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SCREENING OF ANTI-HYPERLIPIDEMIC ACTIVITY IN THE ETHANOLIC EXTRACT OF MEDICINAL PLANTS- *ACALYPHA INDICA AND CROTON BONPLANDIANUS* BAILL

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ABSTRACT:

Hyperlipidemia is a major cause of heart disease and the atherosclerosis-associated condition such as coronary heart disease ischemic cerebrovascular disease and peripheral vascular disease. The present study was carried out to investigate antihyperlipidemic properties of ethanolic stem extract of *Acalypha indica and Croton bonplandianus* baill against hyperlipidemia induced by high fat diet (HFD) in wistar albino male rats. Wistar albino male rats were divided in 7 groups. **Group I** received normal diet. **Group II-** received high fat diet. **Group III-** received atorvastatin 10mg/kg served as standard drug. **Group IV-** received (200mg/kg, p.o.) ethanolic stem extract of *Acalypha indica* (ESAI). **Group V-** received (400mg/kg, p.o.) ESAI, **Group VI-** received (200mg/kg, p.o.) ethanolic stem extract of *Croton bonplandianus* baill (ESCBB) and **Group VII-** received (400mg/kg, p.o.) ethanolic stems extract of *Croton bonplandianus* baill (ESCBB). At the end of experiment The ESAI and ESCBB treatment and Atorvastatin drug significantly lower the body weight by the reduction in the LDL, VLDL levels and increase HDL levels. Oral administration of ethanolic stems extracts significantly reduced the cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins and significantly increased the HDL-cholesterol level as compared with high cholesterol diet induced hyperlipidemic animals.

KEY WORDS- Atorvastatin, Hyperlipidemia, Triglyceride, Cholesterol, Euphorbiaceae

1. INTRODUCTION

Herbal medicines have been playing a vital role in treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. This type of treatment, also known as traditional treatment, was the main source of medical treatment during this time, Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and diseases[1].

Hyperlipidemia is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides in the blood. The extra amount of lipid circulates in blood attached to the protein and this condition is known as hyperlipoptoteinemia. During the circulation the fatty substances remain dissolved. It is a disorder of lipid metabolism caused by elevation of plasma concentrations of the various lipid and lipoprotein fraction, which are the key risks factors for cardio vascular disease (CVD)[2].It also increases the cholesterol esters, phospholipids or triglycerides. Predisposition to coronary, cerebrovascular and peripheral vascular arterial diseases are the most common reason of death in developing and developed nations and they are mostly due to abnormalities in plasma lipids[2]. Obesity is one of the most common health problems and this disorder is associated with abnormal levels of blood lipids (hyperlipidemia) and lipoproteins (hyperlipoproteinemia).In hyperlipidemic conditions, the levels of lipids and cholesterol elevated in the blood and it is a symptom of different disorders of lipoprotein metabolism[3].

Acalypha indica belonging to the family Euphorbiaceae commonly known as haritha manjari, Indian copperleaf and is an annual herb grows naturally in wet, temperate, and tropical areas of India, southern China, tropical and South Africa, Sri Lanka, Pakistan, and Yemen. It is an annual herb with stem dark green, quadrangular and longitudinal furrows and wings. Indian people have the documented records of plant utilization for their traditional medicines as well as conventional medicines[4]. It is traditional used as expectorant, purgative, emetic, gastrointestinal irritant, diuretic, cathartic and anthelmintic, hypertension, lipid lowering, constipation, skin diseases, and ulcers bronchitis. The plant was found to contain alkaloids, flavonoids, glycosides, lactones, terpenoids, cyanogenetic glucosides and glucosinolates, phenantherenes, quinines etc[5].

Croton bonplandianus **baill**, belonging to the family Euphorbiaceae, plant is usually 30-40 cm in height, *Croton bonplandianus*, commonly known as three-leaved caper (English), ban tulasi, jungle tulasi(Bengali), kalabhangre (Hindi), eliamanakkau (Tamil), kukka mirapa (Telugu), alpabedhi soppu (Kannada) which grows in sandy clay soil along road side, railway abandoned field in wide open ravines, and paddy or sugarcane fields. It has been reported that this plant is India, Southern Bolivia, Paraguay, South Western Brazil, North Argentina, Bangladesh, South America, and Pakistan. In India it is widely distributed in the Sub-Himalayan region of West Bengal and desert of Rajasthan. It contains secondary metabolites are alkaloids, saponins, steroids, flavonoids, tannins, terpenoids and phenolic compounds. It is a medicinal herb used in many health related problems like cholera, boils, bowel complaints, diarrhoea, dysentery, insanity, acute constipation, abdominal dropsy, internal abscesses, cold and cough, lungs infection, bronchitis, asthma, jaundice, liver complaints, reduce pain, sprains, headache, high cholesterol and heart diseases.

Fig.-1- *Acalypha Indica* Plantc

Fig.-2- *Croton bonplandianus* baill Plant

2. MATERIALS AND METHODS

Plant material- The plants were collected from university campus of Apex University, Jaipur and tropical areas of Rajasthan. Plant was identified and authenticated at Botany Department of the Apex University, Jaipur.

Extraction of Plant Material-

100g of powdered stem sample of each plant were extracted successively with 500ml of ethanol respectively using Soxhlet extractor until the extract was clear. The extract was evaporated to dryness and the resulting paste form extract were stored in a sterile plastic container.

ANTIHYPERLIPIDEMIC ACTIVITY

Animals

Wistar albino male rats (190-210gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55-65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute Toxicity Studies

Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD)[6] .Animals are fasted prior to dosing (food but not water should be withheld for overnight). After that animals are weighed and the test substance administered. The healthy rats have been taken and divided into 4 different groups. The test substance is administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle. In this study, 4 groups of 6 rats each were given 5, 50 and 300 and 2000 mg/kg of the decoction (p.o.). After drug administration the food is withheld for 3 hours. The animals are observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days, post treatment to observe for any symptoms of toxicity and mortality. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), autonomic effects (salivation, lacrimation, gauntness and piloerection) and central nervous system (gait, tremors and convulsion) were carried out and changes were noted.

Table .1 Acute toxicity study design

GROUP	Number of Animals	DOSE (mg/kg)
Group 1	6	5
Group 2	6	50
Group 3	6	300
Group 4	6	2000

Clinical Observation

All animals were monitored continuously with special attention for 4 hrs after dosing for signs of toxicity. Additional observations are also done for the next 14 days for any other behavioural or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed. LD50 values are established using the formula[7].

Dose Calculation Equation

$$LD_{50} = \text{higher dose} - \Sigma (a \times b)/n$$

Where, **a** = dose difference, **b** = animal died, **n** = No. of animals in each group

$$ED_{50} = \frac{LD_{50}}{10}$$

High-Cholesterol Diet Model

High fat diet induced hyperlipidemic model preparation of feed

Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol l 2%,

Cholic acid 1%, sucrose 40% and coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self-sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals. The animals were administered with the high fat diet for 30 days. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats[8].

Experimental designs

Wistar rats weighing 190-210gm were divided into 7 groups of 6 animals each.

Group I- served +as normal control and were given only vehicle (distilled water)
Group II- received high fat diet served as hyperlipidemic control
Group III - received atorvastatin 10mg/kg served as standard drug
Group IV- received (200mg/kg, p.o.) ESAI
Group V- received (400mg/kg, p.o.) ESAI
Group VI- received (200mg/kg, p.o.) ESCBB
Group VII- received (400mg/kg, p.o.) ESCBB

Estimation of weight gain

During the experimental period, the high cholesterol diet consumed and weight gained by rats was recorded on 0th, 14th and 28th day of ESAI and ESCBB treatment. The pre-weighed food pellets (approximately 30g) were placed inside the hopper of the cage. The food consumed by individual rat was quantified by weighing leftover food in the hopper.

Biochemical Analysis of Serum

After treatment for 29th days with the test drug and on 30th day the rats are kept fasting and the blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes at 2000 r.p.m. and serum samples so collected were used for various biochemical tests. Serum Triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C) and very low-density lipoproteins were estimated by using commercial kits as per the manufacturer instructions[9,11].

Estimation of lipids:

A. Total cholesterol: Cholesterol in serum was estimated by using an Ecoline Diagnostic Kit. Cholesterol and its esters were released from lipoprotein by detergents. Cholesterol esterase hydrolyzes the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H2O2 was formed. This was converted into a colored quinineimine in a reaction with 4-aminoantipyine and phenol catalyzed by peroxidase. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. Cholesterol level in serum was expressed as mg/dL.

B. Triglycerides: Triglycerides level in serum was estimated using Ecoline Diagnostic Kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. Triglyceride level in serum was expressed as mg/dL.

C. HDL cholesterol: The cholesterol was separated from serum after precipitation of LDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant after centrifugation was estimated using Ecoline Diagnostic Kits. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. HDL cholesterol level in serum expressed as mg/dL.

D. LDL cholesterol: LDL cholesterol was calculated by using the formula

LDL cholesterol = Total cholesterol – [HDL cholesterol – Triglycerides/5].

LDL cholesterol level in plasma was expressed as mg/dL.

E. VLDL cholesterol: VLDL cholesterol was calculated by using the formula

VLDL cholesterol = Total cholesterol – HDL cholesterol – Triglycerides- LDL.

Statistical analysis

Data were statically analysed as mean + SEM and expressed as just significant $P<0.05$ and significant $P<0.01$ as the case may be using one way ANOVA followed by Dunnett's multiple comparison test[12,15].

3. RESULTS

Acute Toxicity Study

There was no mortality or signs of toxicity up to the limit dose of 2000 mg/kg in treated rats. All 24 rats were normal throughout the study and survived until the end of the 14-day experiment period. Animal wellness parameters were observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days as per paragraph 24 and 25 of OECD Guideline 423. Experimental observations are recorded systematically for each group. The parameters considered are changes in skin and fur, eyes and mucous membrane and also respiratory and circulatory, autonomic and central nervous system, somatomotor activity and behavioral pattern. Special attention is given for the observations of tremor, convulsion, salivation, diarrhea, lethargy, sleep and coma. No adverse changes and mortality were observed in animals, which orally received ethanolic extract (2000 mg/kg) of *Acalypha indica* and *Croton bonplandianus Baill* Table 1. This indicates that 2000 mg/kg is maximum safe dose. So $1/10^{th}$ and $1/5^{th}$ *i.e.* 200 and 400 mg/kg of body weight of the maximum safe dose were selected for studying *in vivo* antihyperlipidemic activity.

Table 2: Change in wellness parameters observed for *Acalypha indica* and *Croton bonplandianus Baill* stem extract treated wistar rats

S. no	Response	Group1(5mg/kg)		Group 2 (50mg/kg)		Group 3 (300mg/kg)		Group 4 (2000mg/kg)	
		Before	After	Before	After	Before	After	Before	After
1	**Alertness**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	**Grooming**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	**Anxiety**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	**Roaming**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	**Tremor**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	**Convulsion**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	**Depression**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	**Gripping strength**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	**Scratching**	Present	Present	Present	Present	Present	Present	Present	Present
10	**Defecation**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	**Writhing**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	**Pupils**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	**Urination**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	**Salivation**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	**Skin and fur**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	**Lacrimation**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	**Pilo erection**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18	**Nail status**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	**Sleep**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

20	**Gauntness**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	**Lethargy**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
22	**Diarrhea**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
23	**Coma**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	**Mucous membrane**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Antihyperlipidemic activity

Antihyperlipidemic effect of the ethanolic extract of *Acalypha indica* and *Croton bonplandianus Baill* on the high fat diet induced rats, the mean body weight as shown in Table 2.

Table 3: Effect of ESAI and ESCBB on weight gain in hyperlipidemia-induced rats for 4 weeks

Parameter	Test group	Day 0	14th day	28th day
Weight gain	Normal control	126.6±3.10	128± 2.36	128.7± 2.12
	Positive control	215.4±7.00	228.8±8.21	239.8±7.22
	Atorvastatin 10mg/kg	198.3±4.79***	173.3± 5.03***	154±4.55***
	ESAI 200mg/kg	205.5±5.36*	190±7.05***	175.7±5.01***
	ESAI 400mg/kg	193.3±3.78***	176.2±4.68***	167±4.87***
	ESCBB 200mg/kg	200.5±4.33*	185±6.05***	170.7±4.01***
	ESCBB 400mg/kg	178.3±2.77***	171.2±3.58***	162±3.77***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group. * P<0.05, ** P<0.01, ***P<0.001 (one way ANOVA followed by Dunnett test), ESAI= ethanolic stems extract of *Acalypha indica* , ESCBB= ethanolic stems extract of *Croton bonplandianus baill* .

Effect of ESAI and ESCBB on lipid profile in high fat diet induced model

In high fat diet induce model, oral administration of **ESAI and ESCBB** (200 mg/kg and 400mg/kg, p.o.) significantly reduced the serum total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), VLDL-cholesterol levels but significantly increased serum HDL-cholesterol level as compared with positive control group. This study shows serum lipid parameters in animals were significantly reduced (p<0.001,) by 30th days treatment with **ESAI and ESCBB** at dose levels 200 mg/kg and 400 mg/kg, when compared with control group. 400 mg/kg of HANAT group animals has shown very significant (p<0.001) compared with control group Table 3.

Table 4: Effect of ESAI and ESCBB *on* lipid profile in high-cholesterol diet induced hyperlipidemia

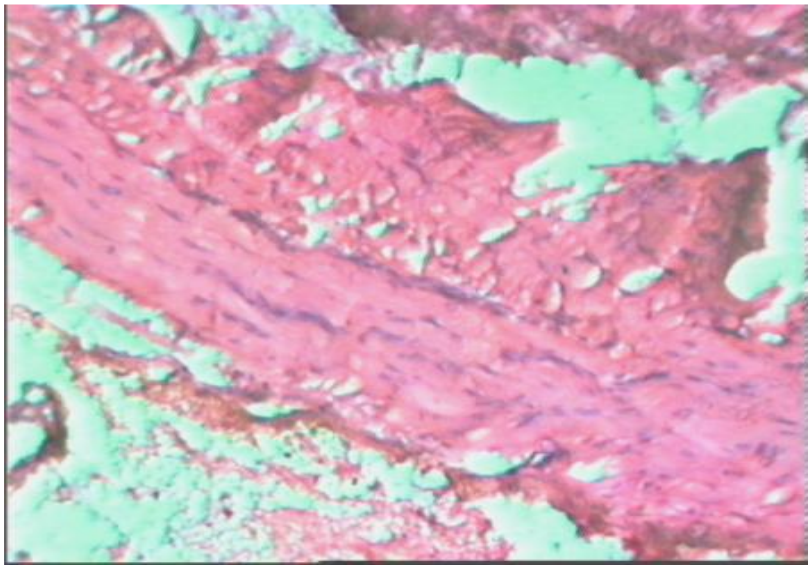
GROUP	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Control	84.1±3.78	44.12±1.32	70.89±2.11	14.79±0.41	28.39±3.52
Positive control	148.62±2.5	36.29±2.72	144.15±3.60	27.73±0.65	66.25±2.62
Atorvastatin 10mg/kg	89.82±1.66	48.33±2.02	101.1±2.33***	22.43±0.44	23.13±2.22**
ESAI 200mg/kg	110.32±2.34	42.81±1.90	118.02±2.10**	25.5±0.32	46.53±2.23

ESAI 400mg/kg	94.35±1.73**	45.49±2.17***	103.06±2.87	22.6±0.50	30.59±1.97**
ESCBB 200mg/kg	105.12±2.22	38.71±1.70	115.02±2.05**	21.4±0.22	41.23±2.11
ESCBB 200mg/kg	90.22±1.53**	4148±2.15***	98.05±3.11	18.6±0.25	26.39±1.95**

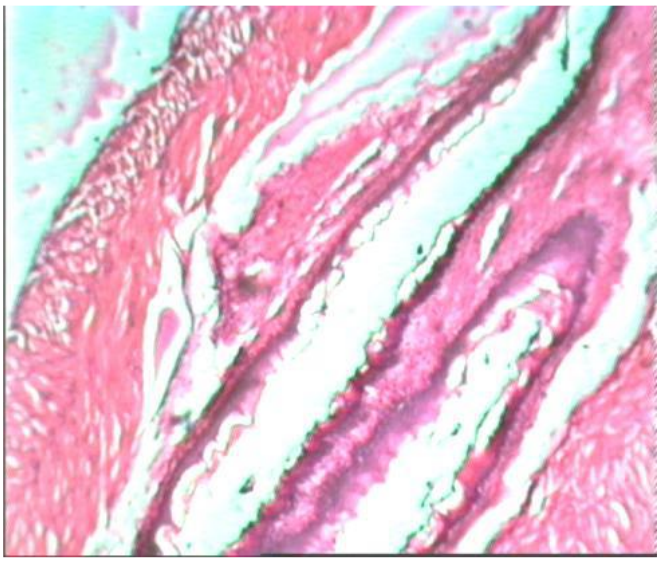
Values were mean ±sd (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01,***P<0.001 Vs hyperlipidemic control using one way ANOVA followed by Dunnet's test ESAI= ethanolic extract of *Acalypha indica* stems, ESCBB= ethanolic extract of *Croton bonplandianus baill* stems.

Histological results of liver

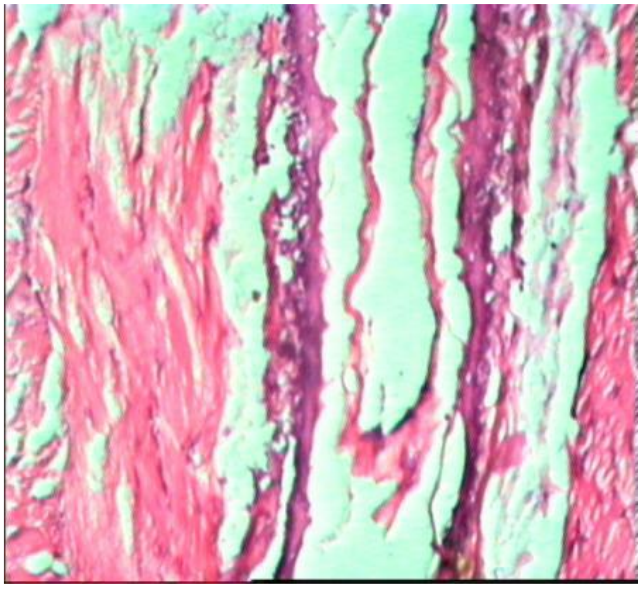
In the histopathological study high cholesterol diet fed rats shows fatty cytoplasmic vaculated cells as compared to normal control. Treatment with ethanolic extract of ESAI shows less fatty cytoplasmic vacuoles as compared to high cholesterol diet fed rats. ESCBB shows focal area of cytoplasmic vacuoles.



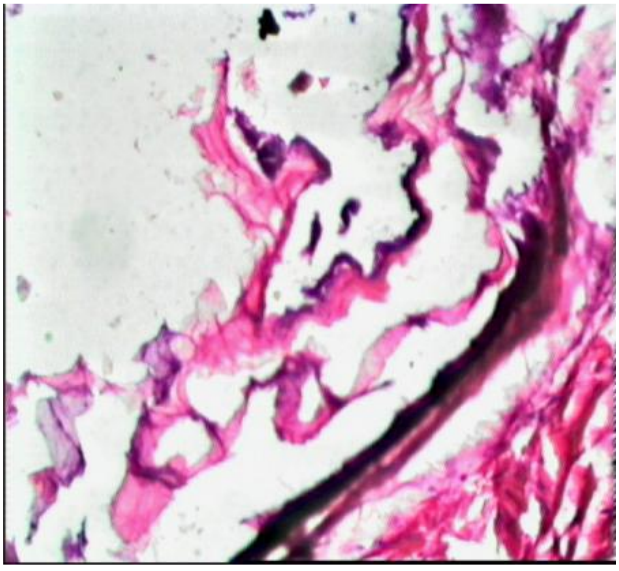
Control



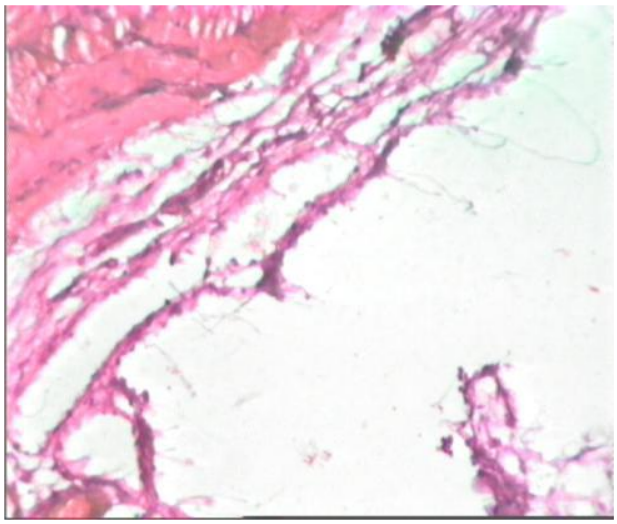
Positive control



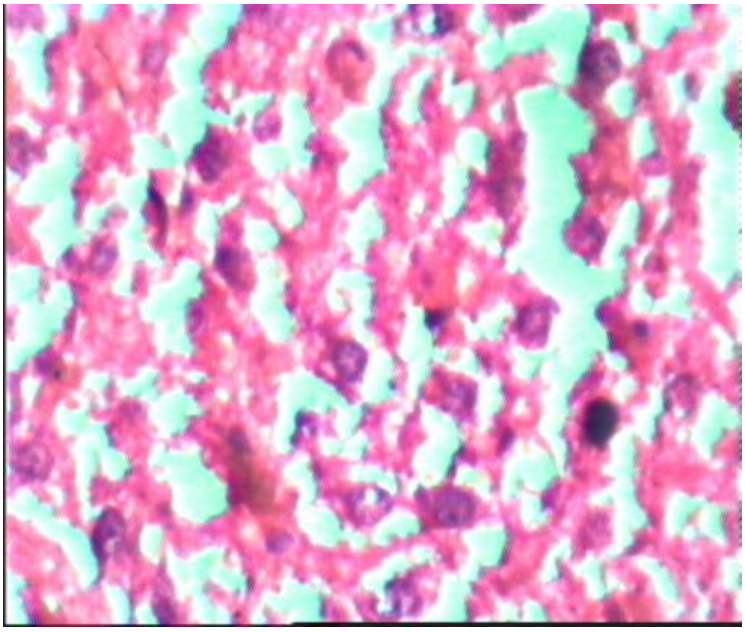
Atorvastatin10mg/kg



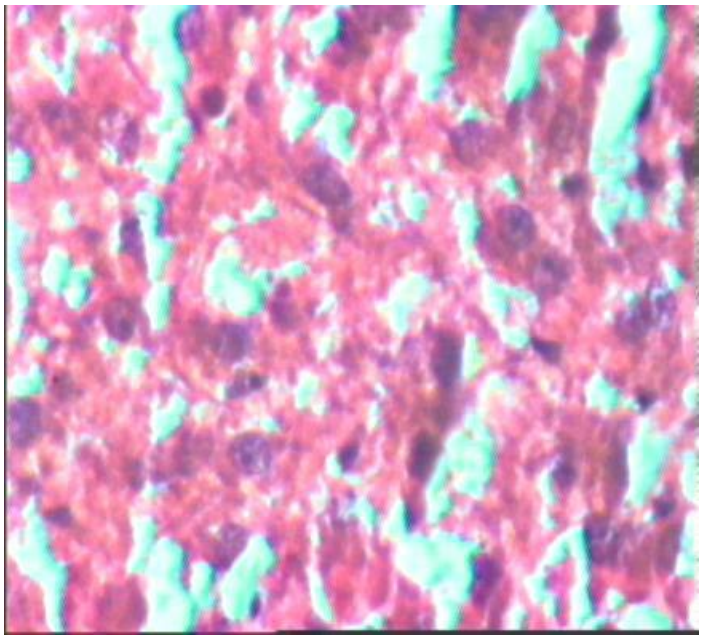
ESAI 200mg/kg



ESAI 400mg/kg



ESCBB 200mg/kg



ESCBB 400mg/kg

4. CONCLUSION

Numerous medicinal plants and their formulations are used for hyperlipidemia in ethno-medical practices and in traditional system of medicine in India. However, we do not satisfactory remedy for hyperlipidemia; most of the herbal drugs speed up the reduction of cholesterol by healthy dietary intake. So, the search for anti-hyperlipidemic activity of high cholesterol induced rats.

Acute phase toxicological studies reports no mortality or signs of toxicity up to the limit dose of 2000 mg/kg in treated rats. All 24 rats were normal throughout the study and survived until the end of the 14-day experiment period. No adverse changes and mortality were observed in animals, which orally received hydro alcoholic extract (2000 mg/kg) of ESAI and ESCBB. This indicates that 2000 mg/kg is maximum safe dose. So $1/10^{th}$ and $1/5^{th}$ *i.e.* 200 and 400

mg/kg of body weight of the maximum safe dose were selected for studying *in vivo* antihyperlipidemic activity. The present study carried out inducing high fat diet for the experimental induction in rats. Cholesterol will deposits in the endothelial cells and transport with lipoproteins in blood stream which leads to increase in the TC, TG, LDL, and VLDL levels and decreases the HDL levels in the body. It is synthesized in the liver and converted into bile acids and excreted through faces and due to hypercholestermia urine failure will occur due to oxidative stress. The assessment of cholesterol function can be made by estimating the body weight and activities of various lipid profiles such as TC, TG, HDL, LDL and VLDL. Body weight can be increased due to the high cholesterol diet induced in hyperlipidemia rats. As the dietary fat, FFAs can be synthesized into many tissues by transport of increased lipoproteins in the blood stream absorbed from the intestine and metabolized in liver[16] . The ESAI and ESCBB treatment and Atorvastatin drug significantly lower the body weight by the reduction in the LDL, VLDL levels and increase HDL levels. Oral administration of ethanolic stems extracts significantly reduced the cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins and significantly increased the HDL-cholesterol level as compared with high cholesterol diet induced hyperlipidemic animals. The results were significant with the p value ($p<0.001$).
In histopathological study we found treatment of *ESAI* significantly decreases the plaque size in aorta and significantly decrease fatty cytoplasmic vaculated cells in Liver parenchyma as well as liver cell necrosis is prevented. But ESCBB of showed no effect in liver compared to HCD rats.

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