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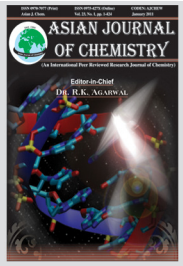

Evaluation of Antiinflammatory and Analgesic Activity of *Ixora coccinea* Flower Extract

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The antiinflammatory and analgesic activity of methanolic flower extract of *Ixora coccinea* Linn. Rubiaceae was investigated in this study. The effect of methanolic flower extract of *Ixora coccinea* was studied using carrageenan induced paw edema, acetic acid induced writhing response and hot plate method for studying antiinflammatory and analgesic activity. The extract at the dose levels of 200 and 400 mg/kg body weight significantly reduces ($P<0.05$) carrageenan induced inflammation in rats and shows analgesic activity, as determined by acetic acid induced writhing response and hot plate method. The effect of methanolic flower extract showed dose dependent reduction in the number of writhing as compared to control drug, which was highly significant. The percentage inflammation protection of methanolic flower extract at 400 and 200 mg/kg was found to be 80.14 and 68.26, which were very close to the standard drug (83.86).

Key Words: *Ixora coccinea*, Antiinflammatory, Analgesic, Methanolic extract, Rubiaceae.

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. The World Health Organization (WHO) estimates that 4 billion people, 80 % of the world population presently use herbal medicine for some aspect of primary healthcare[1]. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental and Native American Indian Medicine. WHO note that of 119 plants derived pharmaceutical medicines; about 74 % are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures.

Ixora coccinea Linn. is a small shrub which is cultivated throughout India. (Flame of Woods in English, Rangan in Hindi and Bengali, Kisukare in Kannada). Its roots and flowers are used for dysentery, dysmenorrhea, leucorrhoea, haemoptysis and catarrhal bronchitis. The leaves are used for diarrhoea. Its roots are also used for hiccups, nausea and loss of appetite and externally for the treatment of sores, eczema and chronic ulcers. Its roots contain an aromatic acrid oil, tannin and fatty acids. Its leaves yield flavonol, kaemferol, quercetin, proanthrocyanidines, phenolic acids and ferulic acids. Its flowers yield cyanidins, flavonol and cooling materials, which are related to quercitin[2-6]. Its roots are ground into pulp, mixed with water and are used as tincture is used for diarrhoea and dysentery[2,3]. Different plant parts of *Ixora coccinea* are used for treatment of various disease conditions some of which are associated with inflammation. A decoction of the flowers is given for haemophytis, acute bronchitis and dysmenorrhoea. Previous studies have reported antiinflammatory effects of aqueous leaf extract of *I. coccinea* using both acute and chronic inflammatory models[7]. The aqueous leaf extract was shown to possess antihistamine and antinociceptive activities[8]. The *Ixora coccinea* leaf extract also exhibited antidiarrhoeal activity[9].

However, there is no scientific evidence for analgesic activity of methanolic extract of *Ixora coccinea* flower. Hence, the present study was undertaken to evaluate the possible anti-inflammatory activity of the flower extract of *Ixora coccinea* Linn.

EXPERIMENTAL

Preparation of plant extract: Flowers of *Ixora coccinea* were collected in October 2009 from Sodhpur area in North 24-Paragana district of West Bengal. Identification and authentication of the plant was done by Dr. P. Sur, Scientist, Botanical Survey of India, Shibpur, India. The voucher specimen was deposited in our herbarium for further information. The shade-dried flowers are coarsely powdered and extracted with mixture of methanol:water (1:1 ratio) by maceration at 45 ºC. The extract was concentrated by using rotatory evaporator and obtained greenish gummy exudates (yield-8.5 %). This crude extract was used for the activity study.

TABLE-1
ACUTE ANTIINFLAMMATORY ACTIVITY OF *Ixora coccinea* FLOWER EXTRACT ON CARRAGEENAN INDUCED RAT PAW OEDEMA.

Group	Dose (mg/kg)	Percentage of inflammation at time (h)				
		1h	2h	3h	4h	5h
Control	10mL/kg	41.56 ± 4.55	81.78 ± 3.28	105.24 ± 6.32	126.65 ± 5.56	132.04 ± 6.44
Indomethacin	10	11.16 ± 3.74*	17.55 ± 3.25*	26.33 ± 4.21*	35.5 ± 3.67*	42.75 ± 5.25*
MEIC-1	100	37.33 ± 5.56	68.34 ± 5.12	87.68 ± 4.86	97.33 ± 5.36	105.44 ± 6.45
MEIC-2	200	24.37 ± 4.38*	38.82 ± 4.64*	66.72 ± 5.22*	75.37 ± 4.43*	81.35 ± 4.75*
MEIC-3	400	15.26 ± 3.55*	24.74 ± 4.78*	31.33 ± 5.31*	42.68 ± 3.14*	48.66 ± 3.74*

*p < 0.05 as compared to control group. Values are mean + SEM: n = 6 in each group. MEIC: Methanolic extract of *Ixora coccinea* flower

Albino rats (150-200 g) of either sex and Albino mice (Swiss strain) weighing 25-30 g either sex were obtained from the Serum Lab, Pune. The animals were maintained at room temperature of 25 ± 2 ºC with relative humidity of 75 ± 5 % under 12 h dark and light cycle. The animals maintained under standard husbandry conditions and had free access to diet and water. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. The ethical committee of the institute approved the protocol of the study.

Antiinflammatory activity

Carrageenan induced paw edema in rats: Pedal inflammation in rats (100-150 g) was described by Winter *et al.*[10]. Oedema was induced by subcutaneous administration of 0.1 mL of 1 % aqueous solution of carrageenan into right hind paws[10]. The test drug is suspended in 1 % solution of gum accacia and diluted with saline. The control group received the vehicle (10 mL/kg body wt.). A test drug suspension (100, 200 and 400 mg/kg) was administered orally for 7 consecutive days prior to the infection of carrageenan paw volume were measured upto 5 h after the carrageenan administration at an interval of 60 min and paw volume was measured with plethysmometer. Indomethacin was used as standard drug[11,12].

Analgesic activity: Two standard methods *viz*. acetic acid induced writhing reflex and hot plate methods were employed to determine the analgesic activity.

Acetic acid induced writhing-reflex method in mice[13]: The analgesic activity was determined by acetic acid induced writhing method using six albino mice (25-30 gm) of either sex selected by random sampling technique. Standard drug nimesulide (50 mg/kg) and the extracts (100 mg/kg, 200 mg/kg and 400 mg/kg) were given intraperitoneally 30 min prior to the administration of the writhing agent (0.6 % v/v aqueous acetic acid, 10 mL/kg). The number of writhings produce in the animal was observed for 30 min. The number of writhing and stretching was recorded and compared with the control drug. The percent was calculated using the following ratio: % of protection = control mean- treated mean × 100/control mean. The analgesic activity data are presented in Table-2.

Hot plate method in mice[14]: Mice of either sex weighing between 25-30 g were kept on a hot plate (55 ± 0.5 ºC), the time for forepaw licking or jumping was taken as the reaction time. Mice showing reaction time between 3-5 s were selected. Animals not responding in this period were discarded. The reaction time was recorded at 30, 60, 90 and 120 min following administration of the test compounds or the standard drug (nimesulide 50 mg/kg s.c.) to determine the onset and duration of action. 1 h after the administration of vehicle, standard drug and extracts treated mice were individually placed on the hot plate of the analgesiometer maintained at 55 ºC. Analgesic activity was determined by comparing with the control group. The analgesic activity data are presented in Table-3.

Statistical analysis: Data are presented as the mean ± standard error of mean (SEM). Statistical analyses used one-way analysis of variance (ANOVA) to account for the different treatment doses and were complemented with unpaired t-test. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The methanolic extract of *Ixora coccinea* significantly (as compared to control) and dose dependently reduced carrageenan induced paw edema in rats. The standard drug indomethacin showed better inhibitory activity than of *Ixora coccinea* as shown in Table-1. The lower the paw volume the better the activity. The inhibitory activity of methanolic extract was very close to Indomethacin.

The analgesic activity of *Ixora coccinea* was dose dependant. The percentage protection of inflammation of methanolic extract of *Ixora coccinea* (MEIC) at 200 and 400 mg/kg b.w. were found to be 68.26 and 80.14 whereas MEIC at 100 mg/kg b.w. was 48.55 as shown in Table-2.

TABLE-2
ANALGESIC ACTIVITY (WRITHING REFLEX METHOD) OF *Ixora coccinea* FLOWER EXTRACTS IN MICE

Group/treatment	Dose (mg/kg)	Writhings ± SEM	% Protection
Control	10 mL/kg	31	-
Nimuselide	50	5.7 ± 0.38	83.86
MEIC-1	100	15.33 ± 2.45	48.55
MEIC-2	200	9.25 ± 0.32	68.26
MEIC-3	400	5.4 ± 0.68	80.14

* p < 0.05 as compared to control group. Values are mean + SEM; n = 6 in each group. MEIC: Methanolic extract of *Ixora coccinea* flower

Analgesic activity of methanolic extracts of *Ixora coccinea* flower increased the response latency in the hot plate test which was significant. The control drug increased the response latencies at various time intervals. The effect of the extract was dose as well as time dependent. Amongst all the doses

TABLE-3
ANALGESIC ACTIVITY (HOT PLATE METHOD) OF METHANOLIC EXTRACTS OF *Ixora coccinea* FLOWER IN MICE

Group/Treatment	Dose (mg/kg))	Reaction Time in Min.			
		30	60	90	120
Control	10 mL/kg	5.67 ± 0.562	6.44 ± 0.871	6.27 ± 0.583	5.84 ± 0.872
Nimuselide	50	3.29 ± 0.394	6.24 ± 0.622	4.31 ± 0.522*	4.51 ± 0.215
MEIC-1	100	3.57 ± 0.179	4.55 ± 0.415	5.72 ± 0.826	6.08 ± 0.755
MEIC-2	200	3.75 ± 0.552	5.26 ± 0.412	6.09 ± 0.336*	5.36 ± 0.352
MEIC-3	400	4.28 ± 0.415	7.13 ± 0.316	8.62 ± 0.655*	5.65 ± 0.477

*$p < 0.05$ as compared to control group. Values are mean + SEM ; n = 6 in each group. MEIC: Methanolic extract of *Ixora coccinea* flower.

used, methanolic extract was most effective at 200 and 400 mg/kg at 90 and 120 min as comparable as the control drug, which was highly significant ($P < 0.05$). The analgesic activity of methanolic extract of *Ixora coccinea* at the dose level of 400 mg/kg showed more significancy than standard drug Nimuselide as shown in Table-3.

The antiinflammatory and analgesic activity of methanolic extract of *Ixora coccinea* (MEIC) flower was investigated in the present study. The carrageenan test was selected because of its sensitivity in defecting orally active antiinflammatory agents particularly in the acute phase of inflammation[15,16]. The intraplantar infection of carrageenan in rats leads to paw edema. Its first phase (0-2.5 h after injection of carrageenan) results from the concomitant release of mediators: histamine, serotonin and kinins on the vascular permeability. The second phase is correlated with leukotrienes. The oral administration of MEIC suppresses inflammation during the second phase. The MEIC at 400 mg/kg shows maximum inhibitory response as compared to other doses and near potent as standard drug. The mechanism for testing analgesic was selected such that both centrally and peripherally mediated effects were investigated. The acetic acid induced abdominal constriction elucidated peripheral and central activity. The hot plate method elucidates peripheral mediated effects[17].

The extract (100 and 200 mg/kg), administered orally, significantly inhibit acetic acid induced writhing in rats. There writhing are related to increase in the peritoneal level of prostaglandins and leukotrienes[18]. The result strongly suggests that the mechanism of action of extract may be linked to lipoxygenase and/or cycloxygenase. In the formalin test there is distinctive biphasic nociceptive response termed neurogenic and inflammatory phases. Drugs that primarily act on central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase[19]. The neurogenic and inflammatory phase is due to the release of substance P, histamine, serotonin, bradykinin prostaglandins and leukotrienes respectively. This test is very useful for not only assessing analgesic drugs but also helping in the elucidation of mode of action. To correlate that the extract had no central analgesic acid, hot plate test[20] were conducted, significant effect noted for 400 mg/kg of the extract in hot plate test were not due to central acting activities of the fraction. This mean there was no opioid receptors involved. The MEIC (400 mg/kg) shows best activity after 5 h than other doses and also near potent to indomethacin.

Conclusion

In present study, the antiinflammatory and analgesic activity of methanolic extract of *Ixora coccinea* flower was investigated by means of carrageenan induced paw edema in rats, acetic acid induced writhing and hot plate method in rats. The oral administration of methanolic extract of *Ixora coccinea* showed suppression of inflammation and mechanism of action of extract might be linked to lipoxygenase and/or cycloxygenase. The Methanolic extract at the dose level of 400 mg/kg body weight showed maximum inhibitory response as compared to other doses. The result strongly suggests that it can be used efficiently as analgesic and antiinflammatory agents.

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ERRATUM

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Synthesis of β-Amino Cyclone Catalyzed by Alkaline Al_2O_3

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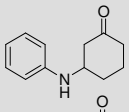
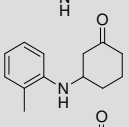
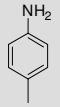
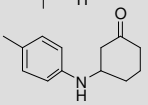
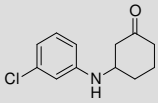
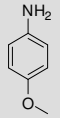
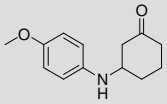
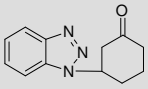
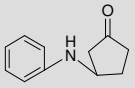
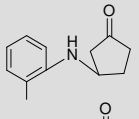
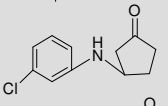
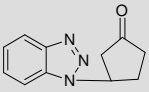
1. Kindly read the correct footnote 'd' in Table-3 read as: Yield refers to HPLC (*n*-hexane:isoprpopanol = 8:1) instead of (1:8).
2. Some structural formula of the products in Table-2 were wrongly published. Please read the following correct Table-2.

TABLE-2
CONJUGATIVE ADDITION OF AROMATIC AMINE TO CYCLOPENTENONE AND CYCLOHEXENONE AT ROOM TEMPERATURE[a]

Entry	Amine	Cyclonene	Product	Time(h)[b]	Isolated Yield%[c]
1				2	98
2				2	95
3				2	95
4				2	96
5				2	98
6				1.5	99
7				2	95
8				2	95
9				2	95
10				1.5	97

[a]All reactions were run at room temperature using cyclohexenone (12 mmol), phenylamine (10 mmol) without solvent; [b]The time of the reaction was monitored by TLC (AcOEt:*n*-hexane = 1:4); [c]Isolated yields after column chromatography (AcOEt:*n*-hexane =1:6).