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Evaluation of analgesic activity of methanolic extract of *Ixora coccinea* stems

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Abstract

Ixora coccinea L. belonging to the family Rubiaceae, is an evergreen shrub or small tree commonly known as Jungle of Geranium or Red Ixora. Pain is defined as an unpleasant complex phenomenon of sensory experiences. Analgesics are the agents which are used to relieve or diminish pain sensation. The evaluation of analgesia is assessed by using various pharmacological screening techniques like thermal stimuli, electrical stimuli or chemical stimuli. In the present study animal models are used to explore the analgesic activity of the methanolic extract of *Ixora coccinea* L. stems by using hot plate method and tail flick method. The two test doses (250mg/kg and 500mg/kg body weight) were compared with standard (Aspirin 20mg/kg body weight) and normal saline (10ml/kg body weight). The methanolic extract of *Ixora coccinea* L. stems have shown to exhibit significant analgesic activity in mice.

Keywords: *Ixora coccinea*, analgesic, aspirin, eddy's hot plate, tail-flick method

Introduction

As stated by the study of International Association 'pain' is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. The pain can be acute or chronic and treatment for chronic pain is crucial public health problem by the results of recurrent use of available drugs that have unpleasant side effects [2]. Regardless of numerous developments in pain remedies, there is a need for safe, effective and potent analgesics for the treatment of pain [3]. The drug which relieves the pain are called as pain killers or analgesics. NSAID's are commonly used for treating mild pain [4].

Ixora is an evergreen shrub belongs to the family Rubiaceae, it is commonly known as Jungle of geranium, Raktaka, Red Ixora. It is naturalized to throughout the India [5]. It contains important phytochemicals i.e., Anthocyanin, Lupeol, Ursolic acid, Rutin, Sitosterol, Glycosides and Quercetin. The plant was reported to possess Antioxidant, Anti-inflammatory, Antimicrobial, Antidiarrheal, Gastro protective, Hepatoprotective and Antinociceptive activities [6]. It has traditional uses in Ayurveda and in the various folk systems of medicine, to treat various diseases [7].

Ixora coccinea L. stem extracts were previously tested for antimicrobial [8], antioxidant [9], antidepressant [10] and antifertility [11] activities. However, the methanolic stem extract was not tested for the analgesic activity formerly, the purpose of this study is to explore the analgesic activity of the stems of *Ixora coccinea* L. plant.

Materials & Methods

Plant material: Stems of *Ixora coccinea* L. was collected from Karimnagar, Telangana, in November. The Plant was authenticated by the Botanical Survey of India. The Herbarium of the plant Specimen has been deposited at BSI in Hyderabad. The voucher specimen no BSI/DRC/2018-19/Tech/554.

Extraction of Plant Material

The Stems of *Ixora coccinea* were washed with water dried in shade. Then the stems were pulverised in grinder and the powder was extracted with methanol by soxhlation. After completion of the extraction, the extract was concentrated at room temperature and residue is made to dryness and stored in desiccator.

Experimental Animal

Male albino mice (25-30gm), six animals per group were used for the study. The Animals were housed in polyvinyl cages and were maintained under standard conditions (12hr light, 12hr dark cycle, 25-20°C, 35-60% Humidity).

The Animal were fed with standard pelleted commercial feed and allowed to water *ad-libitum*, all animals are acclimatized for one week prior to commencement of study. The Animal experimental protocol was approved by the Institutional Animal Ethical Committee.

Study design

The animals were fasted 12hrs before the experiment and were divided into 4 groups, each group containing 6 mice. All treatments were administered intraperitoneally. The first group served as control receiving normal saline solution (10ml/kg). The second group was given standard drug Aspirin (20mg/kg). The third and fourth groups were treated with test doses of methanolic extract of *Ixora coccinea* (250 mg/kg and 500 mg/kg respectively).

Tail flick Method

The analgesic activity was determined by measuring the changes after drug introduction in the sensitivity or tolerability of mice to the radiation or electric current [12]. In this test the pain is induced by applying radiant heat on the tail of the mice 5cm away from the tip of the tail. Mice were hold very loosely during the test [13]. The intensity of the current used in this test naked nichrome wire was 6 amperes [14]. Reaction time was recorded at withdrawal of the tail when exposed to the radiant heat. The maximum reaction time was fixed at 15 secs to prevent tissue damage [15]. The reaction time was recorded at 0, 30, 60, 90,120 and 180 mins.

Eddy's Hot Plate Method

Evaluation of analgesic activity of extract was also carried out by using hot plate method. The animals were placed on a hot

plate with a temperature range of 55-56 °C. The reaction time was determined as the time taken for the animal to react to the heat with responses of paw licking or jumping [16]. The reaction time was recorded at 0, 30, 60, 90, 120 and 180min after administration of dosage. The maximum reaction time was fixed as 15sec for the prevention of paw injury by heat [17].

Acute toxicity studies

Acute toxicity study was carried out using male albino mice as per OECD guidelines. The methanolic extract of *Ixora coccinea* L. was given to different groups of animals at the doses of 50, 300, 1000, 2000, and 3000 mg/kg body weight respectively. The extract produced no death or signs of toxicity after 48 hours, which shows that the extract was well tolerated.

Data analysis

All the obtained values were expressed as mean± SD from six animals, subjected to statistical analysis using one-way analysis of variance followed by Dunnett's test to verify significant difference if any among the groups. $P<0.05^*$, 0.001^{**} was considered as statistically significant.

Results & Discussion

Eddy's Hot Plate Method

The results of the hot plate method of MEIC is represented in table 1. The extract significantly ($P<0.05$) increased the reaction time of thermal nociception in dose dependent manner. The maximum effect of MEIC was observed after 60 mins, which is comparable to the reference drug Aspirin.

Table 1: Values are expressed as mean±SD, (n=6) (compared to control group) by using One Way Analysis of Variance (ANOVA) followed by Dunnett's test $P<0.05^*$

Groups:	0 min	30 min	60 min	90 min	120 min	180 min
Control	3.16±1.4	4.0±0.8	4.8±1.4	5.0±1.8	4.8±1.9	3.5±1.3
Standard	4.83±1.4	5.0±1.09	6.1*±1.3	8.3*±1.3	7.8*±2.1	6.3±1.8
TEST1 (250mg)	5.80±1.4	8.3±1.0	10.6*±0.8	10.6*±1.0	10.5*±1.8	7.3±1.2
TEST2 (500mg)	8.0±1.6	9.0±1.6	10.0*±1.8	11.1*±1.1	9.8*±1.4	7.6±1.3

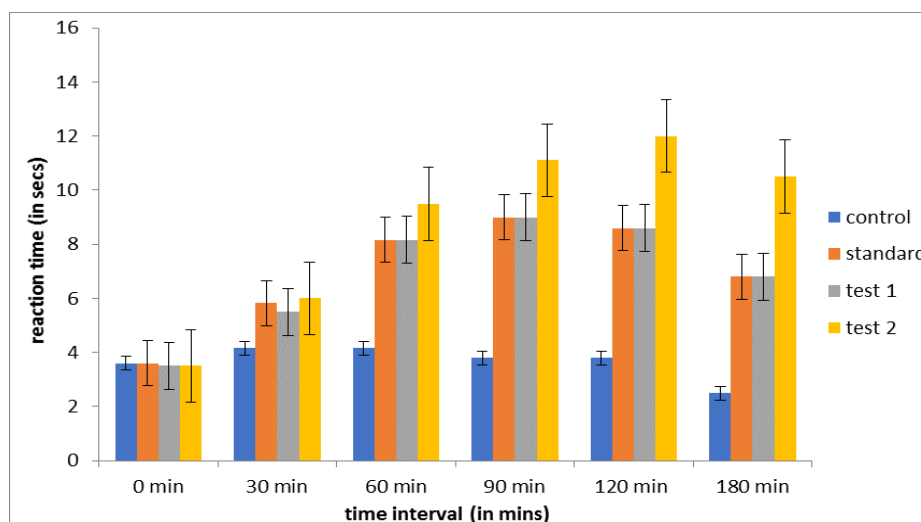


Fig 1: Graphical representation of analgesic effect of Methanolic extract of *Ixora coccinea* compared with standard and control drugs by hot plate method.

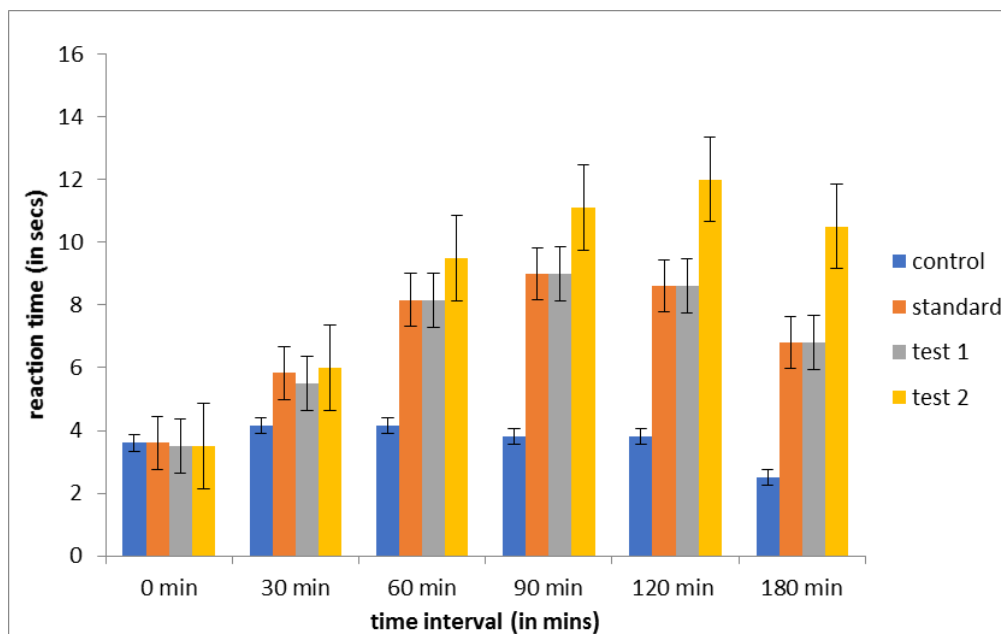
Tail Flick Method

The results of the tail flick method of MEIC is represented in table 2. The extract significantly ($P<0.001$) increased the reaction time of thermal nociception in dose dependent

manner. The maximum effect of MEIC was observed after 60 mins, which is comparable with the reference drug Aspirin.

Table 2: Values are expressed as mean±SD, (n=6) (compared to control group) by using One Way Analysis of Variance (ANOVA) followed by Dunnett's test $P<0.001^{**}$

Groups:	0 min	30 min	60 min	90 min	120 min	180 min
Control	2.83±0.75	4.16±0.75	4.16±0.4	3.8±0.98	3.8±0.98	2.5±0.83
Standard	3.6±1.03	6.0±1.26	9.5**±1.3	11.1**±0.98	12.0**±1.2	10.5±1.76
TEST1 (250mg)	3.8±1.16	5.5±1.04	7.5**±0.5	9.16**±0.14	8.5**±0.54	8.0±1.6
TEST2 (500mg)	3.6±0.5	5.83±0.4	8.16**±8.4	9.0**±0.5	8.6**±1.3	6.8±0.9

**Fig 2:** Graphical representation of analgesic effect of Methanolic extract of *Ixora coccinea* compared with standard and control drugs by Tail flick method.

Conclusion

The methanol extract of *Ixora coccinea* L. stems administered intraperitoneally exhibited significant analgesic activity and it might exert its effect in peripheral pathway. This plant can be further investigated to find out the active components responsible for the analgesic activity and to confirm the possible mechanism of actions for developing potential source of analgesic drug.

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