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Research Paper

HYPOLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *BOMBEX CEIBA*

Asija Rajesh *, Sharma Mohan , Khanijau Rashmi, Soni Tripti

Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India 302020

This study was carried out to evaluate the hypolipidemic activity of ethanolic extracts of seeds of *Bombex Ceiba* in high fat diet induced hyperlipidemic rats. Ethanolic extracts of seeds of *Bombex Ceiba*, orally at the dose of 200 and 400 mg/kg, were given to high fat diet induced hyperlipidemic rats. After eight weeks of dosing, the possible antihyperlipidemic activity was assessed by investigating serum lipid levels. Atorvastatin (10mg/kg p.o.) was used as a standard drug. Results showed that aqueous extracts of Bombex ceibacauses a significant reduction in serum levels of total cholesterol (TC), triglycerides(TG), Low density lipoprotein cholesterol (LDL-C) and Very low density lipoprotein cholesterol (VLDL) and atherogenic index. The results also demonstrated a significant ($p \leq 0.0001$) increase in High density lipoprotein cholesterol (HDL-C).

KEYWORDS: *Bombex Ceiba*, hypolipidemic, high fat diet, atherosclerosis.

INTRODUCTION

Urbanization is characterized by an increase in tendency of people towards consumption of junk food, a decrease in physical activity and an intense level of psychosocial stress, all of which leads to the development of hypertension, hyperlipidemia, etc. Hyperlipidemia is an umbrella term that refers to any of several acquired or genetic disorders that result in a high level of lipids circulating in the blood. A solid link exists between elevated cholesterol levels and cardiovascular diseases (CVD). According to WHO, the prevalence of CVD will double by 2020 and will rank higher than HIV.[1] The underlying primary cause of CVD is believed to be atherosclerosis, a progressive multifactorial disease of the artery walls.[2] Although statins have gained the status of preferred treatment for hyperlipidemia, there are reasons to consider other medications. The consumption of statins causes undesirable effects such as rhabdomyolysis, liver injury, myopathy and acute renal failure. Therefore, it is required to explore the newer lipid lowering drugs with better safety profile. Herbal drugs have proven to be a better choice when compared to synthetic drugs. Literature review shows that *Bombex Ceiba* leaves are known to possess Hypolipidemic activity.[3] Hypolipidemic activity has not yet been reported on its seeds. Epidemiological studies have shown that the risk of heart diseases can be reduced through the consumption of flavonoid rich diets.[4] Saponins also reduce the harmful LDL-cholesterol selectively in the serum of rats, gerbils and human subjects. [5] Presence of

flavonoids and saponin in the seeds has also been reported. Keeping in mind the current status of CVD prevailing world over, this present study was proposed to explore the efficacy of *Bombex Ceiba* leaves against hyperlipidemia.

1. MATERIALS AND METHODS

Collection of plant material

The fresh seeds of *Bombex Ceiba* were collected from the local farms Identification of the leaves of *Bombex Ceiba* Deputy conservator of Forest sikar

Reference no. : **DCF/2022/15**

Plant is authenticated by Bhima ram choudhary . Material was shade dried at room temperature and powdered mechanically and passed through a sieve #40.

Preparation of the extract

The freshly collected leaves of *Bombex Ceiba* were chopped and shade dried at room temperature. The seeds were subjected to size reduction to a coarse powder by using electric blender. 100g of powdered seeds were extracted with ethanol using soxhlet extractor.

Preparation of high fat diet

High fat diet was prepared by mixing cholesterol 1%, cholic acid 0.3% in 10% of coconut oil along with normal diet.[6-8]

Selection of animals

Animals

The study was carried out in rats of Wister strains of either sex weighing 150-200 gm. 2-3 months old. They were procured from animal house of the Maharishi Arvind Institute of Pharmacy, Jaipur .and were kept individually under standard laboratory condition. Food pellets and tap water were provided and libitum. Ethical clearance for experimental studies was obtained from institutional animal Ethical Committee, Accuprec research lab ahmedabd under reg. **1709/Rc/S/13/CPCSEA**

Maintenance of animals

The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. The animals were maintained on standard pellet diet and purified water. The animals were provided with food and water *ad libitum* except during fasting. Each animal in the cage was marked on head or body or tail with permanent marker for their appropriate identification.

Determination of acute oral toxicity[9-12]

Acute oral toxicity study was carried out according to the OECD guidelines – 423. A limit test at one dose level of 2000 mg/kg body weight was administered to a set of three female Wistar rats. The animals were observed individually after the drug treatment for first 30 minutes and then periodically for 4 hours, 24 hours and thereafter for 14 days.

Antihyperlipidemic activity evaluation

The rats were divided into 5 groups each containing 6 rats each.Group I: Normal control

Group II: High fat diet control

Group III: High fat diet + Atorvastatin 10 mg/kg p.o.

Group IV: High fat diet + Ethanolic extract of *Bombex ceiba*(ABCL) 200mg/kg p.o.

Group V: High fat diet + Ethanolic extract of *Bombex ceiba*(ABCL)400mg/kg p.o.

For the induction of hyperlipidemia, high fat diet (1 ml /100g) was given daily through intragastric route to all rats except group 1 for 4 weeks. Blood was collected through retro orbital plexus for the confirmation of induction of hyperlipidemia. Rats showing cholesterol levels above 130 mg/dl were considered hyperlipidemic. After the confirmation of induction of hyperlipidemia, group II continued to receive high fat diet alone while groups III, IV and V received atorvastatin (10 mg/kg p.o.), ABCL (200 mg/kg p.o.) and ABCL (400 mg/kg p.o.) respectively along with high fat diet for the next 28 days. The body weight of each animal in every group was recorded once weekly till the end of the study and percentage change in body weight was determined.

At the end of the study i.e. on 60th day, after overnight fasting, blood was collected from the retro-orbital plexus using micro capillary tube. The serum was separated by centrifugation at 2000 rpm for 10 minutes which was subjected to biochemical evaluation. Plasma has been used for the estimation of lipid peroxide level. The rats were then euthanized by isoflurane (2.5ml) and the aorta was dissected and stored in formalin for histopathologic evaluation.

Serum lipid profile estimation

Total cholesterol, triglycerides, LDL, HDL and VLDL were estimated through diagnostic kits. LDL was calculated by using Friedwald formula. Atherogenic index was calculated by the formula

Atherogenic index = log [TG / HDL]

Hepatic markers

AST and ALT were estimated through enzymatic kits.

Estimation of lipid peroxides in plasma[13-15]

The most commonly used assay for the determination of oxidative stress in plasma is the TBARS (Thiobarbituric acid reactive substances) assay, which measures the concentration of Malondialdehyde produced due to degradation of unstable lipid peroxides. To 0.5µl of plasma, 1.5 ml of 20% Trichloroacetic acid was added. It was centrifuged for 10 minutes at 3400 rpm. To 1 ml of the supernatant, 2 ml of 0.8% Thiobarbituric acid in HCl was added. The contents in the tube were mixed well and kept in the water bath at 65^0 for 20 minutes. After cooling, the solution was centrifuged at 2000 rpm for 10 minutes and the precipitatewas removed. The absorbance of the supernatant was determined at 532 nm against a blank that contained the reagent minus the biological sample. The concentration

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of MDA is calculated using extinction coefficient of MDA-TBA complex which is 1.56 x 105 M^{-1} cm^{-1}. The concentration of MDA in blood plasma was expressed as nmol MDA/ ml.

$$\text{MDA concentration} = \frac{\text{Absorbance at 532 nm}}{1.56 \times 10^{5}}$$

Statistical analysis[17]

All data are expressed as mean ± SEM. Data were statistically analyzed using one-way analysis of variance (ANOVA) using Graph Pad Prism software 7.04. The Dunnett's t-test was applied for the detection of significance between different groups with respect to control and standard. $P < 0.05$ was considered to be significant.

3 RESULTS

Acute toxicity study

The results of acute toxicity studies have shown that the ethanolic extracts of *Bombex ceiba* are non-toxic at the dose of 2000 mg/kg body weight and this dose did not induce any mortality in the treated rats. The behavioral and physical observations of the ethanolic extract of *Bombex ceiba* treated rats are given in Table 1

Table 1: Behavioral and physical observations of the ethanolic extract of *Bombex ceiba* treated rats

Sl 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

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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	**Piloerection**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18	**Nail status**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	**Gauntness**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	**Diarrhoea**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
22	**Sleep**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
23	**Coma**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	**Lethargy**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
25	**Mucous membrane**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

From the observations it was seen that there was no characteristic behavioral or physical changes.

Anthyperlipidemic Activity

Table 2 -Effect of ABCL *on* Lipid Profile in high-cholesterol diet induced hyperlipidaemia

GROUP	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	VLDL mg/dl)	LDL (mg/dl)
Control	82.1±3.79	42.12±1.34	68.89±2.12	12.79±0.42	26.39±3.55
Positive control	146.72±2.5	34.19±2.82	142.18±3.80	25.83±0.75	64.35±2.82
Atorvastatin 10mg/kg	87.92±1.76	46.34±2.02	99.1±2.35***	20.53±0.64	21.23±2.42**
200mg/kg ABCL	108.42±2.35	40.81±2.40	116.02±2.10**	23.5±0.42	44.73±2.33
400mg/kgABCL	92.35±1.63**	43.49±2.18***	101.06±2.89	20.6±0.60	28.59±2.02**

Values were mean ±sd (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01,***P<0.001 Vshyperlipidemic control using one way ANOVA followed by Dunnet's test.

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Table 3 – Effect of ABCL on Total Cholesterol in high cholesterol diet inducedhyperlipidaemia

GROUP	TOTAL CHOLESTEROL (mg/dl)
Control	82.1±3.79
Toxic control	146.72±2.5
Standard (Atorvastatin 10mg/kg)	87.92±1.76
ABCL(200mg/kg)	108.42±2.35
ABCL(400mg/kg)	92.35±1.63**

ABCL - *Alcoholic Extract o f BOMBEX CEIBA Leaves*

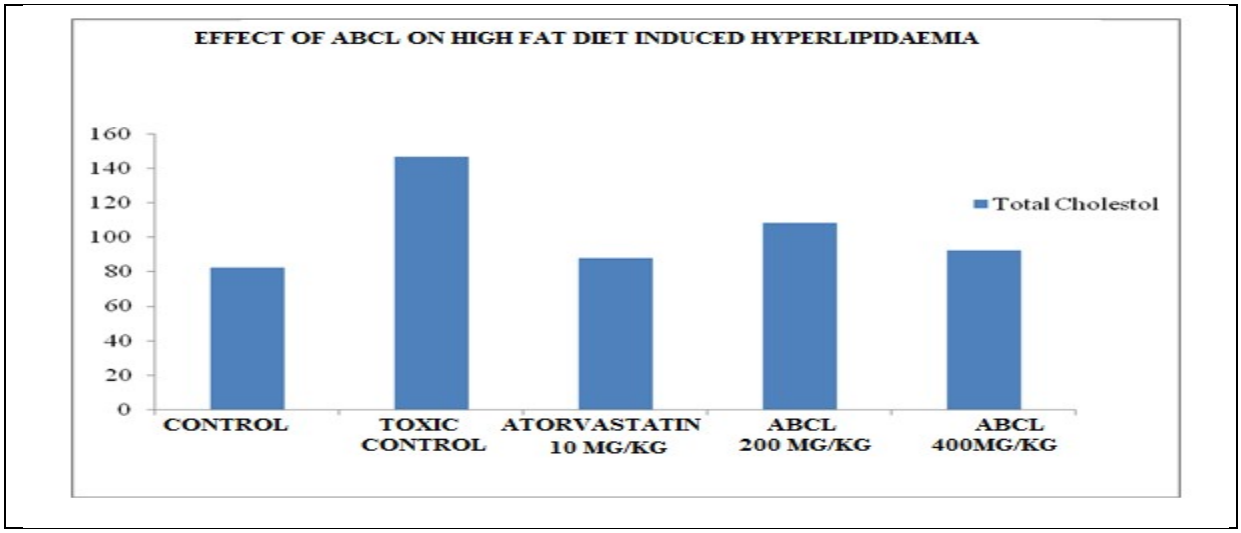

Fig. 1: Effect of ABCL on Total Cholesterol in high cholesterol diet induced hyperlipidaemia

Table 4- Effectof ABCL on HDLin high cholesterol diet inducedhyperlipidaemia

GROUP	HDL (mg/dl)
Control	42.12±1.34
Toxic control	34.19±2.82
Standard (Atorvastatin 10mg/kg)	46.34±2.02
ABCL(200mg/kg)	40.81±2.40
ABCL(400mg/kg)	43.49±2.18***

ABCL - *Alcoholic Extract of Bombex Ceiba Leaves*

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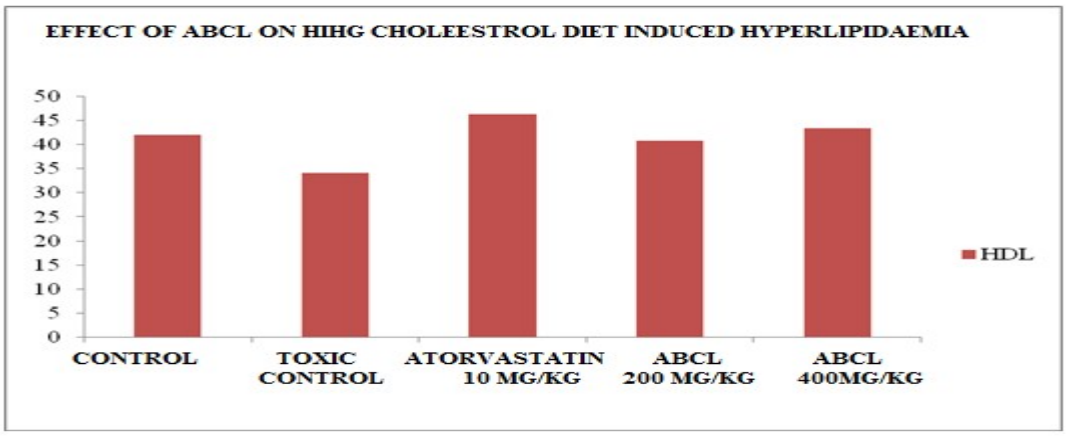

Fig.2: Effect of ABCL on HDL in high cholesterol diet induced hyperlipidaemia

Table 5 - Effect of ABCL on Triglycerides in high cholesterol diet inducedhyperlipidaemia

GROUP	TRIGLYCERIDES (mg/dl)
Control	68.89±2.12
Toxic control	142.18±3.80
Standard (Atorvastatin 10mg/kg)	99.1±2.35***
ABCL(200mg/kg)	116.02±2.10**
ABCL(400mg/kg)	101.06±2.89

ABCL - *Alcoholic Extract of Bombex Ceiba Leaves*

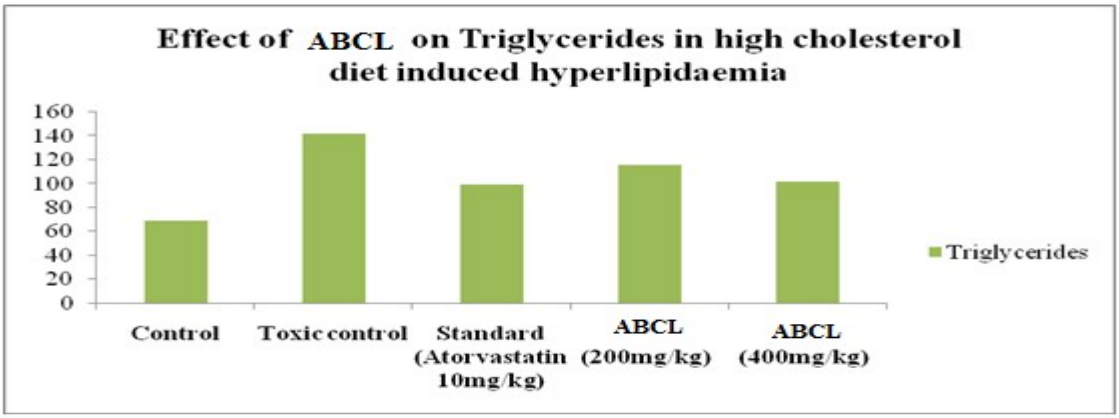

Fig. 9: Effect of ABCL on Triglycerides in high cholesterol diet induced hyperlipidaemia

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Table 6: Effect of ABCL on LDL in high cholesterol diet inducedhyperlipidaemia

GROUP	LDL (mg/dl)
Control	26.39±3.55
Toxic control	64.35±2.82
Standard (Atorvastatin 10mg/kg)	21.23±2.42**
ABCL(200mg/kg)	44.73±2.33
ABCL(400mg/kg)	28.59±2.02**

ABCL - *Alcoholic Extract of Bombex Ceiba Leaves*

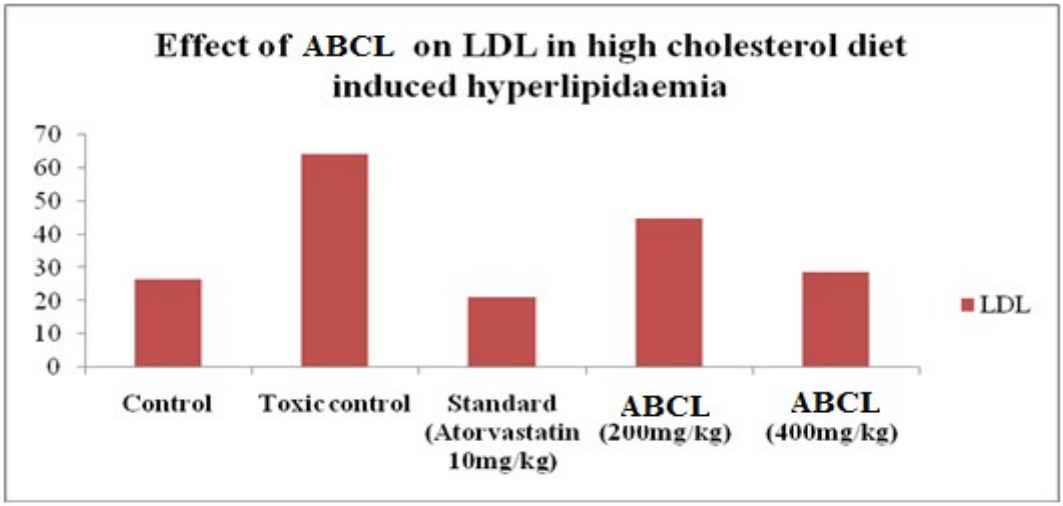

Fig. 3:Effect of ABCL on LDL in high cholesterol diet inducedhyperlipidaemia

Table 7: Effect of ABCL on VLDL in high cholesterol diet induced hyperlipidaemia

GROUP	VLDL (mg/dl)
Control	12.79±0.42
Toxic control	25.83±0.75
Standard (Atorvastatin 10mg/kg)	20.53±0.64
ABCL(200mg/kg)	23.5±0.42
ABCL(400mg/kg)	20.6±0.60

ABCL - *Alcoholic Extract of Bombex Ceiba Leaves*

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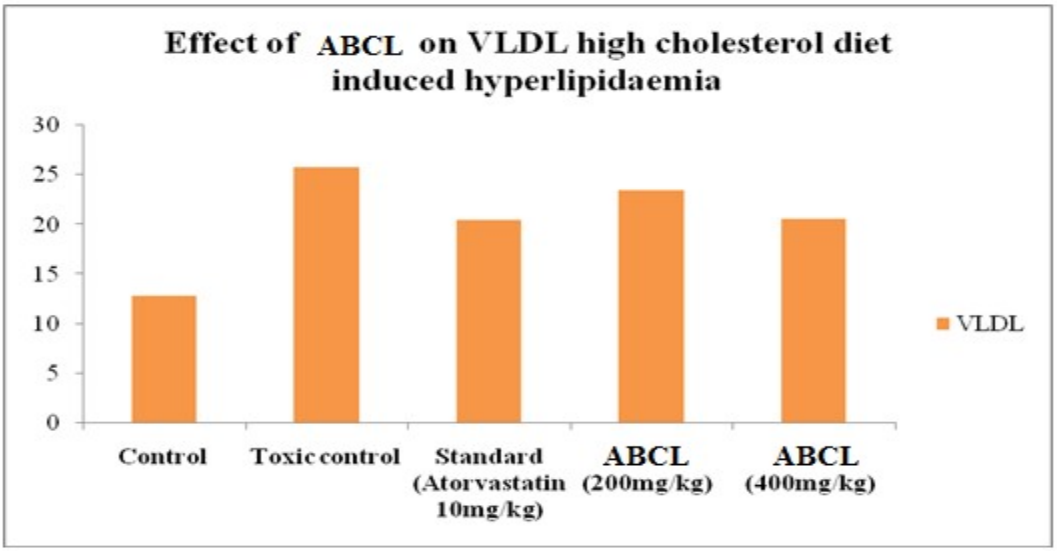

Fig.4: -Effect of ABCL on VLDL in high cholesterol diet induced hyperlipidaemia

4. DISCUSSION

Bombex Ceiba is a large, evergreen tree found generally in Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands. Different parts of the plant, studied revealed antibacterial, anti- inflammatory, antidiabetic, antioxidant and immune modulatory properties.The antioxidant screening shows that that it showed reducing power to DPPH radicals. But the efficiency showed is far below from Vitamin C. Considering super oxide radical scavenging studies, ethanolic extract shows similar percentage of inhibition comparing the standard drug.

Acute phase toxicological studies reports no mortality or signs of toxicity upto 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.The preliminary phytochemical screening of plant *Leaves* extracts indicate in presence of flavonoid, alkaloid, tannins, terpenoids and glycosides may accounts antioxidant and anti-hyprelipidaemic potential.The presences of various phyto-constituents like alkaloids, flavonoids, terpenoids, phytosterols, saponinsetc, were responsible for the specified pharmacological effects. Alcoholic extracts shows more number of phytoconstituents..Oral administration of ethanolic *Leaves* 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 ($p<0.001$).High Fat Diet induces acute hyperlipidemia by raising cholesterol levels raise 2-3 times within 24 hours of administration.. The mechanism of the High Fat Diet induced hypercholesterolemia is thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton to interfere with the uptake of plasma lipids by the tissue high fat diet induced study results shows serum lipid parameters in animals were significantly reduced ($p<0.01$,) by seven days treatment with ABCL at dose levels 200 mg/kg and 400 mg/kg, when compared with control group 400 mg/kg of ABCL group animals has shown significant ($p<0.001$) compared with control group. Atthis time, an increased level of HDL was also observed.

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Conflict of Interest

The authors declare that they have no conflict of interest