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Evaluation of Anti-hyperlipidemic activity of Hydroalcoholic extract of *Ixora coccinea l.* leaves on hyperlipidemic Wistar Albino Rats

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A B S T R A C T

This study was designed to evaluate the possible anti-hyperlipidemic and antioxidant effect of hydroalcoholic leaves extract of *Ixora coccinea L.* in triton (400 mg/kg, bw) and cafeteria diet induced hyperlipidemic rats. Hydroalcoholic leaves extract of *Ixora coccinea* was evaluated for antihyperlipidemic activity in tritonand cafeteria diet induced hyperlipidemic rats. Atorvastatin (10 mg/ kg, bw) used as a standard drug for comparison. The results were expressed as mean± S.EM. and data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett's t-test for multiple comparisons. Values with P < 0.05 were considered as significant. mg/kg) showed a significant (P< 0.05) reduction in the serum total cholesterol, triglyceride

Treatment with *Ixora coccinea* (200 and 400 LDL-cholesterol and glucose level; and a significant (P< 0.05) increase in the serum HDL-cholesterol level in case of both models as compared to hyperlipidemic rats. *Ixora coccinea* leaves extract showed potential to moderate free radicals scavenging activity by reducing elevated MDA level in the liver, heart & serum and increasing in vivo antioxidant enzymes like CAT, SOD, GST, and GPx activities as compared to hyperlipidemic rats.The results demonstrated that hydroalcoholic extract of *Ixora coccinea* leaves has significant antihyperlipidemic activity and hence it could be exploited as an anti-hyperlipidemic therapeutic agent.

KEYWORDS:

Hyperlipidemia, Triton, Cafeteria diet, *Ixora coccinea*, Atorvastatin,

1. 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]

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As none among the available agents fulfil requirements of a desired drug, there is a need to explore the possibility of introducing effective, safe and inexpensive alternatives.

Traditional system of medicine consists of large number of plants with various medicinal and pharmacological importances.One of such plants *Ixora coccinea* which belongs to Rubiaceae family native to Southern India and Srilanka. It is planted worldwide in tropical and subtropical climates.[4-5]It is a densely branched shrub to 3m in height and 3 or 4cm in basal diameter. The Leaves of *Ixora coccinea* are found to have anti-inflammatory, anti-diarrheal, anti- asthmatic, anti-ulcer andanti-nociceptive activities. They are also used forskin diseases, colic, flatulence, diarrhea, indigestion, ulcers, wounds, and used as antiseptic. The flowers are used for the treatment of cancer, leucorrhoea, dysentery, dysmenorrhoea, haemoptysis and hypertension. The roots show wound healing and antimicrobial activity.[6]As there were no previous reports available for the anti-hyperlipidemic activity of this plant, the present study was undertaken to study the anti-hyperlipidemic activity of hydroalcoholic extract of *Ixora coccinea* leaves on triton and cafeteria diet induced hyperlipidemic rats with a view to provide scientific evidence.

2. MATERIAL AND METHODS

Plant material

Ixora coccinea leaves were collected from the local area of Bangalore, India duringJanuary 2014 and authenticated by Mrs. S. Noorunnisa Begum, Senior Research officer in the Institute of Ayurveda and Integrative Medicine in Yelahanka, Bangalore.

Preparation of hydroalcoholic extract

The leaves of *Ixora coccinea* were shade dried at room temperature. The dried leaves were powdered by using grinder, to coarse powder. Then this powder was packed into soxhlet column and extracted with a mixture of ethanol and water (70:30) as solvent and extracted till the solvent becomes colorless. The extract was concentrated under reduced pressure (bath temperature 50°C) and dried. The dried extract was stored in airtight container at 4°C until used. The conventional chemical tests were carried out for the hydroalcoholic extract of *Ixora coccinea* to identify the presence of various chemical constituents.

Animals

Wister Albino rats (100-150 g) of either sex were selected for screening anti-hyperlipidemic activity. The animals were housed under standard conditions of temperature (24±2°C) and relative humidity (50-60%) with a 12:12 light:dark cycle. The rats were allowed free access to feed and water one week before and during the experimental period. The animal protocol was approved by the Institutional Animal Ethical Committee **(IAEC/ABMRCP/2013-2014/01)** and experiments were performed according toguidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), **(Reg no. 997/c/06/CPCSEA)**.

Hyperlipidemia induction:

Cafeteria diet induced

Cafeteria diet was given for inducing hyperlipidemia. The cafeteria diet consisted of 3 diets (condensed milk, 48 g+bread, 48 g), (chocolate, 18 g+ biscuit, 36 g+dried coconut, 36 g) and (cheese, 48 g+boiled potatoes, 60 g). Except normal group, the three diets were presented to each group of six rats on day 1, 2 and 3 respectively and then repeated for 40 days.[7]

Triton induced

Hyperlipidemia was induced on 24 h fasting animals by intraperitoneal injection of Triton at the dose of 400 mg/kg.[8]

Drug and chemicals

Triton X-100 was procured from Sigma Aldrich, USA. Atorvastatin was a gift sample obtained from Micro Labs, Bangalore.Glucose, cholesterol, triglycerides, LDL- cholesterol and HDL- cholesterol kits were purchased from Swemed Diagnostics Ltd. All other chemicals used in the study are of analytical grade.

Preparation of standard drugs

Atorvastatin 10 mg/kg was used as the reference standard drug for evaluating the antihyperlipidemic activity which was made into suspension in distilled water using 1% Tween-80 as a suspending agent.

Experimental Design

Cafeteria diet induced hyperlipidemia

The animals were divided into five groups of six rats each. Group I: Received 0.9% normal saline solution and served as normal control, Group II: Received cafeteria dietand served as cafeteria diet induced hyperlipidemic control,Group III and IV, Received hydroalcoholic extract of *Ixora coccinea* leaves orally at doses of 200 and 400 mg/kg respectively andcafeteria diet, Group V: Received Atorvastatin (10 mg/ kg, po, in 1% tween 80) and cafeteria diet.

Rats were made hyperlipidemic by the oral administration of cafeteria diet for 45 days by mixing with regular pellet diet. The plant extracts were dissolved in distilled water and administered to the rats (Group III & Group IV) once daily in the morning through gastric intubation for 14 consecutive days. Similarly, standard drug Atorvastatin was suspended in tween 80 and given to the rats (Group V) once daily in the morning through gastric intubation for 14 consecutive days. During these days, all these groups including cafeteria diet induced hyperlipidemic control also received cafeteria diet in the same dose as given earlier. The normal control animals were received only saline. At the end of treatment period, the animals were used for the study of various biochemical parameters.[7]

Triton X-100 induced hyperlipidemia

The animals were divided into five groups of six rats each. Group I: Received 0.9% normal salinesolution and served as normal control, Group II: Received saline solution of triton at the dose of 400 mg/kg, ip, and served as triton induced hyperlipidemic control,Group III and IV: Received hydroalcoholic extract of *Ixora coccinea* leaves orally at doses of 200 and 400 mg/kg respectivelyand triton,Group V, Received Atorvastatin (10 mg/ kg, po, in 1% tween 80) and triton. All rats except the normal control group were injected saline solution of triton at the dose of 400 mg/kg intraperitoneally to induce hyperlipidemia in the rats, while the normal control group was received only saline solution. The plant extracts and the standard drug Atorvastatin were administered orally through gastric intubation, the first dose given immediately after triton injection and second dose 20 h later. After 4 h of second dose, the animals were sacrificed.[8, 9]

Parameters tested:

Lipid profile

At the end of the experiment blood was collected by retro-orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500 rpm. Then serum samples were collected and analyzed for serum Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Glucoseby using the biochemical kits.

Antioxidant profile

Tissues (heart or liver) were homogenized with a homogenizer in a volume of 10% of tissues weight with ice-cold phosphate buffer. The homogenate was centrifuged at 5,000 rpm for 15 min at 4°C. The supernatant was taken to carry out biochemical estimations. Malondialdehyde (MDA) in tissue, Malondialdehyde (MDA) in serum, Catalase (CAT), Super Oxide Dismutase (SOD), Glutathione-s-transferase (GST) andGlutathione Peroxidase (GPX)were measured using heart & liver tissue homogenate and **serum** by following standard procedures described below.

Malondialdehyde in tissue

To a sample of 0.2 ml of tissue supernatant (10%, w/v), 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution, 1.5 ml of 0.8% aqueous solution of TBA were added. The mixture was made up to 5 ml with distilled water and then heated at 95°C in oil bath for 60 min. After cooling with tap water, 5 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and the absorbance was measured at 532 nm. The tissue MDA levels were measured from the standard graph of known concentrations of MDA and expressed as nmol/g wet tissue.[10]

Malondialdehyde in serum

To a sample of 0.1 ml of serum, 4 ml of 0.083 N sulphuric acids, 0.5 ml of 10% phosphotungstic acid were added. After keeping at room temperature for 5 min, the mixture was centrifuged at 4000 rpm for 10 min and the sediment was suspended in 2 ml of 0.083 N sulphuric acids and 0.3 ml of 10% phosphotungstic acid and the mixture was centrifuged at 4000 rpm for 10 min. The sediment was suspended in 4 ml of distilled water and to this 1ml of TBA reagent was added. The reaction mixture was heated for 60 min at 95°C. After the mixture was cooled to room temperature, 5ml of n-butanol was added and the mixture was centrifuged at 4000 rpm for 10 min. Then the organic layer was taken and the absorbance was measured at 532 nm. The serum MDA level were measured from the standard graph of known concentrations of MDA and expressed as nmol/ ml.[11]

Catalase

To a sample of 0.1 ml of tissue supernatant (10%, w/v) or serum, 1.9 ml of 50 mM phosphate buffer was added. Then 1.0 ml of freshly prepared 30 mM H_2O_2 was added. A change in absorbance was taken for 3 min at 240 nm at an interval of 30 sec. Reaction mixture without the tissue supernatant or serum was used as blank. The Catalase activity was expressed as µ moles of H_2O_2 metabolized/mg protein/min.[12]

Super Oxide Dismutase

To a sample of 0.1 ml of tissue supernatant (10%, w/v) or serum, 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3), 0.1 ml of 186 µM phenazonium methosulphate, 0.3 ml of 300 µM nitroblue tetrazolium and 0.2 ml of 780 µM NADH were added. Reaction mixture was incubated for 90 s at 30°C and the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. Then the mixture was stirred vigorously and shaken with 4.0 ml of n-butanol and centrifuged at 4000 rpm for 10 min. The absorbance of organic layer was measured at 560 nm against blank. The reaction mixture without the tissue supernatant or serum was used as blank. The Super Oxide Dismutase activity was expressed as Units/mg protein.[13]

Glutathione-S-Transferase

The reaction mixture consisted of 0.1ml of tissue supernatant (10% ,w/v) or serum, 1.0 ml of 0.3 M phosphate buffer (pH 6.5), 0.1 ml of 30 mM CDNB and 1.7 ml of distilled water were added. After incubation the mixture at 37.8ºC for 15 min, 0.1 ml of GSH was added and the change in absorbance was measured at 340 nm for 3 min at an interval of 30 s. The reaction mixture without the tissue supernatant was used as blank. The glutathione-S-transferase activity was expressed as n moles of CDNB conjugated/mg protein/min.[14]

Glutathione Peroxidase

The reaction mixture consisted of 0.2 ml of tissue supernatant(10%, w/v) or serum, 0.2 ml of 0.8 mM EDTA, 0.1 ml of sodium azide, 0.1 ml of 4 mM GSH, 0.1 ml H_2O_2 solution, and 0.4 ml of 0.4 M phosphate buffer (pH 7.0). The mixture was incubated at 37°C for 10 min. The tubes were kept at room temperature and added 0.5 ml of 10% TCA and centrifuged at 2000 rpm for 10 min. After centrifugation the supernatant was taken and added 0.1 ml of 0.04% DTNB solution. Absorbance of the solution was measured at 420 nm against the blank. Reaction mixture without the tissue supernatant was used as blank. The glutathione peroxidase activity was expressed as µ moles of glutathione oxidized/ min/ mg protein.[15]

Statistical Analysis

All the values are expressed as (Mean ± SEM). Results were analyzed by using one way analysis of variance test (ANOVA). Individual group was compared against hyperlipidemic control by using Dunnett's test. P value less than 0.05 were considered as statistically significant.

3. RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of hydroalcoholic extract of *Ixora coccinea* leaves revealed the presence of carbohydrates, anthraquinone glycosides, alkaloids, protein, flavonoids, tannins and phenolic compounds.

Effect of *ixora coccinea*on serum lipid profile

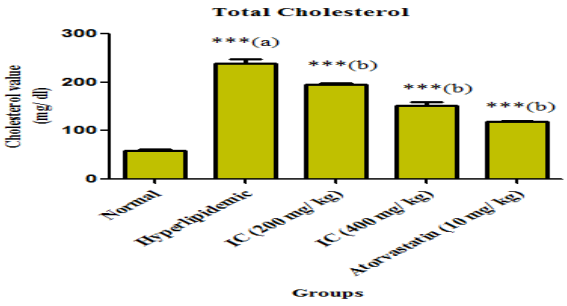
Table-1 Effect of hydroalcoholic extract of *Ixora coccinea* leaves on lipid profile in cafeteria diet induced hyperlipidemic rats

Groups	Dose (mg/kg)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Glucose (mg/dl)
Control	Normal saline	59.20 ±1.02	40.40 ±0.50	23.40 ±0.24	27.72 ±1.02	53.00 ±4.18
Diet induced Hyperlipidemic control	-	239.00 ±3.91 ***a	163.00 ±5.32 ***a	9.80 ±0.58 ***a	196.64 ±3.64 ***a	95.60 ±0.87 ***a
Ixora coccinea extract	200	194.80 ±1.35 ***b	120.40 ±1.86 ***b	13.00 ±1.09 ns b	157.72 ±1.51 ***b	87.00 ±4.68 ns b
Ixora coccinea extract	400	152.41 ±2.92 ***b	95.40 ±1.74 ***b	18.60 ±1.86 ***b	114.72 ±2.0 ***b	79.60 ±1.43 **b
Atorvastatin	10	118.24 ±1.24 ***b	73.40 ±2.31 ***b	25.63 ±0.50 ***b	77.92 ±1.28 ***b	68.60 ±2.44 ***b

The values are expressed as Mean ± SEM. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

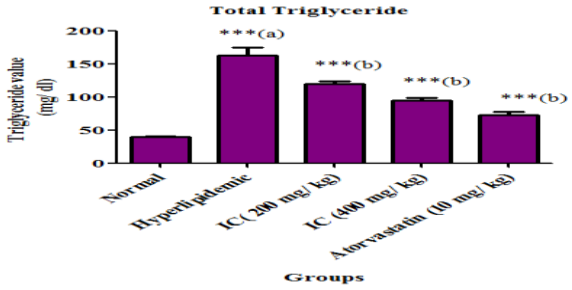
Table 1 shows represent the effect of hydroalcoholic extract of *Ixora coccinea* leaves on lipid profile in Cafeteria diet induced hyperlipidemic rats. There was a significant (P<0.05) increase in serum cholesterol, triglycerides, LDL-C and a decrease in serum HDL-C (good cholesterol) as compared to control group. Treatment of hydroalcoholic extract of *Ixora coccinea* leaves at doses of 200 & 400 mg/kg elicited significantly reduction in serum cholesterol, triglycerides, LDL-C and an increase in serum HDL-C when compared to hyperlipidemic control which was almost comparable to that of the standard drug Atorvastatin (10 mg/ kg) used in treatment.

Graph 1: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on Total Cholesterol in Cafeteria diet induced hyperlipidemic rats.



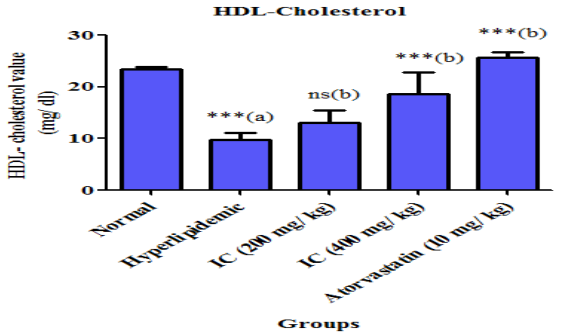
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05.

Graph 2: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on Total Triglyceride in Cafeteria diet induced hyperlipidemic rats



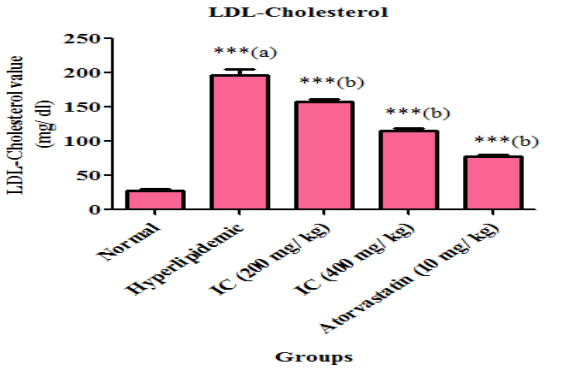
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05.

Graph 3: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on HDL-Cholesterol in Cafeteria diet induced hyperlipidemic rats



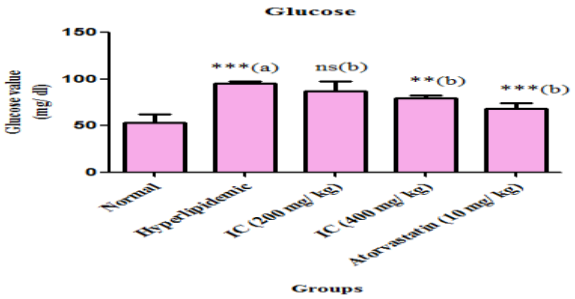
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 4: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on LDL-Cholesterol in Cafeteria diet induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05.

Graph 5: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on Glucose in Cafeteria diet induced



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Table-2 Effect of hydroalcoholic extract of *Ixora coccinea* leaves on lipid profile in Tritoninduced hyperlipidemicrat cholesterol, triglycerides, LDL-C, while increase in serum HDL cholesterol when compared with the hyperlipidemic control.

Groups	**Dose (mg/kg)**	**TC (mg/ dl)**	**TG (mg/ dl)**	**HDL (mg/ dl)**	**LDL (mg/ dl)**	**Glucose (mg/dl)**
Control	Normal saline	59.20 ±1.02	40.40 ±0.50	23.40 ±0.24	27.72 ±1.02	53.00 ±4.18
Triton induced Hyperlipidemic control	400	182.00 ±3.36 ***a	129.80 ±0.86 ***a	11.80 ±0.37 ***a	144.24 ±3.40 ***a	90.20 ±2.43 ***a
Ixora coccinea extract	200	134.00 ±2.08 ***b	97.80 ±1.15 *** b	15.60 ±1.43 ns b	99.64 ±2.14 ***b	81.60 ±1.36 ns b
Ixora coccinea extract	400	98.40 ±1.03 ***b	82.00 ±2.55 ***b	20.40 ±2.11 ***b	61.56 ±2.42 ***b	72.00 ±3.88 *b
Atorvastatin	10	78.00 ±1.90 ***b	63.60 ±2.29 ***b	22.00 ±0.54 ***b	43.48 ±2.25 ***b	61.80 ±3.02 ***b

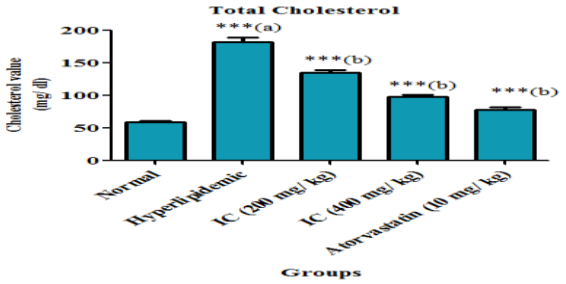
The values are expressed as Mean ± SD. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

From the data presented in Table 2 it is observed that the administration of Triton significantly (P<0.05) induced hyperlipidemia in rats. Concurrent administration of Ixora coccinea leaves extract at doses of 200 & 400 mg/kg showed a significant reduction in the levels of serum total cholesterol, triglycerides, LDL-C, while increase in serum HDL cholesterol when compared with the hyperlipidemic control.

3.3. Effect of Ixora coccineaon glucose:

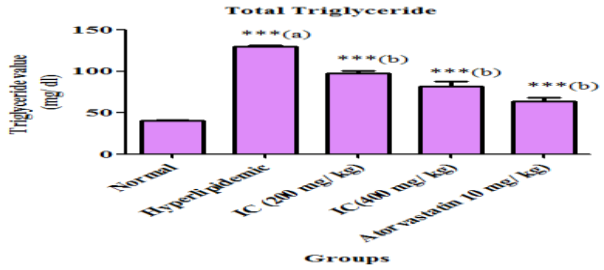
Table 1& 2 also show that rats treated with Cafeteria diet and Triton significantly (P<0.05) induced hyperglycemia. The glucose level has shown significant increased when compared with the normal group in case of both models. A significant decrease in glucose level (from 95.60 to79.60 in case of Cafeteria diet model and from 90.20 to 72.00 in case of Triton model) was observed in animals treated with Ixora coccinea leaves extract at 400 mg/kg dose, when compared to the hyperlipidemic control. However, rats treated with Ixora coccinealeaves extract (200 mg/kg) not shown significant result.

Graph 6: Effect of hydroalcoholic extract of Ixora coccinea leaves on Total Cholesterol in Triton induced hyperlipidemic rats



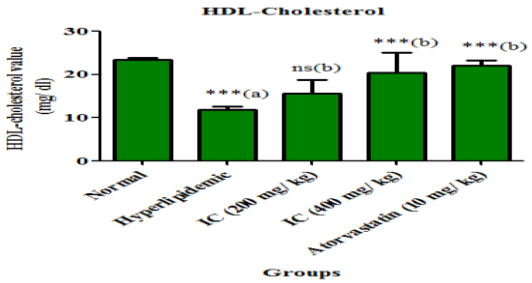
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 7: Effect of hydroalcoholic extract of Ixora coccinea leaves on Total Triglyceride in Triton induced hyperlipidemic rats



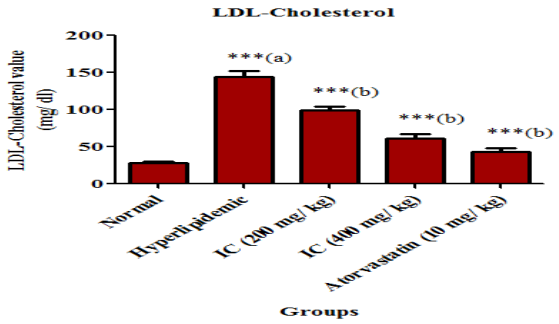
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05.

Graph 8: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on HDL-Cholesterol in Triton induced hyperlipidemic rats.



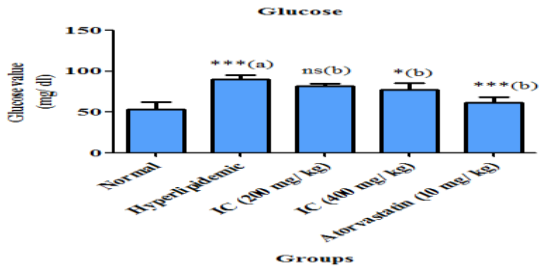
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05.

Graph 9: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on LDL-Cholesterol in Triton induced hyperlipidemic rat



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 10: Effect hydroalcoholic extract of *Ixora coccinea* leaves on Glucose in Triton induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

3.4. Effect of *Ixora coccinea*on Malondialdehyde:

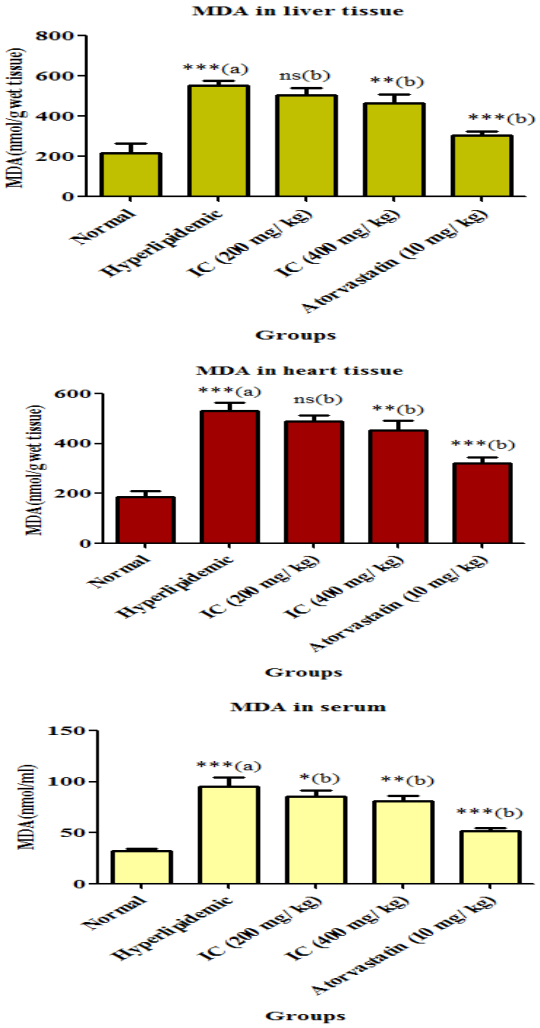
Table 3: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on MDAin Cafeteria diet and Triton induced hyperlipidemic rats

Groups	Dose (mg/ kg)	MDA (nmol/g wet tissue)				MDA(nmol/ml)	
		Liver		Heart		Serum	
		Diet	Triton	Diet	Triton	Diet	Triton
Control	Normal saline	216.20±21.81		185.10±11.51		32.49±0.89	
Hyperlipidemic control	-	552.40 ±11.44 ***a	493.60 ±5.54 ***a	530.80 ±14.86 ***a	465.70 ±11.64 ***a	95.29 ±4.09 ***a	98.80 ±1.97 ***a
Ixora coccinea Extract	200	503.00 ±17.34 ns b	466.52 ±4.16	489.70 ±11.27	429.90 ±6.72	85.32 ±	95.87 ±1.27
	400	464.40 ± 18.94 **b	435.8 ± 3.56 ** b	455.00 ±17.17 **b	411.90 ± 9.80 *b	81.45 ± 2.34 **b	91.44 ± 2.49 *b
Atorvastatin	10	303.71 ±9.73 ***b	426.40 ±11.11 **b	320.11 ±11.74 ***b	397.60 ±21.8 **b	51.82 ±1.25 ***b	57.15 ± 1.07 ***b

*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

There was significant elevation in the level of Malondialdehyde in the in the liver, heart and serum of rats treated with triton and cafeteria diet as compared to normal control (Table 3). Treatment with extract at a dose of 400 mg/kg significantly (P<0.05)decreased level of MDA as compared to hyperlipidemic control in case of both models which may be due to the free radicals scavenging activity of *Ixora coccinea* leaves extract.

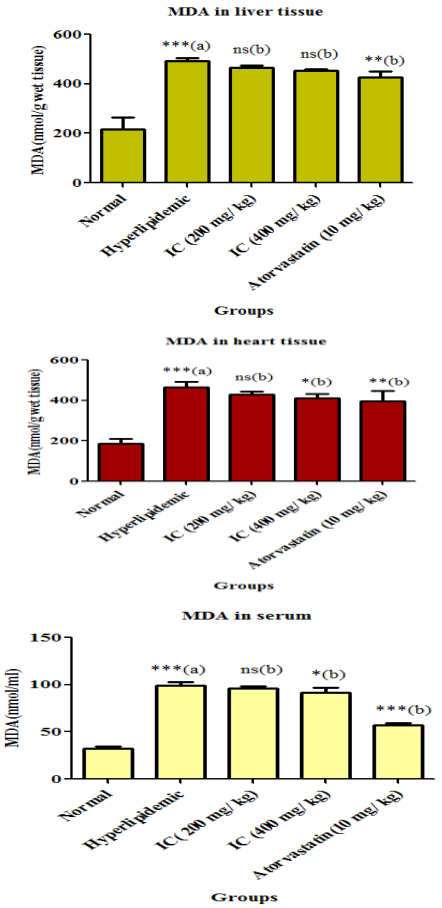
Graph 11: Effect of hydroalcoholic extract of Ixora coccinea leaves on liver, heart tissue and serum MDA in Cafeteria diet induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

There was significant elevation in the level of Malondialdehyde in the in the liver, heart and serum of rats treated with triton and cafeteria diet as compared to normal control (Table 3). Treatment with extract at a dose of 400 mg/kg significantly (P<0.05)decreased level of MDA as compared to hyperlipidemic control in case of both models which may be due to the free radicals scavenging activity of *Ixora coccinea* leaves extract.

Graph 12: Effect of hydroalcoholic extract of Ixora coccinea leaves on liver, heart tissue and serum MDA in Triton induced hyperlipidemic rats.



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

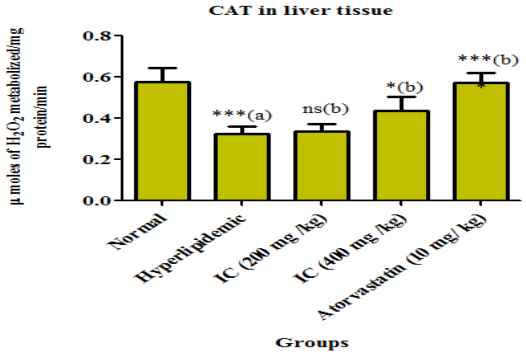
3.4 Effect of *Ixora coccinea*on antioxidant enzymes

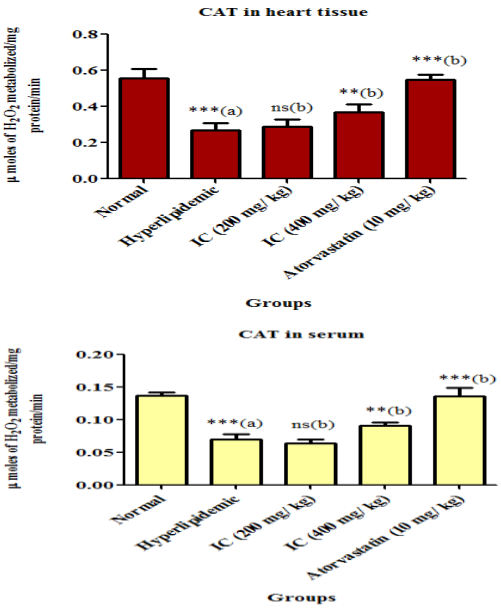
Table 4: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on CAT in Cafeteria diet and Triton induced hyperlipidemic rats

Groups	**Dose (mg / kg)**	**CAT (µmoles of H_2O_2 metabolized/mg protein/min)**					
		Liver		**Heart**		**Serum**	
		Diet	**Triton**	**Diet**	**Triton**	**Diet**	**Triton**
Control	Normal saline	0.57± 0.02		0.55± 0.02		0.13± 0.002	
Hyperlipidemic control		0.32 ±0.01 *** a	0.36 ±0.02 ** a	0.26 ±0.01 ** *a	0.30 ±0.01 ***a	0.06 ±0.003 *** a	0.001 ±0.001 *** a
Ixora coccinea Extract	200	0.33 ±0.01 nsb	0.38 ±0.01 ns b	0.28 ±0.01 ns b	0.33 ±0.005 ns b	0.06 ±0.002 ns b	0.07 ±0.001 ns b
Ixora coccinea extract	400	0.45 ±0.01 **b	0.45 ± 0.01 * b	0.36 ±0.02 ** b	0.42 ±0.01 ** b	0.09 ±0.002 **b	0.08 ±0.002 * b
Atorvastatin	10	0.57 ±0.02 *** b	0.50 ±0.01 ** * b	0.54 ±0.01 ** * b	0.48 ±0.03 *** b	0.13 ±0.006 *** b	0.11 ±0.002 *** b

*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

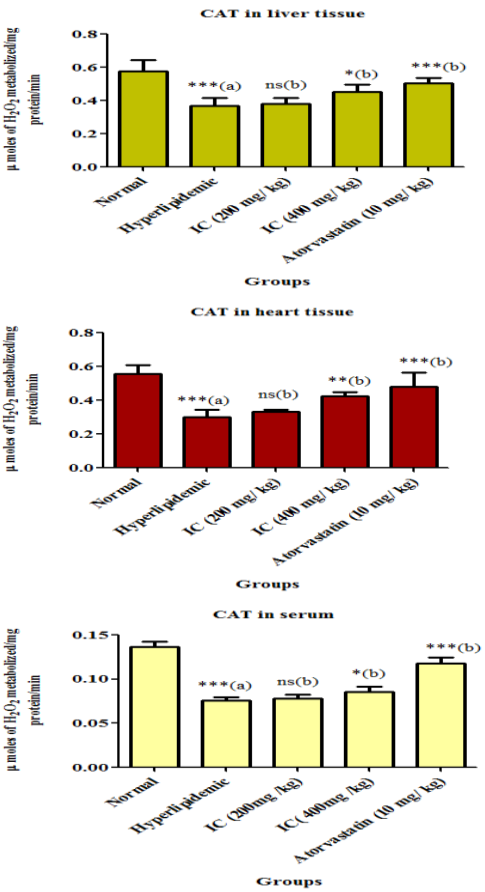
Graph 13: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on liver, heart tissue and serum CAT in Cafeteria diet induced hyperlipidemic rats





*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 14: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on liver, heart tissue and serum CAT in Triton induced hyperlipidemic rats



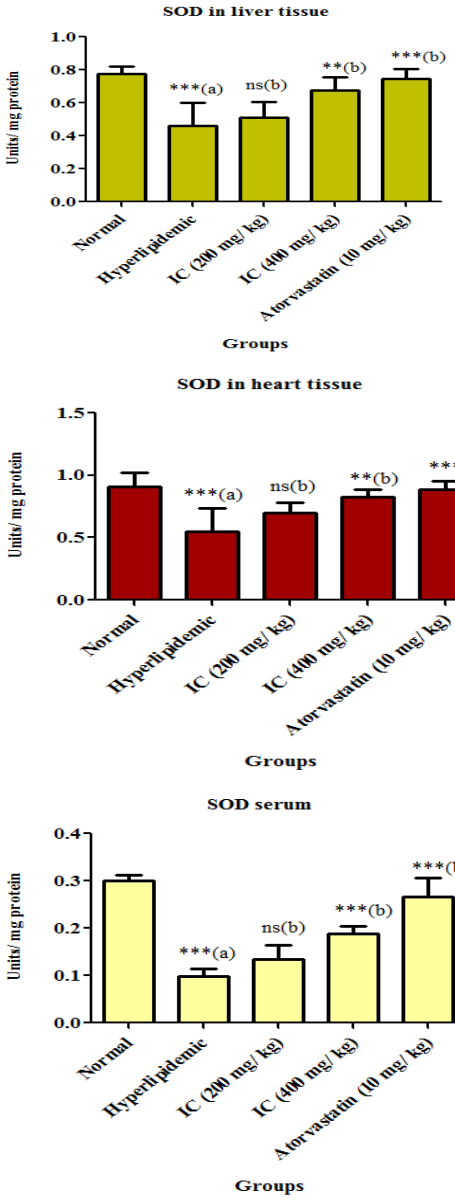
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Table 5: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on SOD in Cafeteria diet and Triton induced hyperlipidemic rats

Groups	**Dose (mg/ kg)**	**SOD (Units/mg protein)**					
		Liver		**Heart**		**Serum**	
		Diet	**Triton**	**Diet**	**Triton**	**Diet**	**Triton**
Control	Normal saline	0.77± 0.01		0.91± 0.04		0.30± 0.005	
Hyperlipidemic control	-	0.46 ±0.06 ***a	0.48 ±0.03 ***a	0.54 ±0.08 ***a	0.57 ±0.03 ***a	0.09 ±0.007 ***a	0.09 ±0.01 ***a
Ixora coccinea Extract	200	0.51 ±0.04 ns b	0.54 ±0.05 ns b	0.70 ±0.03 ns b	0.68 ±0.03 ns b	0.13 ±0.01 ns b	0.13 ±0.02 ns b
Ixora coccinea extract	400	0.67 ±0.03 **b	0.68 ±0.03 **b	0.82 ±0.02 **b	0.80 ±0.04 **b	0.18 ±0.007 ***b	0.16 ±0.007 **b
Atorvastatin	10	0.74 ±0.02 ***b	0.75 ±0.03 ***b	0.88 ±0.03 ***b	0.82 ±0.03 **b	0.26 ±0.017 ***b	0.23 ±0.01 **b

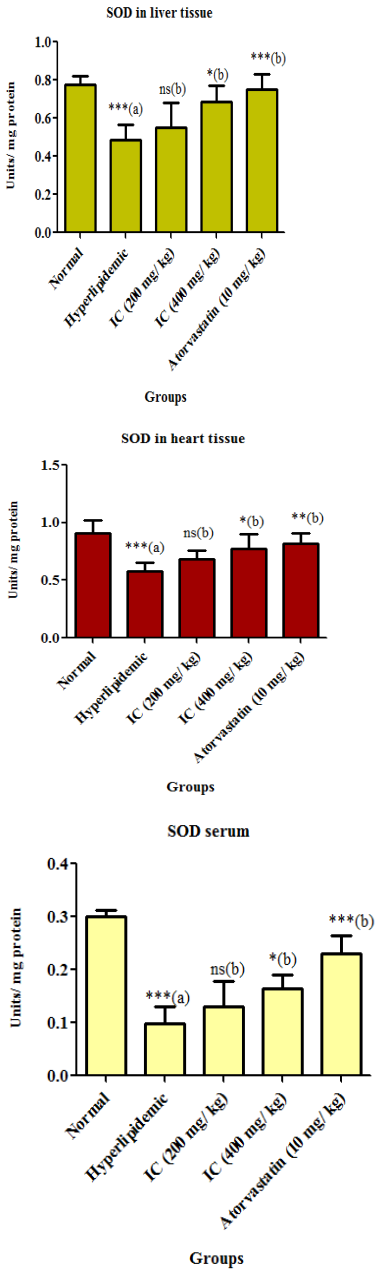
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 15: Effect of hydroalcoholic extracts of *Ixora coccinea* leaves on liver, heart tissue and serum SOD in Cafeteria diet induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 16: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on liver, heart tissue and serum SOD in Triton induced hyperlipidemic rats



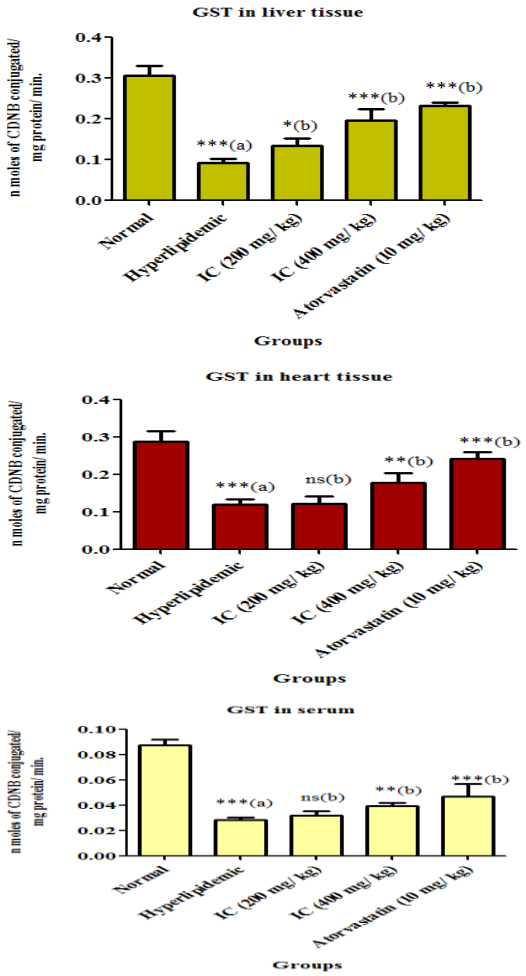
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Table 6: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on GST in Cafeteria diet and Triton induced hyperlipidemic rats

Groups	Dose (mg/kg)	GST (nM of CDNB conjugate formed/min/mg protein)					
		Liver		Heart		Serum	
		Diet	Triton	Diet	Triton	Diet	Triton
Control	Normal saline	0.30± 0.01		0.28± 0.01		0.08± 0.001	
Hyperlipidemic control	-	0.09 ±0.004 ***a	0.09 ±0.003***a	0.11 ±0.006 ***a	0.12 ±0.01 ***a	0.02 ±0.001 ***a	0.03 ±0.002 ***a
Ixora coccinea extract	200	0.13 ±0.008 *b	0.11 ±0.007 ns b	0.12 ±0.008 ns b	0.13 ±0.01 ns b	0.03 ±0.001 ns b	0.03 ±0.001 ns b
Ixora coccinea extract	400	0.19 ±0.01 **b	0.15 ± 0.01 ** b	0.17 ±0.01 **b	0.18 ±0.01 *b	0.03 ±0.001 **b	0.03 ±0.001 * b
Atorvastatin	10	0.23 ±0.003 **b	0.19 ±0.01 *** b	0.24 ±0.007 ** * b	0.24 ±0.01 ** * b	0.04 ±0.004 ** * b	0.05 ±0.001 ** * b

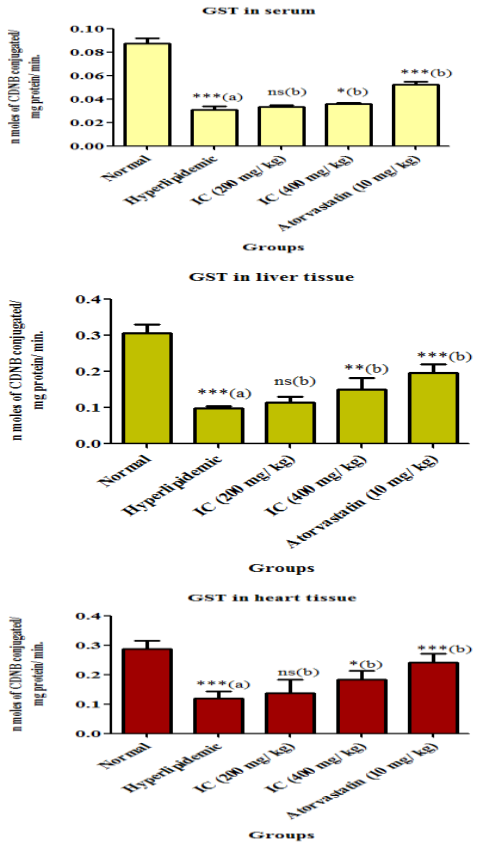
*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 17: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on on liver, heart tissue and serum GST in Cafeteria diet induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 18: Effect of hydroalcoholic extract leaves of *Ixora coccinea* on liver, heart tissue and serum GST in Triton induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

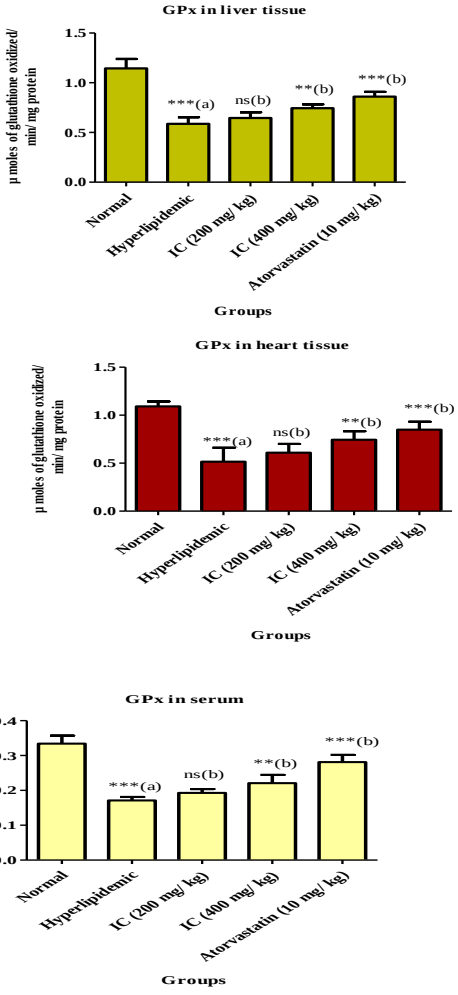
Table 7: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on GPx in cafeteria diet and triton induced hyperlipidemic rats

Groups	**Dose (mg/ kg)**	**GPx (µ moles of glutathione oxidized/min/mg protein)**					
		Liver		**Heart**		**Serum**	
		Diet	**Triton**	**Diet**	**Triton**	**Diet**	**Triton**
Control	Normal saline	1.14±0.04		1.09±0.02		0.33±0.01	
Hyperlipidemic control	-	0.58±0.02***a	0.60±0.02***a	0.51±0.06***a	0.55±0.01***a	0.17±0.01***a	0.18±0.02***a
Atorvastatin	10	0.86±0.02***a	0.86±0.03***a	0.85±0.03***a	0.94±0.03***a	0.28±0.01***a	0.32±0.03***a

*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

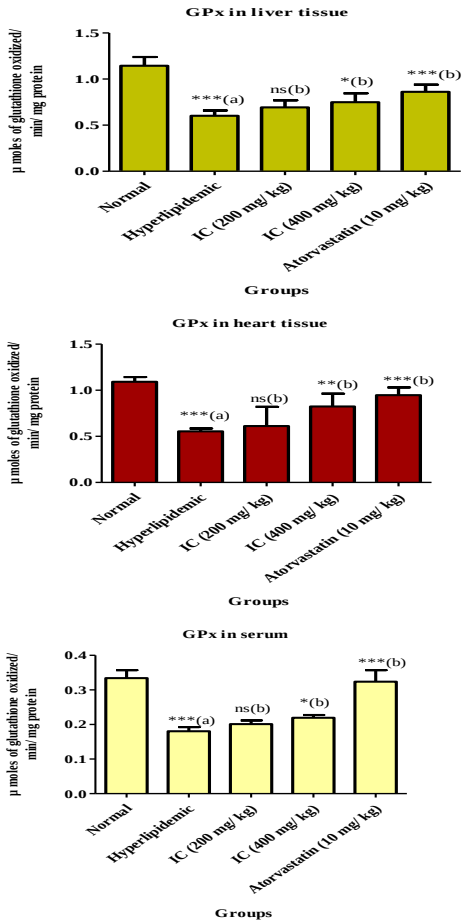
The activities of catalase, superoxide dismutase, glutathione-s-transferase and glutathione peroxidase (Table 4, 5, 6 & 7 respectively) were significantly decreased in the liver, heart and serum of rats when treated with cafeteria diet and triton. While, these antioxidant enzymes level increased with the treatment of *Ixora coccinea*leaves extract as compared to hyperlipidemic control. However, rats treated with 200 mg/kg dose of *Ixora coccinea*leaves extract dose not showed significant level of biochemical changes.

Graph 19: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on liver, heart tissue and serum GPx in Cafeteria diet induced hyperlipidemic rats



*** a Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

Graph 20: Effect of hydroalcoholic extract of *Ixora coccinea* leaves on liver, heart tissue and serum GPx in Triton induced hyperlipidemic rats



*** Values were significantly different from normal control at P < 0.05. *, ** and *** b Values were significantly different from hyperlipidemic control at P < 0.05. ns b = non-significant as compared to hyperlipidemic control at P < 0.05.

4. Discussion:

The results of the present study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate and fat contain cafeteria foods elicited a significant increase in body weight, serum cholesterol, triglycerides, LDL-C and a decrease in serum HDL-C (good cholesterol).

Administration of *Ixora coccinea* leaves extract for 15 days significantly decreased the level of serum cholesterol, triglycerides and LDL-C as compared to hyperlipidemic rats which was almost comparable to that of the standard drug Atorvastatin used in treatment. In addition, no favourable changes in body weight were observed after administration of *Ixora coccinea* leaves extract. This hypolipidemic actions may be due tointerfere with cholesterol biosynthesis or restrict lipoprotein production or increased expression of hepatic LDL receptors leading to an increase removal of LDL from the blood and its increased degradation and catabolism of cholesterol from the body.[16, 17]

In the hyperlipidemic model, ingestion of high cholesterol and fat contain cafeteria foods might be result in accumulation of intracellular cholesterol and its ester in the body tissues.Diet containing saturated fatty acids may be increased the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis which stimulated the cholesterogenesis rate. Moreover, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet, which may leads to elevation of serum LDL-C level.[18]

Similarly, there was significantly increased in the level of serum total cholesterol, triglycerides, LDL-C and decreased in the level of HDL-C in the rats treated with triton. Treatment with extract significantly decreased the level of cholesterol, triglycerides and LDL-C as compared to hyperlipidemic control. Triton accelerates the hepatic cholesterol synthesis through the ability to interfere with the uptake of lipoprotein from the blood circulation by the tissues, resulting in an increase in the level of circulatory lipoproteins.[19] Hence the antihyperlipidemic effect of *Ixora coccinea* leaves extract could be due to interfere with cholesterol biosynthesis.

On the other hand, Serum HDL-C level was significantly raised by the extract (400 mg/ kg) as compared to hyperlipidemic control in case of both models.HDL-C acts likes a scavenger of cholesterol. It mediates the transfer of excess cholesterol from the peripheral tissues to the liver for its catabolism by a pathway termed as ‘‘reverse cholesterol transport”. Hence increased serum HDL-C level may beneficial in lipid disorder and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process. [20, 21]

The lipoprotein abnormalities are also related to the severity of the insulin resistance.[22] The increased free fatty acids also have an effect on beta-cell function. When free fatty acids increased in the beta cell, there is impairment of beta-cell function. There is an increase in the apoptosis or cell death of beta cells that can ultimately lead to a decrease in beta-cell mass. All of this leads to impaired insulin secretion that perpetuates the hyperglycemic state. The increase in the free fatty acids in the muscle causes decrease in the uptake of glucose and a decrease in the glycogenesis in the muscle, which ultimately also leads to the hyperglycemic state.[23]

Studies have shown that there was markedly increased in the level of serum glucose in the hyperlipidemic rats in both models. Treatment with extract (400 mg/kg) significantly decreased the level of serum glucose as compared to hyperlipidemic rats. However, rats treated with *Ixora coccinea* leaves extract (200 mg/kg) not shown significant result.

Hyperlipidemia indicates the onsets of abnormalities in lipid metabolism secondary to the manifestation and progression of atherosclesis.[24] Hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL, protein glycation,

glucose-autooxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects.[25] High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and increased lipid peroxidation.[22]

In the present study, there was significant elevation in the level of Malondialdehyde in the rats treated with triton and cafeteria diet as compared to normal control. Treatment with hydroalcoholic extracts of *Ixora coccinea*leaves (400 mg/kg) significantly reduced Malondialdehyde levels in the liver, heart and serum as compared to hyperlipidemic control in case of both models. However, changes in the 200 mg/kg dose of *Ixora coccinea* leaves extract treated rats are not shown significant result.

The activities of catalase, superoxide dismutase, and glutathione-s-transferase and glutathione peroxidase were significantly decreased in the liver, heart and serum of rats in hyperlipidemic control in both models. While, these antioxidant enzymes level increased with the treatment of extract as compared to hyperlipidemic control.

Cafeteria diet induced hyperlipidemic rats shown better antioxidants potential with the treatment of *Ixora coccinea* leaves extract when compared to triton induced hyperlipidemic rats. The protection of antioxidants activity may be assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalyst, decomposition of peroxides and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. [26]

Ixora coccinea leaves extract exhibited concentration-dependent scavenging activities against hydroxyl radical generated in hyperlipidemic condition. The potential scavenging abilities of phenolic or flavonoids substance might be due to the active hydrogen donor ability of hydroxyl substitution.

All this events either individually or in combination lead to provide antihyperlipidemic and antioxidant effect during the treatment with the hydroalcoholic leaves extract of *Ixora coccinea*.

5. Conclusion: The results obtained from the pharmacological screening have led to the conclusions that, hydroalcoholic extract of *Ixora coccinea* leaves has significant hypolipidemic activity on both Cafeteria diet and Triton induced hyperlipidemic rats, possibly through normalization of serum lipid profile and/or its antioxidant potentials. Hence it can be exploited as an anti-hyperlipidemic therapeutic agent for the management and treatment of hyperlipidemia. Further studies are required to isolate active constituents responsible for antihyperlipidemic activity and to elucidate the possible biochemical mechanism.

6. **CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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REFERENCES

1. North shore pediatric therapy [Internet].2012 [cited 2013 Sep 10]. Available from: http://nspt4kids.com/health-topics-conditions/hyperlipidemia-2/.
2. World Health Organization [Internet].2008 [Updated 2013 Mar; cited 2013 Sep 10]. Available from: www.who.int/mediacentre/factsheets/fs317/.
3. Fedacko Jan ,Singh R, Chaithiraphan S,Vargova Viola, Tomlinson Brian, Meester Fabien, Gvozdjakova Anna, Moesgaard Sven.Clinical manifestations of adverse effects of Statins, oxidative stress and possible role of antioxidants in prevention. Open Nutraceuticals J.2010;3: 154-65.
4. Elumalai A, Eswaraiah C, Venkatesh Y, Kumar BS, Narendar C. Phytochemical and pharmacological profile of *Ixora coccinea* L. Int J of Pharm & Life Sci. 2012;2(2):1563-67.
5. *Ixora coccinea*-US Forest Service [Internet]. [Cited 2014 May 3]. Available from: http://www.fs.fed.us/global/iitf/pdf/shrubs/Ixora%20coccinea.pdf.
6. Kharat AR, Nambiar VV, Tarkasband YS, Pujari RR. A review on phytochemical and pharmacological activity of genus Ixora. International Journal of Research in Pharmacy and Chemistry. 2013;3(3):628-35.
7. Dixit P, Prakash T, Karki Roopa, Kotresha D. Anti-obese activity of *Butea monosperma* (Lam) bark extract in experimentally induced obese rats. Indian J Exp Biol. 2012;50:476-83.
8. Desu BSR, Saileela CH. The anti-hyperlipidemic effect of methanolic extract of *Rhinacanthus nasutus*. International Journal of Research in Pharmacy and Chemistry. 2013;3(3):708-11.
9. Sikarwar MS, Patil MB. Antihyperlipidemic activity of *Salacia chinensis* root extracts in Triton-induced and Atherogenic diet-induced hyperlipidemic rats. Indian J Pharmacol. 2012;44(1):88-92.

10. Ohkawa H, Ohoshi N, Tagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1975;95:351-8.
11. Yagi K. A simple flurometric assay for lipid peroxide in blood plasma. Biochem Med. 1976;15: 212-16.
12. Aebi H. Catalase: Methods of Enzymatic Analysis. 2nd ed. Newyork: Academic Press. 1974; 673-7.
13. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys. 1984; 21: 130-32.
14. Habig WH, Pabst MJ, Jarkoby WB. Glutathione. The first enzymatic step in mercapturic acid formation. J BiolChem. 1974;249: 7130-39.
15. Rotruck JP, Pope AL, Ganther HE, Swason AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179: 588-90.
16. Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors.Science. 1981; 212(4495):628-35.
17. Finkel Richard, Clark Michelle, Cubeddu, Luigi X editors. Lippincott's Illustrated Reviews: Pharmacology. 4th ed. New Delhi: Wolters Kluwer; 2010. p. 253-4.
18. Goldfarb Stanley, Pitot Henry. Stimulatory effect of dietary lipid and cholestyramine on hepatic HMG CoA reductase. J Lipid Res. 1972;13:792-801.
19. Schurr PE, Schultz JR, Parkinson TM. Triton induced hyperlipidaemia in rats as an animal model for screening hypolipidemic drugs. Lipids. 1972;7(1):68-74.
20. Lipoprotein metabolism [Internet]. [Cited 2014 Apr 15]. Available from: http://apbrwww5.apsu.edu/thompsonj/Anatomy%20&%20Physiology/2020/2020%20Exam%20Reviews/Exam%204/CH24%20Lipid%20Metabolism.htm.
21. Zhao XQ, Kerwin WS. Examining the potential of MRI for monitoring cholesterol therapy. Clin Lipidol. 2012;7(3):329-43.
22. Shradha Bisht, Sisodia SS. Diabetes, dyslipidemia, antioxidant and status of oxidative stress. IJRAP. 2010;1(1): 33-42.
23. Diabetes and Dyslipidemia: Interrelationships and clinical implications [Internet]. 2009 [Cited 2014 May 14]. Available from: www.medscape.org/viewarticle/586792.
24. Miller, Thelle DS, Forde OH, Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. Lancet. 1977; 1(8019):965-8.
25. Yang RL, Shi YH, Wei Le G. Increasing oxidative stress with progressive hyperlipidemia in human: Relation between Malondialdehyde and Atherogenic Index. J Clin Biochem Nutr. 2008; 43(3):154-8.
26. Valko Marian, Leibfritz Dieter, Moncol J, Cronin Mark, Mazur Milan, Telser Joshua. Free radicals and antioxidants in normal physiological functions and human disease. Intl J Biochem Cell Biol. 2007; 39(1):44-84.

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