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# Evaluation of Anti-Pyretic Potential of *Indigofera tinctoria* using Brewer's Yeast induced Pyrexia in Albino Rats

**Shubham Bhargava[1], Yogesh Sharma[2]**

[1, 2]Jaipur College of Pharmacy, Sitapura, Jaipur - 302022, Rajasthan, India
[1]Email: *shubham.bhargava1994[at]gmail.com*

**Abstract:** ***Background: Many natural drugs have been found to be having antipyretic potential. Indigofera tinctoria traditionally used to treat fever, diabetes, inflammation etc. Aim: The present study investigates the antipyretic activity of the leaf extracts of Indigofera tinctoria on brewer's yeast induced fever in experimental rats. Methods: 30 albino rats weighing 150-180g were used for study. Brewer's-yeast-induced pyrexia was used to investigate the antipyretic effects. 10ml/Kg (subcutaneous) of 0.5(w/v) suspension of brewer's yeast in Carboxy methylcellulose was injected to induce fever in all the experimental animals. After 18hrs, the rectal temperature was taken and the animals were administered Indigofera tinctoria (100mg/kg, 200mg/kg, and 400mg/kg) and paracetamol (standard group, 30mg/kg) orally. Result: The body temperature of the rats was measured rectally over a period of 3hours. I. tinctoria (200mg/kg and 400mg/kg) significantly reduced yeast induced pyrexia when compared with the standard drug. Thus, this experiment shows that the antipyretic effect of I. tinctoria is dose dependent and the effect is as a result of the phyto-constituents of the extract. Conclusion: These data therefore suggest that extract of I. tinctoria possesses significant antipyretic activity and it mechanism could be by inhibition of release inflammatory mediators and prostaglandins.***

**Keywords:** Anti-pyretic activity, *I. tinctoria* (Neel), Paracetamol, Yeast, albino rats

## 1. Introduction

Over the centuries, plants have been used for their medicinal values. However, many plants have yet to be studied and relatively few scientific researches have been carried out.[1, 2] Therefore, assessment of medicinal plants is of special interest because of rich heritage and the continuous use among large portion of people.[1, 3]

Fever, is known as pyrexia and may occur due to infection, inflammation, or any tissue damage and disease states. It is defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 37°C (98.6°F) (1).Fever is a medical sign rather than a disease and it may be caused as a secondary impact of infection, malignancy or other diseased states. Fever occurs when the body's thermostat (located in the hypothalamus-anterior pituitary) resets at a higher temperature, primarily in response to an infection. From the recent scientific discovery, most of the antipyretic drugs have been developed to reduce elevated body temperature, of which many acts by the mechanism of inhibition of the COX-2 expression to reduce PGE2 biosynthesis.[4-6]

It is currently accepted that prostaglandin E2 (PGE2) is the final fever mediator in the brain, specifically in the pre-optic area of the anterior hypothalamus. Many drugs on chronic usage can able to result a several side effects including gastrointestinal, renal, hepatic, central nervous system and dermatological effects. A rich heritage of knowledge on preventive and curative medicines was available in ancient scholastic work. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant[3, 4]. Many of the plants were found to show the anti-pyretic activity hence the use of traditional plants as relieving agents can be advantageous in many aspects such as easy availability, economical with no or limited side effects.[5, 7, 8]

## 2. Material and Methods

*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.[9-11] It has been naturalized to tropical and temperate Asia, as well as parts of Africa, but its native habitat is unknown since it has been in cultivation worldwide for many centuries. Today most dye is synthetic, but natural dye from *I. tinctoria* is still available, marketed as natural coloring where it is known as tarum in Indonesia and nila in Malaysia.[12-18]

**Plant Taxonomy:** [19-21]

**Table 1:** Scientific classification of *Indigofera tinctoria*

| | |
|---|---|
| Synonyms | Indian indigo, Nili, Anil |
| Kingdom | Plantae |
| Subkingdom: | Tracheophytes |
| Division: | Angiosperms |
| Class: | Dicotyledonae |
| Order: | Fabales |
| Family: | Fabaceae |
| Genus: | *Indigofera* |
| Species: | *tinctoria* |

**Drugs and chemicals:**
Paracetamol was 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.

**Collection & extraction of leaves:**
The fresh leaves of *I. tinctoria* were collected from the local areas of Jaipur district and are air dried under shade for two days and then coarsely powdered

**Defattation of powdered *Indigofera tinctoria* leaf**
180 g coarse leaf powdered were defatted with 400 ml petroleum ether (40-60$^0$C) using soxhlet apparatus. Extraction was continued until a drop of solvent from siphon tube, when evaporated on filter paper, did not leave a greasy spot. After the defattation, mark was taken out from extractor and spreaded as a bed on a clean paper and dried till evaporation of petroleum ether.[21]

**Ethanolic extraction of *I. tinctoria***
The dried mark obtained after defattation was packed in soxhlet apparatus and extracted with 400 ml ethyl alcohol in soxhlet apparatus. Extraction was continued until a drop of solvent from the siphon tube, when taken on TLC plate and sprayed with concentrated sulphuric acid, does not give a black spot. Brownish black extract thus obtained, was collected and solvent was evaporated under reduced pressure. The percentage yield of ethanolic extract was found to be 6.45 % which is comparable with reported standard extractive value.[21-23]

**Animals**
30 albino rats weighing between 150-180g of either sex were used for this experimental study. They were divided into five groups (n=6). To the rats laboratory diet and water *ad libitum was provided and* were kept in polypropylene cages. Which are kept in a constant humidity (55%), temperature at (22± 2$^0$C), and exposed to dark and light {12hr) every day the bedding materials of the cages were changed. The experimental protocol was approved by IAEC (Institutional Animal Ethics Committee), for using animals in this experiment. Animals were fasted overnight with free access to water prior to each experiment.[17, 18]

**Table 2:** Grouping and dose of drug for anti-pyretic activity

| Group | Drug and Doses |
|---|---|
| **Brewer's yeast-induced pyrexia** | |
| Group I | Normal Control (Received 0.5% CMC) |
| Group II | *I tinctoria* 100mg (i.p.) |
| Group III | *I tinctoria* 200mg (i.p.) |
| Group IV | *I tinctoria* 400mg (i.p.) |
| Group V | *Paracetamol* 30 mg/kg (oral) |

**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]

**Drug administration:**
After 18 h of yeast injection 0.5% CMC was administered orally to the control Group I. The extract of leaf of *I tinctoria* was administered at doses of (100, 200 and 400 mg/kg b.w. p.o) to groups II, III, IV animals respectively. Group V animals received the standard drug Paracetamol (30 mg/kg b.w. p.o) and Rats were restrained for recording rectal temperature by at the 18 h, immediately before extracts, normal saline or paracetamol administration, and again at one hour intervals up to the 21 h after yeast injection.

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

## 3. Result and Discussion

Fever is known to be caused by several endogenous pyrogens such as interleukin-1β, interleukin-6, interleukin-8, tumor necrosis factor-α, macrophageprotein-1 and prostaglandins. Prostaglandin synthesis may be activated by tumor necrosis factor-α and phospholipase A2. Brewer's yeast induces both TNF-α and prostaglandin synthesis

**Table 3:** Anti-pyretic Effect of Ethanolic extract of *I tinctoria* on yeast induced pyrexia in rats.

| 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 | - | 37.6±0.24 | 38.8±0.12 | 38.7±0.21 | 38.7±0.04 | 38.7±0.24 | 38.6±0.42 | 38.5±0.24 |
| Paracetamol | 30 | 37.6±0.02 | 38.8±0.24 | 38.1±0.21 | 37.8±0.09 | 37.7±0.04 | 37.6±0.26 | 37.64±0.12 |
| EEWF | 100 | 37.7±0.12 | 38.8±0.45 | 38.4±0.12 | 38.12±0.05 | 37.98±0.16 | 37.90±0.07 | 37.90±0.05 |
| EEWF | 200 | 37.6±0.42 | 38.8±0.16 | 38.3±0.22 | 37.9±0.05 | 37.82±0.04 | 37.76±0.04 | 37.76±0.04 |
| EEWF | 400 | 37.6±0.54 | 38.8±0.52 | 38.2±0.12** | 37.8±0.21** | 37.76±0.12** | 37.70±0.08** | 37.70±0.07 |

Values are expressed as mean ± SEM (n=6). **P<0.01 Statistically

The subcutaneous injection of yeast suspension (Nape of neck) markedly elevated the rectal temperature after 18 h of administration. Treatment with the WF 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 3). 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.

The extracts are likely to reduce fever by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-2 or by enhancement of the production of the body's own antipyretic substances.

The efficacy of the antipyretic effect of dhataki extract was observed to have increased with increased concentration (dose-dependent manner). This can be said to be due to the increased concentration of the component of the extract exhibiting antipyretic effects.

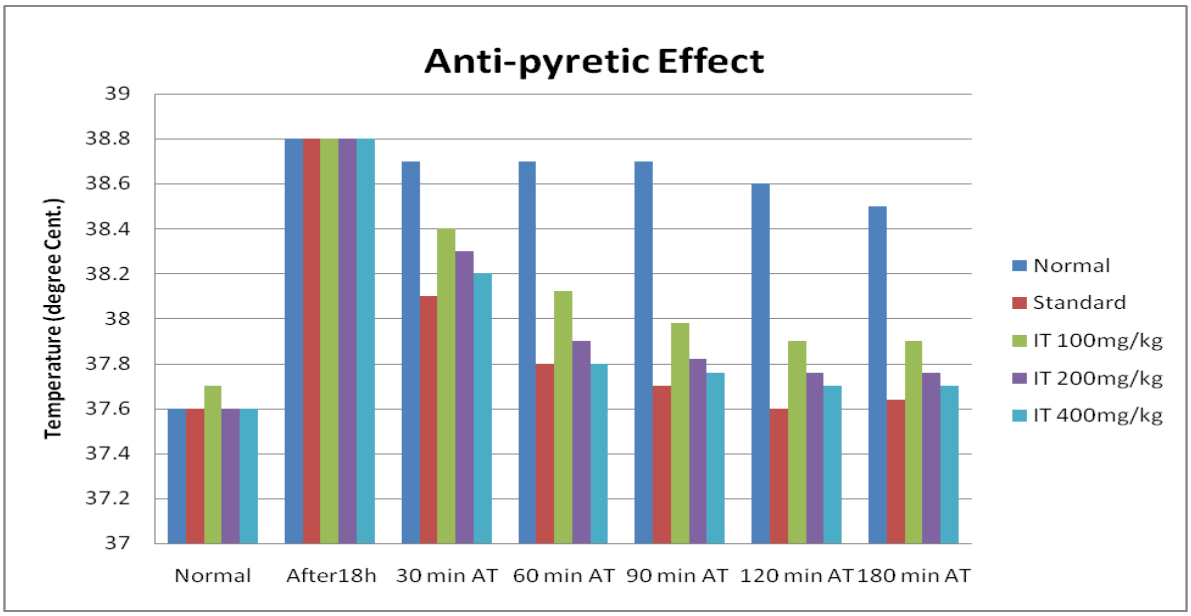


**Figure 1:** Graphical Representation of Anti-pyretic Effect of Ethanolic extract of *I tinctoria* on yeast induced pyrexia in rats

## 4. Conclusion

Antipyretic activity of bioflavonoid from *Woodfordia fruticosa* plant extract was confirmed by the present experimental studies. *W. fruticosa* extract with two different doses of 200mg/kg and 400mg/kg showed the reduced temperature levels in the wistar albino experimental rats when compared to the temperatures of rats of control group. It is through the inhibition of prostaglandin (PGE2). From these results, it can be concluded that the *W. fruticosa* plant extract at the dose level of 200mg/kg has shown marked decrease in temperature to normal. Hence possess antipyretic activity which was comparable with the paracetamol effect. Plant test extract of 100mg/kg was not so efficient as much as the dose level of 200mg/kg.

## 5. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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