



STUDY OF ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF *Impatiens balsamina* LEAVES IN ALBINO RATS

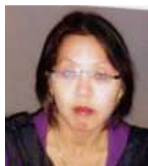
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ABSTRACT

To study the analgesic and anti-inflammatory effects of aqueous extract of *Impatiens balsamina* leaves in suitable animal models, the leaves of *Impatiens balsamina* were extracted with distilled water using Soxhlet apparatus. The extract thus obtained was screened for analgesic and anti-inflammatory activity using tail flick method and carrageenan induced paw oedema method in albino rats respectively. The aqueous extract of the plant produced significant increase in the reaction time at 30, 60 and 120 minutes as compared to respective controls in albino rats ($p < 0.05-0.001$). The aqueous extract of the plant produced significant reduction in the mean increase in paw volume; 0.316 ± 0.04 ($p < 0.01$), 0.266 ± 0.03 ($p < 0.01$) and 0.166 ± 0.04 ($p < 0.01$) at the doses of 500, 1000 and 2000 mg/kg body weight respectively as compared to the control (0.483 ± 0.03). However the extract was found to be less effective than the standard drug. The study demonstrates significant analgesic and anti-inflammatory effects of aqueous extract of *Impatiens balsamina* leaves.

KEYWORDS: *Impatiens balsamina* (IB), analgesic, anti-inflammatory, carrageenan



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INTRODUCTION

The traditional system of medicine has played and will continue to play a very important role in providing preventive/curative services. WHO strongly supports the promotion and development of the rational use of traditional medicines throughout the world¹. Many indigenous plants of India have been subjected to scientific testing and experimentation. The traditional system of medicine is certainly a cheap and potential source of drugs owing to their time tested efficacy and lack of recognizable adverse effects². *Impatiens balsamina* (English name-Rose balsam, Hindi name – (Gul mehendi) locally known as Khujang in Manipuri belonging to the family Balsaminaceae is an ornamental succulent herb commonly found in southern Asia. It comes in a wide variety of flower colours, numbers and forms. The leaves are simple, alternate, ovate-lanceolate and serrate. Different parts of the plant are used to treat disease and skin afflictions; the leaves, seeds and stems are also edible if cooked. *Impatiens balsamina* L. has been used as indigenous medicine in Asia for the treatment of rheumatism, fractures and fingernail inflammation. In Korea, *Impatiens balsamina* has been used in traditional oriental medicine to treat scrofulosis, carbuncles and dysentery³. Juice from the white corolla is painted topically on the skin as an anti-pruritic to treat several types of dermatitis. Flowers are useful when applied to burns and scalds. It acts as an emetic, cathartic and diuretic⁴. It is found abundantly in Manipur and is widely used for its medicinal properties. Even though there are various reports of the uses of *Impatiens balsamina* there is lack of scientific data to substantiate them. Therefore, the present study has been undertaken to evaluate the analgesic and anti-inflammatory properties in suitable animal experimental models.

MATERIALS AND METHODS

The fresh leaves of *Impatiens balsamina* were collected from around the Imphal area during the month of June-July 2010, identified and authenticated by Dr. Athokpam Pinokiyo, Assistant Professor, Department of Botany, DM college of Science, Imphal. Preparation of the aqueous extract was done following the method of Verma and Agarwal⁵ with slight modifications. Eighty grams of the powdered leaves was extracted with distilled water using a Soxhlet apparatus. The brownish extract obtained was evaporated, shade dried, scrapped out, weighed and stored in a glazed porcelain jar. The final yield was 29.5%.

EXPERIMENTAL ANIMALS

Healthy albino rats of Wistar strain of either sex weighing between 100-200gm were collected from Central Animal House, RIMS. The animals were kept in polypropylene cages with 12 hour light-dark cycle in ambient temperature. The animals were maintained on standard laboratory diet and water ad libitum. All the experimental protocols were approved by the Institutional Animal Ethics Committee.

ACUTE TOXICITY STUDY

No adverse effect or mortality was detected in albino rats fed upto 3 gm/kg p.o. of aqueous extract of IB during 24 hour observation period.

DRUGS

The following chemicals and drugs were used-. Pethidine hydrochloride, Gum acacia, Aspirin, Carrageenan.

ANALGESIC ACTIVITY OF AEIB

Tail flick test in albino rats

The method of D' Armor and Smith⁶ as modified by Davies OL et al⁷ was followed to evaluate the analgesic activity of aqueous extract of *Impatiens balsamina* (AEIB) by tail flick method using analgesiometer. The animals were divided into five groups with six animals in each group.

GROUP	DRUG AND DOSES
I (CONTROL)	2% gum acacia in distilled water.
II (TEST)	Aqueous extract of IB (500mg/kg)
III (TEST)	Aqueous extract of IB (1000mg/kg)
IV (TEST)	Aqueous extract of IB(2000mg/kg)
V (STANDARD)	Pethidine (5mg/kg) , i.p

The test drug was suspended in distilled water using 2% gum acacia and administered orally. The volume of medicaments was kept constant at 10ml/kg body weight of the animals. The tail flick latencies (reaction time) of the animals was assessed by analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was kept at 1.5 cm. The site of application of the radiant heat had been fixed at 2.5 cm, measured from the root of the tail of all rats. The time taken by the animals to withdraw

(flick) their tail from the hot wire was noted and taken as the reaction time. The cut off reaction time was fixed at 10 seconds to avoid tissue damage. Reaction time was recorded at 30, 60 and 120 minutes after the drug administration. The average values of reaction time after each time interval were calculated.

ANTI-INFLAMMATORY ACTIVITY

The method of Winter CA et al⁸ was followed to study the anti-inflammatory effects of the aqueous extract of IB in acute inflammation. The animals were divided into five groups with six animals in each group.

GROUP	DRUG AND DOSES
I (CONTROL)	2% gum acacia in distilled water.
II (TEST)	Aqueous extract of IB (500mg/kg)
III (TEST)	Aqueous extract of IB (1000mg/kg)
IV (TEST)	Aqueous extract of IB (2000mg/kg)
V (STANDARD)	Aspirin (100mg/kg),p.o

Acute inflammation was produced by injection of 0.1 ml freshly prepared 1% carrageenan in 0.9% sodium chloride solution in the sub-plantar region of the right hind paw of the rats, 1 hr after oral administration of drugs. The foot volume was measured in unanaesthetised rats by modified plethysmographic method

immediately and at three hours after carrageenan injection and the volume of oedema was recorded as the difference between the two readings. The percentage of anti-inflammatory activity was then calculated by the formula described by Diniz et al⁹.

$$\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where,

V_c = Mean increase in paw volume in control group

V_t = Mean increase in paw volume in drug treated group

STATISTICAL ANALYSIS

All values were expressed as Mean ± SEM. The results were statistically analysed using one way ANOVA followed by Dunnet's "t" test. P values <0.05 were considered significant.

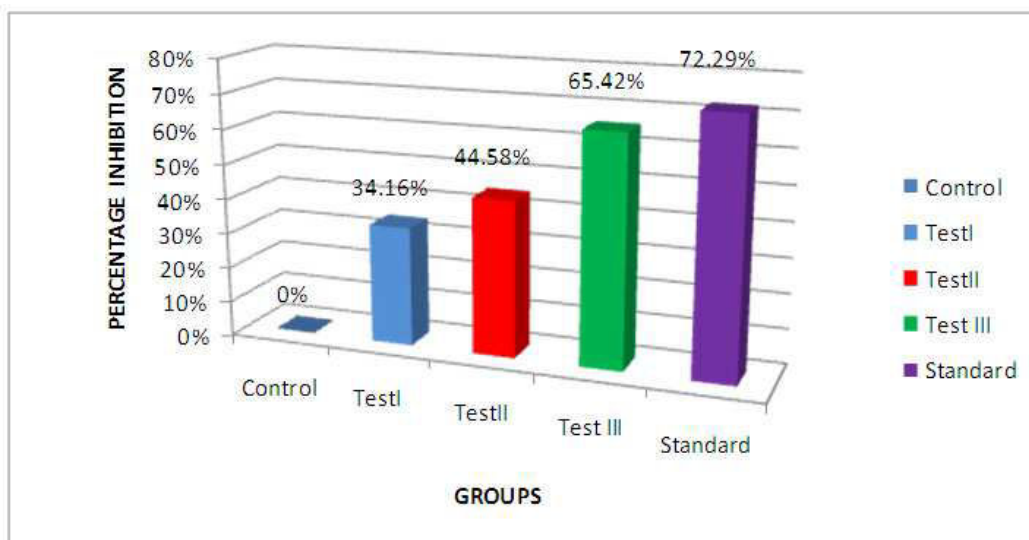
Table II
Anti-inflammatory activity of aqueous extract of IB on carrageenan induced rat paw oedema.

GROUP	DOSE (mg/kg,p.o)	MEAN INCREASE IN PAW VOLUME (MEAN±SEM) IN ML	% INHIBITION OF PAW OEDEMA
I (CONTROL)	10 ml/kg	0.483±0.03	0%
II(TEST)	500	0.316±0.04*	34.16%
III(TEST)	1000	0.266±0.03*	44.58%
IV(TEST)	2000	0.166±0.04*	65.42%
V(STANDARD)	100	0.133±0.02*	72.29%

ONE WAY ANOVA F 13.69
P <0.01
df 4,25

*p<0.001 when compared to control; n= 6 in each group.

Figure 2
Effect of aqueous extract of *Impatiens balsamina* on acute inflammation as tested by carrageenan induced rat paw oedema



DISCUSSION

Tail flick method is a standard model for evaluating central analgesic activity of a drug in albino rats. There was no significant difference between the pre-drug reaction time of the different groups. The animals which showed normal reaction time of 3-4 secs were used for the study. The test drug at doses of 500, 1000 and 2000mg/kg produced significant increase ($p<0.05-0.001$) in the pain threshold after 30,60 and 120 minutes of administration. The standard drug Pethidine (5mg/kg), increased the pain threshold

significantly ($p<0.001$) at 30, 60 and 120 minutes. The results in the control and standard group were comparable to that of Mukherjee A et al¹⁰ The increase in the pain threshold in the tail flick method may be due to the possible partial opioid agonistic effect of the aqueous extract of the leaves of *Impatiens balsamina*. AEIB increased the stress tolerance capacity of the animals and hence also indicates involvement of a higher centre¹¹. The standard drug Pethidine exerts its action through the μ opioid receptors

indicating narcotic involvement¹². Carrageenan induced rat paw oedema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandins and slow reacting substances which peak at 3 hours¹³. The mean increase in paw volume of the control group was 0.483 ± 0.03 which corresponds to the findings of Kurma S Rao and SH Mishra¹⁴ at 0.43 ± 0.01 . The test drug at doses of 500, 1000 and 2000mg/kg produced 34.16%, 44.58% and 65.42% inhibition of paw oedema ($p < 0.001$) compared to 72.29% inhibition produced by 100mg/kg of Aspirin. Thus, the AEIB produced dose dependent and significant inhibition of carrageenan induced paw oedema. The inhibition was however less than that of standard drug, Aspirin. The mean increase in the paw volume of the test group at the dose of 1000mg/kg after 3 hours was 0.266 ± 0.03 which corresponds to the findings of Chakraborty A et al at $0.26 \pm$

0.03 .¹³ The mean increase in paw volume of the standard group was 0.133 ± 0.02 after 3 hours which corresponds to the findings of Mulla WA et al¹⁵ at 0.15 ± 0.03 . The ability of the aqueous extract of the leaves of *Impatiens balsamina* to inhibit carrageenan induced paw oedema suggests that it possesses a significant effect against acute inflammation. The aqueous extract of *Impatiens balsamina* contains anthocyanins, Cox- 2 inhibitory naphthoquinones (lawsone, lawsone methyl ether, methylene- 3,3'-bilawsone), kaempferol glucosides and flavonoids such as quercetin. It has been reported that a large number of flavonoids possess analgesic activity. Flavonoids are known to inhibit the enzyme Prostaglandin synthetase, more specifically the endoperoxidase.¹⁶

CONCLUSION

The findings of the present study show that the aqueous extract of the leaves of *Impatiens balsamina* L. has significant analgesic and anti-inflammatory activity without any significant adverse effects. Further studies on the active constituents present in IB is necessary to understand the mechanism of action

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