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In vitro hypolipidemic activity of *Averrhoa bilimbi* flower extract

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Abstract
Hyperlipidemia and oxidative stress are major risk factors for atherosclerosis, and all three are among the most important risk factors for cardiovascular diseases and conditions. *Averrhoa bilimbi* is a traditional Medicine used as a local remedy for various ailments like hypertension, Diabetes mellitus and dyslipidemia. When used in high concentrations the fruit juice can lead to Acute renal failure due to acute tubular necrosis, owing to its high oxalate contents which results in intratubular oxalate crystal deposition therefore we are focussing on the flower of *Averrhoa bilimbi* Hyperlipidaemia was induced by a single intra-peritoneal injection of Triton X-100 at a dose of 100 mg/kg. body weight. Group 1 was kept as Normal, i.e. no administration of drug and extract follows normal diet. Group 2 was kept as toxic control i.e, no administration of drugs after the introduction of hyperlipidemia. The regression group i.e. the animals of Groups C, D, E, fourty eight hours after inducing Hyperlipidaemia different doses of drug was administered for seven days. For Group C Standard drug- Atorvastatin and for Groups D,E different doses of study drug was given i.e, methanolic extract of *Averrhoa bilimbi* 200mg/kg and 400mg/kg. The results obtained from the present study indicated that *Averrhoa bilimbi*, has potential antioxidant and hypolipidemic activity and it may be the due to the synergistic effect of the major phyto-constituents like flavonoids, tannins, terpinoids and phenols that are highly present in the extract. But further studies are required to provide the exact mechanism of action of the reported activities by using purified fraction of the same.

Keywords: *Averrhoa bilimbi*, hyperlipidaemia, tubular necrosis

Introduction
The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. A complete understanding of medicinal plants involves a number of disciplines including commerce, botany, horticulture, chemistry, enzymology, genetics, quality control and pharmacology. The use of modern isolation techniques and pharmacological testing procedures means that new plant drugs usually find their way into medicine as purified substance rather than in the form of galenical preparations [1].

Hyperlipidemia and oxidative stress are major risk factors for atherosclerosis, and all three are among the most important risk factors for cardiovascular diseases and conditions. Strategies used to prevent and treat atherosclerosis, and to reduce the incidence and severity of associated cardiovascular diseases, mainly include fighting against hyperlipidemia using dietary approaches such as diet rich in fibers, and/or anti-dyslipidemic drugs like atorvastatin. A huge body of population based and experimental evidence shows that high levels of plasma low density lipoprotein (LDL) cholesterol and total cholesterol considerably increase the risk for developing atherosclerosis and associated arterial hypertension. Other changes in lipid parameters associated with atherosclerosis include decreases in high density lipoprotein (HDL) cholesterol and increases in triglycerides. A number of anti-dyslipidemic drugs was developed to slow or prevent atherogenesis, and eventually treat atherosclerosis. Unfortunately, these drugs may have serious undesired effects, and thus, new therapeutics against atherosclerosis are needed in the field.

Averrhoa bilimbi (Oxalidaceae family) commonly known as bilimbi, is an attractive, long-lived tropical tree, The major chemical compounds of extract flower of *Averrhoa bilimbi* were cycloeicosane followed by benzenedicarboxylic acid and benzenepropanoic acid [2]. Fruits have medicinal properties for the effective management of several human ailments Different parts of the plant is used for various conditions. The fruit is administered as a treatment for coughs, beri-beri and biliousness. Syrup prepared from the fruit is taken as a cure for fever and inflammation and to stop rectal bleeding and alleviate internal hemorrhoids.

The leaves are applied as a paste or poultice on itches, swellings of mumps and rheumatism, and on skin eruption. They are applied on bites of poisonous creatures. A leaf infusion is a remedy for coughs and is taken after childbirth as a tonic. A leaf decoction is taken to relieve rectal inflammation. A flower infusion is said to be effective against coughs and thrush. A paste of pickled bilimbi is smeared all over the body to hasten recovery after a fever. Used for obesity & anti diabetic [3]

Averrhoa bilimbi is a traditional Medicine used as a local remedy for various ailments like hypertension, Diabetes mellitus and dyslipidemia. When used in high concentrations the fruit juice can lead to acute renal failure due to acute tubular necrosis, owing to its high oxalate contents which results in intratubular oxalate crystal deposition therefore we are focussing on the flower extract of *Averrhoa bilimbi.*

The present study was designed to evaluate the possible dose dependent effect of methanolic flower extract of Averrhoa bilimbi for its hypolipidemic activity

Materials

Collection of plant materials

The test drug Ilumbanpuli which has been identified pharmacologistically as *Averrhoa bilimbi* Linn of the family Oxalidaceae was collected fresh from the areas of Kottayam in the season of October to January. The plant was identified and authenticated by Dr. M. V. Krishnaraj Department of Botany, Baselius College Kottayam. Kerala the voucher specimen of the plant was kept in Library with register number DPS/MGU/RIMSR/2016/herb.6 for further reference. The flowers of *Averrhoa bilimbi* are dried under shade to avoid over drying.

Animals

Selection of animals

Healthy Wistar strain albino rats of male sex weighing between 200-250 gm was collected from Animal house, Department of Pharmacology, RISMR, Kottayam. (CPCSEA No: 1702/PO/C/13/CPCSEA) The animals were housed in polypropylene cages in room where the congenial temperature 27 °C ±1 °C, 30-60% relative humidity and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days. They were fed on standard pellet diet collected from Hindustan Lever Ltd, Bangalore and water given ad libitum. All procedures and experiments were conducted in day time according to specification of the Indian National Science Academy (INSA).

Extraction

Preparation of extracts using non-polar solvent (chloroform,) and polar solvent (methanol, distilled water) as a solvent. The shade dried flowers were powdered mechanically using commercial electrical stainless steel blender and extracted with different solvents in a soxhlet apparatus. After extraction, the extract was filtered through whatmann filter paper to remove insoluble particles. The extract was concentrated under reduced pressure rotary evaporator and the crude extract obtained was stored in desiccator to avoid contact with atmosphere moisture and used for the Preliminary phytochemical screening to identify various phytoconstituents [4].

Acute Toxicity Study

The acute toxicity was carried out as per the OECD 423 guidelines. Twelve female Swiss Albino Mice weighing between 20 and 25 g and between age eight and twelve weeks were procured for the experimental trial. The animals were maintained under controlled environmental conditions (30 – 70% humidity and temperature – 22 ± 3 ºC) and were exposed to a photoperiod of 12 hours of daylight and 12 hours of night, in an animal house, approved by the committee for the purpose of control and supervision of experiments on animals. The selected animals were fed with standard feed and drinking water and monitored on a regular basis.

The animals were selected randomly and grouped, three animals per group. They were kept fasting four hours prior to the treatment and the test substance was administered in a single dose by the oral route. Subsequently, the dose was gradually increased with each step, starting from 5 mg/kg then to50, 300, 2000 mg/kg. After the substance had been administered, the food could be withheld for a further one-to two hours, in mice.

The animals were observed for changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic, and central nervous systems, and somatic motor activity and behaviour pattern. They were noted individually after dosing, at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first four hours, daily, a and thereafter for a total of 14 days [5].

Study Design

Triton X-100 (a non-ionic detergent, iso octyl polyoxy ethylene phenol, form aldehyde polymer) was obtained from Laboratory Chemicals, Ernakulam. All other chemicals were of analytical grade and obtained locally. Triton leads to the rapid increase of various lipid fractions and cholesterol, this increase is confined to the blood itself and not due to any intestinal alterations [6].

- **Mechanism to induce hyperlipidemia by Triton X-100**

Triton is a non-ionic detergent of polymeric structure that has been successfully used in several studies to induce hypercholesterolemia. Direct inhibitor effect on the lipoprotein lipase in muscle and adipose tissue Triton X-100 act as surfactant and cause structure modifications in circulatory lipoproteins suppress the action of lipases and as a consequence block the uptake of circulatory lipids by extra hepatic tissues, resulting in increased blood lipid concentration [7].

Experimental Design

Treatment protocol

Group 1

Served as control and received normal feed and water ad libitum

Group 2

Served as Hyperlipidemic control Triton X -100 100mg\kg body weight and water ad libitum

Group 3

Served as reference standard I and received Atorvastin 1.2 mg/kg p.o. in 1% sodium CMC for a period of 7days.

Group 4

Animals received AECA 200mg/kg p.o. in 1% Sodium CMC for a period of 7 days

Group 5

Animals received AECA 400mg/kg p.o. in 1% Sodium CMC a period of 7 days.24

Procedure

Thirty six albino rats of male sex weighing about 200 – 250 gm. were selected in the present study. The animals were kept under

observation for one week and grouped into six in such a way that each group consisted of six animals. For all except normal group Hyperlipidaemia was induced by a single intra-peritoneal injection of Triton X-100 at a dose of 100 mg/kg. body weight. Group 1 was kept as Normal, i.e. no administration of drug and extract follows normal diet. Group 2 was kept as toxic control i.e, no administration of drugs after the introduction of hyperlipidemia. The regression group i.e. the animals of Groups C, D, E, fourty eight hours after inducing Hyperlipidaemia different doses of drug was administered for seven days. For Group C Standard drug- Atorvastatin and for Groups D,E different doses of study drug was given i.e, methanolic extract of *Averrhoa bilimbi* 200mg/kg and 400mg/kg [6].

Table 1: Grouping of animals

Groups	Triton X 100	Drug dose & duration
Group A	No administration of triton x 100 serves as normal group	No drug was given
Regression groups	**Section 1**	
Group B	Single ip injection at a dose of 100mg/kg body weight serves as toxic control group	No drug was given
Group C	Single intraperitoneal injection at a dose of 100mg/kg body weight	Standard drug atorvastatin at effective dose 1.2 mg/kg was given orally for 7 days
Group D	Single intraperitoneal injection at a dose of 100mg/kg body weight	Animals received MEAB200mg/kg per orally in 1% sodium CMC for a period of 7days
Group E	Single intraperitoneal injection at a dose of 100mg/kg body weight	Animals received MEAB 400mg/kg/po in 1% sodium CMC for a peroid of 7days

The blood samples from the animals were collected on the 8th day of drug administration. Blood was collected from retro-orbital of the rats and serum was separated in coolint centrifuge by centrifuging at 2500rpm for 15 min. The efficacy of the drug was mainly assessed by Biochemical parameters.

Result & Discussion

Preliminary phytochemical screening

Phytochemical screening shows methanolic extract contain Flavanoids, Tannins, Terpinoids, Phenolic compounds. The result is as follows

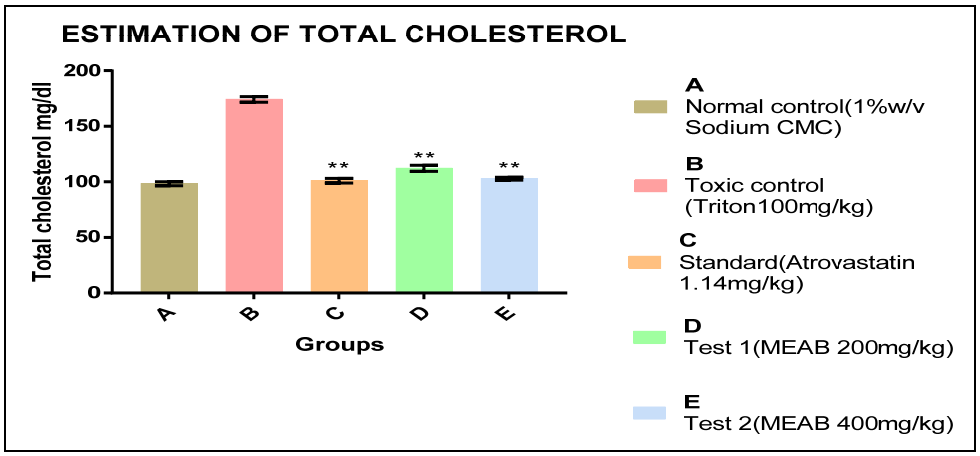
Table 2

Si. No	Constituents	Result
1	Flavonoids ➢ Shinoda test ➢ Lead acetate	+ve
2	Tannins ➢ Ferric chloride ➢ Lead acetate	+ve
3	Alkaloids ➢ Mayer's Test ➢ Wagner's Test	-ve
4	Saponins	-ve
5	Phenols ➢ Ferric chloride Test	+ve
6	Terpenoid	+ve
7	Steroids	-ve
8	Resins	-ve
9	Protein ➢ Biurette test	-ve
10	Cardiac glycosides	-ve

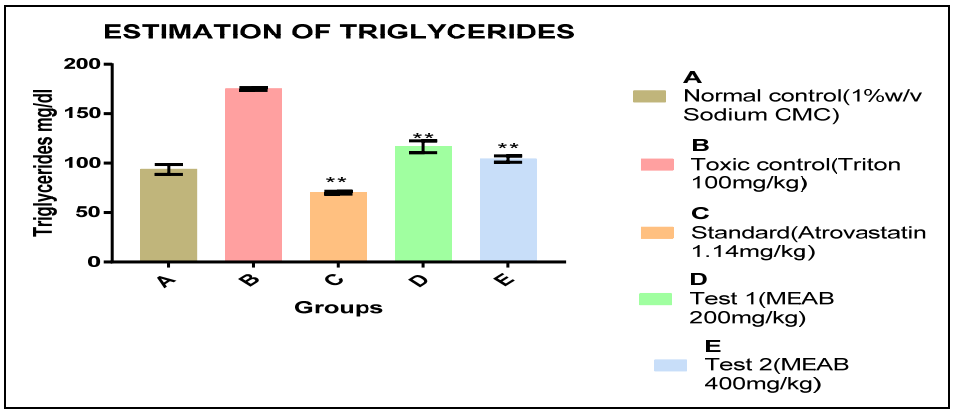
Hypolipidemic Activity

Table 3

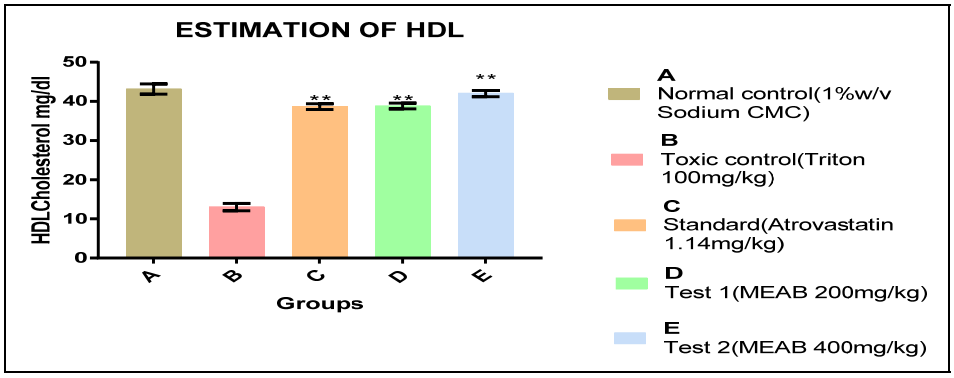
Groups	Total Cholest Rol	Hdl	Tgl	Ldl	Vldl	Ai
NORMAL Control (1% CMC)	98.33±1.74	43.16±1.35	93.66±5	36.16±2.90	18.66±5	1.261±.04
Toxic Control (Triton 100mg/kg)	174.16±2.52	13±0.930	175±2.35	126.16±2.7	35±0.5	12.71±0.93
Standard (Atorvastatin 1.2mg/kg)	101±2.160**	38.66±1**	70.16±1**	48.33±1**	14±0.25**	1.61±1**
Test 1 (MEAB 200mg/Kg)	112.16±2.7**	38±0.74**	116±5.1**	49.6±2.3**	23.3±2.6**	1.8±0.05**
Test 2 (MEAB 400mg/Kg)	102.8±1.30**	42±0.81**	104.1±3**	39.8±2**	21±1.7**	1.5±0.63**



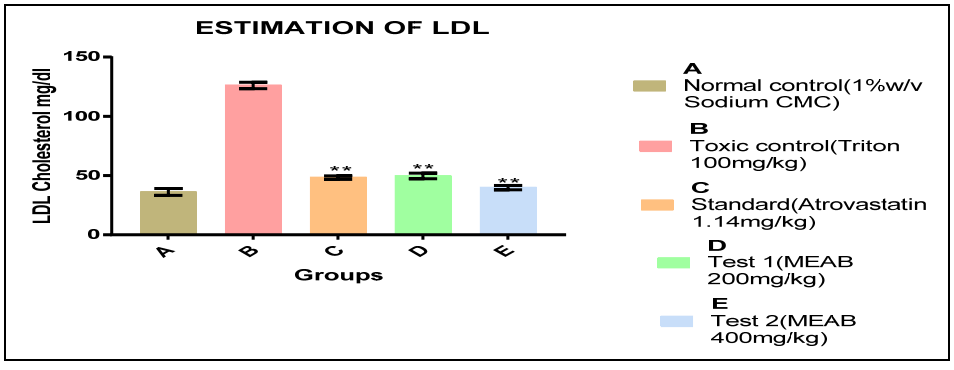
Graph 1: Effect of MEAB on Total cholesterol in hyperlipidemic rats.



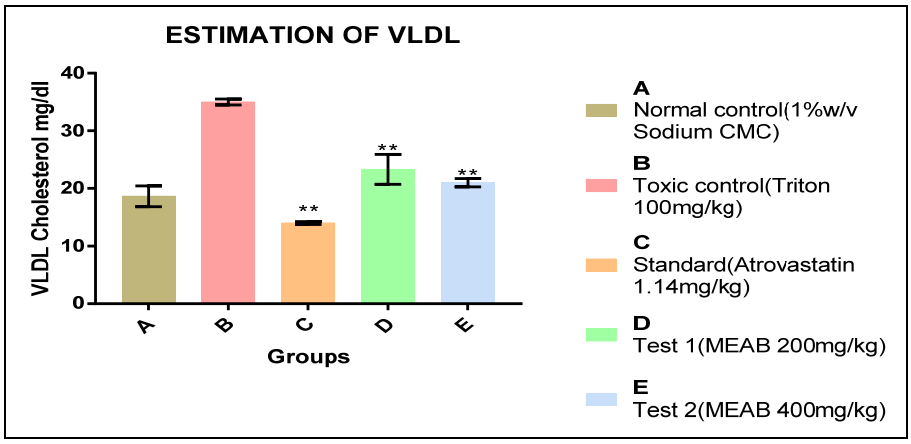
Graph 2: Effect of MEAB on Triglycerides in hyperlipidemic rats.



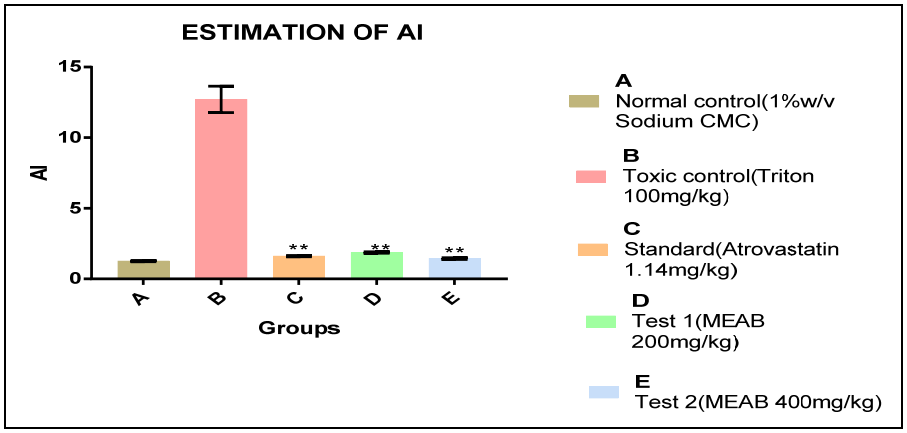
Graph 3: Effect of MECA on HDL cholesterol in hyperlipidemic rats.



Graph 4: Effect of MECA on LDL in hyperlipidemic rats



Graph 5: Effect of MEAB on VLDL in hyperlipidemic rats.



Graph: 6 Effect of MEAB on Atherogenic index in hyperlipidemic rats.

Averrhoa bilimbi is a traditional medicine used as a local remedy for various ailments like hypertension, diabetes mellitus and dyslipidemia [3]. When used in high concentrations the fruit juice can lead to acute renal failure due to acute tubular necrosis, owing to its high oxalate content which results in intratubular oxalate crystal deposition. By using flower extract *Averrhoa bilimbi* we can overcome this harmful effect [8].

In our experiments the rats treated with triton showed a significant increase in serum cholesterol level from 98.33mg/dl in normal rats to 174.16mg/dl in triton treated groups. Triglycerides level from 93.66mg/dl in normal rats to 175mg/dl. VLDL level is 18.6mg/dl in normal group and 35mg/dl in triton treated grop. LDL level 36.16mg/dl in normal rats and 126.16mg/dl in triton treated group. HDL level is decreased from 43.16mg/dl to 13mg/dl in triton treated group. Treatment with different doses of methanolic extract ie 200mg/kg&400mg/kgreduced the TC,TG,LDL&VLDL and increased HDL levels when compared to the triton group. Administration of methanolic extract of AVB at a dose of 400mg/kg and standard drug 1.14mg/kg. The cholesterol level is 102.83mg/dl. Triglycerides 104.16mg/dl and HDL cholesterol is 42mg/dl as compare to standard drug Atorvastatin were decrease of cholesterol level is by 101mg/dl. Triglycerides level is by 70.16, VLDL level is 14mg/dl and HDL level is in 38.66mg/dl. A significant increase in HDL-C which was related to a significant reduction of atherogenic index.

In the present study atorvastatin was used as a positive control because it is a potent hypolipidemic drug with known mechanism of action. Increased plasma levels of LDL& VLDL cholesterol is often found in hypertension and diabetes mellitus and is a risk factor for cardiovascular diseases. In this study we observed a significantly lower plasma LDL and VLDL cholesterol levels in the treated animals. In this study the methanolic extract of *Averrhoa bilimbi* increase plasma HDL cholesterol level. Atherogenic index are powerful indicators of the risk of heart disease; methanolic extract of *Averrhoa bilimbi* significantly reduced atherogenic index. The results obtained from the present study indicated that *Averrhoa bilimbi*, has potential antioxidant and hypolipidemic activity and the phytochemical results showed the presence of phenols, tannins, flavanoids and terpenoids were already reported from various other plant sources have all been independently reported to exert hypocholesterolemic effect so our present activity may be due to the synergistic effect of the major phyto-constituents like flavonoids, tannins, terpinoids and phenols that are highly present in the extract. But further studies are required to provide the exact mechanism of action of the reported activities by using purified fraction of the same.

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