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TO STUDY THE ANTI-INFLAMMATORY PROPERTIES OF AQUEOUS EXTRACT OF LEAVES OF *IMPATIENS BALSAMINA* IN EXPERIMENTAL ANIMAL MODELS

Debashree Ningthoujam ^{* 1} and Aruna Soibam ²

Department of Pharmacology ¹, Jawaharlal Institute of Medical Sciences (JNIMS), 2 Demonstrator, Department of Pharmacology ², Imphal - 795140, Manipur, India.

Keywords:

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Correspondence to Author: Dr. Debashree Ningthoujam

Assistant Professor,
Department of Pharmacology,
Jawaharlal Institute of Medical
Sciences (JNIMS), Imphal - 795140,
Manipur, India.

E-mail: debashree27@gmail.com

ABSTRACT: The present study was undertaken to study the 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 the Soxhlet apparatus. The extract thus obtained was screened for anti-inflammatory activity using Cotton pellet implantation method, Granuloma pouch method, and formaldehyde arthritis method in albino rats. The aqueous extract of the plant produced significant inhibition of granuloma formation, significant inhibition of exudate formation and significantly inhibited rat paw oedema induced by formaldehyde as compared to respective controls in albino rats ($p < 0.05 - 0.001$). The aqueous extract of the plant produced significant inhibition of granuloma formation 18.79% ($p < 0.01$), 29.13% ($p < 0.001$) and 44.83% ($p < 0.001$) at concentrations of 500 mg/kg, 1000 mg/kg and 2000 mg/kg respectively. The aqueous extract produced 36.34%, ($p < 0.01$), 50% ($p < 0.001$) and 68.3% ($p < 0.001$) inhibition of exudate formation at 500 mg/kg, 1000 mg/kg and 2000 mg/kg respectively. The aqueous extract also significantly inhibited rat paw oedema induced by formaldehyde ($p < 0.05 - 0.001$). However, the extract was found to be less effective than the standard drug. The study demonstrates significant anti-inflammatory effects of aqueous extract of *Impatiens balsamina* leaves.

INTRODUCTION: Medicinal plants represent one of the most important fields of traditional medicine all over the world and are a natural source of nutraceuticals. A crucial factor in medicinal plant research and in clinical practice is sustainability. The term “sustainable medicines” describes the importance of considering the long-term use of both traditional medicines and synthetic

drugs from a perspective of reliable and non-destructive sourcing for the future. This is of great importance since the population and the use of traditional medicines are growing fast, globalization of products is in increasing demand, and climate change may affect the growth of traditional medicines ¹. Medicinal plants have been used worldwide as various kinds of therapeutic agents since ancient times. Since old times before modern medicine, people became ill and suffered from various ailments.

In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research. In the western world also, the use of

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herbal medicines is steadily growing with approximately 40 percent of the population reporting use of herbs to treat medical illnesses in the past few years². WHO strongly supports the promotion and development of the rational use of traditional medicines throughout the world. According to a recent estimate of WHO, 70- 80% of the world population, especially in developing countries, rely on traditional medicine, mostly plant drugs, for their primary health care needs³.

The traditional system of medicine is certainly a cheap and potential source of drugs owing to their time-tested efficacy and without recognizable adverse effects⁴. The lack of potent anti-inflammatory drugs now actually in use prompted studies to be conducted on various plants. *Impatiens balsamina* is one of the traditional plants with a wide range of medicinal properties. *Impatiens balsamina* locally known as Khujang in Manipuri, belonging to the family Balsaminaceae, is a succulent ornamental herb with a thick but soft stem. It is commonly found in southern Asia. It is commonly known as garden balsam and is an annual herb possessing a wide range of medicinal uses. It comes in a wide variety of flower colours and forms, ranging from bright single flowers to double flowers.

The flowers are red, pink, purple, or white and the fruits are pale green dehiscent capsules containing small globose reddish seeds. The 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 to treat rheumatism, fractures, and fingernail inflammation. In Korea, *Impatiens balsamina* has been used in traditional oriental medicine to treat scrofulosis, carbuncles, and dysentery⁵. It is found abundantly in Manipur and is widely used for its medicinal properties. The seeds contain 36-38% fixed oil, with proteins, alkaloids, saponins and essential oils making up the rest of the composition. Although seed extract or oil has been reported to possess antimicrobial activity, antioxidant activity, antitumor activity, and a stimulatory effect on the immune system, its full potential as an antimicrobial agent has not been exploited⁶. Even though there are various reports of *Impatiens balsamina*, there is a lack of scientific data to

substantiate them. Therefore, the present study has been undertaken to evaluate the anti-inflammatory properties in suitable animal experimental models.

Aims and Objects: To evaluate the anti-inflammatory properties of *Impatiens balsamina* Linn. in experimental animal models.

MATERIALS AND METHODS:

Plant Material: 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. A plant sample was deposited at the Department herbarium, Manipur University, and allocated Account no. 004225 of MUH.

Preparation of the Plant Extract: The leaves were cleaned, dried under shade, powdered by a mechanical grinder, and stored in an airtight container for future use. Preparation of the aqueous extract was done following the method of Verma and Agarwal⁷ with slight modifications. Eighty grams of the powdered leaves were 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 for future use. The final yield was 29.5%.

Toxicity Testing: The aqueous extract of *Impatiens balsamina* was administered at doses 100, 200, 400, 800, 1600, and 3000 mg/kg, per orally to groups of mice, each group consisting of 10 mice and mortality was observed for 24 h. No sign of toxicity was observed up to the dose of 3000 mg/kg. The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences (RIMS), Imphal after getting approval of the Institutional Ethics Committee, Regional Institute of Medical Sciences, Imphal.(No.1596/GO/a/12/CPCSEA).

Anti-Inflammatory Activity: Anti-inflammatory agents have been evaluated by studying the inflammatory response produced in the animals by injecting foreign or noxious agents. These responses mostly comprise the development of oedema and/or the formation of exudates and granuloma⁸.

The term Anti-inflammatory has many connotations that multiplicity of assays is required to affirm this property in any chemical compound⁹.

Method: Healthy albino rats of Wistar strain of either sex weighing between 100-200 gm were used throughout the present experiment.

They were collected from Central Animal House, RIMS. Animals were kept for 3 days under laboratory conditions before any experimental work.

Animals were fed a standard pellet diet and water *ad libitum* and maintained at 24-28 °C temperature and 12 hour day and night cycle. The rats were divided into five groups, with six animals in each group. This arrangement was used throughout the present experiment. The drugs were suspended in 2% gum acacia and administered orally at a uniform volume of 10 ml/kg body weight of the animals.

Group	Drug Dose
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

The volume of medicaments was kept constant at 1 ml/100 gm bodyweight of the animals. Both control and treated groups in a particular series received the same phlogistic agent. Paw volume was measured by the modified plethysmometric method described by Singh H and Ghosh MN⁸

Determination of Percentage of Inhibition of Oedema: The calculation of percentage inhibition of oedema formation after premedication with standard drug and indigenous drug under study was done according to the method adopted by Tomar A *et al.*⁹.

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

Where, V_c = Mean increase in paw volume in control group
 V_t = Mean increase in paw volume in drug-treated group

Tests on Sub-Acute Inflammation:

Cotton Pellet Implantation: The method as described by Ghosh MN and Singh H¹⁰ was followed.

Materials: Animal - Albino rats, Drugs - Gum acacia, test drug, Aspirin, Penicillin, Streptomycin, ether. Equipment - Oven, incubator, electronic weighing machine, Others - Cotton roll.

Method: Cotton pellets cut from dental rolls to size approximately 0.5 × 0.5 cm and weighing 20 ± 1 mg were sterilized at 120 °C for 2 h. They were then soaked in 0.2ml distilled water containing Penicillin (0.1 mg) and Streptomycin (0.13 mg) and then inserted one in each axilla into anesthetized rats (with ether). Groups of rats as described before were treated with the test drug, standard, and control for 6 days.

On the seventh day, after ether anaesthesia, the cotton pellets and the granuloma were dissected carefully and dried in an incubator at 37 °C for 24 h. Any increment in the dry weight was taken as a measure of granuloma formation. Then the percentage inhibition of granuloma formation was calculated for the various doses of the test drug and Aspirin as compared to the control group.

Granuloma Pouch: The method described by Selye H¹¹ with slight modification was adopted.

Materials: Animal - Albino rats, Drugs - Gum acacia, test drug, Aspirin, ether, Equipment - hypodermic syringe with needle, isolation cages, Chemicals - Turpentine oil.

Method: Rats were anesthetized with ether, and a subcutaneous dorsal pouch was prepared in between the shoulder blades by injecting 20ml of air. Then, 0.5 ml of turpentine oil was injected into the pouch. Groups of rats were treated with varying doses of the test drug, Aspirin and control per oral for six days beginning from the day of pouch formation.

On the seventh day, the pouch was opened under ether anesthesia and the exudates were sucked out and the amount measured. The percentage inhibition was then calculated for the different groups of drugs as compared to the control group.

Tests on Chronic Inflammation:

Formaldehyde Arthritis: The method described by Selye H12 with slight modification was used.

Materials: Animal - Albino rats, Drugs - Test drug, gum acacia, Aspirin, Chemicals - 2% formalin, Equipment - Plethysmometer, isolation cages, feeding tubes

Method: A subcutaneous injection of 0.1ml of 2% formalin was given under the plantar aponeurosis of the right hind foot of the rats. The paw volume was measured plethysmometrically for 13 days to assess the degree of inflammation. Groups of animals, as described before, were treated with varying doses of test drug, gum acacia and Aspirin per oral daily for 13 days.

Statistical Analysis: All values were expressed as Mean \pm SEM. The results were statistically analyzed. The data thus obtained were subjected to statistical analysis using one-way anova followed by Dunnet's "t" test for significant differences

between different groups. p-value of <0.05 was considered as significant.

RESULTS AND DISCUSSION:**Anti-Inflammatory Activity:****A) Tests on Sub-Acute Inflammation:**

Cotton Pellet Implantation: Table I shows the activity of the aqueous extract of the leaves of *Impatiens balsamina* on sub-acute inflammation as tested on cotton pellet implantation.

The mean dry weight of the granuloma in the control group was 80.66 ± 3.88 , test (500 mg/kg) 65.5 ± 3.46 ($p < 0.01$), test (1000 mg/kg) 57.16 ± 2.53 ($p < 0.001$), test (2000 mg/kg) 47.5 ± 2.14 ($p < 0.001$) respectively. The test drug in concentrations of 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced 18.79%, 29.13% and 44.83% inhibition of granuloma formation. Both the test and standard drug produced highly significant inhibition of granuloma formation in comparison to control.

TABLE 1: EFFECTS ON SUB-ACUTE INFLAMMATION OF AQUEOUS EXTRACT OF IMPATIENS BALSAMINA ON COTTON PELLET IMPLANTATION

Group	Drug Dose (Mg/Kg,P.O)	Mean Dry Weight Granuloma (Mean \pm Sem) In Mg	% Inhibition of granuloma Formation
I (Control)	10 ml/kg	80.66 ± 3.88	0%
Ii (Test)	500	$65.5 \pm 3.46^*$	18.79%
Iii (Test)	1000	$57.16 \pm 2.53^{**}$	29.13%
Iv (Test)	2000	$44.5 \pm 3.64^{**}$	44.83%
V (Standard)	100	$47.5 \pm 2.14^{**}$	41.11%

One way anovaf (df) 20.89 (4,25) $p < 0.01$ n = 6 in each group, * $p < 0.01$, ** $p < 0.001$ when compared to control.

Granuloma Pouch: Table 2 shows the anti-inflammatory activity of aqueous extract of *Impatiens balsamina* on sub-acute inflammation by granuloma pouch method. The volume of exudate in the control group was 3.66 ± 0.33 , test (500 mg/kg) 2.33 ± 0.42 ($p < 0.01$), test (1000 mg/kg) 1.83 ± 0.22 ($p < 0.001$), test (2000 mg/kg) 1.16 ± 0.28 ($p < 0.001$) and Aspirin (100 mg/kg) 1 ± 0.22 ($p < 0.001$) respectively.

The test drug in concentrations of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg produced 36.34%, 50%, and 68.3% inhibition of exudate formation compared to 72.67% produced by 100mg/kg of the standard drug, Aspirin. Increasing concentration of the test drug produced increased inhibition of exudate formation. Both the test and standard drug produced highly significant inhibition of exudate formation compared to the control.

TABLE 2: EFFECTS ON SUB-ACUTE INFLAMMATION OF AQUEOUS EXTRACT OF IMPATIENS BALSAMINA ON GRANULOMA POUCH

Group	Dose (Mg/Kg,P.O.)	Mean Volume of Exudate (Mean \pm Sem) In Ml	% Inhibition of Exudate Formation
I (Control)	10ml/kg	3.66 ± 0.33	0%
Ii (Test)	500	$2.33 \pm 0.42^*$	36.34%
Iii (Test)	1000	$1.83 \pm 0.22^{**}$	50%
Iii (Test)	2000	$1.16 \pm 0.27^{**}$	68.3%
Iv (Standard)	100	$1 \pm 0.22^{**}$	72.67%

One Way Anovaf (Df) 11.25(4,25) $P < 0.01$ * $P < 0.01$, ** $P < 0.001$ When Compared To Control; N = 6 In Each Group.

Tests on Chronic Inflammation:

Formaldehyde Arthritis: Table 3 shows the anti-inflammatory activity of aqueous extract of *Impatiens balsamina* on chronic inflammation by formaldehyde arthritis method. The mean increase in paw volumes in the control group were 0.43 ± 0.06 , 0.48 ± 0.02 , 0.47 ± 0.08 , 0.33 ± 0.05 and 0.32 ± 0.05 respectively on the 3rd, 5th, 9th, 11th and 13th day. On pre-treatment with aqueous extract of *Impatiens balsamina*, the mean increase in paw volumes were: test (500 mg/kg) 0.22 ± 0.03 ($p < 0.001$), 0.25 ± 0.05 ($p < 0.001$), 0.33 ± 0.07 ($p < 0.05$), 0.18 ± 0.03 ($p < 0.01$) and 0.1 ± 0.02 ($p < 0.01$); test (1000 mg/kg) 0.2 ± 0.02 ($p < 0.001$), 0.23 ± 0.04 ($p < 0.001$), 0.3 ± 0.04 ($p < 0.01$), 0.15 ± 0.02 ($p < 0.001$) and 0.08 ± 0.03 ($p < 0.001$); test

(2000 mg/kg) 0.18 ± 0.03 ($p < 0.001$), 0.22 ± 0.03 ($p < 0.001$), 0.25 ± 0.02 ($p < 0.001$), 0.13 ± 0.02 ($p < 0.001$) and 0.04 ± 0.05 ($p < 0.001$). Aspirin (100 mg/kg) pre medication showed mean increase in paw volume of 0.16 ± 0.04 ($p < 0.001$), 0.2 ± 0.04 ($p < 0.001$), 0.23 ± 0.02 ($p < 0.001$), 0.12 ± 0.02 ($p < 0.001$) and 0.03 ± 0.08 ($p < 0.001$) on the 3rd, 5th, 9th, 11th and 13th day respectively.

Both test and standard drugs produced significant inhibition of paw oedema when compared to control. The result shows that there was no significant reduction in paw volume in the control groups of rats even on the 13th day. On the other hand, the aqueous extract of *Impatiens balsamina* showed a significant reduction in paw volume.

TABLE 3: EFFECTS OF AQUEOUS EXTRACT OF *IMPATIENS BALSAMINA* (AEIB) ON FORMALDEHYDE INDUCED ARTHRITIS

Group	Dose(mg/kg, p.o)	Mean increase in paw volume (Mean \pm SEM) in ml				
		3 rd day	5 th day	9 th day	11 th day	13 th day
I(Control)	10 ml/kg	0.43 \pm 0.06	0.48 \pm 0.02	0.47 \pm 0.08	0.33 \pm 0.05	0.32 \pm 0.05
Ii (Test)	500	0.22 \pm 0.03***	0.25 \pm 0.05***	0.33 \pm 0.07*	0.18 \pm 0.03**	0.1 \pm 0.02***
Iii (Test)	1000	0.2 \pm 0.02***	0.23 \pm 0.04***	0.3 \pm 0.04**	0.15 \pm 0.02***	0.08 \pm 0.03***
Iv (Test)	2000	0.18 \pm 0.03***	0.22 \pm 0.03***	0.25 \pm 0.02***	0.13 \pm 0.02***	0.04 \pm 0.05***
V (Standard)	100	0.16 \pm 0.04***	0.2 \pm 0.04***	0.23 \pm 0.02***	0.12 \pm 0.02***	0.03 \pm 0.08***

One Way F(Df) 9.27 (4,25) 55.23(4,25) 5(4,25) 7.6(4,25) 73.14(4,25) ANOVA $p < 0.01$ < 0.0 < 0.01 < 0.01 < 0.01
 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to control; n =6 in each group



FIG. 1: PICTURE SHOWING AERIAL PARTS OF *IMPATIENS BALSAMINA*

The cotton pellet implantation was done by the method of Ghosh MN, and Singh.⁸ The mean dry weight of granuloma of the control group was 80.66 ± 3.85 , which corresponds to the findings of Onyegbule *et al.* 13 at 81.44 ± 6.10 ; and D Souza *et al.* 14 at 91.01 ± 0.17 . The test drug in concentrations of 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced 18.79% ($p < 0.01$), 29.13% ($p < 0.001$) and 44.83% ($p < 0.001$) inhibition of granuloma formation. The standard drug Aspirin produced 41.11% ($p < 0.001$) inhibition of granuloma

formation. The effect of the test drug (2000mg/kg) was comparable to that of the standard drug Aspirin (100 mg/kg). The mean dry weight of the granuloma of the standard group was 47.5 ± 2.14 , which was comparable to that of Devi RKB *et al.* 4 at 40.5 ± 1.28 . The cotton pellet granuloma bioassay is considered as a model of the exudative and proliferative type of inflammation. It is mediated mostly by kinins. The phases of cotton pellet implantation are 1) transudative phase, during the first 3 h 2) exudative phase, between 3 and 72 h and 3) proliferative phase, between 3 and 5 days.

The cotton pellet granuloma model has been adopted to assess the anti-inflammatory activity of IB on the proliferative phase of sub-acute inflammation. It is probable that *Impatiens Balsamina* possesses anti-kinin activity and inhibits the proliferative phase of subacute inflammation⁴. AEIB produced a significant reduction of granuloma formation in rats in the cotton pellet induced granuloma model. During the repair process of inflammation, macrophages, neutrophils,

fibroblasts and multiple small blood vessels are the basic sources of forming a highly vascularized, reddish mass termed granuloma¹⁵. The AEIB inhibited the formation of this granuloma. The granuloma pouch experiment was done by the method of Selye H¹⁶. The anti-inflammatory activity of *Impatiens balsamina* was profound and highly significant in the granuloma pouch model. Since granuloma represents the late exudative and proliferative phases of inflammation, the present

study indicates that AEIB possesses anti-inflammatory activity against the subacute inflammatory condition. AEIB has shown potent inhibitory action on exudates formation. Kinin is the main mediator of granuloma, as it not only vasodilates but also increases the vascular permeability in the early stages of inflammation¹⁷. The mean value of exudate in the control group was 3.66 ± 0.33 (Mean \pm SEM), which corresponds to the findings of VM Shastry *et al* (2.68 ± 0.08)¹⁸.

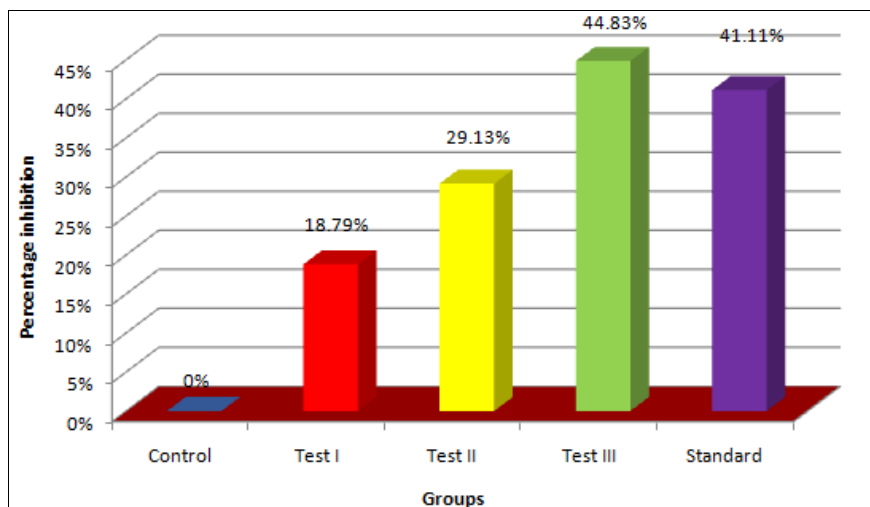


FIG. 2: EFFECT OF AQUEOUS EXTRACT OF IMPATIENS BALSAMINA LINN. ON SUB-ACUTE INFLAMMATION AS TESTED BY COTTON PELLET IMPLANTATION

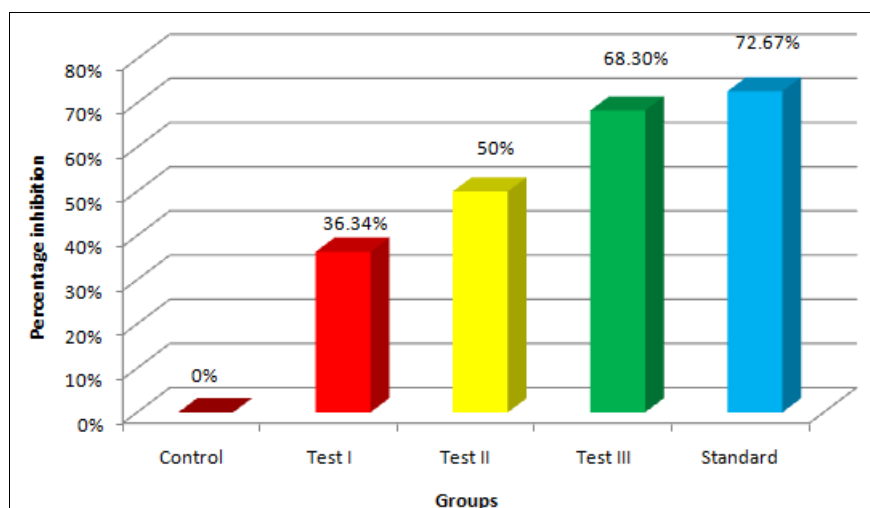


FIG. 3: EFFECT OF AQUEOUS EXTRACT OF IB ON SUB-ACUTE INFLAMMATION AS TESTED BY GRANULOMA POUCH METHOD

The test drug in concentrations of 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced 36.34 % ($p < 0.01$), 50% ($p < 0.001$) and 68.3% ($p < 0.001$) inhibition of exudates formation respectively. The standard drug Aspirin produced 72.67% ($p < 0.001$) inhibition of exudates formation. Thus, the test drug possesses significant anti-inflammatory

activity against sub-acute inflammation but is lesser than that produced by the standard drug. Formaldehyde arthritis was done by the method of Selye¹⁰. The mean increase in paw volume in the control group was found to be 0.43 ± 0.06 (Mean \pm SEM) on the 3rd day and 0.47 ± 0.08 on the 9th day. The test drug in concentrations of 500 mg/kg, 1000

mg/kg and 2000 mg/kg increased the paw volume from 0.22 ± 0.03 to 0.33 ± 0.07 , 0.2 ± 0.02 to 0.3 ± 0.04 and 0.18 ± 0.03 to 0.25 ± 0.02 respectively from 3rd day to 9th day followed by decrease in the paw volume to 0.1 ± 0.02 , 0.08 ± 0.03 and 0.04 ± 0.05 respectively on the 13th day. The paw volume in the standard group treated with Aspirin (100

mg/kg) increased from 0.16 ± 0.04 on the 3rd day to 0.23 ± 0.02 on the 9th day, followed by a decrease to 0.03 ± 0.08 on the 13th day. From the present study it is evident that the test drug at doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg significantly inhibited rat paw oedema induced by formaldehyde ($p < 0.05-0.001$).

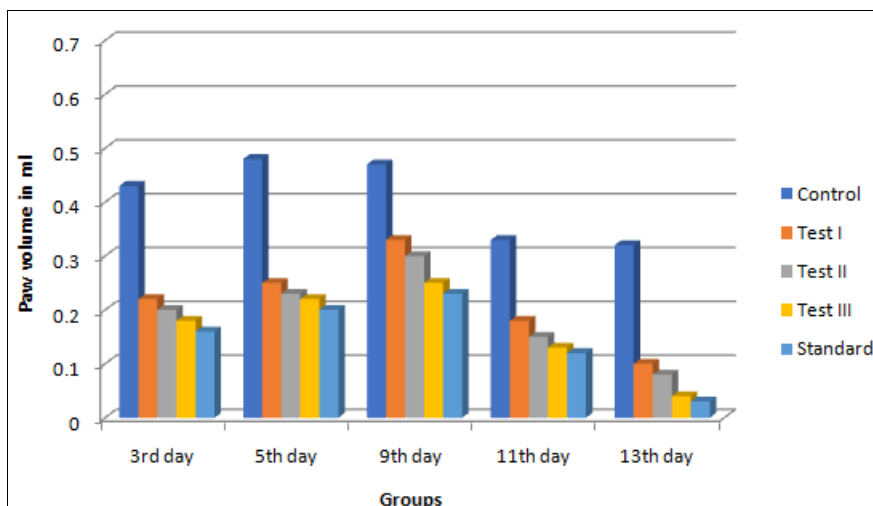


FIG. 4: ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF IMPATIENS BALSAMINA LINN. ON CHRONIC INFLAMMATION BY FORMALDEHYDE ARTHRITIS METHOD



FIG. 5: PICTURE SHOWING COTTON PELLET IMPLANTATION

The significant reduction of paw volume by aqueous extract of leaves of *Impatiens balsamina* Linn may suggest its effectiveness in arthritis. Inhibition of formalin induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritis and anti-inflammatory agents as it closely resembles human arthritis. Injection of formalin subcutaneously into the hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is diphasic, an early neurogenic component followed by a later tissue

mediated response. Thus formalin-induced arthritis is a model used for evaluation of an agent with probable anti-proliferative activity¹⁵. Therefore the AEIB appears to be effective against formalin-induced arthritis. AEIB contains anthocyanins, Cox-2 inhibitory naphthoquinones, kaempferol glycosides, and flavonoids such as quercetin. Flavonoids have been shown to possess various biological properties related to antioxidant, anti-nociception and anti-inflammatory mechanisms by targeting reactive oxygen species and

Prostaglandins, which are involved in the late phase of acute inflammation and pain perception¹⁹. It can be concluded that the anti-inflammatory and anti-nociceptive action of the aqueous extract of *Impatiens balsamina* may likely be due to the presence of these compounds, either singly or in combination. However, further studies are needed to isolate the active constituents responsible for the observed effect and to reveal the possible

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



FIG. 6: PICTURE SHOWING GRANULOMA POUCH

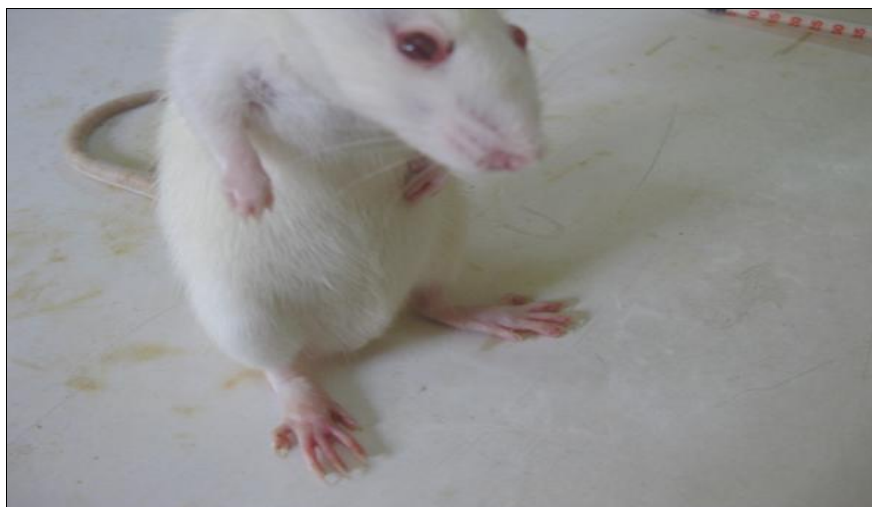


FIG. 7: PICTURE SHOWING PAW OEDEMA

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CONFLICTS OF INTEREST: Nil

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