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Lipid Lowering Activity of *Ixora coccinea* leaves in Hyperlipidemic Rats

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ABSTRACT

Hyperlipidemia is defined as increase in the lipid content in blood. Abundant evidence exists to proof the link between hyperlipidemia and atherosclerosis. *Ixora coccinea* (Rubiaceae) is one member of this family which is traditionally used in dysentery, dysmenorrhoea and in urinary dysfunctions of females. In Indo-China, an infusion of leaves is given for fevers. Flowers and leaves of *I. coccinea* are reported to have antimicrobial, hepatoprotective, anticancer and anti-inflammatory property. The lipid lowering activity of *I. coccinea* leaf extracts has been studied in Triton-X-100 induced hyperlipidemia in rats. The results of our study demonstrated that *I. coccinea* extracts caused a decrease in the plasma lipid levels in Triton-induced hyperlipidemic rats. These results proved that both lipid lowering and antioxidant activities are present in leaf extraxts of *I. coccinea*, which could help prevention of hyperlipidemia and related diseases.

Keywords: atherosclerosis, hyperlipidemia, oxidative stress

INTRODUCTION

Disorders of lipid metabolism, hyperlipidemia, hypertension and obesity are associated with increased oxidative stress and over-production of oxygen free radicals (Zalba *et al.* 2001). An excess of superoxide anions (O_2^-) is further converted into other reactive oxygen species and among them the hydroxyl radical (OH^-) is most damaging to lipids and lipoproteins (Rehman *et al.* 2003). Moreover, hyperlipidemia following oxidative stress may cause oxidative modifications in low density lipoproteins (LDL), which play an important role in the initiation and progression of atherosclerosis and related cardiovascular diseases (Parthasarthy *et al.* 1992). Furthermore, there have been reports that the lipid lowering drugs – fibrates, statins and bile acid sequestraints – used for the treatment of hyperlipidemia-associated disorders do not posses antioxidant property and they are also not free from toxic side effects (Chattopadhyaya *et al.* 1996). Therefore, on the basis of above facts there is an urgent need to have a drug having the dual property of lowering lipid level and antioxidant activities together; a natural product would be the best option.

Plants belonging to the Rubiaceae family are generally a rich source of substances of phytochemical interest. Members of plants from this family are used in traditional systems of medicine. *Ixora coccinea* is one member of this family which is traditionally used in dysentery, dysmenorrhoea and in urinary dysfunctions of females. In Indo-China, an infusion of leaves is given for fevers (Kirtikar and Basu 2001). Flowers and leaves of *I. coccinea* are reported to have antimicrobial, hepatoprotective, anticancer and anti-inflammatory property (Latha and Panikkar 1998; Latha and Panikkar 1999; Annapurna *et al*. 2003).

After getting very positive results in an *in vitro* model of the antioxidant property of *I. coccinea* against various free radicals (Sankhadip *et al.* 2008), now an attempt has been taken to investigate the dual property of lowering lipid level and antioxidant activities together of the same plant extracts through an *in vivo* lipid lowering model against hyperlipidemic rats.

MATERIALS AND METHODS

Plant material

The leaves of *I. coccinea* were collected from the herbal garden, Gupta College of Technological Sciences, Asansol, India. The collected leaves were identified and authenticated by Mr. M. S. Mondal, Joint Director, Botanical Survey of India, Shibpur, Kolkata. A voucher specimen (Specimen no. CNH/I-I/(201)/2007/Tech.II/2) has been deposited at the office of the Central National Herbarium, Botanical Survey of India, Shibpur, Kolkata.

Preparation of extracts

The leaves were shade dried at room temperature for 15 days, powdered and used for extraction. A required quantity of powder (100 g each) was extracted with 50% hydro-alcohol and ethyl acetate separately by continuous hot percolation in a Soxhlet apparatus for 72 hrs. Both extracts were concentrated in reduced pressure below 40°C by using a rotary flash evaporator to get two separate extracts: hydro-alcoholic (27.2% w/w) and ethyl acetate extract (14.34% w/w). Separately another quantity of powder (100 g) was extracted with distilled water (600 ml) by cold maceration and the extract was concentrated under reduced pressure below 40°C to get a separate aqueous extract (13.17% w/w). All extracts were stored at 4°C until use.

Hyperlipidemia inducer

Triton-X-100 (LR Grade, Spectrochem Pvt. Ltd., Mumbai, India) was used to induce hyperlipidemia in experimental rats.

Animal treatments

Wistar albino rats of 180-200 g body weight were used in this study. Animals were procured from the Institutional Animal House of Gupta College of Technological Sciences, Asansol. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27°C and 60-65% relative humidity with 12:12 light: dark cycles). Food was provided in the form of dry pellets (INTOX Pvt. Ltd., Pune) and water *ad libi-*

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tum. All the experiments were conducted between 9 am and 3 pm. The animals were allowed to acclimatize to laboratory conditions for 7 days before the commencement of the experiment. Food was withdrawn 18 hrs prior to the commencement of the experiment. All experiments involving animals complied with the ethical standards of animal handling and were approved by the Institutional Animal Ethics Committee (Regd. No. – 955/a/06/CPCSEA).

Induction of hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats (Institutional Animal House of Gupta College of Technological Sciences, Asansol) by single intraperitoneal (i.p.) injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h (Moss *et al.*1971; Vogel *et al.* 1997; Mohale *et al.* 2008).

Dose preparation and administration of extracts

All three extracts were dissolved in distilled water and administered orally to the animals by gastric intubation, three times a day with a 3-hr interval, just after intra-peritoneal administration of Triton-X-100.

Protocol for lipid-lowering activity

In the experiment a total number of 36 rats (30 hyperlipidemic rats and six normal) were used. The rats were divided into six groups of six each. Group I: Normal distilled water treated (oral dose); Group II: Triton-X-100 (100 mg/kg) (i.p. dose); Group III: Triton-X-100 (100 mg/kg) (i.p. dose) + Lovastatin (cholesterol-lowering drug, Brand name 'Mevacor', Merck Co. Inc) (10 mg/kg) (oral dose); Group IV: Triton-X-100 (100 mg/kg) (i.p. dose) + hydro-alcoholic extract of leaves (oral dose); Group V: Triton-X-100 (100 mg/kg) (i.p. dose) + ethyl acetate extract of leaves (oral dose); Group VI: Triton-X-100 (100 mg/kg) (i.p. dose) + aqueous extract of leaves (oral dose).

Biochemical analysis in plasma

Total cholesterol (TC), triglyceride (Tg), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c) and very-low-density lipoprotein (VLDL-c) were determined according to the standard methods (references).

Statistical analysis

The values are expressed as means ± S.E.M. with n = 6. The Triton control was compared with the normal and experimental results were compared with the Triton control. Prism 3.0 software was used to perform one-way ANOVA followed by Dunnett's test to determine the significance of the result.

RESULTS AND DISCUSSION

The present study was planned because various hypolipidemic drugs are available in market, like statins, fibrates and bile acids sequestraints have numerous side effects. Therefore, to overcome these adverse effects there is an urgent need for the development of hypolipidemic drugs from natural resources.

The results of our study demonstrated that *I. coccinea* extracts and Lovastatin caused a significant decrease in the plasma lipid levels in Triton-induced hyperlipidemic rats (**Tables 1-5**). Triton-X-100 acts as surfactant and causes

Table 1 Effect of *I. coccinea* extracts on total cholesterol level of triton-induced hyperlipidemia in rats.

Groups	Treatments	Total cholesterol (mg/dl)
I	Normal (normal saline)	73.89 ± 0.59
II	Triton control (100 mg/kg)	276.27 ± 1.60
III	Triton + Lovastatin (10 mg/kg b.w.)	183.42 ± 0.77
IV	Triton + hydro-alcoholic extract (10 mg/kg b.w)	211.75 ± 0.87**
V	Triton + ethyl acetate extract (10 mg/kg b.w.)	235.26 ± 2.06**
VI	Triton + aqueous extract (10 mg/kg b.w.)	250.08 ± 1.03**

$^{**}P < 0.01$, very significant; $^{*}P < 0.05$, significant; $^{ns}P > 0.05$, non-significant, one-way ANOVA followed by Dunnett's test.

Table 2 Effect of *I. coccinea* extracts on triglyceride level of triton-induced hyperlipidemia in rats.

Groups	Treatments	Triglycerides (mg/dl)
I	Normal (normal saline)	65.11 ± 0.74
II	Triton control (100 mg/kg)	157.13 ± 1.16
III	Triton + Lovastatin (10 mg/kg b.w.)	86.36 ± 0.62
IV	Triton + hydro-alcoholic extract (10 mg/kg b.w)	118.17 ± 2.15**
V	Triton + ethyl acetate extract (10 mg/kg b.w.)	127.75 ± 1.56**
VI	Triton + aqueous extract (10 mg/kg b.w.)	133.77 ± 1.34**

$^{**}P < 0.01$, very significant; $^{*}P < 0.05$, significant; $^{ns}P > 0.05$, non-significant, one-way ANOVA followed by Dunnett's test.

Table 3 Effect of *I. coccinea* extracts on HDL-c level of triton-induced hyperlipidemia in rats.

Groups	Treatments	HDL-c (mg/dl)
I	Normal (normal saline)	28.72 ± 1.23
II	Triton control (100 mg/kg)	13.54 ± 1.01
III	Triton + Lovastatin (10 mg/kg b.w.)	27.83 ± 0.73
IV	Triton + hydro-alcoholic extract (10 mg/kg b.w)	21.08 ± 0.73**
V	Triton + ethyl acetate extract (10 mg/kg b.w.)	24.20 ± 0.80**
VI	Triton + aqueous extract (10 mg/kg b.w.)	27.80 ± 0.55**

$^{**}P < 0.01$, very significant; $^{*}P < 0.05$, significant; $^{ns}P > 0.05$, non-significant, one-way ANOVA followed by Dunnett's test.

Table 4 Effect of *I. coccinea* extracts on LDL-c level of triton-induced hyperlipidemia in rats.

Groups	Treatments	LDL-c (mg/dl)
I	Normal (Normal saline)	32.15 ± 1.17
II	Triton control (100 mg/kg)	230.90 ± 0.23
III	Triton + Lovastatin (10 mg/kg b.w.)	140.82 ± 5.38
IV	Triton + hydro-alcoholic extract (10 mg/kg b.w)	162.04 ± 10.44**
V	Triton + ethyl acetate extract (10 mg/kg b.w.)	185.51 ± 1.5**
VI	Triton + aqueous extract (10 mg/kg b.w.)	195.53 ± 1.05**

$^{**}P < 0.01$, very significant; $^{*}P < 0.05$, significant; $^{ns}P > 0.05$, non-significant, one-way ANOVA followed by Dunnett's test.

Table 5 Effect of *I. coccinea* extracts on VLDL-c level of triton-induced hyperlipidemia in rats.

Groups	Treatments	VLDL-c (mg/dl)
I	Normal (normal saline)	12.93 ± 0.08
II	Triton control (100 mg/kg)	31.43 ± 0.23
III	Triton + Lovastatin (10 mg/kg b.w.)	17.27 ± 0.12
IV	Triton + hydro-alcoholic extract (10 mg/kg b.w)	23.63 ± 0.43**
V	Triton + ethyl acetate extract (10 mg/kg b.w.)	25.55 ± 0.31**
VI	Triton + aqueous extract (10 mg/kg b.w.)	26.75 ± 0.26**

$^{**}P < 0.01$, very significant; $^{*}P < 0.05$, significant; $^{ns}P > 0.05$, non-significant, one-way ANOVA followed by Dunnett's test.

structural modifications in circulatory lipoproteins, suppresses the action of lipases and as a consequence blocks the uptake of circulating lipids by extra hepatic tissues, resulting in an increase in blood lipid concentration (Schurr *et al.* 1972). Treatment with *I. coccinea* extracts caused a significant reversal in this level (**Tables 1-5**). Earlier, we successfully proved that all three extracts (hydro-alcoholic, ethyl acetate and aqueous extract) of *I. coccinea* are significantly active to scavenge all types of free radicals through *in vitro* antioxidant models. Hydroxyl radicals play a major role in peroxidative damage to lipids and lipoproteins and this, in turn, may be responsible for initiation and progression of atherosclerosis in hyperlipidemic subjects (Parthasarthy *et al.* 1992). Thus, the antioxidant property of the above plant extracts may help in preventing the oxidative modifications of various vital biomolicules, including lipids and lipoproteins, in hyperlipidemic subjects.

In conclusion, it may be stated that the results of the present study demonstrated new properties of *I. coccinea* leaves as a potent lipid lowering and antioxidant agent and these beneficial activities may contribute to its cardio protective and antiatherosclerotic role. Moreover, further studies by using isolated compounds are under progress to substantiate the present findings.

REFERENCES

Annapurna J, Amarnath PV, Amar Kumar D, Ramakrishna SV, Raghavan KV (2003) Antimicrobial activity of *Ixora coccinea* leaves. *Fitoterapia* **74 (3)**, 291-293

Chattopadhyaya R, Pathak D, Jindal DP (1996) Antihyperlipidemic agents – A review. *Indian Drugs* **33**, 85-97

Kirtikar KR, Basu BD (2001) *Indian Medicinal Plants* (2nd Edn, Vol 6) Oriental Enterprises, Dehradun, Uttaranchal, India, 1783 pp

Latha PG, Panikkar KG (1998) Cytotoxic and antitumour principles from *Ixora coccinea* flowers. *Cancer Letter* **130 (1-2)**, 197-202

Latha PG, Panikkar KG (1999) Modulatory effects of *Ixora coccinea* flower on cyclophosphamide-induced toxicity in mice. *Phytotherapy Research* **13 (6)**, 517-520

Mohale DS, Dewani AP, Saoji AN, Khadse CD (2008) Antihyperlipidemic activity of isolated constituents from the fruits of *Lagenaria siceraria* in albino rats. *International Journal of Green Pharmacy* **2 (2)**, 104-107

Moss JN, Dajani EZ (1971) Antihyperlipidemic agents. In: Turner RA, Hebben PA (Eds) *Screening Methods in Pharmacology*, Academic Press, New York, pp 121-143

Parthasarthy S, Steinberg D, Seitztum JL (1992) The role of oxidized low-density lipoprotein in the pathogenesis of atherosclerosis. *Annual Review of Medicine* **43**, 219-225

Rehman S, Mahdi AA, Hasan M (2003) Trace metal induced lipid peroxidetion in biological system. *Society for Free Radical Research – India Bulletin* **2**, 12-18

Bose S, Bose A, Maji S, Chakraborty P (2008) *In vitro* antioxidant property of leaf extracts of *Ixora coccinea* L. *International Journal of Biomedical and Pharmaceutical Sciences* **2 (2)**, 84-87

Schurr PE, Schultz JR, Parkinson TM (1972) Triton induced hyperlipidemia in rats as an animal model for screening of hypoliperimic drugs. *Lipids* **7**, 68-74

Vogel G, Vogel WH (1997) Influence of lipid metabolism. In: *Drug Discovery and Evaluation Pharmacological Assay*, Springer-Verlag, Berloin, pp 604-608

Zalba G, San JG, Moreno MU, Fortuno A, Beaumont FJ, Diez J (2001) Oxidative stress in arterial hypertension – Role of NADPH oxidase. *Hypertension* **38**, 1395-1399