Open Access Research Paper
November - December 2025, Vol. 01 (3), 21-29
INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND MEDICINAL PLANTS
(An International Peer Reviewed Journal)

# EVALUATION OF *IN-VIVO* ANALGESIC AND ANTIPYRETIC POTENTIAL OF *INDIGOFERA TINCTORIA* IN ALBINO WISTAR RATS

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**ARTICLE INFO:**

**Received:** 01st Nov. 2025; **Received** in revised form: 24th Nov. 2025; **Accepted:** 19th Dec. 2025; **Available online:** 31st December 2025.

**Keywords:** *Indigofera tinctoria*, Leaves, Analgesic, Antipyretic, Anti-inflammatory, Pyrexia, Paracetamol.

**DOI:**
https://doi.org/10.61280/journalmedicinalplants.v1i3.216

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**ABSTRACT**

**Objective:** The leaves of the *Indigofera tinctoria* plant has not been studied for its potential antipyretic, anti-inflammatory, or analgesic effects. Thus, the purpose of the current research is to support the traditional usage of this plant by scientifically evaluating these aforementioned actions of the plant *I tinctoria* on experimental animals.

**Methods:** Fever was induced by injecting 10ml/Kg (subcutaneous) of 0.5(w/v) suspension of brewer's yeast in Carboxy methylcellulose below the nape of the neck. Analgesic activity in rats determined by hot-plate method.

**Results:** The initial and final rectal temperatures (°C) in the group treated with ethanol extract (200 mg/kg body weight) and ethanol extract (400 mg/kg body weight) were found to be 37.75±0.04 and 37.70±0.07 respectively, compared to 37.64±0.12 in paracetamol (reference drug) treated group. For analgesic potential the result shows that the latency period of ethanolic leaves extract 200 and 400 mg/kg was significantly good when compared to control ($p<0.001$).

**Conclusion:** The result of the present preliminary study confirmed the antipyretic and analgesic activity of *I tinctoria* in rats. However, further investigation is required to separate the active fraction(s)/constituent(s) responsible for the activity and to ascertain the mechanism(s) of action.

## Introduction:

The most prevalent debilitating symptoms that a person may endure during their lifetime are pain, inflammation, and fever.[1,2] Numerous medications are available on the market for treating these symptoms, many of which are offered over the counter. The majority of the time, nonsteroidal anti-inflammatory drugs, often known as NSAIDs, are prescribed to patients in order to treat these conditions.[2,3]

Fever, is known as pyrexia, may occur due to infection, inflammation, or any tissue damage and disease states. Normally, the infected or damaged tissue initiates the enhanced formation of pro-inflammatory

mediators like cytokines which further increases the synthesis of prostaglandin E2 (PgE2) near the hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature. Pain is a signal in your nervous system that something may be wrong.[3,4] It is an unpleasant feeling, such as a prick, tingle, sting, burn, or ache. Pain may be sharp or dull. It may come and go, or it may be constant.[5]

Because of the extensive usage of NSAIDs, the side effects of these medications have grown more common. The two primary adverse drug reactions (ADRs) linked with NSAIDs pertain to the gastrointestinal tract and renal system.[6, 7] These side effects are dose-dependent, and in many instances, they are severe enough to put the patient at risk for ulcer perforation and upper gastrointestinal bleeding.[8]

Now a day there is revival of interest with herbal based medicines in developed countries is mainly due to increasing the realization of the health hazards with the indiscriminate use of modern medicines in providing effective treatment for chronic diseases and emergence of multi drug resistance bacteria and parasites. [9,10]

As stated in the literature review of various plants in the traditional Indian medical system, we are going to present study on plant ***Indigofera tinctoria*** that can treat fever and pain. Since these activities of the leaves of ***Indigofera tinctoria*** have not been assessed and scientifically confirmed.

***Indigofera tinctoria***, also called **true indigo**, is a species of plant from the bean family that was one of the original sources of indigo dye. The extracts of *Indigofera tinctoria* showed the presence of carbohydrates, gums, flavonoids, sterols and phenolic compounds/tannins. Indigofera tinctoria is used in constipation, liver disease, heart palpitation and gout. The roots, stems and leaves are **Anti pyretic**, **Analgesic**, bitter, thermogenic, laxative, trichogenous, expectorant, anthelminthic, tonic, naturopathy, splenomegaly, echolalia, cardiopathy, chronic bronchitis, asthama, ulcers, skin diseases, diuretic and are useful for promoting growth of hair.[11-14]

**Material and Methods:**

**Collection, Procurement and Authentication of plant material:**

In the present study, the Leaf of ***Indigofera tinctoria*** was collected from local and nearby area of Alwar district, Rajasthan, with the help of field botanist. The plant of *Indigofera tinctoria* has been authenticated from botanist.

**Materials:**

Paracetamol and Diclofenac Sodium were purchased from local market. The solvents and other chemicals of analytical grade were used and obtained from the institute's central store. Brewer's yeast was also purchased from local market.

**Quantitative Studies:**

The quantitative microscopy such as vein-islet number, vein- terminal number, stomatal number, and stomatal index were determined, and the results were tabulated. Quantitative studies for foaming index and swelling index were also performed.[16]

**Methods: Preparation of extract:** Dried course powder of the leaves was extracted with alcohol (90%) and water by using soxhlet apparatus separately until the extraction solvents becomes colorless.

**Preliminary Phytochemical Screening:**

Extract of ***Indigofera tinctoria*** leaves will be subjected to preliminary quantitative phytochemical investigation for the detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, gums and mucilages, fats & fixed oils using the following standard methods.[11-12]

**Determination of physicochemical parameters:**

Physicochemical values such as the foreign organic matter, moisture content, ash value as well as extractive value were determine as per the official methods (Anonymous,1996) as well as per WHO guidelines of quality control method for medicinal plant materials [17]

**Animals:** The ethical clearance obtained via the institutional Animal Ethics Committee (CPCSEA registration number-1659/PO/a/ CPCSEA.) before the experiment. For acute toxicity study and for pharmacological activity evaluation Albino rats, weighing 150-200gm, were used for study, the animal were fasted whole night before the experiment starts for various extracts. Animal were kept in a constant humidity (55%), temp at (22± 20C), and exposed to dark and light {12hr) every day the bedding materials of the cages were changed.[14]

**Brewer's yeast suspension method**

**Experimental method:** [18]

- Taken rats avg wt (150 -200 gm)
- Body temperature by rectal thermometer
- 15% brewer's yeast+0.9% saline sol inject rat subcutaneously &placed for 18 hr
- When body temperature rises test drug is given
- Record the response at 30, 60, 120, 180 min

**Experimental design:**

Animal will be randomly divided into 5 groups of 6 each and assigned as below.

Group I: Vehicle control (normal saline 5ml/kg)

Group II: Animals were administered with 100mg/kg ethanol extract

Group III: Animals were administered with 200mg/kg ethanol extract

Group IV: Animals were administered with 400mg/kg ethanol extract

Group V: Animals were treated with Paracetamol (30mg/kg)

**Induction of Pyrexia:** Fever was induced by injecting 10ml/Kg (subcutaneous) of 0.5(w/v) suspension of brewer's yeast in Carboxy methylcellulose below the nape of the neck. The temperature was measured after 18hours using rectal thermometer.24-25

**Evaluation of Analgesic activity in rats by hot-plate method:** [19-20]

Animals will be randomly divided into four groups of six animals each. Group I will serve as negative control, treated with 2% gum acacia (10 ml/kg), group II-III will be treated with flower extract of *I. tinctoria* in the dose 200mg/kg (minimum dose) and 400mg/kg (maximum dose) b.w. p.o. and group IV will be treated with diclofenac sodium (20mg/kg) standard drug.

**Group I** - will receive 2% gum acacia (10ml/kg), serve as negative control

**Group II** - will receive extract (200mg/kg bw p.o.)

**Group III** - will receive extract (400mg/kg bw p.o.)

**Group IV** - will receive Diclofenac Sodium (20 mg/kg, i.p.) standard drug and serve as positive control

**Statistical Analysis:** Results are expressed as mean ± SEM. Statistical analysis of data was performed using ANOVA to study differences among the means.

**Results and Discussion:**

**Quantitative Studies:**

**Table 1: Quantitative evaluation of the leaf of *I. tinctoria***

| S.No. | Plant constants | Values |
|---|---|---|
| 1 | Vein islet no. | 8/sq mm |
| 2 | Vein termination no. | 6/sq mm |
| 3 | Stomatal number (upper) | 16.16 |
| 4 | Stomatal number (lower) | 28.66 |
| 5 | Stomatal index (upper) | 6.311 |
| 6 | Stomatal index (lower) | 8.703 |

**Table 2: Quantitative studies of *Indigofera tinctoria* leaf**

| S.No. | Estimation | Observation |
|---|---|---|
| 1. | Foaming Index | < 100 |
| 2. | Swelling Index | >1 |

**Physico-chemical parameters:**

**Table 3: Extractive values and color of extract in different solvent**

| Extract | Color of Extract | Extractive value (% w/w) |
|---|---|---|
| Petroleum ether (60-80°C) | Pale green | 2.24 |
| Hexane | Green | 1.2 |
| Acetone | Greenish Brown | 4.86 |
| Ethanol | Greenish Brown | 6.45% |
| Aqueous/Water | Brownish green | 5.12% |

The result of proximate analysis of crude powder of ***Indigofera tinctoria*** leaf is shown in Table 4 The average values are expressed as percentage of air-dried material. The loss on drying was 2.2%. Total ash was 5.12%, acid insoluble ash was 3.83% and water soluble ash was 2.45%. The extractive value of crude powder was maximum in ethanol (6.45%), followed by water (5.12%) and minimum was in hexane (1.2%). pH and melting point of methanol extract was 3.8 and 107°C respectively.

**Table 4: Physico-chemical parameter of crude powder of *Indigofera tinctoria* leaf**

| | |
|---|---|
| Loss on drying | 3.32% |
| Total ash | 5.12% |
| Acid insoluble ash | 3.83% |
| Water soluble ash | 2.45% |
| pH of Ethanol extract | 3.8 |
| Melting point ethanol extracts | 107°C |

**Extraction of *Indigofera tinctoria*:**

The percentage yield of ethanolic extract was found to be 6.45% which is comparable with reported standard extractive value. This extract and fractions were stored in airtight container for further studies. Ethanolic extract used for Phyto-chemical analysis and for the evaluation of Analgesic and Anti-pyretic activity of leaf as ethanol extract contain maximum no of phyto-constituents.

**Qualitative phytochemical investigation of *Indigofera tinctoria:***

In crude powder and ethanol extract maximum amount of Terpenoids and Flavanoids were present. Alkaloid, phlobotannins, saponins and triterpenes were present in moderate amount. Mucilages and steroids were absent in crude powder as well as in ethanol extract. Terpenoids and Flavanoids are mainly responsible for anti-pyretic potential of herbal medicine and leaf of *Indigofera tinctoria* contain higher amount of these constituents.

**Table 5: Preliminary qualitative phytochemical analysis of *Indigofera tinctoria***

| Test | Drug powder | Petroleum ether extract | Benzene extract | Chlorofom extract | Ethanol extract | Aqueous extract |
|---|---|---|---|---|---|---|
| Sterols | - | + | - | - | - | - |
| Terpenoids | ++ | - | - | - | +++ | + |
| Carbohydrate | ++ | - | - | - | ++ | ++ |
| Flavanoids | ++ | - | - | - | +++ | + |
| Proteins | - | - | - | - | - | - |
| Alkaloids | ++ | - | - | - | ++ | - |
| Glycosides | + | - | - | - | ++ | + |
| Saponins | + | - | - | - | ++ | ++ |
| Tannins | + | - | - | - | + | + |
| Mucilages | - | - | - | - | ++ | + |
| Volatile oil | + | - | - | - | ++ | + |

**Pharmacological screening of extracts for evaluation of anti-pyretic activity of *Indigofera tinctoria:***

**Acute oral toxicity Study:**

The results of acute toxicity studies were recorded. The therapeutic dose was calculated for the purpose of anti-pyretic investigations. The $LD_{50}$ value determined by the method as per guidelines of Organization for Economic Co- operation Development (OECD) was found to be 2000 mg/kg b.w. by oral route.

**Brewer's yeast-induced pyrexia in Rats:**

The subcutaneous injection of yeast suspension (Nape of neck) markedly elevated the rectal temperature after 18 h of administration. Treatment with the *I tinctoria* at doses 200 and 400 mg/kg significantly ($p<0.01$) decreased the rectal temperature of the rats in dose dependent manner up to 3 h after pyrexia when compared with control (Table 6). Effect of 100mg/kg extract was negligible. The antipyretic effect was sustained throughout the remaining period of the experiment in a manner similar to the reference drug Paracetamol 30mg/kg, b.w. p.o.

**Table 6: Anti-pyretic Effect of extract of *I. tinctoria* on yeast induced pyrexia**

| Treatment | Dose mg/kg | Rectal temp. ºC before and after treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Normal | 18h | 30 min after treatment | 60 min after treatment | 90 min after treatment | 120 min after treatment | 180 min after treatment |
| Control | - | 37.7±0.15 | 38.9±0.12 | 38.7±0.21 | 38.7±0.04 | 38.7±0.24 | 38.5±0.42 | 38.4±0.24 |
| Paracetamol | 30 | 37.6±0.02 | 38.8±0.24 | 38±0.21 | 37.8±0.09** | 37.7±0.04** | 37.6±0.26** | 37.64±0.12** |
| EEIT | 100 | 37.7±0.12 | 38.8±0.45 | 38.4±0.12 | 38.18±0.05 | 38.02±0.16 | 37.96±0.07 | 37.90±0.05** |
| EEIT | 200 | 37.6±0.32 | 38.8±0.16 | 38.3±0.22 | 37.9±0.05 | 37.8±0.04** | 37.8±0.06** | 37.75±0.04** |
| EEIT | 400 | 37.7±0.42 | 38.8±0.52 | 38.2±0.12** | 37.8±0.21** | 37.76±0.12** | 37.7±0.08** | 37.7±0.07 |

In antipyretic study the experimental rats showed a mean increase of about 1.15 °C in rectal temperature 18 h after Brewer's yeast injection. Ethanol extracts showed significant ($p < 0.05$) antipyretic activity and reduction of the elevated rectal temperature after 3 hr of treatment (Table 7.15) in a dose dependent manner. The initial and final rectal temperatures (°C) in the group treated with ethanol extract (200 mg/kg body weight) and ethanol extract (400 mg/kg body weight) were found to be 37.75±0.04 and 37.70±0.07 respectively, compared to 37.64±0.12 in paracetamol (reference drug) treated group. At the dose of 200 and 400 mg/kg body weight the ethanol extract reduced 79% and 82.2% respectively of the elevated rectal temperature as compared to reference drug paracetamol (97.6 %) after 3 h. Hence, the ethanol extract in dose of 200mg/kg and 400mg/kg was found to be effective to treat fever compare to paracetamol.

**Discussion:**

The presence of proteins in yeast is linked to fever via inflammatory reaction in this method. The production of prostaglandins, mainly the most potent pyretic agent, PGE2 appears to be a final pathway responsible for fever production induced by several pyrogens. The antipyretic potential of alcoholic extracts of *I.tinctoria* might be linked to the prevention of prostaglandin formation. Antipyretic drugs such as acetylsalicylic acid reduce body temperature by inhibiting the synthesis of prostaglandin in hypothalamus. Ethanol extracts of *I.*

*tinctoria* dose dependently exhibited significant ($p < 0.05$) antipyretic activity in yeast-induced elevation in body temperature in rats and the effects are comparable to the reference antipyretic drug (paracetamol). The ethanol extract in 400mg/kg was found to be slightly potent than 200mg/kg b.w. It might be likely to conclude that the *I. tinctoria* extract prevents the prostaglandins synthesis.[21]

**Pharmacological investigation of ethanol crude extracts for Analgesic activity:**

The result of the effect of ethanolic and aqueous leaves extracts of *I. tinctoria* on the hot plate method is presented in below table. The standard drug diclofenac sodium showed a significant increase in analgesic activity when compared with the control ($p<0.001$). The result shows that the latency period of ethanolic leaves extract 200 and 400 mg/kg was significantly good when compared to control ($p<0.001$). The ethanolic leaves extracts 100 mg/kg did not showed significance effect when compared to Group II ($p<0.05$).

**Table 7: Reaction time in seconds among groups in Eddy's hot plate method**

| Treatment | Dose mg/kg | Reaction time in seconds | | | |
|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min |
| Control Normal saline | - | 17.1±2.2 | 18.12±1.1 | 16.4±2 | 16.8±0.6 |
| Diclofenac Sodium | 25 | 18.5±2.3 | 25.8±1.9 | 35.2±2.2*** | 36.3±1.2*** |
| EEIT | 100 | 16.2±2.1 | 20.2±3.6 | 22.1±1.5 | 18.8±2.2 |
| EEIT | 200 | 18.6±1.8 | 21.6±2.9 | 26.3±3.4 | 29.7±2.7*** |
| EEIT | 400 | 17.5±2.7 | 22±1.8 | 28.1±2.8 | 30.5±3.5*** |

**Discussion:** Eddy's hot plate method involves higher brain functions and pain is produced by the supraspinal mediated response. In this study, ethanolic extracts of *I. tinctoria* reduces the number of writhes and increases the reaction time in hot plate test. These findings strongly suggest that ethanolic leaves extracts of this plant possess peripheral and central analgesic properties.

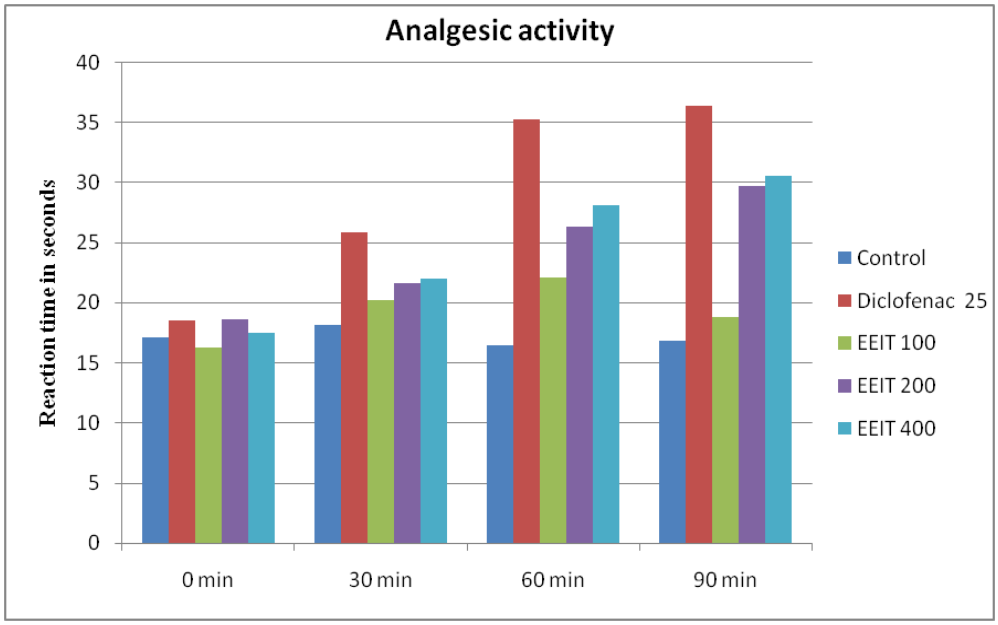


**Fig. 1 Analgesic Effect of Ethanolic extract of *I tinctoria* Eddy's Hot plate method induced pain in rats**

## Conclusion:

The ethanol extract and its different fractions from leaf of *Indigofera tinctoria* displayed significant peripheral potential antipyretic property. The central antinociceptive activity was absent. Since this is a pioneer work further studies are necessary to validate this result and other detailed studies on compound identification and isolation and underlying mechanism for the observed effect are essential to guarantee its clinical use.

In vivo studies showed significant analgesic activity in the plant extract at the doses tested. Thus, present study validated the use of the plant in the management of pain and fever, providing the pharmacological basis for its traditional use.

The result of the present preliminary study confirmed the antipyretic and analgesic activity of *Indigofera tinctoria* in rats. However, further investigation is required to separate the active fraction(s)/constituent(s) responsible for the activity and to ascertain the mechanism(s) of action.

## Conflict of interest:

No conflicts of interest are mentioned by the researchers.

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**How to cite this article:** Dr Praveen Kumar Goyal, & Dr Gurpreet Singh. (2025). Evaluation of In-Vivo Analgesic and Antipyretic Potential of Indigofera tinctoria in Albino Wistar Rats. *International Journal of Pharmaceutical Research and Medicinal Plants*, *1*(3), 21–29. https://doi.org/10.61280/journalmedicinalplants.v1i3.216

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