbit of either sex were taken and divided into four groups, 5 rabbit in each and treated as follows-

- 1) **Normal Control**- received normal diet.
- 2) **Experimental Control**- received high fat diet at a dose of 10 ml/Kg body weight per day mixed with food.
- 3) **Test drug** - received high fat diet mixed with food plus ethanolic extract of *Aegle marmelos* at a dose of 500 mg/Kg/day orally.
- 4) **Standard Drug**- received high fat diet mixed with food plus Atorvastatin at a dose of 2.1 mg/Kg/day orally<sup>8</sup>.

All the animals used for the experiment were kept under observation for daily food intake, general health and behaviour. The drugs were administered to the animals in the doses given above orally, once daily, for 12 weeks by means of intra-gastric feeding tube in the volume of 5ml/kg body weight.

At the end of the 12 weeks, all the animals were kept fasting for 18 hours. Animals were anaesthetized by using proper dose of ether inhalation. Then, animals were dissected and blood was collected from inferior vena cava in different vials for biochemical analysis. Two cm piece of abdominal aorta was sent in 10% formalin for making slide and histopathological examination to Pathology department, Assam Medical College and Hospital. Then animals were euthanized by proper dose of ether and then animals were sent for incineration to the Central Incineration House, Assam Medical College and Hospital.

#### BIOCHEMICAL ESTIMATION

##### Lipid profile estimation

Total cholesterol was measured by CHOP/PAP method<sup>9</sup>, Triglyceride was measured by GPO/PAP method<sup>10</sup>, HDL- Cholesterol was measured by PEG precipitation method<sup>11</sup> by using colorimetric method and LDL-Cholesterol was calculated by using Freidewald's formula<sup>12</sup>.

#### Antioxidant activity assessment

##### Catalase estimation

Catalase was measured in blood by Continuous Spectrophotometric Rate determination by Beers and Sizer method for antioxidant status<sup>13</sup>.

##### Superoxide dismutase (SOD) estimation

Superoxide dismutase was measured according to Kakkar method<sup>14</sup>.

##### Malondialdehyde (MDA) estimation

Malondialdehyde (MDA) level was measured by colorimeter using thiobarbituric acid reactive substance by TBA method<sup>15</sup> for antioxidant status.

##### Atherogenic Index

Atherogenic Index was calculated by using the following formula <sup>16</sup>,

$$AI = \text{Total serum cholesterol} / \text{Total HDL cholesterol}$$

#### STATISTICAL ANALYSIS

The statistical significance between groups was analysed separately using One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. The significance was expressed by 'p' values, as mentioned in the tables. 'p' values of <0.05 were considered as significant.

#### RESULTS

Acute toxicity test- There was no mortality and no sign-symptom of toxicity reported among the animals upto 2000mg/kg. So the LD<sub>50</sub> was calculated more than 2000 mg/kg body weight.

There was a significant (p < 0.05) decrease in serum cholesterol, triglyceride, low density lipoprotein (LDL), atherogenic index (Table 1), and malondialdehyde (MDA) (Table 2) level in test drug and standard group compared to experimental group which showed a significant (p < 0.05) increase as compared to normal control.

**Table 1: Effect of *Aegle marmelos* on Lipid Profile Test**

GROUPS	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Atherogenic Index
Normal Control	38.74±3.428	76.02±5.702	11.23±1.108	10.52±1.602	3.44
Experimental Control	77.46±1.550*	187.0±6.226*	7.600±0.6013*	24.64±3.456*	10.11
Test drug	50.26±2.286**	108.6±2.464**	17.08±0.704**	10.33±0.4326**	2.76
Standard Drug	60.89±2.160**	118.4±5.483**	18.20±0.8602**	16.22±0.7599**	3.36
ANOVA	F	76	32.52	9.945	
	df	3,16	3,16	3,16	
	p	<0.0001	<0.0001	<0.0001	<0.0001

Values are expressed as MEAN ± SEM (n=5), ANOVA followed by Dunnett's test. \*p <0.05, when compared to the Normal control. \*\*p <0.05, when compared to the Experimental Control

**Table 2: Effect of *Aegle marmelos* on Catalase, Superoxide Dismutase and Malondialdehyde**

Groups	Catalase (μmol /min /ml)	Superoxide dismutase (U/ml)	Malondialdehyde (nmol/ml)
Normal Control	206.0±2.302	7.3±0.08	3.533±0.1291
Experimental Control	179.4±5.077*	2.96±0.13*	5.914±0.0484*
Test drug	332.3±6.261**	5.2±1.13**	2.352±0.0572**
Standard Drug	268.5±7.298**	5.12±0.03**	3.031±0.2013**
ANOVA	F	448.7	151.6
	Df	3,16	3,16
	p	<0.0001	<0.0001

Values are expressed as MEAN ± SEM (n=5), ANOVA followed by Dunnett's test. \*p <0.05, when compared to the Normal control. \*\*p <0.05, when compared to the Experimental control.

There was a significant (p < 0.05) increase in serum high density lipoprotein (HDL) cholesterol (Table 1), catalase and SOD (Table 2) activity in test drug and standard group compared to experimental group which showed a significant (p < 0.05) decrease as compared to normal control.

Histopathological examination of abdominal aorta in normal control group had shown no abnormality (figure 1). Animals in experimental control group shown intimal thickening, separation of tunica media from intima and presence of lipid laden macrophages (foam cells) (figure 2). Animals in test and standard group shown near normal histological architecture (figure 3, 4) compared to experimental group.

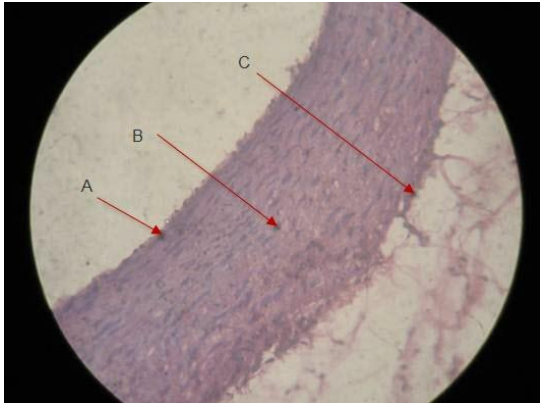


Figure 1: Normal control

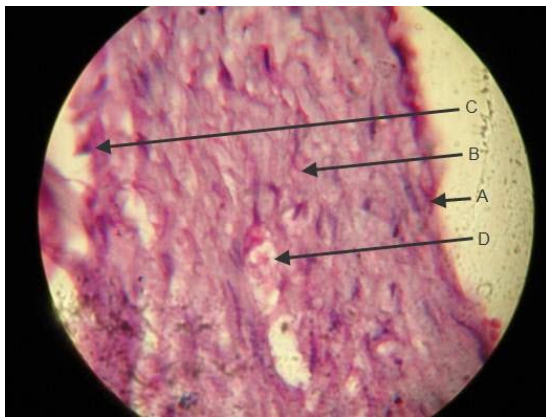


Figure 2: Experimental control

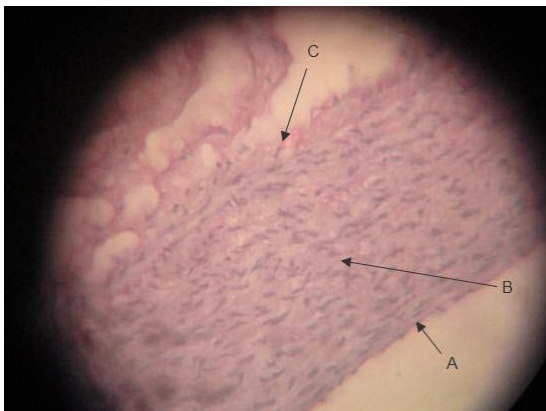


Figure 3: Test drug

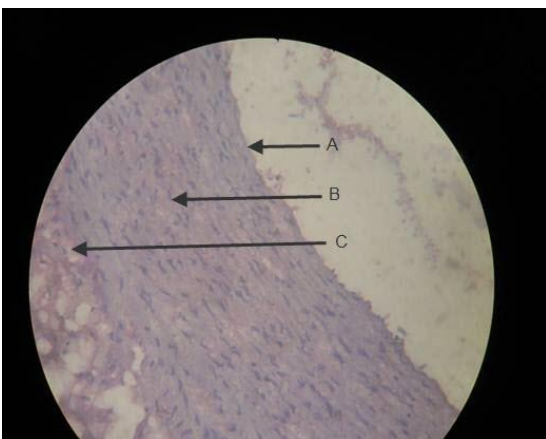


Figure 4: Standard drug

## DISCUSSION

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas e.g., obesity, diabetes mellitus, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health<sup>17</sup>.

Aegeline-2, an alkaloidal amide isolated from the leaves was found to regulate the lipid level<sup>18</sup>. It could be suggested that the constituents of *Aegle marmelos* extract, may act as inhibitors for enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in de novo cholesterol biosynthesis as has been suggested for some plants earlier<sup>19</sup>. However, further more study is required to evaluate its exact mechanism of action as anti-hyperlipidemic.

Anti-oxidants, as the name implies, are substances, which counter the effect of oxidants. Oxidants being derived from normal aerobic metabolism are also products of the inflammatory response. They are mostly of the nature of "Free radicals," which are highly reactive molecules and can cause dyslipidemia, coronary artery disease, atherosclerosis and so many other diseases. In human anti-oxidants are enzymes like Catalase, Glutathione peroxidase, Superoxide dismutase or non-enzymatic like Vitamin A, C, E and minerals like selenium, zinc, copper etc<sup>20</sup>. Here, eugenol and marmesinin present in this plant have anti-oxidant activity<sup>4</sup> and thus prevent dyslipidemia and atherogenesis.

Results of atherogenic index and histopathological examination proved ethanolic extracts of *Aegle marmelos* have anti-hyperlipidemic and antioxidant activity and thus prevents atherogenesis.

It can be concluded from the above study that ethanolic extract of *Aegle marmelos* Linn. has antihyperlipidemic, antioxidative and antiatherogenic activities.

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