

# IN VITRO AND IN VIVO STUDIES FOR ANTIHYPERLIPIDEMIC ACTIVITY OF ANTIDESMA LACINIATUM AND BIOTA SEMPERVIRENS ETHANOLIC EXTRACTS IN EXPERIMENTALLY INDUCED HYPERLIPIDEMIC MODELS

Dr, Mehnoor Farheen\*\*\*, Ajmeri begum\*\*, Aqsa Amtul\*, Afrose Fatima\*

\*\*\*Head of Department - Pharmacology,

Shadan women's college of pharmacy- Khairatabad- 500004- Hyderabad.

## Abstract:

This project is focused on the study of anti-hyperlipidemic effects of the leaves extract of "Antidesma laciniatum" and "Biota sempervirens" on High fat diet and Triton induced albino wistar rats. To evaluate the anti-hyperlipidemic activity of ethanolic extract of antidesma laciniatum & biota sempervirens, tested by phytochemical analysis, In-vitro and In-vivo screening models. Chronic hyperlipidemia is influenced by giving HFD in rats for about 21 days. Oral dosing of EEAL & EEBS in different dosing alleviates the blood serum levels of lipoproteins like LDL, VLDL, triglycerides, total cholesterol and HDL compare to the standard drug (atorvastatin) & normal control. The histopathological studies are carried out by dissecting out the liver of one animal from each group.

**Keywords:** Hyperlipidemia, Antidesma laciniatum, Biota sempervirens, High Fat Die, Atorvastatin.

## INTRODUCTION

Herbal drugs are biologically active natural products which have been used since ancient times but the pharmacological and biological properties are still unknown. [1] Atherosclerosis is the major risk factor of hyperlipidemia. It is the metabolic disorder characterized by high or elevated levels of lipids or cholesterol in the body. Lipids are carried by lipoproteins like LDL, VLDL, HDL, TG etc. Irregularities in plasma lipids result in coronary, cerebrovascular and peripheral vascular arterial diseases due to predisposition of fat. Atherosclerosis is formation of plaque due to narrowing and stiffening of arteries which reduces blood flow due to accumulation of fat. Hyperlipidemia is classified into two types

1. Based on lipids: a) Hypercholesterolemia b) Hypertriglyceridemia
2. Based on causing or affecting factor: a) Primary hyperlipidemia b) Secondary hyperlipidemia

## SYMPTOMS:

Chest pain, MI, Stroke are the most common symptoms, obesity, enlargement or swelling of liver, pancreas or spleen, abscess like abrasion across the body etc.

## ORGAN:

**LIVER-** It is the largest of the human body. Located in the upper right quadrant region of the abdomen below the diaphragm, under the lower rib cage. It mainly involves drug biotransformation, bile secretion, storage of vitamins and minerals (such as vitA, D, E, K and copper, zinc, magnesium, iron), Fe and B12 deposition. Production of plasma proteins, Formation of urea by removal of amino acids, Prothrombin and fibrinogen formation, Cholesterol production etc.

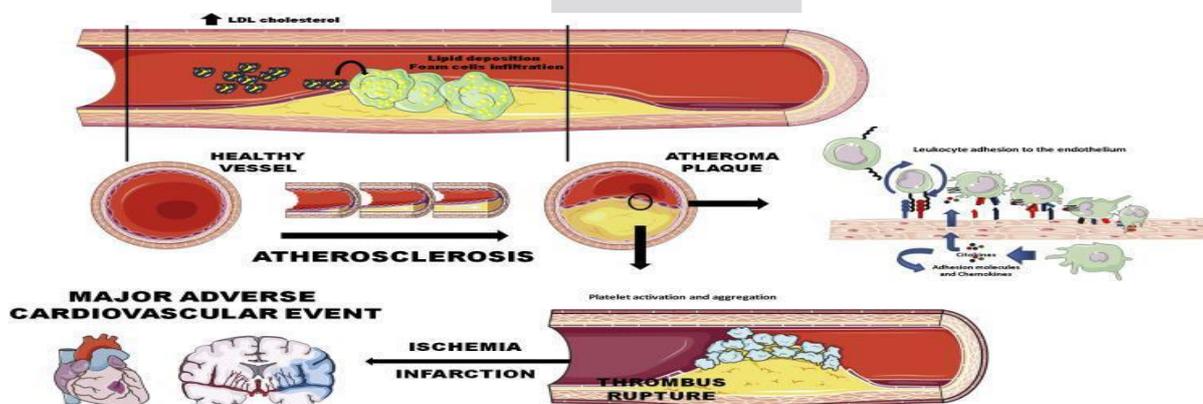


Fig 1 : Lipid Metabolism in Hypercholesterolemia

**Plant Profile:****Antidesma laciniatum:****Fig 2:** Antidesma laciniatum

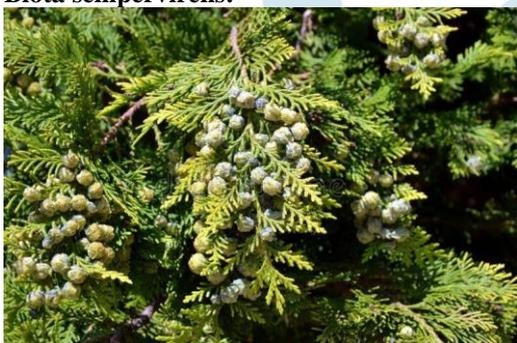
**Synonym:** Antidesma chevalieri Beille

**Family:** Phyllanthaceae

It is a shrub 2-3 meters or small dioecious tree with a height of 15 meters. They are found in dense forest, moist deciduous forests, rain forests, and forests from sea level to 1200 meters above sea levels.

**Chemical constituents:** Tannins, terpenoids, flavonoids, carbohydrates, alkaloids, phenols, etc. Ester benzyl benzoate and geranyl acetate are two important constituents which provide sweet balsamic odor to the oil.

**Uses:** medicinal properties include cytotoxic, antiplasmodial, antioxidant, analgesic, antipyretic, enema, antiviral activities.

**Biota sempervirens:****Fig 3:** Biota sempervirens

**Synonym:** Cupressus sempervirens

**Common name:** Mediterranean cypress

**Family:** Cupressaceae

It is mostly cultivated in northern America, southern Europe, north Africa, Asia, etc. Grows tall up to 30m, leaves are dark green and obtuse of 0.5 to 1 mm, size of both female and male cones are 25-40 mm and 4-8 mm.

**Chemical constituents:** Tannins, saponins, flavonoids, phenols, alkaloids, etc.

**Uses:** It is used in the treatment of hemorrhoids and venous circulation disorder as an ointment, bronchitis, antiseptic, antispasmodic, diuretics, enuresis, anti-viral, anti-cancer, osteogenic, antifungal and antibacterial effect.

**METHODOLOGY****Plant Material:**

The dried leaves of *Antidesma laciniatum* and *Biota sempervirens* are obtained and authenticated by Dr. Madhava Chetty, Assistant Professor of Botany, (Dept. of Pharmacognosy), Sri Venkateswara College, Tirupati.

Standard Drug: Atorvastatin

**Other chemicals:**

- Ethanol
- Normal saline
- Triton-X-100
- High Fat Diet
- Ethanolic Extract of *Antidesma laciniatum*
- Ethanolic extract of *Biota sempervirens*
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**Ethanolic Extracts Preparation:** Maceration process is carried out for extraction .

**Solvent:** Ethanol(99.9%)

**Maceration Process:**The dried and finely powdered plant leaves are taken,an extraction jar is used for extraction in which ethanol ( 99.9%v/v) is added in a ratio of 1:2 to the finely powdered kept in contact for about 7 days and stirred occasionally. Separation of solid liquid biphasic at the end of maceration process. Then filtration is carried out and filtrate is collected subject to evaporation in open area to evaporate ethanol and obtain a concentrate extracts.This extract is use for animal testing as per the dose determined by acute toxicity testing. Normal saline is used to reconstitute the extract at the time of dosing to animals.

**Phytochemical evaluation :** It is carried to identify the secondary metabolites of plant extracts such as carbohydrates, glycosides, flavonoids, terpenoids, tannins, saponins, steroids, polyphenols, etc.

**Experimental Animals:** Male albino wistar rats of 180-200g of body weight are taken. Five days prior to use animals are acclimatized in an animal house. They are placed in polypropylene cages with 12:12hr light and dark cycle at temperature of 25°C and standard pellet fed, water ad libitum. CPCSEA guidelines- care and handling of animals. Permission and approval of animal studies was obtained from IAEC.



**Fig 4:** Experimental Animals

**Experimental Design:**

Male wistar rats were taken and divided into nine groups of 6 animals each and doses are given respectively as mentioned below:

Groups	Wt. Animals(gm)	Treatment	Dose
Group 1	150 - 200	Normal control (normal saline)	1 ml of 0.9% w/v p.o
Group 2	150 - 200	Hyperlipidemic control + Triton X-100	10 ml/kg i.p
Group 3	150 - 200	Hyperlipidemic control + standard control (Atorvastatin)	10 mg/kg o.p
Group 4	150 - 200	Hyperlipidemic control + EEAL	200 mg/kg o.p
Group 5	150 - 200	Hyperlipidemic control +	400 mg/kg o.p

		EEAL	
Group 6	150 - 200	Hyperlipidemic control + EEBS	200 mg/kg o.p
Group 7	150 - 200	Hyperlipidemic control + EEBS	400 mg/kg o.p
Group 8	150 - 200	Hyperlipidemic control + EEAL +EEBS	200 mg/kg o.p
Group 9	150 - 200	Hyperlipidemic control+ EEAI+EEBS	400 mg/kg o.p



**Fig 5:** Experimental design

#### Screening Models:

##### In Vitro method:

**HMG CoA reductase inhibition:** The conversion of HMG CoA to mevalonate from acetyl CoA is the rate limiting step for biosynthesis of cholesterol. The enzyme involved in this conversion is HMG CoA Reductase enzyme. Cholesterol biosynthesis is stopped due to enzyme inhibition. Statins and lipid lowering drugs have the ability to inhibit HMG CoA due to this plasma level of cholesterol decreases as LDL receptors get expressed. The kit usually consists of enzyme, NADPH, assay buffer, standard blocking agent - Atorvastatin. It is mainly used in measurement of NADPH utilized, that is measured by absorbance of radiation at 340 nm. The % inhibition is measured using below equation:

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

##### In vivo method:

##### High fat diet induced hyperlipidemic model:

In HFD induced hyperlipidemia, plasma lipoproteins level increases and this aggravates prostatic parametrial fat. To stimulate hyperlipidemia animal models are used. Individually and in combination the therapeutic effect of herbal plants is studied.

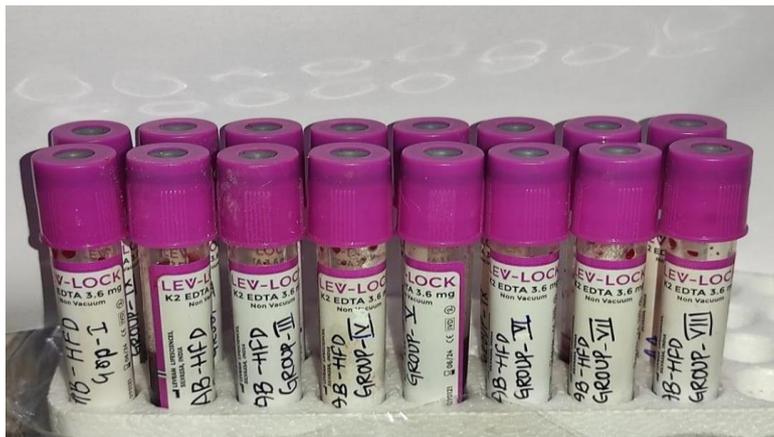
**Induction of Hyperlipidemia and its Treatment :** Acclimatization of wistar rats were one for about 7 days and are divided into nine groups of six in each. The standard chow diet was given to the normal control group, 0.9% w/n of normal saline and water, HFD were given to all other group for about 21 days. After 7 days of high fat diet vehicles were given to hyperlipidemic control group animals, atorvastatin 10mg/kg by p.o route were given to standard control group and different doses of extract individually or in combination were given to group IV, V, VI, VII, VIII, IX as per the experimental design through p.o route using oral gavage for about 14 consecutive days. Blood is collected by retro-orbital puncture under anesthesia for serum analysis of lipid profile.

**Body weight(gm):** On day 1, day 7, day 14, it is recorded for evaluation of hyperlipidemia in the normal and experimental group rats. Weighing balance is used for this.

Difference in body weight or the % increase is calculated using below formula:

$$(\text{final.wt} - \text{initial b.wt}) / \text{initial b.wt} \times 100$$

**Blood Collection:** Blood is collected after 21 days of the study, for blood collection animals was fasted overnight and retro orbital puncture done for collecting blood using capillary tubes under anesthesia in coagulant ampules for estimation of serum levels.



**Fig 6:** Blood collection

**Biochemical Estimations:** The samples were sent to the diagnostic center for various laboratory estimation of biochemical parameters such as LDL, VLDL, HDL, Total cholesterol, cholesterol ratio.

$$LDL-c = [TC - \{HDL-c + (TG/5)\}]$$

$$VLDL-c = (HDL-c + LDL-c) - TC$$

$$\text{Atherogenic index (AI)} = \log(TG/HDL-c)$$

**Histopathological studies:** Single rat from each group anesthetized and liver is dissected out and this was kept in 10% formalin solution (10ml of formalin in 100ml of NS) stored further for laboratory testing.



**Fig 7:** Liver of rats for histopathological study

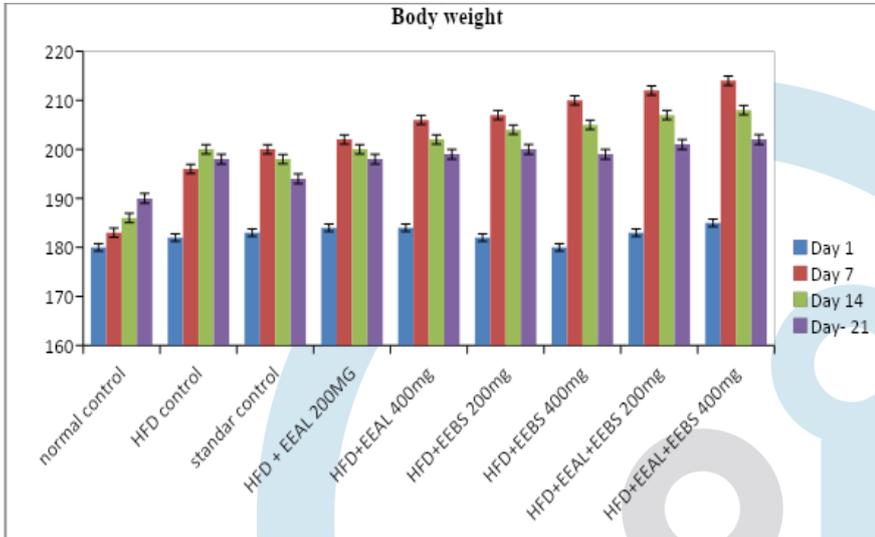
**RESULTS:**

**Phytochemical analysis**

Phytochemical Tests	Result for Antidesma laciniatum	Results for Biota sempervirens
Alkaloids	++	+++
Saponins	++	+
Flavonoids	+++	+++
glycosides	++	++
Terpenoids	+	+++
Carbohydrates	+	++

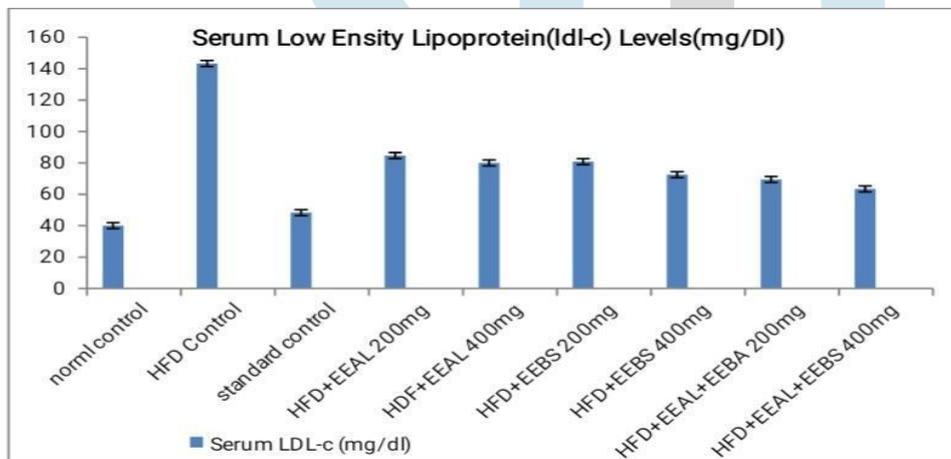
Quinines	++	++
Proteins and amino acids	++	+++
Reducing sugars	++	++

**Anthropometric parameters:  
Assessment of body weight (gm):**

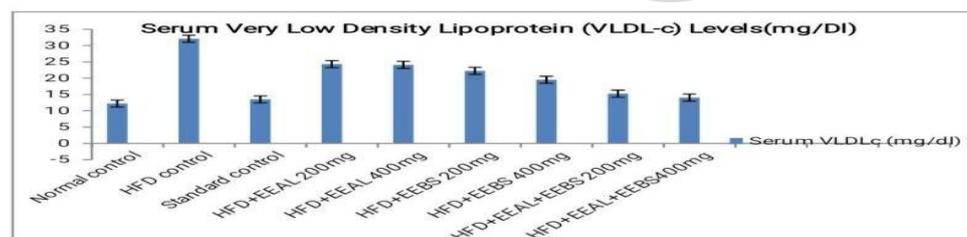


**Graph 1:** Body weight

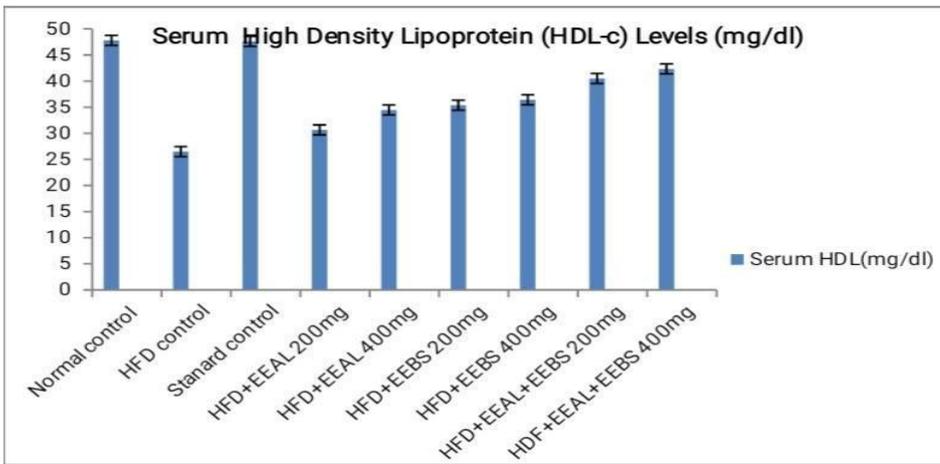
**Effect of EEAL and EEBS on serum lipid profile in HFD induced hyperlipidemic rats**



**Graph 2:** Effect of EEAL and EEBS in serum LDL-c(mg/dl) of HFD induced hyperlipidemic rat



**Graph 3:**Effect of EEAL and EEBS on serum VLDL-c(mg/dl) of HFD induced hyperlipidemic rat.

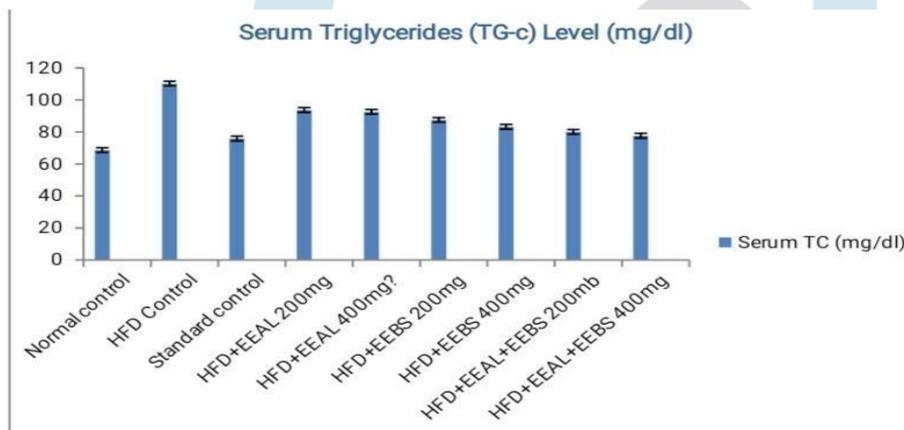


**Graph 4:** Effect of EEAL and EEBS on serum HDL-c(mg/dl) in HFD induced hyperlipidemic rat.

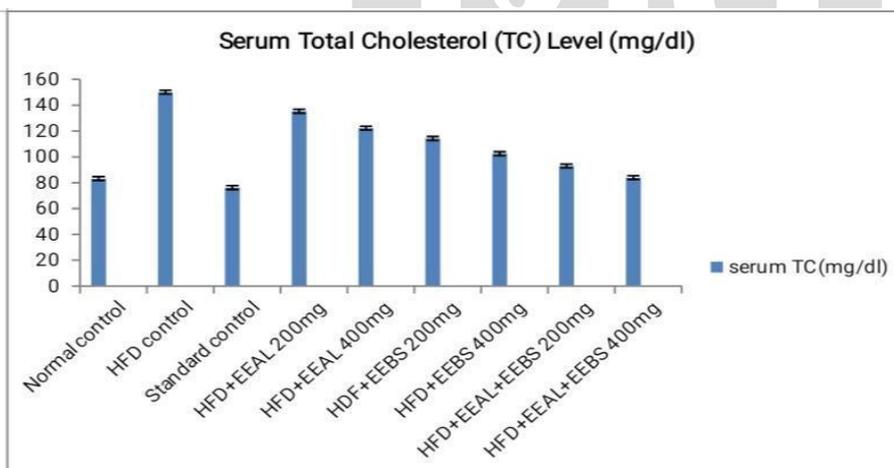
Data was expressed as mean ±S.E.M, n=6 rats in each group.

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

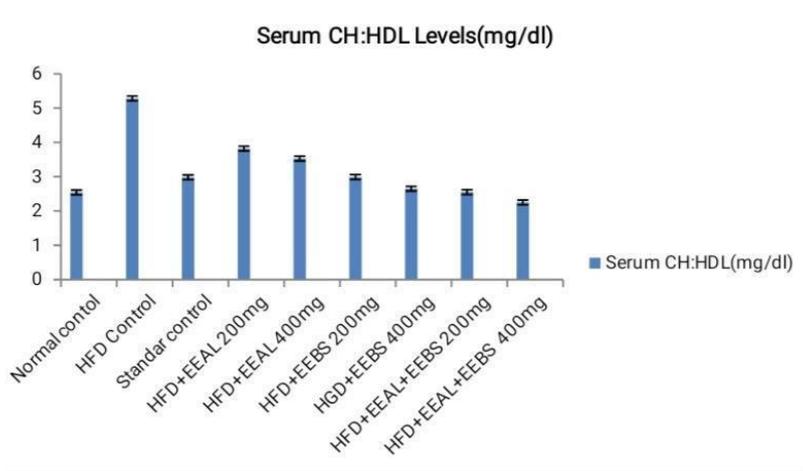
**Effect of EEAL and EEBS on serum TG, TC, CH-HDL Ratio of HFD**



**Graph 5:** Effect of EEAL and EEBS on serum TG of HFD induced hyperlipidemic rats.



**Graph 6:** Effect of EEAL and EEBS on TC of HFD induced hyperlipidemic rats.

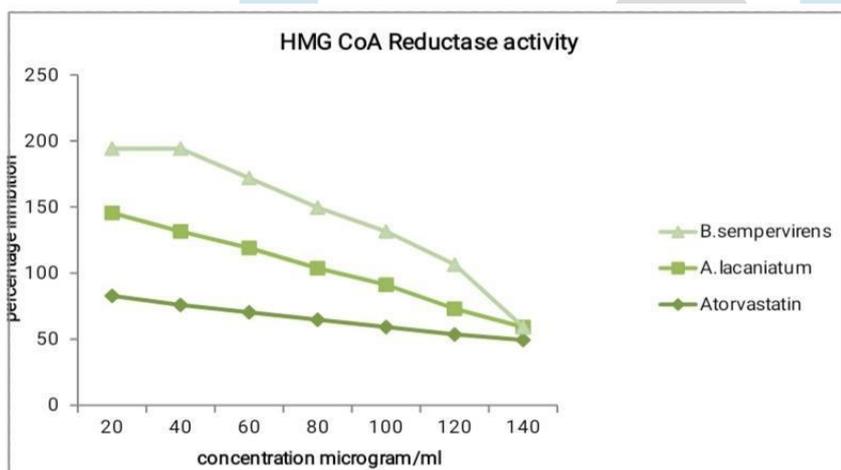


**Graph 7:** Effect of EEAL and EEBS on CH:HDL of HFD induced hyperlipidemic rats.

Data was expressed 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. Group 3,8,9 are found to be more significant among all treatment groups.

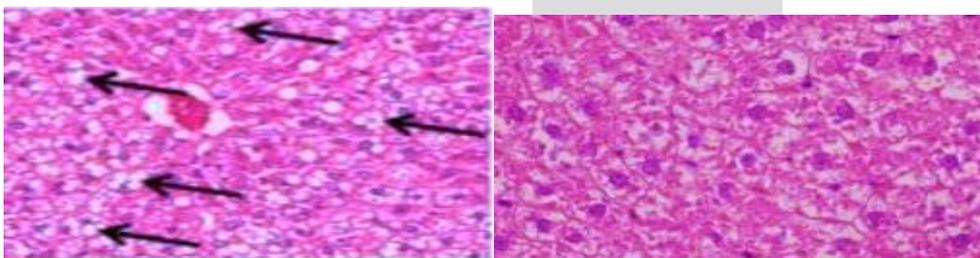
**HMG CoA Reductase activity:**



**Graph 8:** HMG CoA reductase activity, a plot between concentration and percentage inhibition.

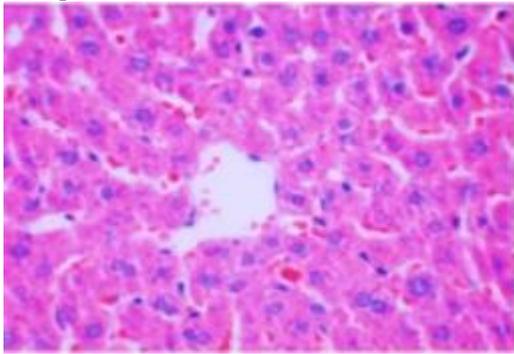
**Microscopic Examination of Liver:**

Group 1: Normal control

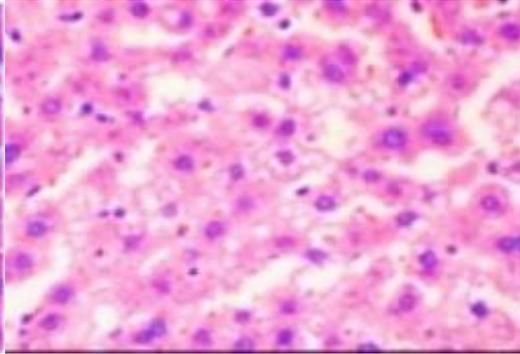


Group 2: Toxic control -HFD

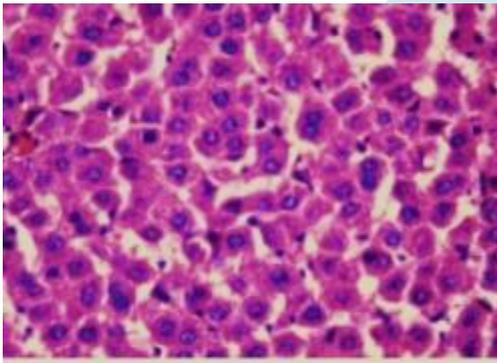
Group3: Standard control



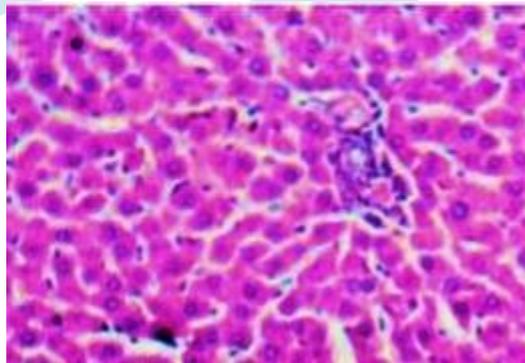
Group4:HFD + (plant 1 Dose 1)



Group 5: HFD + Plant1 Dose 2



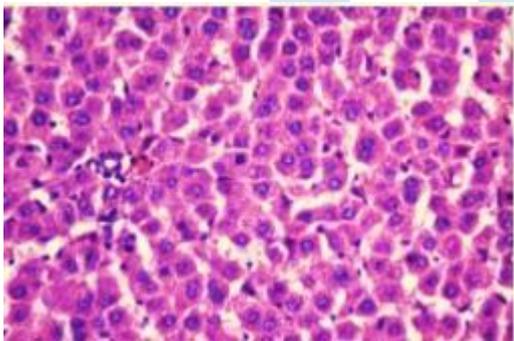
Group 6: HFD + Plant 2 D



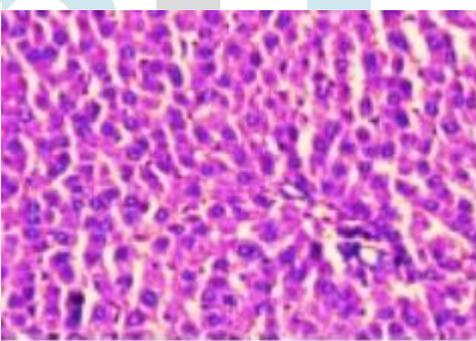
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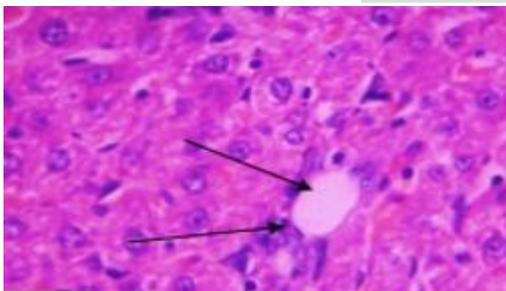
Group 7: HFD + Plant 2 Dose 2



Group 8: HFD + Combination-1 Dose 1



Group 9: HFD + Combination-2 Dose 2



**Fig:8:** Microscopic Examination of rat liver cells

**1 NORMAL CONTROL:** Microscopic liver of normal group rat shows normal structure of liver.

**2 TOXIC CONTROL:** Different histological changes and unusual hepatic architecture are identified by dilation of portal and central vein in the liver of the toxic group of rat. Vacuoles can be seen (H&E x 400).

**3 STANDARD CONTROL:** liver of group 3 treated with atorvastatin showed less fatty infiltration and granular degeneration and is similar to normal group rat.

**4 EEAL-DOSE-1:** Group 4 treated with low dose of EEAL indicates cellular degeneration, droplets of lipids and fatty infiltration are found.

**5 EEAL-DOSE-2:** Group 5 treated with high dose of EEAL indicates less dilation, mild fatty infiltration and dilation and congestion of blood sinusoids, congested portal vein.

**6 EEBS-DOSE-1:** Group 6 treated with low dose of EEBS indicates Dilation and congestion of portal & hepatic vein, cellular aggression.

**7 EEBS-DOSE-2:** Group 7 rat liver is treated with high dose of EEBS indicates minute dilation and congestion of blood sinusoids, vacuole has been displayed in some hepatocytes. Normal hepatic cells (most).

**8 EEAL + EEBS-DOSE-1:** Group 8 rat liver is treated with a low combination dose of EEAL & EEBS indicates mild liver cell steatosis and mild congestion and dilation of blood sinusoids, central vein, portal vein.

**9 EEAL + EEBS-DOSE-2:** Group 9 rat liver is treated with a high combination dose of EEAL & EEBS indicating normal hepatocytes with negligible congestion and dilation of central and portal vein, negligible fatty infiltration. Normal blood sinusoids.

#### DISCUSSION:

Hyperlipidemia is a chronic disease characterized by elevated levels of lipids over a longer period of time. This is mainly due to intake of improper diet, change in lifestyle etc. Associated with other disease conditions and improves cardiovascular risks. It may further lead to liver damage and angina pectoris, MI, if not treated. The main factor CHD is hyperlipidemia but due to occurrence of DM the hyperlipidemia will be at risk. As it forms atherosclerotic plaque if not treated may cause heart attack, stroke. The two important enzymes that help in fat metabolism are HMG CoA reductase and Lipoprotein lipase activation. *Antidesma laciniatum* is known for antibacterial, antipyretic, anti oxidant, anticancer, antimicrobial activities etc. *Biota sempervirens* is known for anthelmintic, antibacterial, antiviral and antioxidant action etc. The ethanolic leaves extracts of *Antidesma laciniatum* and *Biota sempervirens* were prepared and tested for phytochemical constituents such as flavonoids, terpenoids, and glycosides showed positive results of plant A.L which thought to be main active constituents for hyperlipidemic activity and B.S possesses glycosides, alkaloids, carbohydrates etc but its effect is due to increase amount of alkaloids. Discussion is focused on 400mg/kg between AL and BS individually and combination of both administered orally and found competitive against potent drug atorvastatin at 10 mg/kg body weight observed in HFD. The study outcome is compared with toxic v/s standard and high dosage form is best for each plant and combination, The histopathology of liver showed appropriate difference in slides showing clear visuals of liver.

#### CONCLUSION:

The present study shows significant antihyperlipidemic effect by *Antidesma laciniatum* and *Biota sempervirens* with their potent effect. So, the conclusion is that oral administration of ethanolic extract of both plants exhibit antihyperlipidemic activity. The rats belonging to the group-7 produced significant effect and maximum was exhibited with a combination of both *Antidesma laciniatum* and *Biota sempervirens*.

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