ORIGINAL RESEARCH PAPER

Pharmacology

STUDY OF THE HYPOLIPIDEMIC EFFECT OF SOLANUM MELONGENA LINN AND EMBLICA OFFICINALIS ON RABBITS RECEIVING HIGH FAT DIET

KEY WORDS: Hypolipidemic, Clofibrate, Lipid profile, Solanum melongena Linn, and Emblica officinalis

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ABSTRACT

Solanum melongena Linn, and *Emblica officinalis* has been traditionally used as hypolipidemic and both are edible. The present work was carried out to investigate their hypolipidemic effect on rabbits. Thirty rabbits of either sex were taken and divided into five groups six in each as : Normal Control (fed with standard diet), Experimental Control (fed with high fat diet), Standard Drug (fed with high fat diet plus Clofibrate 33mg Kg^{-1} day^{-1}), Test drug first group (fed with high fat diet plus 10% *Solanum melongena* water extract per day orally) and Test drug second group (fed with dry *Emblica officinalis* fruit 1 g kg^{-1} orally) for 16 weeks and then blood sample collected and lipid profile were measured. Data were statistically analysed by one way ANOVA followed by multiple Dunnett's test. It was observed that *Solanum melongena* Linn and *Emblica officinalis* significantly decreased serum lipids towards normal levels. Hypolipidemic effect of both *Solanum melongena* Linn and *Emblica officinalis* was observed.

INTRODUCTION:

Lipemia *(Lipid + G. Haemia, Blood)* is the presence of abnormally large amount of lipids in the circulating blood. It is also called hyperlipoidemia, lipidema, lipoidemia [1]. Elevated serum cholesterol level is the leading cause of morbidity and mortality from Coronary heart disease (CHD) which cause 25 - 30% of death in most of industrialized countries and had been declared as a modern epidemic by WHO [2][3].

It is now well documented that CHD is preventable. Modification of diet, life style changing and measurement for modifications of risk factors are major point for prevention. Lowering of elevated lipid levels is also one of the important steps to prevent emergence of CHD. The benefits of lipid lowering therapy on CHD have been clearly established in many clinical trials on primary and secondary prevention [4]. *Solanum melongena* Linn. (Brinjal; Solanaceae), a culinary vegetable, has been in use in the Indian system of medicine. Various parts of the plant are useful in the treatment of inflammatory conditions cardiac debility, neuralgia, ulcers of nose, cholera bronchitis and asthma. Its antioxidant, analgesic and hypolipidemic activities have been reported [5]. It has been reported that the flavonoids isolated from *Solanum melongena* (brinjal) showed potent antioxidant activity [6]. The elevated levels of glutathione and significantly stimulated activity of catalase may be responsible for the antioxidant effect of these flavonoids [7][8]. Hypolidemic potentials of fresh, ripe fruits of *Solanum melongena* and *Solanum gilo* were reported [9].

Emblica officinalis (Amalaka, Amla, Avla, Indian gooseberry) an extensively available Indian herb has been used as valuable ingredient of various medicines. The great authority on Ayurveda, considers it the best of all acid fruits and most useful for health and in treating diseases [10]. The fruit of *Emblica officinalis* is commonly used in the treatment of burning sensation anywhere in the body, anorexia, constipation, anti-hyperlipidemic, urinary discharges, inflammatory bowels, cough, hemorrhoids, fever, thirst, and toxicity of the blood. *Emblica officinalis* is highly nutritious and could be an important dietary source of Vitamin C, minerals and amino acids [11]. It has been found that percentage of glutamic acid, proline, aspartic acid, alanine, and lysine in *Emblica officinalis* are 29.6, 14.6, 8.1, 5.4 and 5.3 respectively of the total amino acids [12]. Composition of the pulpy portion of fruit, dried at 100°C and freed from the nuts is: gallic acid 1.32%, tannin, sugar 36.10%; gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%. Amla fruit ash contains chromium, 2.5 ppm; zinc, 4ppm; and copper, 3 ppm [13]. Presence of chromium is of therapeutic value in diabetes. Fruit also contains phyllemblin. The fruit contained 482.14 units of superoxide dismutase/g fresh weight, and exhibited antisenescent activity. The seed oil contains 64.8% linolenic acid and closely resembles linseed oil [11]. *Emblica officinalis* juice is an effective hypolipidemic agent and can be used as a pharmaceutical tool in hyperlipidemic subjects [14]. The tissue lipid levels including Serum cholesterol, TG, phospholipid and LDL showed a significant reduction following *Emblica officinalis* juice administration [14] [15].

In modern practice, the agents used for lipid lowering are costly and these drugs do not fulfill the WHO guidelines of essential drugs. Today the herbal products and herbal medicine not even in India, but in abroad are becoming more and more popular due to its natural properties, which are homogenous to body and is easily assimiable as well as absorbable in our body [15]. So, the present study has been designed to evaluate hypolipidemic activity of both the fruits.

MATERIAL AND METHOD

Methods of Extraction:

1. Preparation of *Solanum melongena* Linn. extract:

Solanum melongena Linn is extracted by *Soxhlet* extraction. 250gm ripe *fruit* of *Solanum melongena Linn (Eggplant)* local variety is collected from a local market of Tezpur. Then ripe *fruit* are washed with cold water and allowed to get dry. Care is taken to wash away all impurities and also to maintain absolute cleanness. The dried *Egg plants* are powdered and made ready for extraction.

Menstrum used in our study is distilled water: The drug and menstrum ratio is 1:3. The obtained extracts are kept in cold place in tight and light resistant container. These extracts are used throughout the study and are prepared freshly when required.

Dose of *Solanum melongena* Linn is calculated on basis of 10% *Solanum melongena* per kg body weight daily.

Extraction Procedure:

The drug to be extracted is packed in a paper cylinder made from a filter paper and it is placed in the body of the soxhlet extractor, the solvent is placed in the flask.

When solvent *(Menstrum)* is boiled on heating the flask, it gets converted into vapours. These vapours enter into the

condenser through the side tube and get condensed into hot liquid which falls on the column of the drug. When the extractor gets filled with the solvent, the level of syphon tube is also raised up to its top. The solvent containing active constituents of the drugs in the syphon tube syphon over and run into the flask thus emptying the body of extractor. This alteration of filling and emptying the body of extractor goes on continuously. The soluble active constituent's .The soluble active constituents of the drugs remain in the flask while the solvent is repeatedly volatilized. The process of filling and emptying of extractor is repeated until the drug is exhausted. Normally the process is repeated 15 times for complete exhaustion of the drugs [16].

2. *Preparation of dry Emblica officinalis fruit*

250 g of *Emblica officinalis fruit (Locally known as Amla)* is purchased from a local market in Guwahati. Impurities are separated and washed thoroughly in tap water. Washed *fruit* is dried in shade at room temperature. Dry and cleaned *Emblica officinalis fruit* is taken into mortar and powdered. Dose of *Emblica officinalis* Linn is calculated on basis of 1 g kg^{-1} body weight daily [17].

Drug

Clofibrate was obtained from Lupin LTD., Kartholi, Jammu, India. Chemical used in our experiments is cholesterol *extra pure* ($C_{27}H_{46}O$) was procured form E Merck (India) limited, Worli, Mumbai - 400 018.

Chemicals

HDL-Cholesterol Kit, Total Cholesterol Kit and Triglyceride Kit were obtained from of Dr. Reddy's dependent on enzymatic method with end point, suitable for colorimetric estimation.All the biochemical estimations were done by using *Dade Behring Dimension Clinical Chemistry system and Boehringer 4020 Photometer (Semi –Automatic).*

High fat diet

This was prepared by mixing 2% cholesterol. [Merck, E. Merk (India) Limited] and 3% coconut oil (edible). It was given to the rabbits at a dose of 10 ml Kg^{-1} body weight per day mixed with food.

Animal

Healthy adult New Zealand white rabbits *(Lupas or Oryctolagus cuniculus)* of weight 1 kg to 2kg *(Average 1.5 kg)* were selected for our study from Central Animal House of Gauhati Medical College (Registration No 351/CPCSEA). They were kept in animal house under natural photoperiod at 25 ± 1°C temperature in a well-ventilated condition. They were provided with standard diet (SD) and water *ad libitum.* The Bengal gram is restricted to all animals, as it has been shown to be hypercholesterolemia [18].*Before commencing the work permission from the Institutional Animal Ethical Committee was taken.*

Acute oral toxicity studies

Acute oral toxicity test was done with both test drugs according to the OECD guidelines 4257. One arbitrary dose of 500 mg kg^{-1} was selected for the study, as the extract was found safe even at doses more than 2000 mg kg^{-1}. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days without any sign of toxicity or mortality.

Experimental Design

Thirty numbers of rabbit of either sex were taken and divided into five groups, 6 rabbit in each and treated as follows:

Group A = Fed standard diet. The average diet given per rabbit is 100 g day^{-1}. ("SD").

Group B = Fed cholesterol diet at a dose of 10 ml Kg^{-1} body weight per day mixed with food.("AD").

Group C = Fed "AD" + Clofibrate 33 mg Kg^{-1} day^{-1} [19].

Group D = Fed "AD" + *Solanum melongena* (10% water extract).

Group E = Fed "AD" + Dry *Emblica officinalis* fruit 1 g kg^{-1} [17].

Group A animals are taken as control for Group B animals and Group B animals are taken as control for Group C, D & E. Finally, Group C is taken as standard drug control. Both drugs and vehicle are administered daily orally for 16 weeks by means of intra-gastric feeding tube in the volume of 5ml kg^{-1} of body weight.

Collection of blood: Blood samples are collected from marginal ear vein of the animal after overnight fasting at the 0^{th} week, at the 4^{th} week and at the 16^{th} week. During collection of blood, care is taken to prevent haemolysis of blood. Amount of collected blood is around 1.5 ml and was collected in sterile empty vial *(SEV)*. Lipid parameters are estimated by kit methods.

Duration of experiment for hypolipidemic effect was carried out for 16 weeks i.e., all animals were given treatment for 16 weeks. The serum of each animal of all groups were estimated for cholesterol, triglyceride, VLDL, LDL and HDL.

Estimation of serum lipid profile:

All the biochemical estimations were done by using Dade Behring Dimension Clinical Chemistry system and Boehringer 4020 Photometer (Semi - Automatic).

Estimation of cholesterol:

The method used for estimation of serum total cholesterol was adaptation of the cholesterol oxidase peroxidase method which is the Allain et al (1974) modificaiton of original Richmond (1972) and Flegg (1973) enzymatic method [20].

Estimation of triacylglycerol:

Serum triacylglycerol is estimated by the reagent kits of Dr. Reddy's based on glycerol-3 - phosphate oxidase/peroxidase method developed by Fossati and Prencipe (1982) which is a modification Bucolo and David (973) enzymatic method [21].

Estimation of High Density Lipoprotein:

HDL Cholesterol was measured by PEG precipitation method by using colorimetric method. LDL and VLDL-Cholesterol was calculated by using Freidewald's formula [22].

Statistical analysis:

Levels of serum lipids are compared statistically over the six groups comprising baseline (0^{th} week) and at 4^{th} week and 16^{th} week determinations. This is performed using a one-way Analysis of Variance (ANOVA) with Dunnett's t-test for multiple comparisons. For all tests $p < 0.05$ is used for significance level and $P < 0.01$ is taken as highly significant.

RESULTS:

Acute toxicity test

The LD 50 was calculated more than 2000 mg kg^{-1} body weight. No mortility among the animals was observed.

Mean lipid profile at 0^{th} week, 4^{th} week and 16^{th} week (Table 1) are shown in Figure 1, Figure 2 and Figure 3 respectively. In all the groups the lipid levels are within normal limits at the beginning of the study. Again in group A all the values of the lipid profile at 4^{th} week are more or less within normal limits.

In group B, fed with A.D, there is significant rise of mean serum cholesterol level at 4^{th} week which is 105 ± 11.18 and at 16^{th} week it is 180.16 ± 6.85.

In group C, fed with A.D + Clofibrate, there is also significant

rise of mean cholesterol level which is 84.83 ± 4.70 at 4th week and 119.5 ± 4.85 at 16th week, but this is less than that of the group B value.

In group D, fed with A.D + *Solanum melongena*, there is also significant rise of mean cholesterol level which is 86.16 ± 5.63 at 4th week and 127.5 ± 4.84 at 16th week, but this is less than that of the group B value.

Table 1: Mean Lipid Profile at 0th, 4th and 16th week

Week	Group	Cholesterol (mg/dl) mean	Triglyceride(mg/dl) mean	VLDL (mg/dl) mean	LDL (mg/dl) mean	HDL (mg/dl) mean
0th	A	67.33± 5.71	81.33± 5.27	16.26± 1.05	26.23± 3.80	24.83± 2.78
	B	64.33± 4.45	80.16± 4.73	16.03± 0.93	24.8± 4.42	23.5± 3.08
	C	68.83± 8.30	75.5± 5.81	15.1±.96	29.9± 4.80	23.83± 3.76
	D	66± 4.85	77.33± 5.81	15.4± 1.17	24.5± 4.18	26± 5.14
	E	69.33± 5.0	77.33± 6.15	15.46± 1.23	27.86± 5.30	26± 5.62
4th	A	64.33± 6.08	78.16± 5.84	15.63± 1.16	23.36± 4.04	24.83± 2.78
	B	105± 11.18	99± 6.66	19.8± 1.33	64.7± 5.01	20.5± 5.35
	C	84.83± 4.70	94.16± 4.95	18.83± .99	33±3.30	33±2.75
	D	86.16± 5.63	89± 5.47	17.8± 1.09	39.86±1.71	28.5±4.64
	E	85.5± 5.82	89.16± 6.70	17.83± 1.33	39± 3.01	28.66± 4.35
16th	A	69.33± 7.47	85± 5.83	17± 1.16	27.33± 2.58	25± 4.28
	B	180.16± 5.82	115.5± 5.78	23.25± 1.07	138.25± 5.72	18.66± 2.87
	C	119± 4.85	101± 5.21	20.33± .96	64.5±4.73	34.16±3.31
	D	127± 4.84	95.16± 5.84	19.03± 1.16	76.46±.86	32±4.73
	E	128± 3.74	95.33± 6.65	18.87± 1.21	77.95±3.53	31.8± 4.35

Figure 1

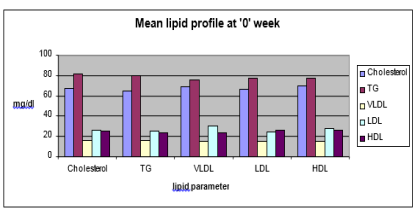


Figure 2

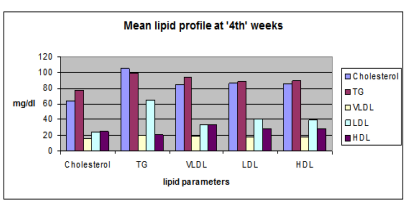
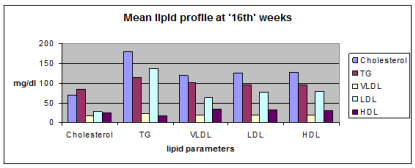


Figure 3



From One Way ANOVA and Dunnett's Multiple Comparison analysis, significant ($p < 0.05$) reduction of cholesterol, triglyceride, VLDL, LDL levels at 4th week and that at 16th week in groups A, C, D and E when compared with group B was observed (Table 2). However no significant changes in cholesterol, triglyceride, VLDL, LDL level in group D and group E was observed compared to clofibrate treated group.

Table 2: ANOVA for cholesterol, triglyceride, VLDL, LDL and HDL level in 4th and 16th week in all groups of animals.

Values are expressed as Mean SEM (n=5)

	Week	Source	Sum of square	Mean of square	DF	F -ratio	P -value
Cholesterol	4th	Between group	4971.33	1242.83	4	24.84	<0.01
		Error	1250.84	50.033	25		
		Total	6222.17	-	29		
	16th	Between group	37156.92	9289.23	4	82.27	<0.01
		Error	2822.86	112.91	25		
		Totel	39979.78	-	29		
Triglyceride	4th	Between group	1440.86	360.22	4	9.88	<0.01
		Error	911.42	36.46	25		
		Total	2352.28	-	29		
	16th	Between group	2991.92	747.98	4	21.32	<0.01
		Error	877.10	35.08	25		
		Total	3869.02	-	29		
VLDL	4th	Between group	57.67	14.42	4	9.55	<0.01
		Error	37.72	1.51	25		
		Total	95.39	-	29		
	16th	Between group	128.56	32.14	4	23.28	<0.01
		Error	34.52	1.38	25		
		Total	163.08	-	29		
LDL	4th	Between group	5621.98	1405.49	4	108.53	<0.01
		Error	323.85	12.95	25		
		Total	5945.83	-	29		
	16th	Between group	38256.38	9564.09	4	111.53	<0.01
		Error	2143.72	85.75	25		
		Total	40400.10	-	29		
HDL	4th	Between group	527.50	131.88	4	8.15	<0.01
		Error	404.45	16.18	25		
		Total	931.95	-	29		
	16th	Between group	984.57	246.14	4	15.23	<0.01
		Error	403.96	16.16	25		
		Total	1388.53	-	29		

The change of HDL at 4th week and at 16th week in group A and group B are highly significant. There are no significant changes in HDL level in group D and group E compared to clofibrate treated group. At 16th week in HDL change is significant in group A, and B when is taken other groups as control, but not significant in group C, D and E compared to each other.

The result thus obtained is compared with that of the standard drug Clofibrate and was found to be statistically significant. The action of lipid lowering action was found to be more or less similar to that of the Clofibrate at both the weeks of the study.

It can be concluded from the above study that extract of Solanum melongena and Emblica officinalis has hypolipidemic activities.

DISCUSSION:

Hypolipidemic activity of *Solanum melongena* (brinjal) is probably due to the flavonoids that showed potent antioxidant activity. The elevated levels of glutathione and significantly stimulated activity of catalase may be responsible for the antioxidant effect of these flavonoids.

Hypolipidemic activity of *Emblica officinalis* is probably due to Vitamin C, tannin, crude cellulose and phyllemblin. The mechanism of action of tannins is to inhibit lipid absorption and or activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors [12]. *Emblica officinalis* treated rabbits excreted more cholesterol and phospholipids, due to cellulose suggesting that the mode of absorption was affected. Oxidants derived from aerobic metabolism are products of the inflammatory response. They are mostly nature of "Free radicals," which are highly reactive molecules and cause dyslipidemia, coronary artery disease, atherosclerosis and many other diseases. Both the plants contain Vitamins C, E, carotene, selenium, bioflavonoids etc. [10][15]. Thus antioxidant activity of both plant also prevent dyslipidemia and atherogenesis. Tannins were reported to be involved in growth regulations [14]. The weight lowering potential of *Emblica officinalis can* be partially attributed to the presence of tannins found in the plant. The hypolipidemic response of Solanum melongena and *Emblica officinalis* are more or less similar with that of Clofibrate almost throughout the study.

It can be concluded from the above study that extract of Solanum melongena and Emblica officinalis has hypolipidemic activities.

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