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Research Article

Immunomodulatory activity of methanolic extract of flowers of *Ixora coccinea*

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ABSTRACT

The aim of this study was to investigate the immunomodulatory potential of an methanolic extract of flowers of *Ixora coccinea* (Rubiaceae) in rats. *Ixora coccinea* was administred orally at doses of 100, 200 and 400 mg/kg to healthy rats divided into five groups consisting of six animals each. The effect of the methanolic extract of the flowers of the plant *Ixora coccinea* (MEIC) was evaluated by the cell mediated and humoral immunity response for a specific immune response. The effect of *Ixora coccinea* on the neutrophil activation was evaluated by the neutrophil adhesion test for a nonspecific immune response. On oral administration of the extract, a significant increase in neutrophil adhesion was observed. The data was analyzed by one-way ANOVA followed by Dunnet's comparision test. In Carbon clearance assay at doses of 100, 200 and 400 mg/kg/bw showed a significant increase in the phagocytic index, which determines the stimulation of reticuloendothelial system. The study demonstrates that *Ixora coccinea* triggers both specific and non-specific responses to a better extent. The study comprised the acute toxicity and preliminary phytochemical screening of *Ixora coccinea*. From the results obtained and phytochemical studies the immunostimulant effect of *Ixora coccinea* might be attributed to the alkaloids & flavonoids content.

Key words: *Ixora coccinea,* Immunomodulatory activity, Immune system, Neutrophil, Carbon Clearance

INTRODUCTION

Natural products of plant and animal origin offer vast resources of newer medicinal agents with potential in clinical use [1]. Some of these are believed to promote positive health and maintain organic resistance against infection by re-establishing the body's equilibrium and conditioning the body tissue [2]. The historic use of herbal medicines to treat and prevent infectious disease has been supplanted with the emergence of specific synthetic drugs and antimicrobial agents. However, the use of plant remedies, known to possess natural antioxidant, immunomodulatory and other activities, has increased in the last decade in human and animal medicine, as it is perceived as a natural approach to treat disease. Intensive farming system rely heavily on the use of pharmaceuticals, but there is increasing public concern regarding their use, mostly for the emergence of drug resistance [3], the associated risk of developing antibiotic resistance in human pathogens [4] and contamination in the food chain[5]. *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[6]. etc. However, there is limited scientific evidence to verify these claims. There is a dearth of reports on the immunomodulatory effects on the flowers of this plant. It has been reported that alkalois and flavonids in this plant possess various biological activities such as anti-tumor, anti – oxidative and anti – inflammatory activities. In view of this, the current study was designed to evaluate the

immunomodulatory activities of the methanolic extract of the flowers of *I. coccinea* in rats.

MATERIALS AND METHODS

Plant material

The plant *Ixora coccinea* was collected from Palvoncha, Khammam (Dist), A.P, India and it is authenticated by the botanist and the voucher specimen(number 0040) was deposited in the Department of Pharmacology of KLR pharmacy college, Paloncha, Khammam(D.t)., A.P., India. These flowers were shade dried and powdered

Preparation of extract

The powdered flowers of selected plants were subjected to successive solvent extraction. The extraction was carried out for 16 h with the following solvents in the increasing order of polarity i.e.,

1. Petroleum ether.
2. Chloroform.
3. Methanol.

Preparation of petroleum ether extract:

About 250 g of the dried powdered flowers were extracted with 2 lt of Petroleum ether by soxhletion method for 16 h. The extract was concentrated to $1/4^{th}$ of its original volume by distillation. The concentrated extract was taken in a beaker and evaporated to a thick paste on water bath, maintained at around 50^0c to get Petroleum ether extract.

Preparation of chloroform and methanolic extracts:

The above dried marc was extracted successfully with chloroform and methanol as solvents to get chloroform and methanolic extracts respectively.

Experimental animals

Adult male Wister rats of 150 to 200 g of either sex were used for the study. They were provided with a standard diet (Pranav Agro, India) and water *ad libitum* in animal house facility and maintained under standard laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of KLR Pharmacy College, Palvancha, Khammam (dist), with CPCSEA Reg. No: 1516/PO/a/11/CPCSEA dated 01-11-2011.

Qualitative chemical evaluation

The above prepared extracts were subjected to qualitative chemical examination for identification of various plant constituents.

Chemicals

Indian ink (Camel industries, Mumbai), Cyclophosphamaide (Endoxan injection, Elder Pharmaceuticals, Mumbai). Levamisole was purchased from Khandelwal Labs (Mumbai, India), all other reagents used were of analytical grade.

Neutrophil Adhesion Test[7]

Adult male Wister rats were weighing about 150 to 200 g were divided into 5 groups of 6 animals each. The dosages of drugs administered to the different groups were as follows:

Group I – Vehicle Control (received distilled water 1 ml/kg)

Group II – Standard Control (received Levamisole 2.5 mg/kg)

Group III – Animals receiving methanolic extract of flowers of *Ixora coccinea* (100 mg/kg p.o) for 14 days

Group IV – Animals receiving methanolic extract of flowers of *Ixora coccinea* (200 mg/kg p.o) for 14 days

Group V – Animals receiving methanolic extract of flowers of *Ixora coccinea* (400 mg/kg p.o) for 14 days

All group of rats were administred orally with vehicle, standard and extract for 14 days as per the above prescribed design. On day 14, blood samples were collected from the retro-orbital plexus into heparinised vials and analyzed for differential leukocyte count. After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 10 min at 37°C. The incubated blood samples were again analyzed for DLC. The percentage of neutrophils in the nylon treated fiber and nylon fiber untreated blood was determined and the difference was taken as index of neutrophil adhesion.

Carbon Clearance Test[8]

Adult male Wister rats were weighing about 150 to 200 g were divided into 5 groups of 6 animals each. The dosages of drugs administered to the different groups were as follows:

- **Group I –** Vehicle Control (received distilled water 1 ml/kg)
- **Group II –** Standard Control (received Levamisole 2.5 mg/kg)
- **Group III –** Animals receiving methanolic extract of flowers of *Ixora coccinea* (100 mg/kg p.o) for 10 days
- **Group IV –** Animals receiving methanolic extract of flowers of *Ixora coccinea* (200 mg/kg p.o) for 10 days
- **Group V –** Animals receiving methanolic extract of flowers of *Ixora coccinea* (400 mg/kg p.o) for 10 days

Eight groups of normal Albino rats (Wistar Strain) consisting of 6 rats each group were used for studying. Albino rats were treated with the extract or vehicle orally for 10 days. After 48 hr of the last dose of the drug, animals were injected 0.1 ml of Indian ink via the tail vein. Blood samples were withdrawn at 0 and 15 min after injection. A 50 µl blood sample was mixed with 4 ml of

0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm.

The phagocytic index K was calculated using the following equation:

$$K = (\text{Log}_e OD1 - \text{Log}_e OD2)/15$$

Where, OD1 and OD2 are the optical densities at 0 and 15 min respectively

Statistical analysis:

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's 't' comparison test by using Graphpad prism5 trail version. The values are expressed as mean ± SEM and P<0.05 was considered significant.

RESULTS AND DISCUSSION

Qualitative chemical tests for extracts prepared from flowers of *Ixora coccinea*

The extracts of *flowers* of *Ixora coccinea* was qualitatively tested for the presence of various phytoconstituents. The petroleum ether and chloroform extracts of *Ixora coccinea* gave positive tests for steroids and glycosides. The methanolic extract of *Ixora coccinea* gave positive tests for steroids, triterpenes, alkaloids, tannins, glycosides, saponins, flavonoids, carbohydrates and proteins.

Effect on neutrophil adhesion test

Incubation of neutrophils with nylon fibres produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibres. Methanolic extract of *Ixora coccinea* showed significant increase in the neutrophil adhesion when compared to control. The neutrophil count in untreated blood was also increased by all the treatments (100 mg/kg, 200 mg/kg, 400 mg/kg) (fig. 1).

Effect on carbon clearance test

Methanolic extract of *Ixora coccinea* at (100 mg/kg, 200 mg/kg, 400 mg/kg) doses showed significant increase in the phagocytic index when compared to control indicating that there was increase in the clearance of colloidal carbon from the blood after administration of these drugs (table 2).

Neutrophil Adhesion Test

Table No.1: "Effect on Neutrophil Adhesion in rats"

S. No.	Treatment	% Neutrophil		Difference (A-B)
		UB(A)	NFTB(B)	
1.	Normal Control (1ml/Kg,po.)	48.0 ±0.04	39.0 ±0.04	9.0 ±0.61
2.	Standard (2.5mg/kg, po)	57.53 ±0.23	38.05 ±0.04	19.48 ±0.20***
3.	MEIC (100mg/Kg,po.)	54.75 ± 0.79	41.37±0.16	13.2 ±0.87***
4.	MEIC (200mg/Kg,po.)	57.2 ± 0.22	40.27 ± 0.08	17.03±0.18***
5.	MEIC (400mg/Kg,po.)	65.03±0.04	41.5 ±0.56	23.8 ±0.06***

n=6 significant at p<0.05*, 0.01** and 0.001. MEIC: Methanolic extract of Ixora coccinea UB: Untreated blood, NFTB: nylon fiber treated blood***

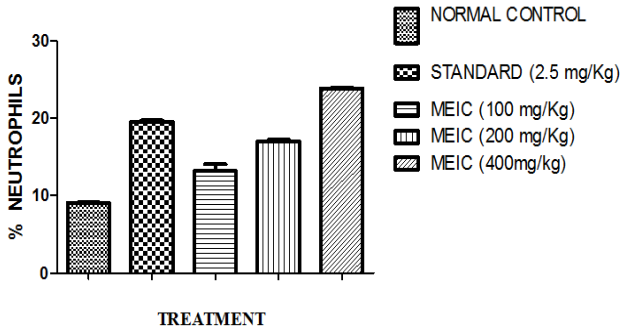

Figure 1: Histogram showing effect of MEIC on neutrophil adhesion

Table 2: Effect of MEIC on phagocytic inedex and % increase in phagocytic index in normal albino rats

S.No.	Treatment	Phagocytic Index	% Increase in phagocytic index
1.	Normal Control (1ml/Kg,po.)	0.0595±0.0004282	-
2.	Standard (2.5mg/kg, po)	0.0920±0.0002582***	53.37±1.165
3.	MEIC (100mg/Kg,po.)	0.07383±0.0005426***	23.37±1.668
4.	MEIC (200mg/Kg,po.)	0.08533±0.0003333***	43.30±0.63
5.	MEIC (400mg/Kg,po.)	0.09178±0.00005426***	53.05±1.936

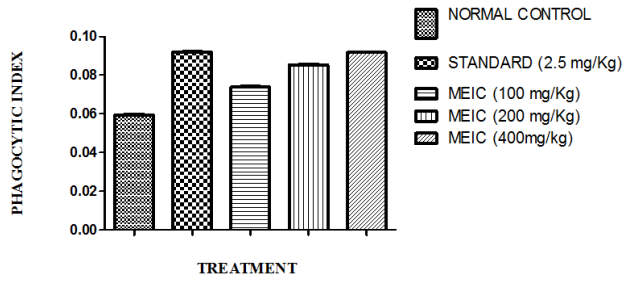

Figure 2 Histogram showing effect of MEIC on Phagocytic index in carbon clearance assay

Immune system dysfunction is responsible for various diseases like arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer and infectious diseases[9]. The degree to which the patient becomes abnormally susceptible to infections by this microbial environment depends on the extent of immunosuppression. The suppression of the immune system is characterized by reduction in the number and phagocytic function of the neutrophils and macrophages, as well as an impairment of the intracellular bactericidal capacity of these cells. This immunosuppression allows opportunistic pathogens to overwhelm the host to cause secondary infections. This problem can be overcome by boosting the immune system by the use of immunomodulatory drugs. Use of plants and plant products as immunomodulators is still in a developing stage. It has been reported that *Piper longum*[10], an immunopotentiating plant, enhances the total bone marrow cells. *Tinospora cordifolia* which is widely used in

Indian system of medicine has been reported for its immunomodulatory and antitumor activities[11]. Curcumin, which is present in the plant *Curcuma longa*, has shown to stimulate the immune system in animals[12]. It has also been reported to reduce the leukocytopenia in radiation and chemotherapeutic drug treated animals. The present study was an endeavor to evaluate Immunomodulatory activity of methanolic extract of *Ixora coccinea* flowers on animal models. Chronic administration of MEIC significantly increased Neutrophil adhesion (in normal rats) and Phagocytic index, in a dose dependent manner. The adhesion of neutrophils to nylon fibres describes the margination of cells in the blood vessels and the number of neutrophils reaching the site of inflammation[13, 14]. MEIC at three doses (100, 200 and 400mg/kg) showed a significant increase in the neutrophil adhesion to nylon fibres. This might be due to the up regulation of the β2 integrins, present on the surface of the neutrophils through which they adhere firmly to the nylon fibres. Hence, it was inferred that MEIC causes stimulation of neutrophils towards the site of inflammation. The carbon clearance test was carried out to evaluate the effect of drugs on the reticulo- endothelial system (RES). This is a diffuse system comprising of phagocytic cells, fixed tissue macrophages and mobile macrophages. The phagocytic cells in this system comprise the mononuclear phagocyte system (MPS), and the macrophage is the major differentiated cell in the MPS. Cells of the RES and MPS are known to be important in the clearance of particles from the bloodstream[15]. Once particulate material is ingested into phagosomes, the phagosomes fuse with lysosomes and the ingested material is then digested. Thus, it is not only ingesting and removing microorganisms but also malignant cells, inorganic particles and tissue debries[16]. When colloidal ink containing carbon particles is injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation. Methanolic extract of *Ixora coccinea* at three doses (100, 200, and 400 mg/kg) showed a significant increase in the phagocytic index. Hence, these agents may stimulate the reticuloendothelial system.

CONCLUSION

Methanolic extract of *Ixora coccinea* flower extracts showed profound immunostimulant activity in male albino rat model. The immunomodulatory activity might be attributed to the presence of flavonoids, alkaloids, steroids and tannin compounds. The present study demonstrated and provided evidence for the traditional uses of *Ixora coccinea*. Further studies might be required to determine detailed mechanisms and active phytochemicals responsible for immunomodulatory activity.

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