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Hypolipidemic activity and HPTLC analysis of *Ixora coccinea* L. Leaves

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ABSTRACT

The present research was made to investigate the potential hypolipidemic effect of *Ixora coccinea*. Hypolipidemic activity is screened by inducing hyperlipidemia with the help of atherogenic diet in wistar albino rats and serum levels of biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol were determined. Atherogenic index shows the measure of the atherogenic potential of the drugs. Ethanol extract showed significant ($p < 0.05$) hypolipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and HDL level which was similar to the standard drug Atorvastatin. Ethanol extract exhibited significant atherogenic index and percentage protection against hyperlipidemia. Preliminary phytochemical analysis revealed the presence of phytoconstituents such as alkaloids, tannins, flavonoids, carbohydrates, protein and amino acids and reducing sugars which is further confirmed by HPTLC (high performance thin layer chromatography). The overall experimental results suggests that the biologically active constituents such as flavonoids, alkaloids, tannins and glycosides in the ethanol extract, of *Ixora coccinea*, may be responsible for the significant hypolipidemic activity and the results justify the use of *Ixora coccinea* as a significant hypolipidemic agent.

Keywords: *Ixora coccinea* L., Atherogenic diet, Atorvastatin, Ethanol.

INTRODUCTION

The use of medicinal plants in the management of various illnesses is due to their phytochemical constituents and dates back to antiquity (Yakuba *et al.*, 2007). However, during the last decade, an increase in the use of medicinal plants has been observed in metropolitan areas of developed countries (Hamack *et al.*, 2001). Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter *et al.*, 1982). During diabetes a profound alteration in the concentration and composition of lipid occurs (Sochar *et al.*, 1995). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver. Diabetes mellitus is known to cause hyperlipidemia through various metabolic derangements. Among several metabolic derangements, insulin deficiency has been known to stimulate lipolysis in the adipose tissue and give rise to hyperlipidemia and fatty liver. Thus, in diabetes hypercholesterolemia and hypertriglyceridemia often occur (Hardman *et al.*, 2001). *Ixora coccinea* Linn is a small shrub which is cultivated throughout India. It is called as 'Flame of the Woods' in English, 'Rangan' in Hindi and Bengali and 'Kisukare' in Kannada. Its roots and flowers are used for the treatment of dysentery, dysmenorrhoea, leucorrhoea, haemoptysis and catarrhal bronchitis. Its leaves are used for the treatment of diarrhea. Its roots are also used for the treatment of hiccups, nausea and loss of

appetite and externally for the treatment of sores, eczema and chronic ulcers. Its roots contain aromatic acrid oil, tannin and fatty acids. Its leaves yield flavonoids, kaemferol, quercetin, anthocyanidines phenolic acids and ferulic acids. Its flowers yield cyanidins, flaconboides and cooling materials which are related to quercetin. Its roots are ground to a pulp, mixed with water and are used as a tincture for diarrhea and dysentery (Vadivu *et al.*, 2010; Satyavathi *et al.*, 1976; Cooke., 1901). However there is limited scientific evidence to verify these claims. There is a dearth of reports on the hypolipidemic effects on the leaves of this plant. In view of this, the current study was designed to evaluate the hypolipidemic activities of the aqueous extract of the leaves of *I. coccinea* in rats.

MATERIAL AND METHODS

Plant material

The leaves of *I.coccinea* were collected from the garden of Genba Sopanrao Moze College of Pharmacy, Pune, Maharashtra, India, during the period from March-June 2009. The identity of this plant was authenticated by the experts of Botany Department, Pune University, Maharashtra and the voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy of Genba Sopanrao Moze College of Pharmacy, Pune, Maharashtra, India.

Preparation of the extract

The collected plant material was shade dried and subjected to size reduction to a coarse powder by using dry grinder and they were passed through a sieve. The powdered leaves (50 gm) of *I.coccinea* was extracted to exhaustion using soxhlet apparatus (Kokate., 1993) with 50% ethanol. Ethanol was Separated under reduced pressure on rotavapor to obtain a dark-brown crude extract. Extract was stored in sterile glass containers at -4°C.

Preliminary phytochemical analysis

The extract which was obtained, was subjected to various qualitative tests for the identification of the constituents which were present, by using simple and standard qualitative methods (Harborne *et al.*, 1984); it revealed the presence of alkaloids, tannins, flavonoids, carbohydrates, protein and amino acids and reducing sugars in the extract. Preliminary thin layer chromatography studies also confirmed these constituents (Wagner *et al.*, 1996).

HPTLC analysis

Fifteen µl of ethanol extract of *I. coccinea* Linn., was spotted on pre-coated silica gel TLC plate of dimension (10X6 cm) (E.Merck) after activation at 105°C. Then the spotted plate was developed in a pre-saturated chamber containing the solvent system of Toluene: Ethylacetate: Acetic acid (7.5:2.4:0.3) as the mobile phase conditions for separation. Developed plate was air dried and scanned under UV 254 nm using Camag densitometer and the chromatogram was recorded.

Animals

Healthy wistar albino rats of either sex, which weighed about 150-200 g, were used. The animals were housed in polypropylene cages and were maintained under standard conditions (12h light:12h dark cycle;25±20 C,35-60% humidity). They were fed with standard laboratory food and *ad libitum*. The experiments were performed after the experimental protocols were approved by the Institutional Animal Ethics Committee, India 2010. Animals were regularly checked throughout the investigation for any infection and if found infected, the animals were isolated and treated. Animals were treated intermittently with antibiotic and antihelminthic suspensions as a prophylactic measure.

Experimental design

The animals were divided into four groups with six animals in each group. In order to render the rat's hyperlipidemia, they were given an atherogenic diet comprising of corn flour base, milk powder, butter, salt, groundnut oil, sucrose and vitamin mixture. In addition 400 mg of cholesterol powder/kg body weight was dissolved in coconut oil and administered orally for 45 days.

- Group 1 : Control or intact: They received 0.5% sodium carboxy methyl cellulose.
- Group 2 : Atherogenic group: They received atherogenic diet
- Group 3 : Atherogenic diet + *I. coccinea* ethanol leaves extract (200 mg/kg body weight)
- Group 4 : Atherogenic diet + Standard drug, Atorvastatin (1.2 mg/kg body weight)

At the end of 45th day, blood serum was withdrawn from the retro orbital plexus after overnight fasting for the study of biochemical parameters. Serum was estimated for the total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol by using standard kits (Lopes-Virella *et al.*, 1977; McGowan *et al.*, 1983; Lowry *et al.*, 1951). Atherogenic index (AI), which is a measure of the atherogenic potential of an agent, was calculated using the formula and the results were tabulated.

Atherogenic Index = Total serum triglycerides/Total serum HDL-C

% Protection = $\frac{\text{AI of control} - \text{AI of treated group}}{\text{AI of control}} \times 100$.

Statistical analysis

Results were presented as mean±SD. The significance of difference among the groups was assessed using one way analysis of variance (ANOVA) followed by Dunnet's test. P<0.05 was considered significant.

RESULTS

Phytochemical screening

The preliminary phytochemical screening revealed the presence of phytoconstituents such as alkaloids, tannins, flavonoids, carbohydrates, protein and amino acids and reducing

sugars in the ethanol extract of *Ixora coccinea* Linn., leaves. HPTLC analysis also confirmed these phytoconstituents. HPTLC analysis also confirmed these phytoconstituents. HPTLC profile is shown in Table 1. The HPTLC finger print for ethanol extract is shown in Fig 1.

Table 1. HPTLC profile for ethanol extract of *Ixora coccinea*.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.27	1.6	0.29	16.3	5.31	0.30	13.4	244.1	2.59	Unknown*
2	0.34	9.8	0.36	24.9	8.12	0.38	10.1	626.3	6.63	Unknown*
3	0.39	8.4	0.41	13.6	4.44	0.43	3.4	288.6	3.08	Unknown*
4	0.49	0.8	0.51	15.3	4.98	0.52	3.4	178.1	1.89	Unknown*
5	0.54	6.8	0.59	67.4	21.97	0.63	5.7	2236.1	23.70	Unknown*
6	0.69	4.9	0.74	79.0	25.78	0.79	29.3	3939.5	41.78	Unknown*
7	0.84	10.7	0.85	15.5	5.05	0.88	3.7	153.7	1.83	Unknown*
8	0.91	0.1	0.95	74.8	24.37	0.97	0.2	1767.8	18.74	Unknown*

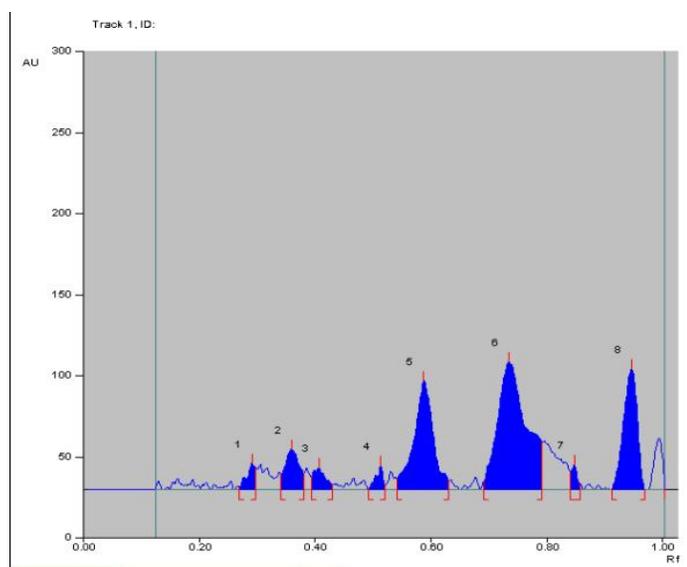


Fig 1. Chromatogram of ethanol extract.

Hypolipidemic activity

A marked increase in the level of serum cholesterol, triglycerides, LDL and VLDL were found in the animals which received atherogenic diet and HDL levels were decreased. Administration of chloroform extract at the dose of 200 mg/kg shows significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard Atorvastatin and are almost near the levels of normal control. A potent hypolipidemic effect of ethanol extract was evident by a significant reduction in the level of serum cholesterol, LDL, VLDL and triglycerides in the cholesterol treated animals and also marked increase in the HDL level (Table 2.). Lipid profile of serum total cholesterol, triglycerides, HDL, LDL and VLDL is represented diagrammatically in Fig 2. The Atherogenic index was considerably decreased in the plant extract group which was also comparable with the standard group Atorvastatin against hyperlipidemia. The percentage protection against hyperlipidemia in the plant extract treated group was 63.3%, whereas the standard group protection is 68%. which

further confirms the significant protective effect of the plant extract against hyperlipidemia (Table 3).

Table 2. Effect of *Ixora coccinea* on biochemical parameters.

Treatment groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control I	101.4 ± 0.16	121.2 ± 0.82	36.3 ± 0.07	80.9 ± 0.5	19.4 ± 0.16
Atherogenic diet II	242.4 ± 0.31	160.8 ± 0.12	25.3 ± 0.06	135.2 ± 0.13	30.6 ± 0.05
Ethanol extract III	112.6 ± 0.17**	112.6 ± 0.42***	34. ± 0.11***	82.9 ± 0.15**	18.9 ± 0.09**
Standard Atorvastatin IV	107.1 ± 0.08***	107.3 ± 0.03***	32.2 ± 0.07***	85.0 ± 0.25***	20.6 ± 0.10***

Values expressed as mean ± SEM. Levels of significance- Group II compared with Group I, III and IV. **p<0.05, ***p<0.001, ****p<0.0001.

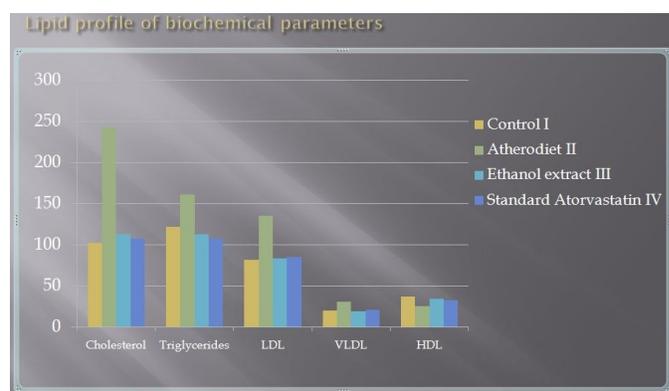


Fig 2. Lipid profile of biochemical parameters.

Table 3. Atherogenic index of *Ixora coccinea*.

Treatment groups	Atherogenic Index (A.I)	% Protection
Control I	3.34	-
Atherogenic diet II	6.36	-
Ethanol extract III	2.33	63.3%
Standard Atorvastatin IV	2.25	68%

DISCUSSION

The aim of the present study was to test the effect of the *I. coccinea* extract on serum cholesterol and triglyceride concentrations. Ethanol extract showed significant hypolipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard drug Atorvastatin. Chloroform extract exhibited significant Atherogenic Index and percentage protection against hyperlipidemia. Hyperlipidemia has been implicated in the development of atherosclerosis (Kaplan *et al.*, 1989; Witzum, 1994; Alexander, 1995). The underlying mechanism of the lipid-lowering activity of *I.coccinea* could be the inhibition of lipid absorption due to the presence of tannins in the ethanolic extract (Goyal *et al.*, 2003). LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of cholesterol and LDL levels achieved by administration of ethanol

extract, demonstrates a possible protection against hypercholesterolemia.

CONCLUSION

In accordance with these results, it may be confirmed that due to the presence of phytoconstituents such as flavonoids, alkaloids, tannins and glycosides in the ethanol extract, it could be responsible for the observed significant hypolipidemic activity. In conclusion, it can be said that the ethanol extract of *Ixora coccinea* exhibited a significant hypolipidemic effect at the dose of 200 mg/kg body weight. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hypolipidemic efficacy of this valuable medicinal plant and further studies are required to establish the efficacy of the *I. coccinea* as a hypolipidemic drug.

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