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EVALUATION OF ANTI-HYPERLIPIDEMIC ACTIVITY OF LEAVES OF IXORA CHINENSIS IN ALBINO RATS

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Abstract:
Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein (HDL) concentration. Hyperlipidemia is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death in developed as well as developing nations. And hence only in this approach, intention has been to evaluate the anti- hyperlipidemic and antioxidant activity of Ixora chinensis leaf extract on High fat diet-induced hypercholesterolemia and triton induced hyperlipidemia models. . It has been observed from our experimental research, there has been a remarkable and significant activity observed, against the anti-hyperlipidemic activity. Futuristic scope of this study will be extended to the investigation and isolation of the specific components responsible for antihyperlipidemic trait.

Keywords: *Anti-hyperlipidemic activity, Leaves, ixora chinensis, Albino rats*

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INTRODUCTION:

Hyperlipidemia is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. This is also referred to as high blood cholesterol and triglycerides. It is one of the major risk factors for atherosclerosis, and other heart diseases including coronary artery disease, high blood pressure, stroke and other problems. [1] According to World Health Organization (WHO), in 2008, 17.3 million people died from Cardio vascular diseases (CVDs), of these deaths, 7.3 million were due to coronary heart disease and 6.2 million were due to strokes.CVDs are projected to remain the single leading cause of death representing 30% of all global deaths. [2] Currently available hypolipidemic drugs though have good efficacy, are also associated with adverse effects. [3]

Ixora is a large genus of about 400 species of popular evergreen shrubs and small trees, many of which are native to southern china, India and Srilanka and belong to the taxonomic family rubiaceae. A few of these species are grown in gardens for their vibrantly coloured flowers produced in large clusters at the tips of branches. Various species of Ixora are otherwise called as West Indian Jasmine, Jungle Flame, Flame of the World, Jungle Geranium etc., but are very popular by the genus name, Ixora. Ixora is a compact, multibranched evergreen shrubs or small tree, commonly 4 to 6 feet in height, but can reach up to 12 feet height. Leaves are glossy, leathery, oblong, about 4 to 6 inches long and are arranged in opposite pairs. A decoction of the root is used after child birth. A decoction is used against bronchial disorders. An infusion of the fresh flowers is said to be a remedy against incipient tuberculosis and haemorrhage. A decoction is prescribed in the treatment of amenorrhoea and hypertension. An infusion of leaves or flowers is used against headache.

To successfully evaluate the anti-hyperlipidemic and antioxidant activity of *Ixora chinensis leaf extract on* High fat diet-induced hypercholesterolemia andtriton induced hyperlipidaemia models. The objectives are to conduct a literature survey for establishing the relevance of the study, [4-8] tcollect and authenticate *Ixora chinensis* leaves to characterize the antioxidant property of the extract

MATERIALS AND METHODS:

Solvent Extraction and Phytochemical Screening:

Plant collection and extraction *Ixora chinensis* were collected from the regions of ananthagiri hills vikarabad, Telengana. After that the plant parts such as leaf and was coarsely powdered and subjected to successive solvent extraction using soxhlet apparatus.

The leaves were initially separated from the main tree and rinsed with distilledwater and shade dried and then homogenized into fine powder and stored in air tight bottles. Calculated amount of powder in contrasting number of organic solvents in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it wasfiltered with the help of muslin cloth and centrifuged. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume[88-92]. It was stored at 40 °C in air tight bottles for further studies.

Phytochemical screening Qualitative phytochemical screening with the extract of both the plants *Ixora chinensis* was determined as follows:

Phytochemical Test:

Maeyer's reagent:

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0g of Potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Dragendorff's reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 mlof distilled water. Both solutions (A and B) were mixed in1:1 ratio.

Test for alkaloid

About 0.5 to 0.6 g of the plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's).

Test for steroid

About 0.5 g of the extract was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

Test for terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of CHCl3 in a test tube. 3ml of concentrated H2SO4 was carefully added to the mixture to form a layer.

Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentratedHydrochloric acid were added and boiled for five minutes.

Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

Test for Phytosterol

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the testtube.

Test for Phytosterol

Foam Test: 5 ml of the test solution taken in a test tube was shaken well for fiveminutes.

Test for glycosides

Keller -Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuricacid was added along the side of the test tube carefully.

Hydroxyanthraquinone Test; To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

Animals:

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House of the study centre are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) and animal care wastaken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute Toxicity Studies:

Acute toxicity studies for the extracts were conducted as per OECD guidelines423 using female Albino rat. Each animal was administered the extracts by oral route.

The animals were observed for mortality up to 72 hrs. The ethanolic and aqueous extract was found to be safe up to 2000 mg/kg body weight.

Experimental design:

In the experiment, the rats were divided into three groups of eight rats each. Group I rats received 5% CMC and considered as controls, Group II rats were treated with Triton WR-1339 (400 mg/kg body weight with Ethanolic extract) and Group III rats were treated with Triton WR-1339 (400 mg/kg body weight with aqueous extract) and ethanolic extract of *Ixora chinensis* leaf (200mg/kg body weight) andStandard fenobirate (100mg/kg body weight).

At the end of 8th day, rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected, and serums were separated by centrifugation. Liver tissues were excised immediately and rinsed in ice-chilled normal saline, 500mg ofthe tissues were homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH, 7.4). Biochemical estimations were carried out in serum and liver tissues, parameters such as cholesterol (Zak's, 1977), phospholipids (Rouser *et al*., 1970), triglycerides (Rice, 1970), LDL (Friedwald Levy and Frederickson, 1972), VLDL (Henry *et al*., 1998) and HDL (Varley *et al*., 1980) were analyzed.

Statistical analysis:

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnnet t test using Graph Pad prism software package 9.05. Results were expressed as mean ± SD from 8 rats in each group. *P* values <0.05 were considered as significant.

RESULTS AND DISCUSSION:

Table 1. Photochemical test

S.No	PHYTOCHEMICAL TEST	EXTRACTS	
		Aqueous	Ethanolic
1	Alkaloids	-	-
2	Tannins	-	+
3	Flavonoids	-	-
4	Steroids	-	+
5	Phenols	+	+
6	Glycosides	+	+
7	Terpenoids	+	+
8	Anthraquinones	-	+
9	Saponins	+	-
10	Caridiac glycosides	-	-

The toxicity for the aqueous and ethanolic extracts leaf of *Ixora chinensis* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies. There was no sign of toxicity for first 48 hours and no animal died on 14 days of study at a dose of 2000 mg/kg.

Table 2. Cholesterol induced diet model

Sample	Treatment (mg/dl)				
Serum	Control	HC	HC+ Ethanolic Extract	HC+ Aqueous Extract	HC+ Standard (Fenofibrate)
TC	152.6 ± 2.0	219.0 ± 9.0	179.86±0.98	165.3±0.94	156.6 ± 5.2ab
TG	138.3 ± 23.0	225.0 ± 8.0	165.0 ± 7.0	142.9 ± 6.5	139.0 ± 7.0
LDL	80.9±0.27	136.±0.63	107.05±0.82	94.51±0.37	84.59±0.56
HDL	45.0 ± 2.4	38.0 ± 4.4	39.0 ± 3.1	42.3±0.56	44.2±0.17

HC- High Cholesterol; GMF- Gemfibrozil; TC-total cholesterol; TG- triacylglycerol; LDL- low density lipoprotein; HDL- High density lipoprotein

Table 3. Blood parameters

Sample	**Treatment (mg/kg/b.wt)**				
Serum	Control	HC	HC+ Ethanolic Extract	HC+ Aqueous Extract	Standard (GMF)
RBC (x 10^6/Ml	7.56±0.33	5.64±0.17	6.11±0.34	6.89±0.45	7.34±0.48
Hb (g/dl)	12.6±0.46	8.56±1.76	10.55±0.58	13.22±0.78	13.03±0.56
PCV (%)	38±1.56	29.33±0.77	36.3±1.43	35.89±0.77	36.77±0.34
MCV (pg)	91±0.34	70.33±0.87	90.4±0.47	89.83±0.29	92.38±0.58
MCH (pg)	29.30±0.56	25.14±0.46	26.31±1.87	27.37±0.63	30.62±1.20
MCHC (g/dL)	30.34±0.87	26.95±1.56	30.34±0.56	29.57±0.33	31.56±0.45
WBC (x 10^3/ µL	6.97±1.56	9.97±0.04	9.22±0.56	9.12±0.54	7.59±0.53
Neutrophils (%)	54.36±1.20	34.70±0.42	40.89±0.76	45.35±0.78	50.64±0.74
Monocytes (%)	6.00±2.03	4.99±0.13	5.68±0.57	6.07±0.67	5.43±0.66
Lymphocytes (%)	29.30±0.78	54.0±0.56	48.87±0.56	39.40±0.41	32.67±0.62
Eosinophils (%)	1.90±0.03	2.03±0.32	1.67±0.79	2.05±0.55	1.78±0.21
Basophils (%)	0.24±0.04	0.35±0.23	0.29±0.13	0.25±0.54	0.23±0.50
Platelets (x10^3/µL	325±1.56	647.0±0.45	415.0±0.58	392.0±0.82	341.4±0.16

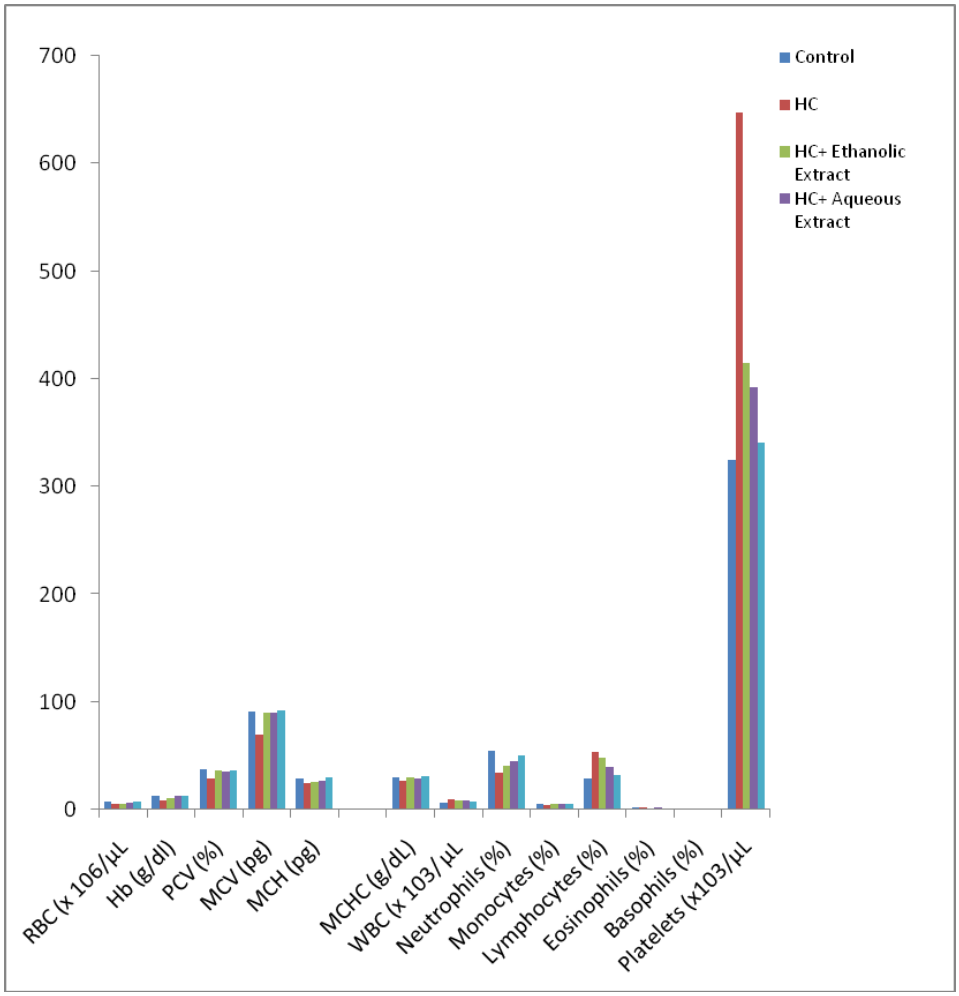

Figure 1: Haematological Parameters

Table 4: Effect of *Ixora chinensis*on changes in the levels of cholesterol and phospholipids in serum and liver tissue of control and experimental animal

Groups	Cholesterol		Phospholipids	
	Serum	Liver	Serum	Liver
Control	078±2.12	071±1.85	052±0.45	083±1.87
Ethanolic extract+Triton (200 mg/Kg)	098±0.33	097±0.54	064±0.58	092±0.33
Aqueous extract+ Triton(400 mg/Kg)	084±0.87	077±0.33	056±0.67	088±0.76
Standard Fenofibrate	078±0.23	072±1.59	051±1.62	085±1.62

Each value is mean ± SD for eight rats in each group, one way ANOVA followed byDunnett's test.

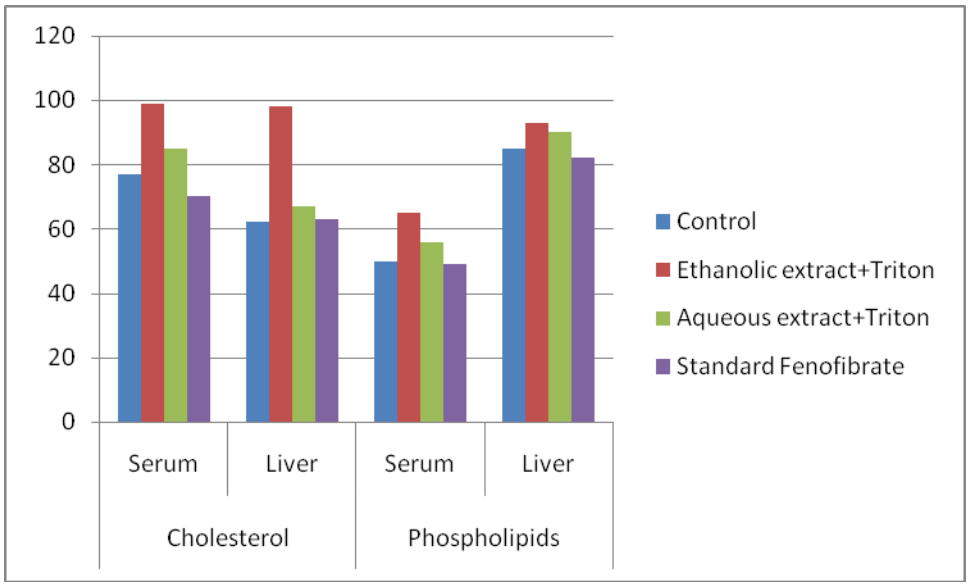

Figure 2: Scheme of Effects of Cholesterol and Phospholipids

Table 5: Effect of *Ixora chinensis*on changes in the levels of triglyceridesand LDL in serum and liver tissue of control and experimental animal

Groups	Triglycerides		LDL	
	Serum	Liver	Serum	Liver
Control	075±1.98	061±2.00	038±1.44	021±0.76
Ethanolic extract+Triton	096±0.65	093±0.78	065±0.39	035±0.58
Aqueous extract+Triton	088±0.57	064±1.98	060±1.52	030±1.77
Standard Fenofibrate	069±2.01	058±0.73	035±0.42	018±0.36

Each value is mean ± SD for eight rats in each group, one way ANOVA followed byDunnet t test.

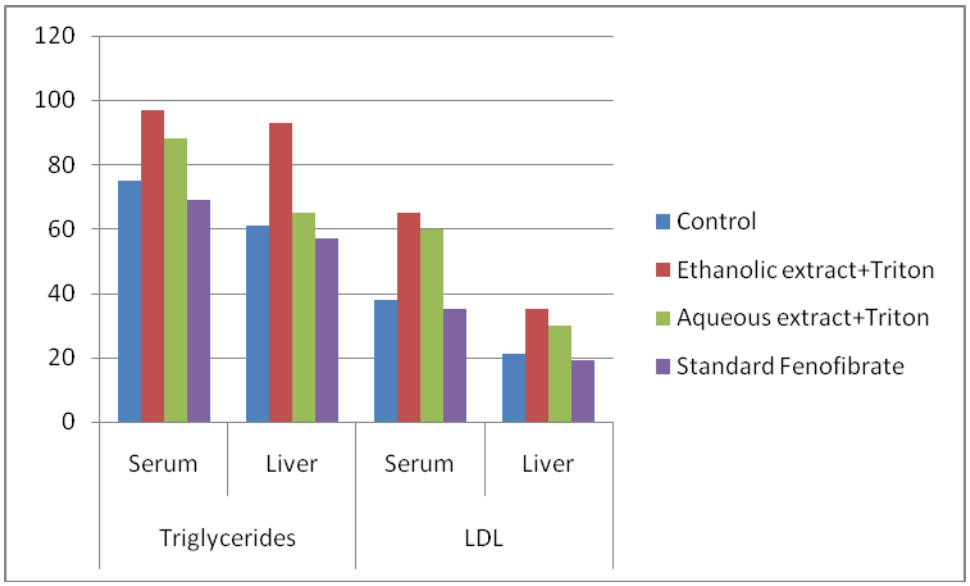

Figure 3: Scheme of Effects of triglycerides and LDL

Table 6: Effect of *Ixora chinensis* on changes in the levels of VLDL andHDL in serum and liver tissue of control and experimental animal

Groups	VLDL		HDL	
	Serum	Liver	Serum	Liver
Control	016±2.60	013±2.05	039±1.62	030±0.24
Ethanolic extract+Triton	015±0.55	014±0.73	045±1.56	036±0.56
Aqueous extract+Triton	014±1.83	011±0.56	055±0.47	030±0.35
Standard Fenofibrate	012±0.92	010±0.37	057±1.83	047±0.28

Each value is mean ± SD for eight rats in each group, one way ANOVA followed byDunnett's test.

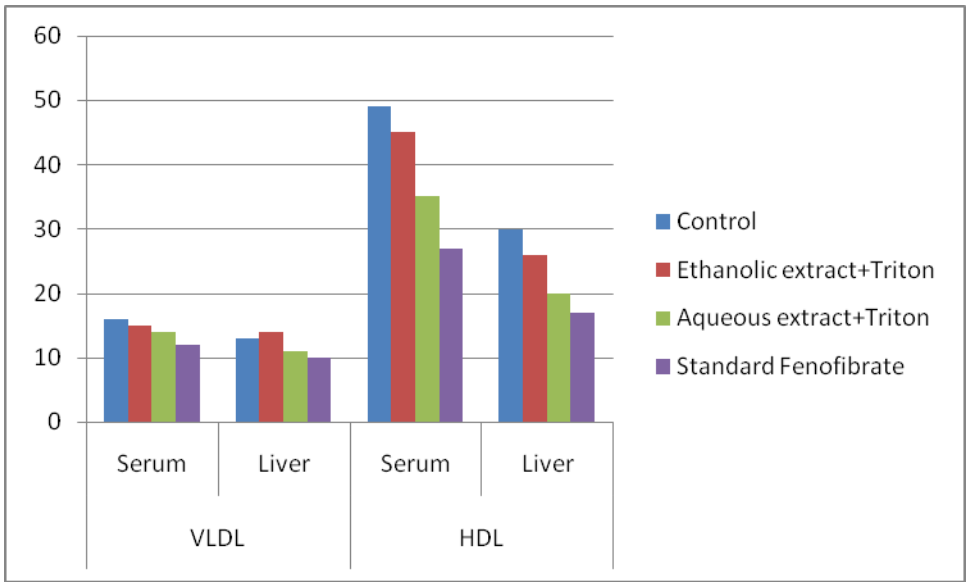

Figure 4: Scheme of Effects of VLDL and HDL

EFFECT ON NORMOCHOLESTEREMIC RATS:
The hypolipidemic effects of the extracts were evaluated in 4 groups fasted for 18hours and these studies were carried out as described for antihyperlipidemic effects.

The rats were treated orally for 7 days. After the end of the stipulated period of drug treatment, all the animals were starved for 20 hours and blood samples were collectedfrom the puncture of retro-orbital plexus and analyzed for blood lipid profile.

Table 7. Effect of the extracts on blood lipid profile

Groups	Blood lipid profile			
	Cholesterol	**Triglycerides**	**HDL**	**LDL**
Control	149.6 ± 2.0	221.0 ± 9.0	79.86±0.98	85.3±0.94
Ethanolic extract	136.3 ± 23.0	227.0 ± 8.0	87.0 ± 7.0	122.9 ± 6.5
Aqueous extract	81.9±0.27	137.±0.63	104.05±0.82	94.51±0.37
Standard Fenofibrate	46.0 ± 2.4	39.0 ± 4.4	129.0 ± 3.1	86.3±0.56

In statistical analysis the extract treated groups have been compared with theirrespective control.
P<0.01 (ANOVA followed by Dunnett's t-test)

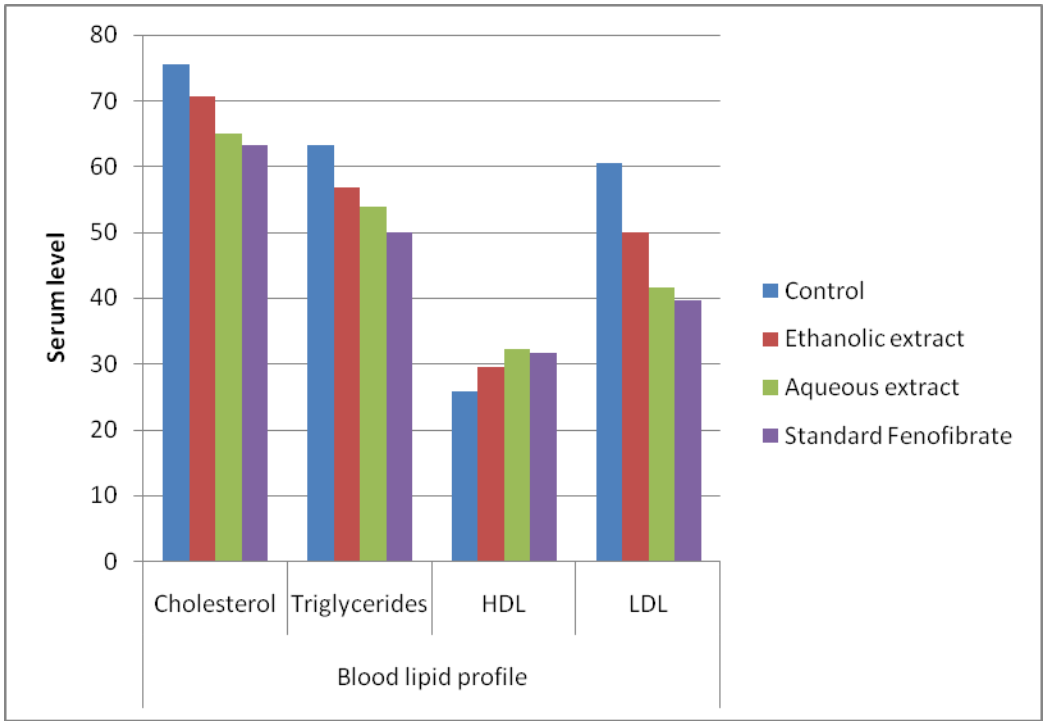

Figure 5: Scheme of Blood lipid profiles

DISCUSSION:

The antihyperlipidaemic and antioxidant activity of the plant leaf extract isstudied and the significance is evaluated. Aqueous Extract of the taken sample was found to be best.

Table 8. Superoxide Radical Scavenging activity

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	73.50±0.70	46.46±0.34	24.23±1.45
50	77.63±3.12	56.47±0.86	38.01±0.34
75	82.33±0.96	65.00±0.87	48.44±0.14
100	85.93±0.79	70.23±1.46	59.36±1.11
150	93.30±0.02	73.56±1.76	68.36±1.08
200	94.54±0.06	75.22±0.60	70.57±0.59

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed freeradicals with the constituents present in both the samples.

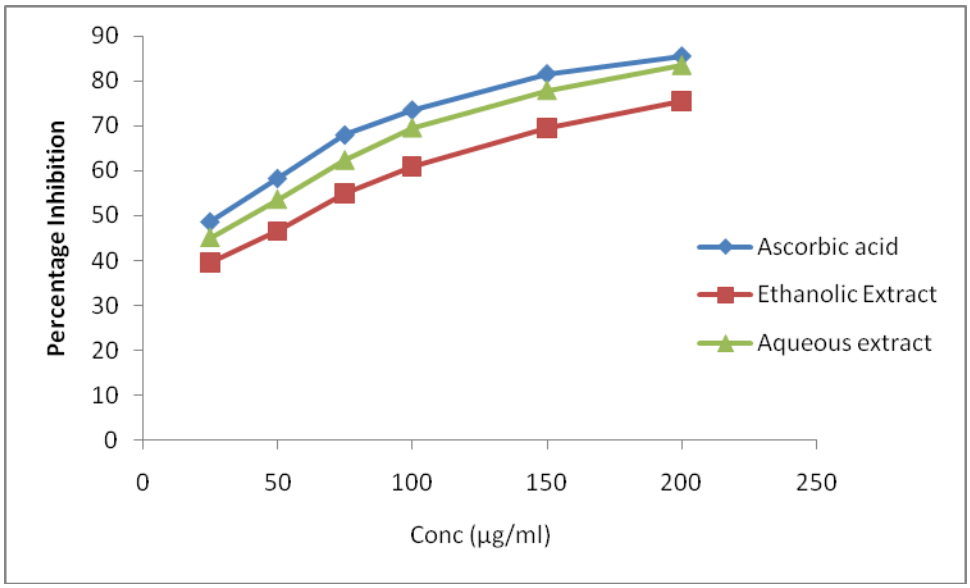

Figure 6: Schematic representation of superoxide radical scavengingactivity of all the extracts

Table 9. DPPH Assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	67.20±0.22	62.25±0.29	66.24±0.90
50	71.44±0.57	63.54±0.62	68.52±0.13
75	77.22±0.67	64.22±0.56	72.44±2.76
100	79.44±0.29	68.77±0.56	77.34±1.56
150	88.47±1.05	75.24±2.33	85.59±1.99
200	93.02±0.02	77.23±0.24	88.33±1.97

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibitionAll the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed freeradicals with the constituents present in both the samples.

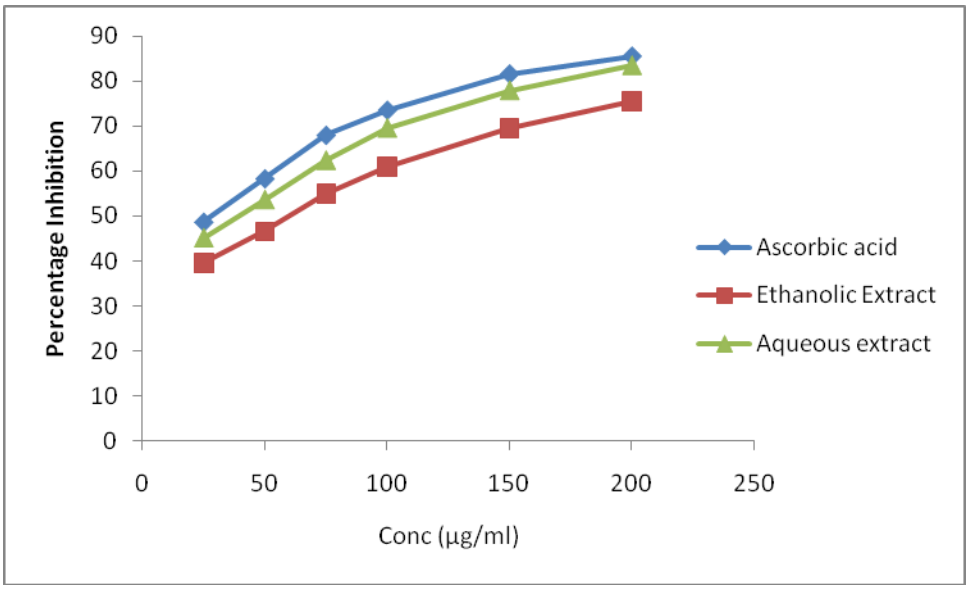

Figure 7: Schematic representation of DPPH activity of all the extracts

Table 10. Lipid per oxidation Assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	69.52±2.97	45.33±3.45	54.55±1.36
50	74.56±1.93	53.34±1.25	61.50±1.50
75	81.47±0.48	61.45±1.34	69.45±1.78
100	84.32±1.62	67.77±1.36	74.38±0.56
150	88.52±0.35	74.28±3.46	83.67±1.91
200	89.67±0.46	78.54±0.87	86.33±0.73

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibitionAll the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standardascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed freeradicals with the constituents present in both the samples.

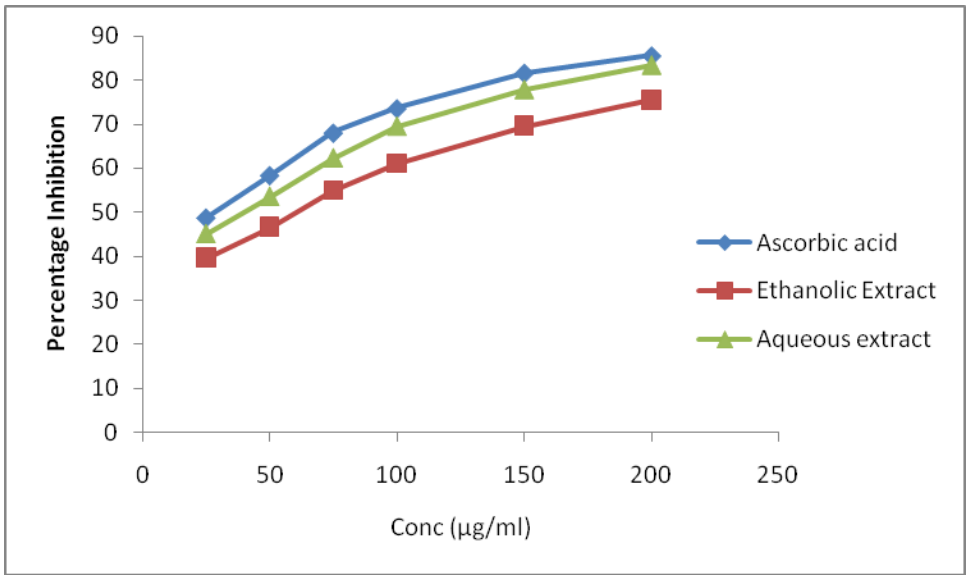

Figure 8: Schematic representation of Lipid per oxidation Assay of all theextracts

Table 11. Nitric oxide scavenging assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	43.50±0.62	30.62±0.56	35.33±2.56
50	55.36±1.57	36.57±0.33	49.44±0.45
75	68.22±0.32	42.67±1.56	60.67±0.77
100	80.12±0.66	50.28±1.36	72.53±1.32
150	91.56±0.23	60.12±0.38	87.62±1.66
200	95.33±2.34	69.88±0.34	93.44±0.65

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibitionAll the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standardascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed freeradicals with the constituents present in both the samples.

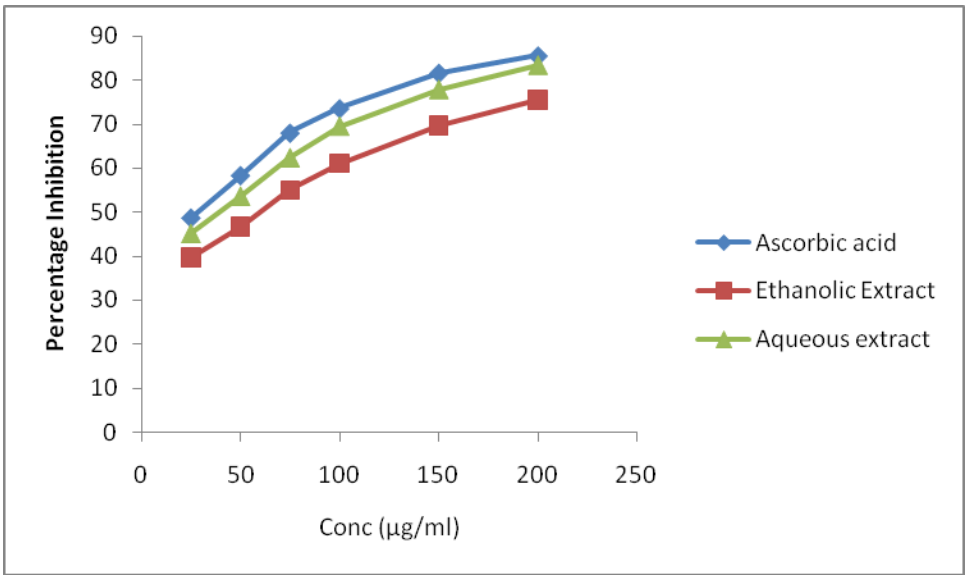

Figure 9: Schematic representation of Nitric oxide scavenging assay of allthe extra

Table 12. Hydrogen per oxide assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	48.66±0.22	39.56±0.34	45.09±0.42
50	58.32±0.68	47.58±1.26	54.60±1.52
75	67.99±1.66	55.88±1.62	62.36±1.67
100	74.56±0.12	60.97±1.42	69.56±0.79
150	81.62±0.26	69.49±1.31	76.93±1.74
200	85.57±0.12	75.45±0.38	84.49±0.57

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibitionAll the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed freeradicals with the constituents present in both the samples.

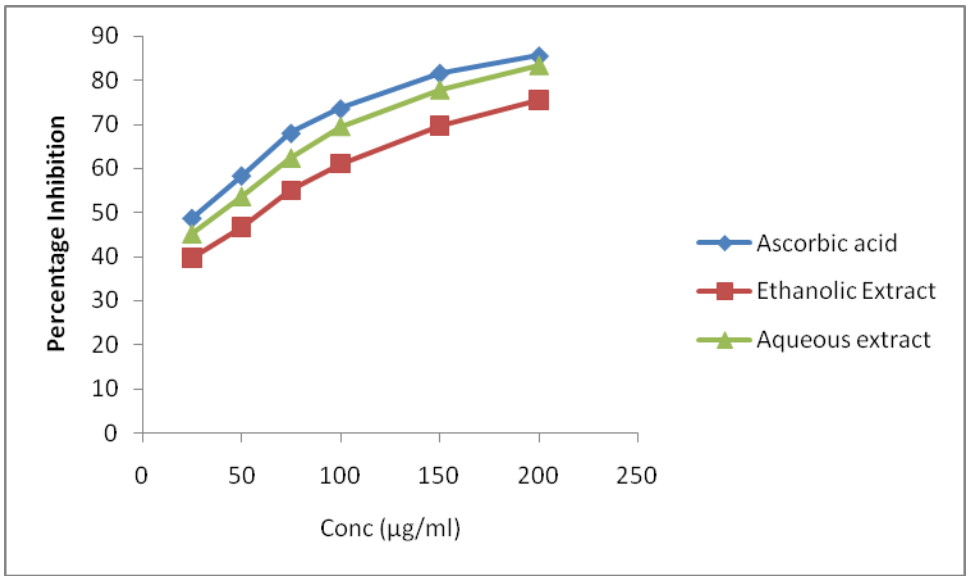

Figure 10: Schematic representation of Hydrogen peroxide assay of all theextracts

CONCLUSION:

In this current approach, we have selected *Ixora chinensis*, where its different part of plants has been in exploitation since ancient times for various benefits, including inflammation, constipation, and it has been that, in our current approach we have been investigating on the aspect and perspective of hypolididemic activity. It has been observed from our experimental research, there has been a remarkable and significant activity observed, against the anti-hyperlipidemic activity. Futuristic scope of this study will be extended to investigation and isolation of the specific components responsible for antihyperlipidemic activity.

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