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Invivo & Invitro Hypolipidemic Effect of Herbal Extracts on Triton X-100 Induced Hyperlipidemia

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Abstract:
Atherosclerosis is a disease with multiple etiology of which hyperlipidemia and oxidative stress are two major factors, the present study was proposed to evaluate the antihyperlipidemic activity of ethanolic extract of Aconitum heterophyllum & Commiphora mukul, which is tested by in-vivo and in-vitro screening models. In in-vivo model triton & high fat diet induced hyperlipidemia rat model is used. Acute hyperlipidemia is persuaded in rats by giving a single I.P dose of triton X-100 at 100mg/kg b.wt. & chronic hyperlipidemia is persuaded by feeding high a fat diet for about 21 days. Oral dosing of EEAH & EECM in different doses alleviates the blood serum level of different lipoprotein like LDL, VLDL, triglycerides, total cholesterol & HDL compared to the normal control & standard drug atorvastatin (10mg/kg). The in-vitro studies of extracts (EEAH &EECM) are done for HMG CoA reductase activity & IC_{50} is found to be 84.02, 66.39 µg/ml compared to standard atorvastatin 9.3nm. The rat liver was dissected out from one animal of each group for histopathology examination for evaluation of fatty infiltration, congestion of arteries and lipid droplet formation. The liver microscopy of toxic, standard and treatment group is compared and it was found that with the plant extract the cell degeneration is repairing when compared to toxic but is less compared to standard control.

Keywords*: Hyperlipidemia, Aconitum heterophyllum, Commiphora mukul, Triton X-100, High Fat Diet, Atorvastatin.*

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I. Introduction

The biological uses and pharmacological properties of many herbal plants are still unknown. Importance of herbs and its traditional uses are always an issue to resolve in wellness programme of world.Hyperlipidemia is an abnormal disorder i.e. commonly differentiated by increased serum levels of Total Cholesterol, Low-Density & Very Low-Density Lipoprotein Cholesterol (VLDL-C) Triglycerides and Decreased Levels of High-Density Lipoprotein Cholesterol (HDL-C).[1][2]

Hyperlipidemia is the solitary risk factors i.e. contributing to the prevalence & severity of cardiovascular disorder such as atherosclerosis and coronary heart disease. The WHO has reported that High Blood Cholesterol contributes to approx. 56% cases of CVD worldwide and leads to about 4.4 million deaths each year.[3]

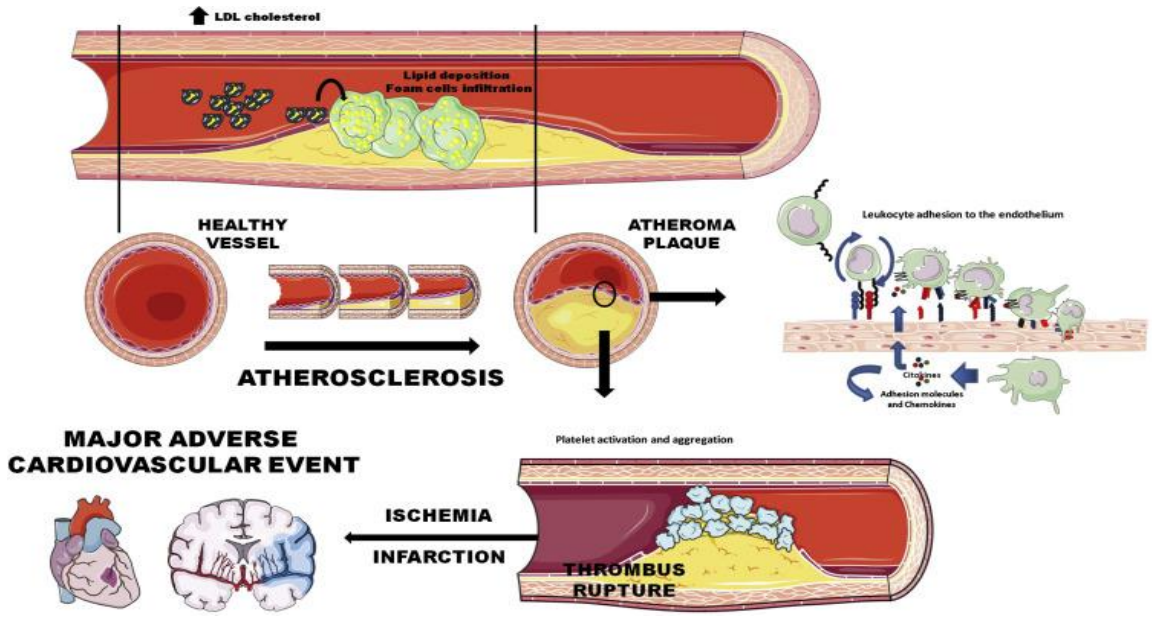


Fig 1: Lipid Metabolism in hypercholesteremia

Current figures suggest that by 2020, India will be at the top among world population in case of CVD. One of the major initiations to this disease is oxidative stress or oxidative damage to the cholesterol component of LDL, known as LDL oxidation, which forms atheromatous plaque leading to CVD. The WHO have found that eighty percentage of people in many developing areas depends on holistic medicines, mostly herbal drugs for their initial health care needs. India is well known for its rich traditional systems of medicines that is Ayurveda, Siddha & Unani. Various synthetic drugs in use such as statin, fibrates & resins, decreases blood cholesterol levels, either by blocking endogenous synthesis or by decreasing cholesterol absorption from the duodenum. Due to their ill effects, people are looking forward for much safer alternatives, & the investigation for new drug molecule that have ability to reduce & regulate serum cholesterol level has gained interest resulting into various research reported on significant activities of natural drugs.[4]

The current study is focused on identifying the individual and combination, antihyperlipidemic effect of two different plant. The plants used here are Aconitum heterophyllum (family: Ranunculaceae) and Commiphora mukul (family: Burseraceae). Here the root part of Aconitum heterophyllum is used and resin of Commiphora mukul is used depending on the chemical constituents presents in them. Basically, for hypolipidemic activity the chemical constituents responsible are flavonoids, resins, tannins and mostly steroids which are found in them based on the literature review. The plants have also been chosen due to their indigenous nature. The above herbal drug extract is given to wistar rats for evaluation of effect of curative plants The In-vivo screening method used for evaluation of hypolipidemic activity isTriton X-100 induced

Commiphora mukul (Gumresin Extract)	**Aconitum heterophyllum (Root Extract)**
 **Family:(Burseraceae)**	 **Family:(Ranunculaceae)**
Phytoconstituents: guggulsterone, eugenol, ellagic acid, quercetin, stigmasterol, and campesterol. Other constituents: E and Z guggulsterone, dihydro guggulsterone, flavonoids, lignans, sugars, amino acids, diterpenoids and other steroids etc.	**Phytoconstituents**: Diterpene alkaloids, atisine, atidine,heterophylline, heterophyllidine, hetratisine, hetisine, hetidine, hetisinone, isoatisine, F-dihydro atisine. Other constituents: Tannic acid, starch, fat, a mixture of fatty acid and their glycerides, carbohydrates
Biologicaluses: anti-inflammatory, anti-spasmodic, anti-supportive, nervine tonic, thyroid stimulant,cardiovascular disorder, anthelmintic, depurative, pyorrhoea, skin disorder, hypertension, antiseptic liver tonic and demulcent etc.	**Biological uses**: hepatoprotective, antipyretic and analgesic, antioxidant, alexipharmic, anodyne, anti-atrabilious, anti-flatulent, anti-periodic, anti-phlegmatic and carminative properties.

hyperlipidemia. The In-vitro screening method for hypolipidemic activity evaluation is HMG CoA reductase activity.Various examination is done to identify the hypolipidemic activity such as biochemical estimation of blood samples for lipid profile testand microscopic examination of liver for conversion in the liver cells.

MATERIALS: The dried root of *Aconitum heterophyllum & Commiphora mukul* were assorted and wastaxonomically recognised and identified by Dr. Madhava Chetty, Assistantprofessor of botany, (Dept. of pharmacognosy), Sri Venkateshwara College, Tirupati.
Standard drug: Atorvastatin is used as a reference standard drug. It is used as a lipid lowering drugs and it also reduces oxidative stress.

Other chemicals:

i. Ethanol: Used as a solvent for extraction.
ii. Triton X-100: To induce acute hyperlipidemia.
iii. Normal saline: For reconstitution of plant E.D.
iv. Tween 80: For reconstitution of gum resin E.D.
v. Ethanolic extract of Aconitum heterophyllum.
vi. Ethanolic extract of Commiphora mukul.

II. Methodology

1. Extraction Method

For extraction of plant maceration technique is used. Requirements of this are as follows:
Solvent: Ethanol (99.9 %) is used as solvent.
Apparatus: Maceration Assembly.

1.1 aceration Process:

In this process, the dried & finely powdered plant is used, the extraction is done in an extraction jar in which ethanol (99% v/v) is added in a ratio of 1:2 to the finely powdered plant & kept in contact for about 7 days and it is reconstituted or stirred occasionally. At the end of maceration process, there is separation of solid-liquid bi-phase. Then it if filtered out and the filtrate is collected and made to evaporate the ethanol and get a concentrate extract. Then this extract will be used for animal testing at a portion determined by acute toxicity testing. It is reconstituted with normal saline at the time of dosing to animals.

Process of maceration

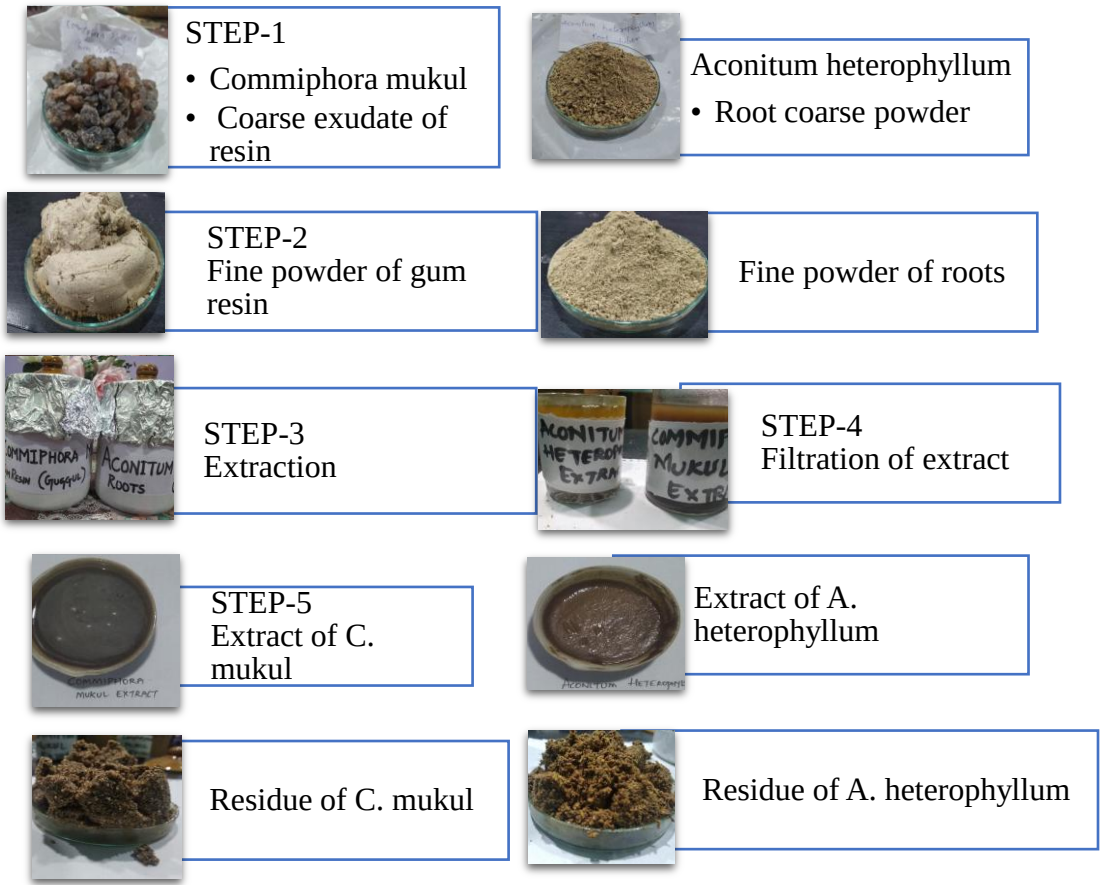


Fig 3: Process of maceration

PHYTOCHEMICAL TESTING: It is done for plant extract in order to evaluate the chemical constituents present in the extract such as flavonoids, carbohydrates, glycosides, terpenoids, steroids, polyphenol, resins, tannins, saponins, flavonoids etc., by standard methods present in practical pharmacognosy by K.R. Khandelwal and C.K. Kokate. All the chemical and reagents used are of analytical grade.

Experimental Animals

The experimental study is performed on male wistar rats of 180-200g of body weight are procured from Jeeva life sciences, Uppal, Hyderabad. All the animals are acclimatized for about 5 days prior to use in animal house. They are kept in polypropylene cages with 12hr light: 12 hrs dark cycle at a temp of 25º C and are fed with standard pellet diet and water adequately. Care and handling of the animals are done according to the CPCSEA guidelines. Permission and approval of animal studies was obtained from IAEC, after submission of form B and protocol no. (IAEC-012/SES/2019/007).

Fig 4: Experimental animals (wistar rats)

Acute Toxicity Studies[5]

Selection of Animals and Housing

GROUPS	Age of animals (weeks)	Weight of the animals (gm.)	Treatment	Dose
GROUP-I	12	150-200	(Normal control) normal saline	1ml of 0.9% w/v p.o.
GROUP-II	12	150-200	(Toxic control) Triton X100	400 mg/kg i.p.
GROUP-III	12	150-200	(Standard control) Atorvastatin	10 mg/kg p.o.
GROUP-IV	12	150-200	Toxic control + EEAH	200 mg/kg p.o.
GROUP-V	12	150-200	Toxic control + EEAH	400 mg/kg p.o.
GROUP-VI	12	150-200	Toxic control + EECM	200 mg/kg p.o.
GROUP-VII	12	150-200	Toxic control + EECM	400 mg/kg p.o.
GROUP-VIII	12	150-200	Toxic control + EEAH + EECM	200 mg/kg p.o.
GROUP-IX	12	150-200	Toxic control + EEAH + EECM	400 mg/kg p.o.

Generally, acute toxicity studies are performed on female rats because of their higher sensitivity than male rats. The animals are housed five days prior to toxicity testing for acclimatization and temp is maintained 25ºC and 12:12 hrs light and dark cycle, provided with sufficient food and water.

Acute Oral Toxicity Study

To find the effective dose or LD_{50} toxicity studies was done as per OECD guidelines No 425 of CPCSEA for acute oral toxicity. No mortality and no sign of toxicity were identified after the dosing of animal with 200 mg/kg; 400mg/kg; 800mg/kg; 1600mg/kg and 2000mg/kg of extract by oral route. No death observes up to dose of 2000mg/kg. Therefore, the LD_{50} of ethanolic roots extract was found to beyond 2000 mg/kg. In the current study the $1/10^{th}$ of LD_{50} i.e., 200mg/kg is taken as lower dose and double of that 400mg/kg is taken as higher dose for both the extracts.

Experimental Design

Table 1: Experimental design

Male wistar rats are divided into nine groups of six (6) animals in each group and are given respective dose as mentioned below:

Screening Models for Anti-Hyperlipidemic Activity

Triton Induced Hyperlipidemic Model(Invivo Study)[6]

It is an acute model for screening of lipid lowering agents. It is a 7 days study. The rats are fasted overnight. Here, all the group except normal control group was given 10% solution of triton X-100 at a portion of 400mg/kg by i.p. route. II group is administered with vehicle & III group was given atorvastatin 10 mg/kg through p.o. Whereas group II, V, VI, VIII, IX were given different doses of extract for 7days as per protocol. The blood was withdrawn on 8^{th} day for lipid profile.

INVITRO ANTI-HYPERLIPIDEMIC MODEL

HMG CoA Reductase Activity [7]

HMG CoA conversion to mevalonate is rate limiting step in biosynthesis of cholesterol from acetyl CoA. The HMG CoA reductase enzyme aid in conversion of HMG CoA to mevalonate by reduction of the former to latter. Due to inhibition of the enzyme, cholesterol biosynthesis is stopped. Therefore, invitro screening models are developed in order to evaluate the activity of new drug on HMG CoA enzyme activity. In present lipid lowering drug, statins have the ability to block HMG CoA reductase activity and due to this the LDL receptor get expressed an there is a decrease in plasma level of cholesterol. This reaction is depending on NADPH reaction. The assay kits for HMG CoA reductase activity are commercially available. The kit consists of assay buffer, enzyme, NADPH, and standard blocking agent such as atorvastatin. This kit is used in measurement of NADPH utilised, that is measured by alleviation of absorbance of radiation at 340nm. By this the activity of the experimental drug as an enzyme inhibitor and as well as the activity of purified enzyme i.e.,

HMG CoA (3- Hydroxy, 3-methyl glut acyl Co-enzyme A) is possible. The % inhibition is measured using the below equation:

$$\% \text{ inhibition} = \frac{\text{absorbance of enzyme} \times \text{absorbance of enzyme in presence of inhibitor} \times 100}{\text{absorbance of enzyme}}$$

Blood Collection: After 7days of doing the blood is collected, for this the animals are fasted overnight and on 8 day after dosing the blood is collected by retro orbital puncture under ether anaesthesia using capillary tube in coagulant ampule for estimation of serum levels of different parameters such as lipid profile.[8]

Fig 8: collection of blood samples by retro-orbital puncture.

Biochemical estimations: The samples were sent to Vijaya Diagnostic Centre, Boduppal, Hyderabad for various laboratory for estimation of biochemical parameters in the serum samples such as LDL, VLDL, HDL, total cholesterol, cholesterol: HDL ratio.

Histopathological studies

One rat from each group was anesthetized using ether anaesthesia and the liver is dissected out and this was kept in 10% of neutral buffered formalin solution (10ml of formalin in 100ml of normal saline) stored for further laboratory testing. In the laboratory tissue are cleansed with xylene, impregnated and embedded in paraffin wax. Several sections of 2-3cm cut was made using microtome and stained with eosin dye. The conversion in the liver due to obesity are analysed using 40X lens in compound microscope.

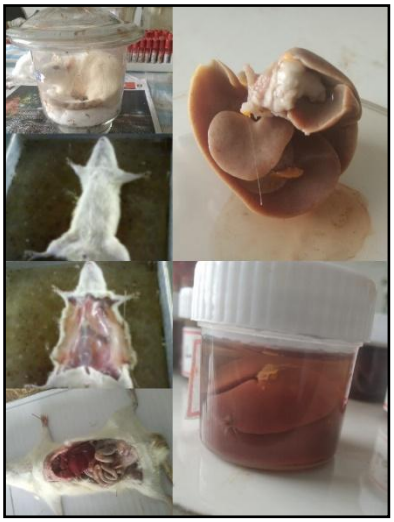


Fig 9: Dissection of animal for histopathological studies

Statistical Analysis: The results were depicted as mean of ± S.E.M and P values are $P<0.01$**, $P<0.001$*** were considered significant between standard and various treatment group. In the enzymatic invitro assay the graphs or curves are drawn using GraphPad Prism 8 software.

III. Results

Phytochemical Evaluation of Eth. extract of *Aconitum heterophyllum*

Phytochemical tests	Results for Aconitum heterophyllum	Results for *Commiphora mukul*
Alkaloids	+++	+
Flavonoids	+++	+++
Glycosides	++	++
Terpenoids	+	+++
Carbohydrates	+	++
Quinones	++	++
Proteins and amino acids	++	++
Saponins	++	+
Resins and its compounds	_	+++

Table 2: Phytochemical analysis of Eth. extract of*Aconitum heterophyllum&Commiphora mukul*

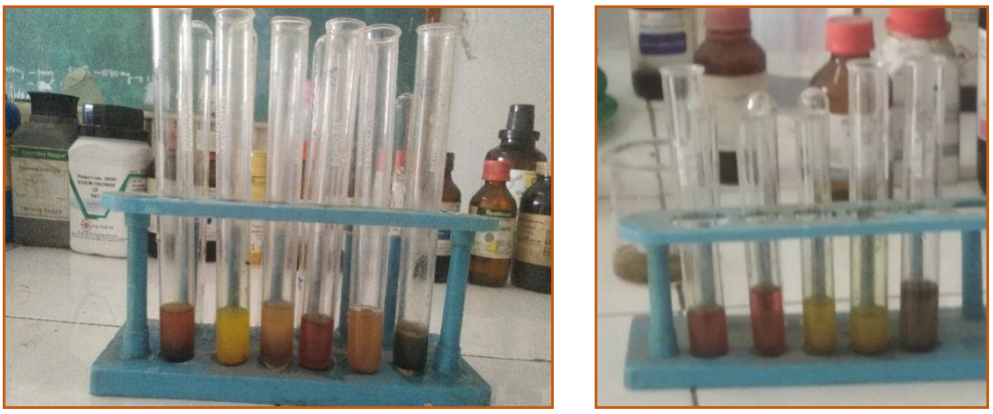


Fig 10: Phytochemical tests of Aconitum heterophyllum &Commiphora mukul

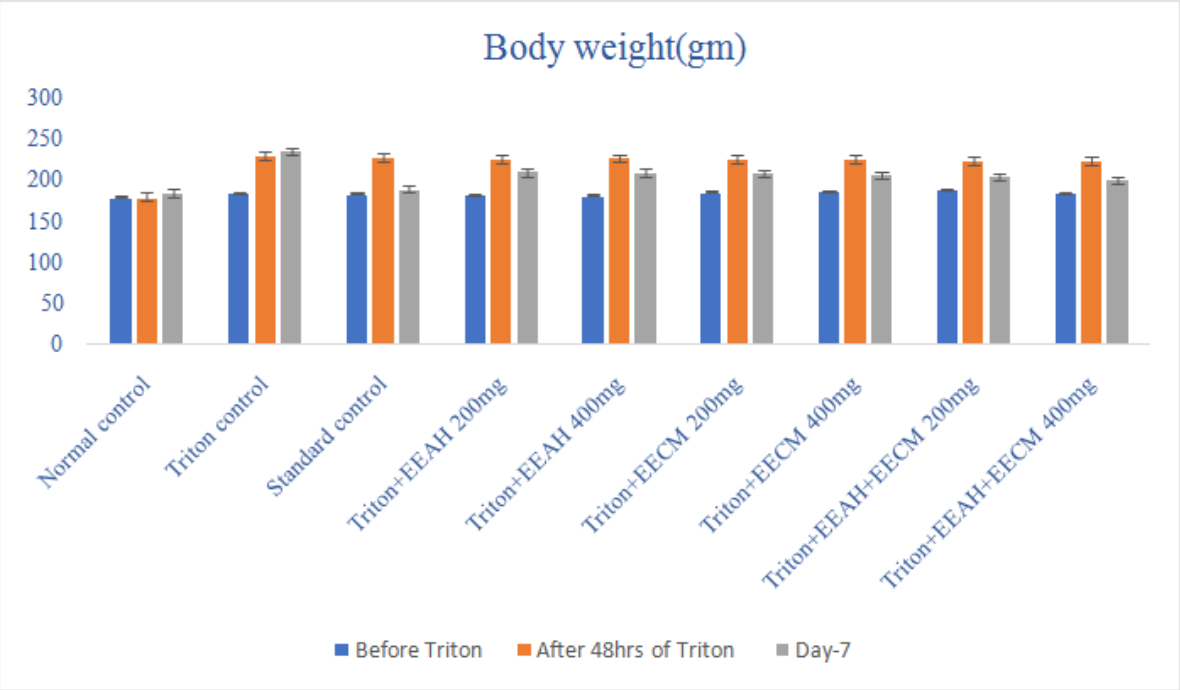
ANTHROPOMETRIC PARAMETERS

Effect of Triton X-100 on Body Weight of Animal.

S.no	Treatment Group	Before Triton	After 48hrs of Triton	Day-7
1	Normal control	180	180	185
2	Triton control	185	230	235
3	Standard control	184	228	190***
4	Triton+EEAH 200mg	183	225	210*
5	Triton+EEAH 400mg	182	227	209*
6	Triton+EECM 200mg	186	226	208**
7	Triton+EECM 400mg	187	225	206**
8	Triton+EEAH+EECM 200mg	189	224	204***
9	Triton+EEAH+EECM 400mg	185	223	200***

Table 3: Effect of EEAH and EECM on body weight of Triton induced hyperlipidemic rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group* = P<0.05, ** = P<0.001, *** = P<0.0001 are considered significant when compared with hyperlipidemic control group. Among all the treatment groups, 3, 8, 9 are found to be more significant.

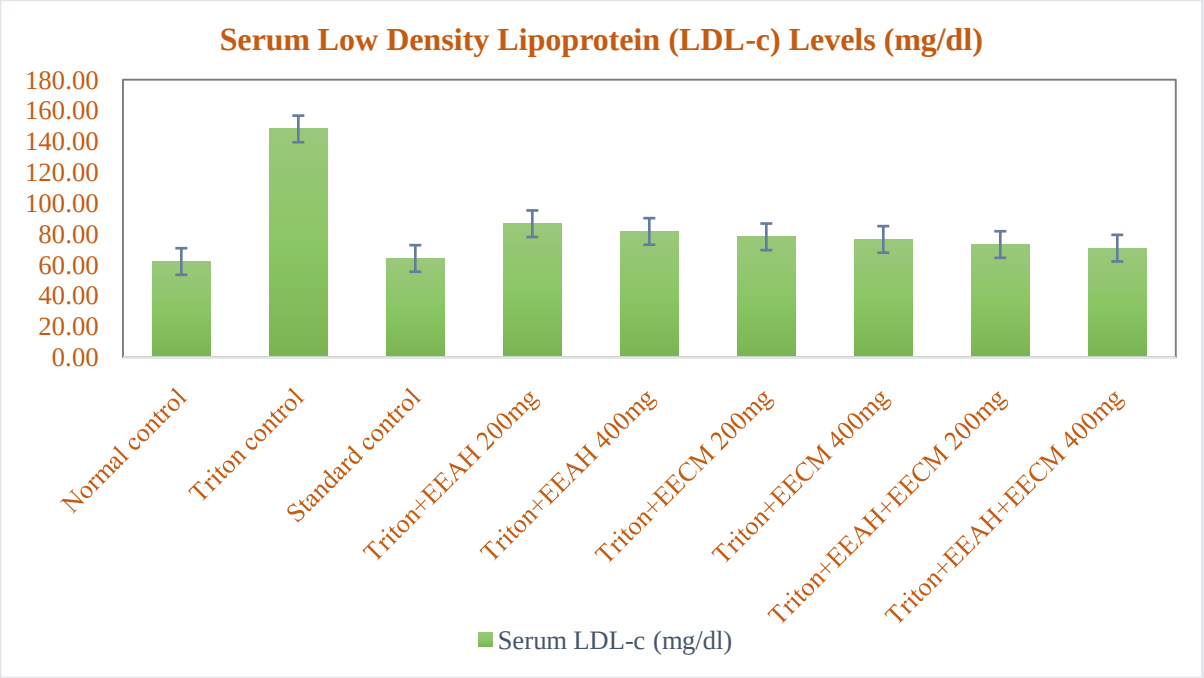


Graph 1: Effect of EEAH and EECM on body weight of Triton induced hyperlipidemic rat.

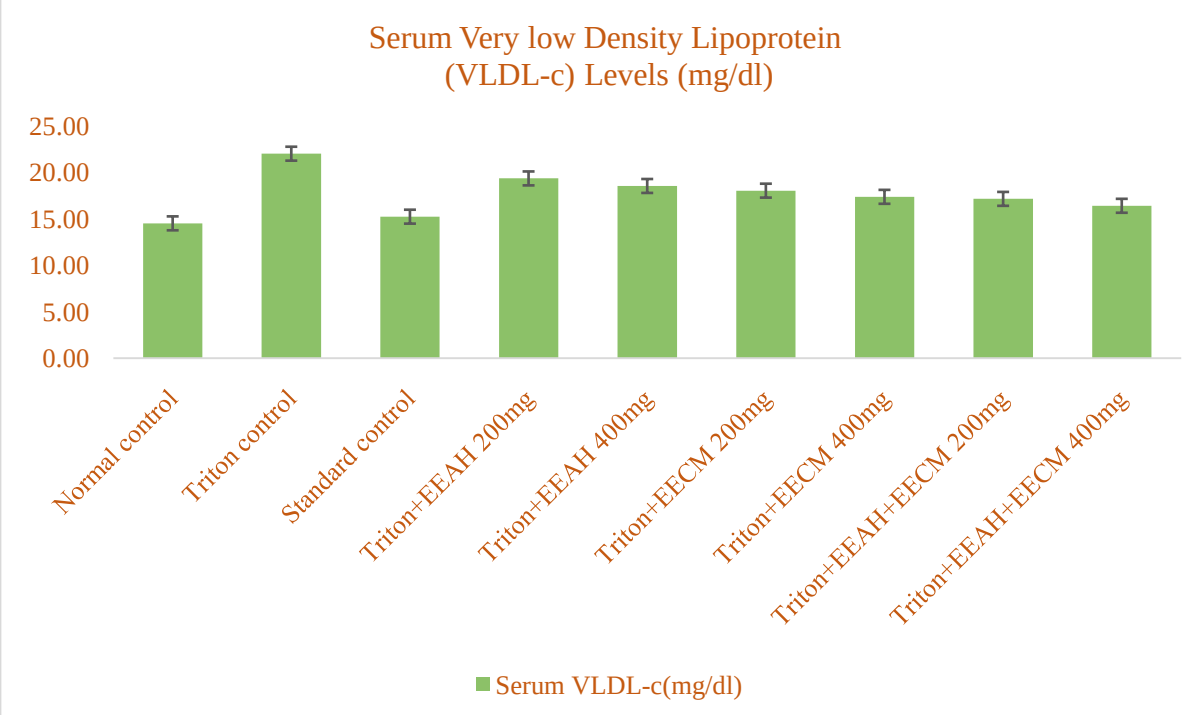
Effect of EEAH & EECM on Lipid profile serum in Triton-X 100 Induced Hyperlipidemic Rats

S.no	Treatment Group	Serum LDL (mg/dl)	Serum VLDL (mg/dl)	Serum HDL (mg/dl)
1	Normal control	70.66 ± 0.51	14.49 ± 0.08	48.67 ± 0.88
2	Triton control	148 ± 0.57	21.97 ± 0.11	28.83 ± 0.87
3	Standard control	64 ± 0.57***	15.20 ± 0.18***	40.33 ± 0.66***
4	Triton+EEAH 200mg	86.5 ± 0.76*	19.31 ± 0.27*	31.5 ± 0.76*
5	Triton+EEAH 400mg	81.5 ± 0.76*	18.50 ± 0.11*	33.83 ± 0.79*
6	Triton+EECM 200mg	78 ± 0.93**	18 ± 0.21**	35.5 ± 0.56**
7	Triton+EECM 400mg	76.33 ± 0.61**	17.33 ± 0.13**	36.33 ± 0.55**
8	Triton+EEAH+EECM 200mg	73 ± 0.96***	17.12 ± 0.081***	37 ± 1.03***
9	Triton+EEAH+EECM 400mg	70.66 ± 0.88***	16.37 ± 0.08***	38 ± 0.57***

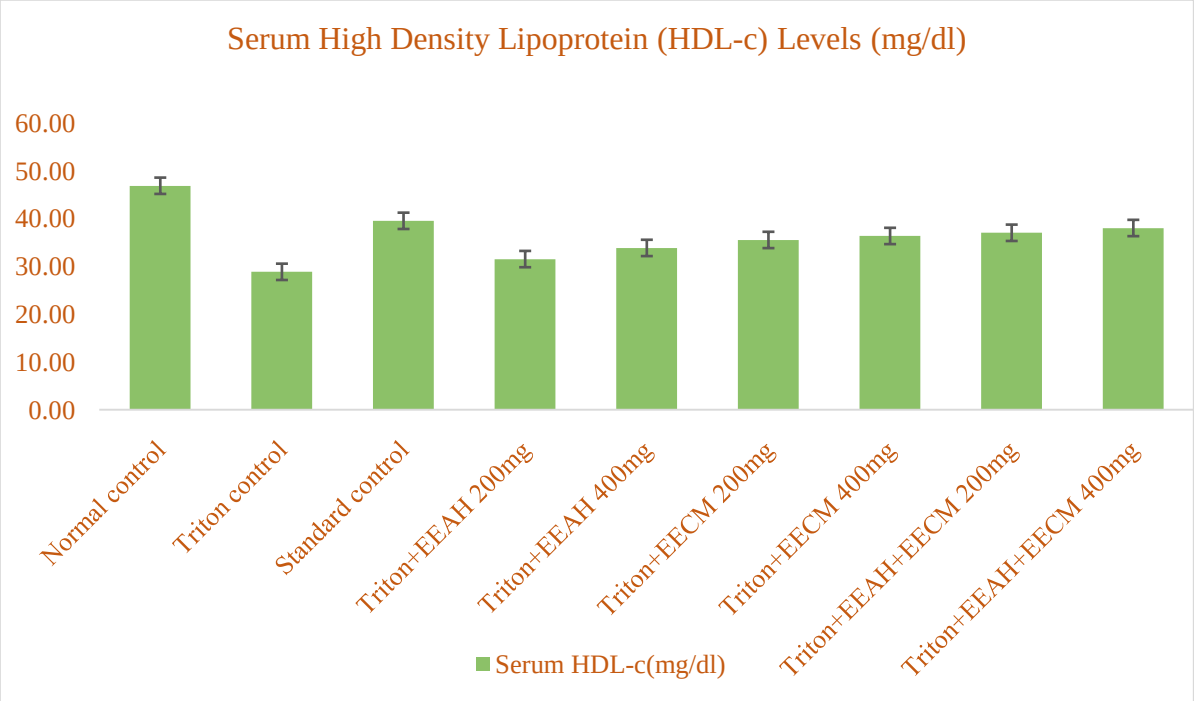
Table 4: Effect of EEAH & EECM on Lipid profile serum of Triton induced Hyperlipidemic rats.
Data was demonstrated as Mean ± S.E.M, n=6 rats in each group
* = P<0.05, ** = P<0.001, *** = P<0.0001 are considered significant when compared with hyperlipidemic control group. Among all the treatment groups, 3, 8, 9 are found to be more significant.



Graph 2: Effect of EEAH and EECM on Serum LDL-c (mg/dl) of Triton induced hyperlipidemic rat.



Graph3: Effect of EEAH and EECM on Serum VLDL-c (mg/dl) of Triton induced hyperlipidemic rat.



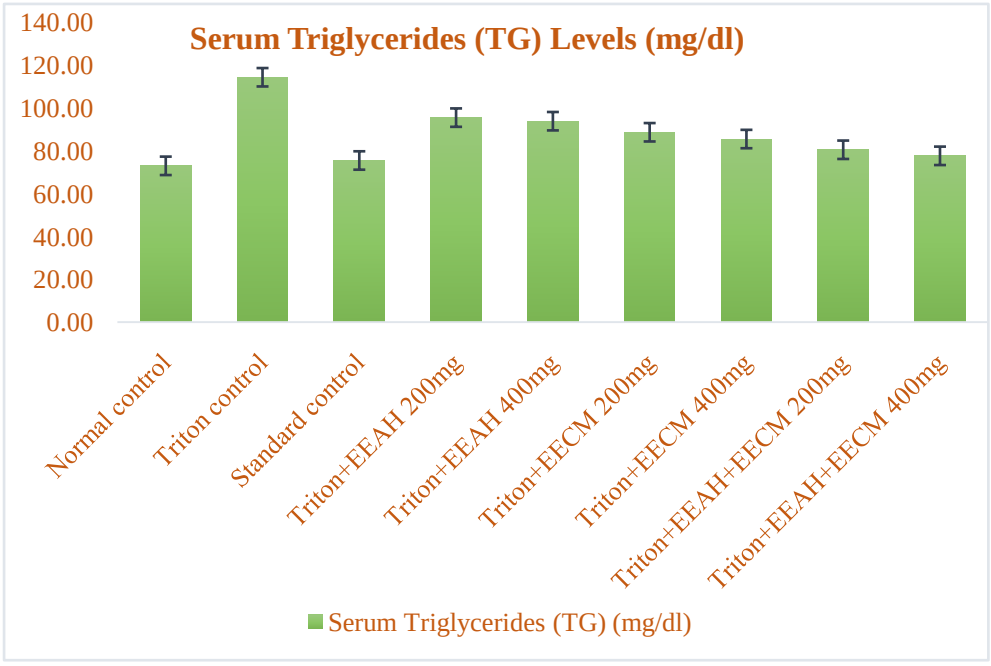
Graph 4: Effect of EEAH and EECM on Serum HDL-c (mg/dl) of Triton induced hyperlipidemic rat.

Effect of EEAH & EECM on Serum TG, TC, CH:HDL ratio of Triton-X100 induced hyperlipidemic rats.

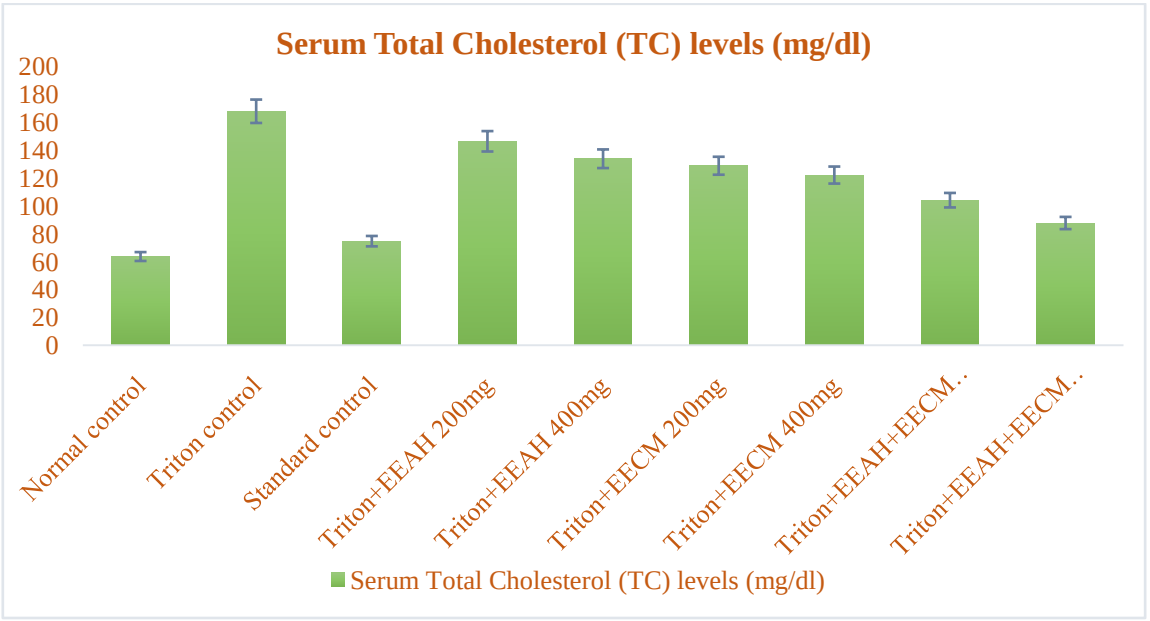
S.no	Treatment Group	Serum TG (mg/dl)	Serum TC (mg/dl)	Serum CH: HDL (mg/dl)
1	Normal control	73 ± 0.57	63.5 ± 0.76	3.41 ± 0.06
2	Triton control	114.33 ± 1.05	167.5 ± 1.11	6.76 ± 0.04
3	Standard control	75.5 ± 0.42***	74.5 ± 1.60***	3.78 ± 0.07***
4	Triton+EEAH 200mg	95.5 ± 0.76*	146 ± 0.57*	6.25 ± 0.04*
5	Triton+EEAH 400mg	93.83 ± 0.60*	133.5 ± 0.76*	5.56 ± 0.11*
6	Triton+EECM 200mg	88.66 ± 1.05**	128.5 ± 0.76**	5.35 ± 0.07**
7	Triton+EECM 400mg	85.5 ± 0.84**	121.83 ± 0.94**	4.8 ± 0.05**
8	Triton+EEAH+EECM 200mg	80.5 ± 0.76***	103.83 ± 1.64***	4.35 ± 0.07***
9	Triton+EEAH+EECM 400mg	77.66 ± 0.80***	87.5 ± 1.54***	4 ± 0.06***

Table 4: Effect of EEAH & EECM on Serum TG, TC, CH:HDL ratio of Triton-X100 induced hyperlipidemic rats.Data was demonstrated as Mean ± S.E.M, n=6 rats in each group

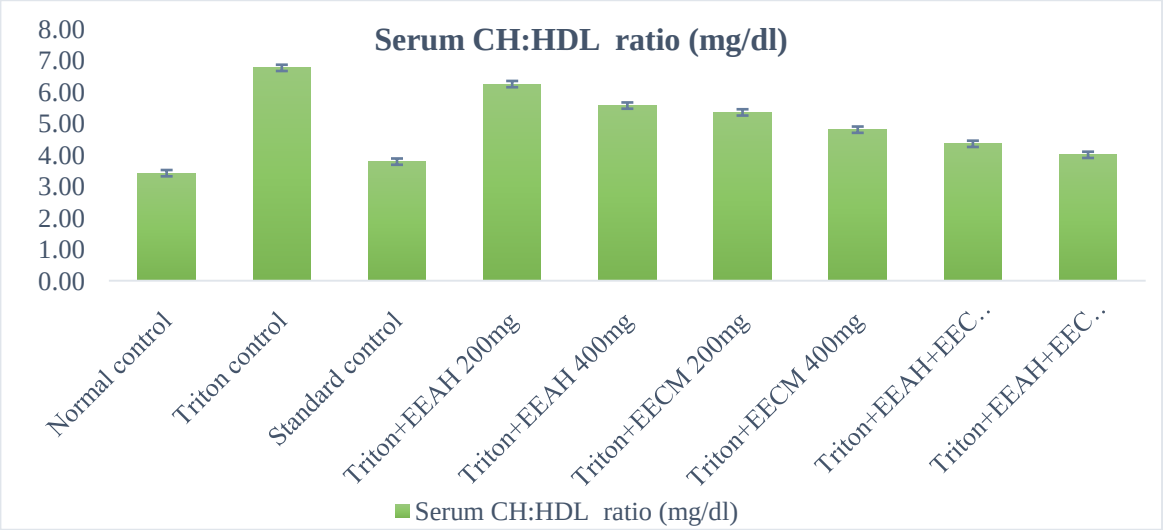
* = P<0.05, ** = P<0.001, *** = P<0.0001 are considered significant when compared with hyperlipidemic control group. Among all the treatment groups, 3, 8, 9 are found to be more significant.



Graph 5: Effect of EEAH and EECM on Serum Triglycerides (TG) levels (mg/dl) of Triton induced hyperlipidemic rat.



Graph 6: Effect of EEAH and EECM on Serum Total Cholesterol (TC) levels (mg/dl) of HFD induced hyperlipidemic rat.

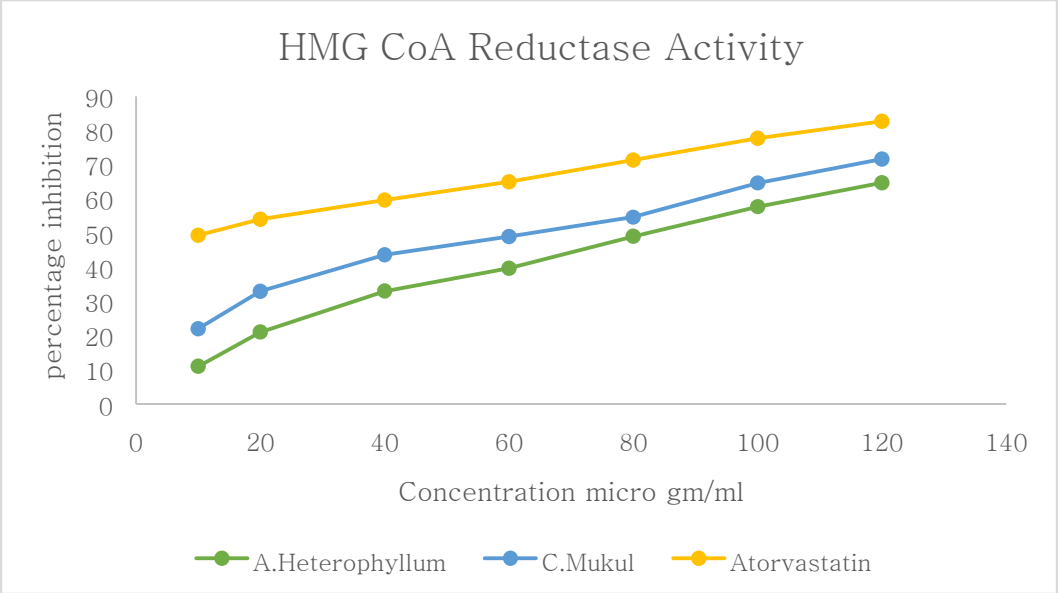


Graph 7: Effect of EEAH and EECM on Serum CH: HDL ratio levels (mg/dl) of Triton induced hyperlipidemic

In-vitro HMG CoA Reductase Activity

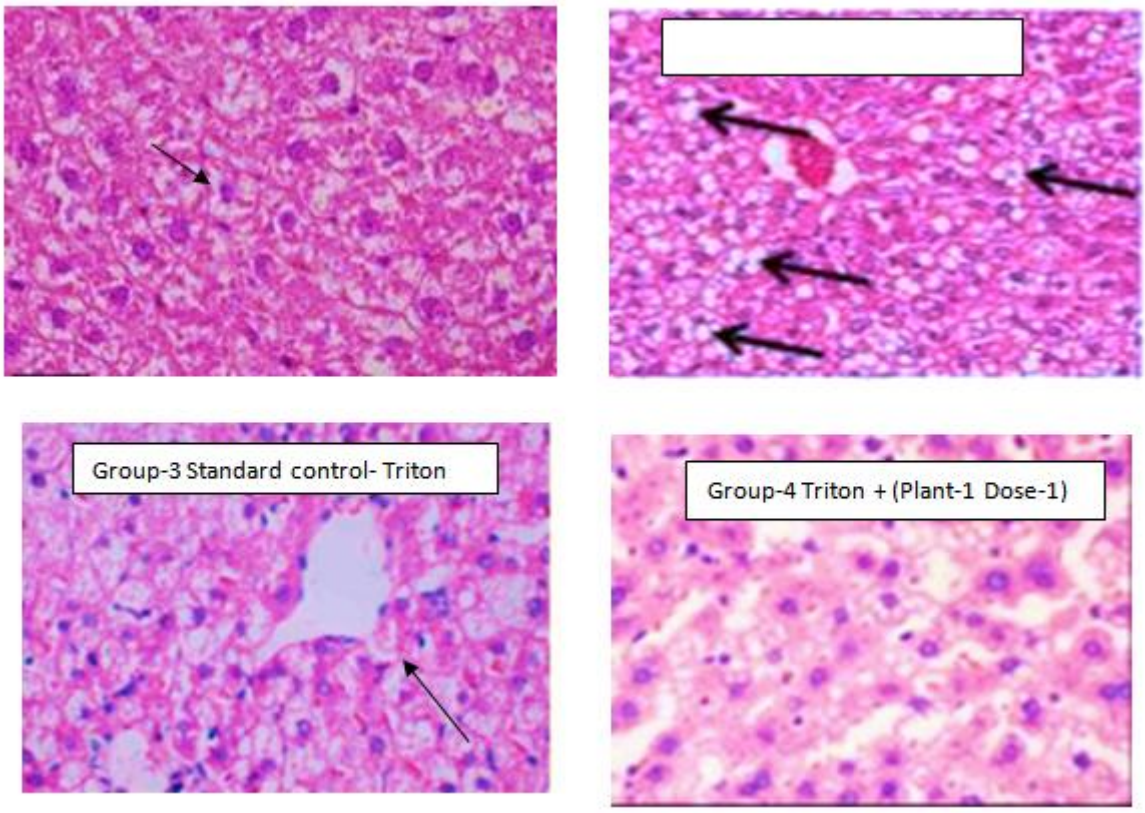
S.no.	Concentration (µg/ml)	% inhibition (mean ± S.E.M)	IC_{50} (µg/ml)
Standard Drug (Atorvastatin)			
1	120	82.66 ± 1.20	**9.3**
2	100	77.66 ± 1.45	
3	80	71.33 ± 1.45	
4	60	65 ± 1.15	
5	40	59.66 ± 0.88	
6	20	54 ± 1.15	
7	10	49.33 ± 0.33	
Plant-1 (A. heterophyllum)			
1	120	64.66 ± 1.76	**84.02**
2	100	57.66 ± 1.45	
3	80	49 ± 2.08	
4	60	39.66 ± 1.20	
5	40	33 ± 1.73	
6	20	21 ± 2.08	
7	10	11 ± 0.57	
Plant-2 (C. mukul)			
1	120	52 0.57	**66.39**
2	100	64.66 ± 2.02	
3	80	54.66 ± 1.76	
4	60	49 ± 0.57	
5	40	43.66 ± 0.88	
6	20	33 ± 1.15	
7	10	22 ± 1.52	

Table 5: HMG CoA reductase activity of standard drug and plant extracts



Graph 8: HMG CoA Reductase activity, a plot between concentration and percentage inhibition

MICROSCOPIC RESULTS OF LIVER HISTOLOGY



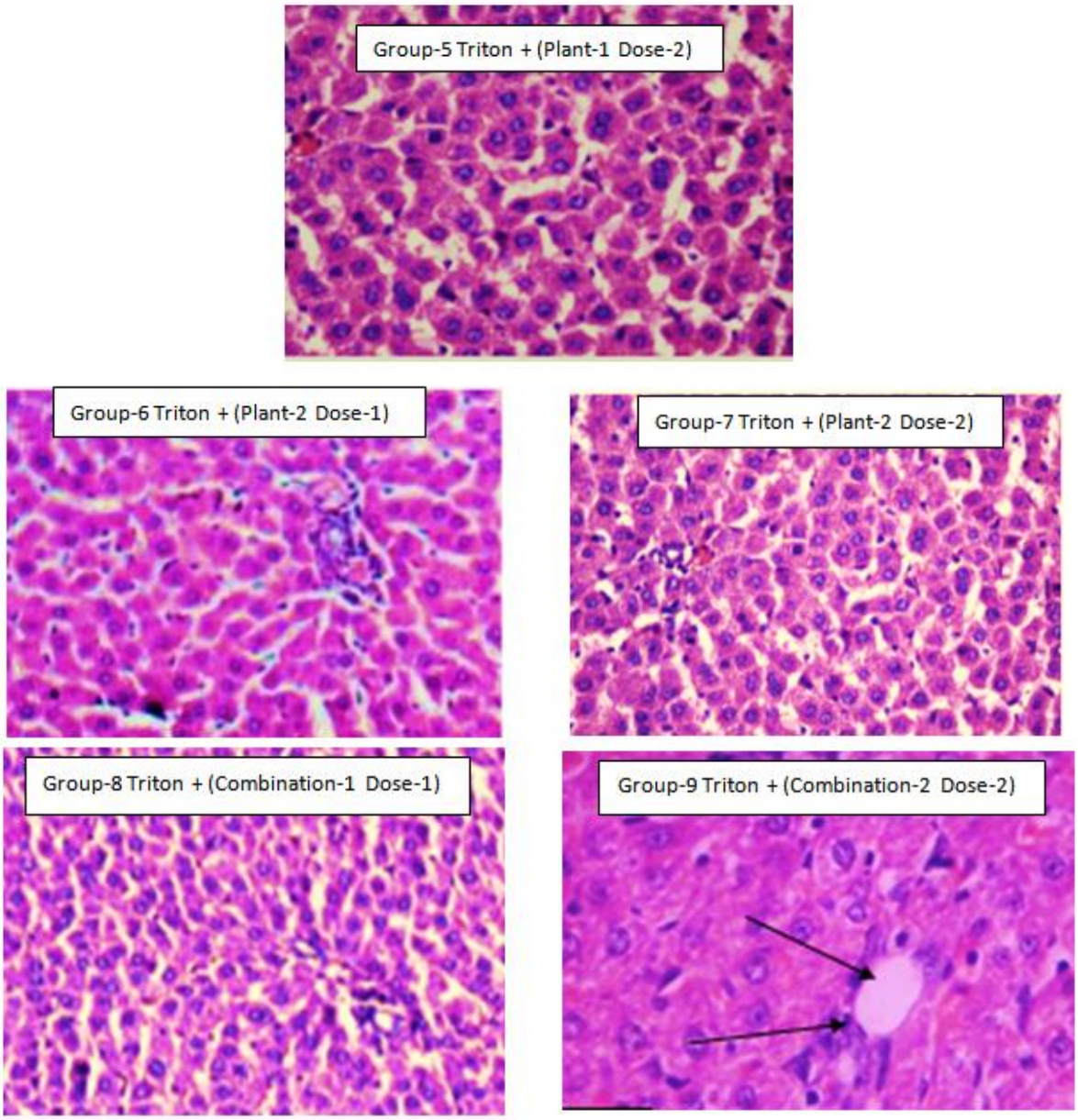


Fig 11: Microscopic examination of rat liver cells

1. **NORMAL CONTROL:** Microscopic liver tissue section of normal group rat shows normal structure of liver i.e,. hepatic lobule with intra lobular vein .It also contain bile duct, and hepatic artery which are present in between the lobules. Blood sinusoids are also present with Kuffer cells (stained with H & E × 400 magnification)
2. **TOXIC CONTROL:** The rat liver from toxic group showed different histologic changes & had unusual hepatic architecture, identified by the dilation of the central vein, portal vein. Blood sinusoids also got dilated along with this, there is formation of lipid globules which can be found beside the central vein & in between the portal vein & hepatocytes. Vacuoles of hepatic cells can also be seen (H & E × 400).
3. **STANDARD CONTROL:** liver of Group-3 treated with atorvastatin (standard drug) showed lesser fatty infiltration Rat and granular degeneration and is similar to normal group rat liver (H & E × 400).
4. **EEAH-DOSE-1**: Rat liver of Group-4 treated with low dose of EEAH showed cellular degeneration, fatty infiltration and droplets of lipids are found. Infiltration are seen in between and around the central vein, portal vein. Noticeable number of vacuoles are found. Dilated and congested portal vein, blood sinusoids are found in the liver (H & E × 400)
5. **EEAH-DOSE-2:** Rat liver of Group-5 treated with high dose of EEAH showed mild fatty infiltration and lesser dilated and congested portal vein and blood sinusoids are dilated and congested. Branched central

vein are found and steatosis can be seen in case of triton induced hyperlipidemia. Droplets of lipids are non-significant (H & E × 400).

6. **EECM-DOSE-1:** Rat liver of Group-6 treated with low dose of EECM showed cellular aggression. Dilation and congestion of portal veins, dilation and congestion of the hepatic vein. Infiltration between the hepatocytes and it also wide spread up to the bile ductulus, dark stained nuclei can also be seen (H & E × 400).
7. **EECM-DOSE-2:** Rat liver of Group-7 treated with high dose of EECM showed minimal dilation and congestion of blood sinusoids some hepatocytes contains vacuole. Fatty filtration is insignificant in hepatocytes. Lesser steatosis around components of portal vein. Most of the hepatic cells are found to be normal (H & E × 400).
8. **EEAH + EECM-DOSE-1**: Rat liver of Group-8 treated with low combination dose of EEAH & EECM shows mild liver cell steatosis and normal hepatic duct with apparently mild dilation and congestion of portal vein, central vein, blood sinusoids, some ballooned vacuoles can be seen (H & E × 400).
9. **EEAH + EECM-DOSE-2**: Rat liver of Group-9 treated with high combination dose of EEAH & EECM shows negligible fatty infiltration & normal hepatocytes with negligible dilation and congestion of central vein and portal veins. Blood sinusoids are also normal.

Proliferation of some hepatocytes can be seen (arrow), dark stained nuclei can beseen.

IV. Discussion

The present study was proposed in order to identify the antihyperlipidemic and antioxidant activity of Commiphora mukul & Aconitum heterophyllum. The phytochemical tests have been performed and it was found to consist of chemical constituents such as carbohydrates, flavonoids, saponins, terpenoids, resinoids, triterpenoids, steroids, tannins etc. Based on the previous studies the active chemical constituents that are responsible for its lipid lowering activity are flavonoids and steroids which decreases the LDL levels and it also contains quercetin which has an antioxidant property. By the HPLC and GC-MS analysis studies other specific chemical constituents are confirmed. The In-vivo & In-vitro screening of the plant is done using the ethanolic extract of root and resin of the respective plants which is done by Maceration technique. For lipid lowering activity TritonX-100 is used as inducing agent and respective screening methods are followed for anti-hyperlipidemic activity. Dosage selection is done after performing toxicity studies following the OECD guidelines 425 for oral toxicity.

Triton induced hyperlipidemia model is an acute model for hyperlipidemia, triton acts as surfactant & decreases the activity of LPL to block the uptake of LPP from blood circulation by extrahepatic tissues, which causes an increase in blood lipid conc. In this study the observations are similar to those of earlier findings, Ansarullah, *et al*:[9] Triton elevates total cholesterol, triglycerides, plasma non-HDL and alleviates the HDL levels in the blood. Satyavati [10] stated the outcome of guggul in lipoprotein levels in obesity in rabbits. Following her research, an expanded research in done in order to confirm the lipid lowering activity of gum guggul and it has been identified that some compounds like E and Z isomers of guggulsterone are the active lipid lowering agents. Several mechanisms have been proposed to find the mechanism of action of plant. Research for the efficacy of guggul has given contrast results and the study till now showed that it may be effective for lowering TC and TG in people with non-western diet.

EFFECT ON PLASMA LIPID PROFILE IN TRITON RAT MODELS

In the present study, the Triton induced hyperlipidemic rat model when administered with the extract of EEAH and EECM at different dosage, they are found to decrease the elevated levels of VLDL at a percentile of 59, 66, 78, 81 in different treatment group, when compared with the rapid decrease in std group at 84 %. The LDL level was found to decrease in treatment groups at rate of 59, 68, 75, 79 percent when compared to significant decrease in standard group 86%. The HDL levels are increased in treatment group at a percentage of 68, 76, 82, 85 in contrast with the standard group 88%. The Triglyceride levels are found to decrease at 62, 66, 71, 76 in contrast to 83% decrease in standard group animals. Total cholesterol (TC) level is decreased at a rate of 52, 57, 63, 72 in contrast to standard group 78% decrease.

INVITRO ANTIHYPERLIPIDEMIC ACTIVITY

The invitro study was also carried out for antihyperlipidemic & antioxidant activity in order to find the IC50 value of both the extracts. For hyperlipidemia HMG CoA reductase activity is done, because it is rate determining enzyme in cholesterol synthesis inhibition of this will have direct effect on cholesterol synthesis and lipoprotein metabolism. The invitro testing is done following the standard methodology and IC50 of standard, plant-1 and plant-2 are found to be 9.3, 84.02, 66.39 micro gm/mlrespectively. By this it can be established that plant 2 is having better inhibitory activity on HMG CoA reductase enzyme than plant-1 but is lesser in activity than the standard drug.

V. Conclusion

The present study was proposed to evaluate the anti-hyperlipidemic and antioxidant activity of *Commiphora mukul* and *Aconitum heterophyllum* ethanolic extract. In In-vivo models i.e., triton induced hyperlipidemic model, hyperlipidemia is successfully induced by triton X-100 which is examined by increase in the body weight, BMI and serum levels of lipoproteins, triglycerides, total cholesterol which is treated by the different concentrations of extracts that is in single dose of 200, 400mg/kg and in combination dose of 100 & 200mg each. After dosing for 7 days the serum evaluation is done and it is found that there is decrease in the levels of LDL, VLDL, triglycerides TC and increase in HDL levels. which confirms that the plant extract is effective against treating hyperlipidemia. Based on these beneficial effects of EECM and EEAH are appreciable in preventing lifestyle borne diseases of cardiovascular system. The phytochemical analysis shows presence of terpenoids, steroids, flavonoids which are said to have lipid lowering, based on previous studies it was found that guggulsterone is the active constituent which is responsible for its lipid lowering activity and other constituents are also present such as eugenol, quercetin which are antihyperlipidemic in nature. EECM decrease the LDL effectively but it has less effect in increasing the HDL levels of blood, but in combination with EEAH it effectively increases the HDL-C levels of blood serum. In the invitro analysis the IC50 of each extract is evaluated and EECM found to more effective than EEAH as it has lesser IC50 value than EEAH. After the completion of the dosing the animals are sacrificed and liver histological examination done and the results is interpreted that fatty infiltration can be seen in toxic and other group but when compared with the toxic group the histology of the standard and combination are more significant. In EECM histology is better than EEAH the cells showed lesser fatty infiltration. The results are promising and encouraging for further study on the combinatorial study of these extract to find the mechanism of action and identify the active components involved. As the herbal drug are needed to be administer in more dose and for prolong period of time, so studies need to be carried out to fill the side effects if any on prolong use. Although it doesn't show any side effect in short period time administration. In the previous studies I was found that guggul shows periodic syndrome on prolong usage that should be analysed in case of combination use.

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