

# Analgesic and Antipyretic Activities of Ethanolic Extract of *Hamelia Patens* Leaf in Animal Models

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## Abstract

**Aim:** The research was carried out to investigate the analgesic and antipyretic effects of ethanol extract of *Hamelia patens* leaves.

**Materials:** In order to assess the analgesic effects by Formalin induced writhing response model. However at the doses of 50-200 mg/kg analgesic activities were observed in the early and late phases of formalin induced paw licking test in rats. The latency in the hot plate test was increased from 2.2 + 0.3 to 5.7 + 0.3 second ( $P < 0.05$ ). Likewise, the early and the late phases of formalin test were reduced from 7.4 + 9.4 and 57.8 + 8.4 to 36.6 + 4.4 and 29.2 + 4.4 second respectively.

**Results:** The result confirms the analgesic and antipyretic activities of *Hamelia patens* leaf extract.

**Conclusion:** we recommended further research on this plant leaves for possible isolation and characterization of the various active chemical substances which has the toxic and medicinal values.

**Keywords:** *Hamelia patens*; NSAIDs; Labium

**Abbreviations:** NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; SEM: Standard Error Of Mean; COX: Cyclooxygenase

## Introduction

Medicinal plants or their active components are used in the prevention and treatment of chronic diseases is based on experience from traditional systems of medicine from various ethnic societies. During the past decade, a large number of natural products and dietary components have been evaluated as potential chemo preventive agents [1]. The application of chemo preventive agents to cancer prevention and control is attractive because conventional therapy alone has not been fully effective in combating either the high incidence or the low survival rate of several forms of cancer [2,3]. Inflammation is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by five cardinal signs, redness, swelling, heat, pain and loss of function. The inflammatory process involves a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, antigen-antibody interaction and thermal or physical injury [4].

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better anti-inflammatory drugs and indigenous medicinal plants could be a logical source to find these. No steroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of rheumatoid arthritis and various types of inflammatory conditions. The target for these drugs is cyclooxygenase (COX), a rate limiting enzyme involved in the conversion of arachidonic acid into inflammatory prostaglandins. The two isozymes of COX involved in prostaglandin biosynthesis are COX-1 and COX-2. COX-1 is known as a housekeeping enzyme and constitutively expressed in all tissues, while COX-2 is constitutively expressed only in kidney, brain and ovaries. COX-

2 is increasingly expressed during inflammatory conditions by pro-inflammatory molecules such as IL-1, TNF- $\alpha$ , LPS and agents such as carrageenan [5].

*Hameliapatens* Jacque. (Rubiaceae) is a small to large tree found all over India. It is a large perennial shrub or small tree in the coffee family, Rubiaceae that is a native to the American subtropics and tropics. Common names such as Fire bush, Hummingbird bush, Scarlet bush. It is also cultivated in garden as an ornamental tree [6].

*Hamelia patens* have been studied chemically. It is known to contain pentacyclic indole alkaloids. Also, the plants are used in folk medicine against a range of ailments. Number of active compounds have been found in fire bush, such as alienin, ephedrine, flavanones, isomarinine, isopteropodine, maruquine, narirutin, indole alkaloids, palmirine, pteropodine, rosmarinic acid, rumberine, rutin, seneciophylline, speciophylline, isopteropodine, stigmast-4-ene-3,6-dione and tannin (Duke, 2007). It has been used in the indigenous system of medicine for the treatment of various ailments such as, dental disease, burning sensation, uterine disorders, ulcers, cardiac diseases, fever and the plant has been used as diuretic, astringent and aphrodisiac. Photochemical evaluation showed the presence of alkaloids, tannins, ursolic acid, steroids, quercetin, and mixtures of triterpenoid saponins. However there have been no published reports on the anti-inflammatory, analgesic and antipyretic activities of *Hamelia patens*. Thus the present study was undertaken to investigate the analgesic and antipyretic activities of alcohol extract of *Hamelia patens* leaf.

### Materials and Methods

The leaves of *Hamelia patens* (HP) were collected from mature trees grown locally. The leaves of the plant were authenticated in the Department of Botany, Andhra Loyola College, Vijayawada. Experiments were carried out on Wistar rats weighing 150-200 g at Vasavi Institute of Pharmacy, Tadepalligudem. Animals were housed in standard polypropylene cages for one week to acclimatize to laboratory conditions before starting the experiment at a temperature of  $24 \pm 2^\circ$  and relative humidity of 30-70%. A 12:12 light: dark cycle was followed. They were given free access to standard rat feed and water *ad libitum*, but 12 h prior to an experiment; the rats were deprived of food but not water. The animal study protocols were approved by the institutional animal ethical committee.

### Extraction and preparation of sample

The leaves were dried, until free from moisture. Then, they were subjected to size reduction to get coarse powder of desired particle size. The powdered material was subjected to successive extraction in a Soxhlet apparatus using solvent petroleum ether (40-60 $^\circ$ ) and alcohol. Temperature was maintained on an electric heating mantle with a thermostat control. The extract was then concentrated to 3/4<sup>th</sup> of its original volume using a rotavapour apparatus. The concentrated extract was then

transferred to a china dish and evaporated on a thermostat-controlled water bath till it formed a thick paste. This thick mass was vacuum dried in a desiccator till it is free from moisture. The extract was subjected to chemical tests for the detection of phytoconstituents. Alcohol extract of HP was administered orally as suspension by triturating with 5% Tween 80.

### Analgesic studies by the hot plate test and Formalin induced paw licking test

**5.2.1. The hot plate test:** This was carried out by slightly modifying the method described by Woolf & MacDonald [7]. The rats were placed on a confined hot plate maintained at  $55^\circ\text{C} \pm 1^\circ\text{C}$ . The time taken for the rats to respond to the thermal stimulus (usually by jumping) was noted as the latency (in seconds). The rats were divided into 5 groups (A to E), each made up of 5 rats. Rats in group B, C, and D were given extracts of HP orally after 12 hours fast. The doses were 50, 100 and 200 g / kg for the rats in groups B, C and D respectively; representing low medium and high doses. The rats in group A and E were given equivalent doses of normal saline (10 ml / kg) and Diclofenac sodium (5 mg / kg) respectively. Each of the rats was placed on the hot plate and the latency was recorded. The mean latency  $\pm$  standard error of mean (S.E.M) was determined for each group. This short lasting stimulus elicited from the hot plate surface causes little or no damage at all to paw tissues, so it can be followed immediately by the formalin test.

**5.2.2. The Formalin induced paw licking test:** The formalin induced paw-licking test was carried out in accordance with the method described by [8]. 100  $\mu\text{l}$  of 4 % formalin was injected subcutaneously into the plantar surface of the left hind paw of the rats, one hour after oral administration of the extract, Diclofenac sodium or saline. The same groups of rats used in the hot plate test and also, the same doses of extract, Diclofenac sodium or saline were also used in the test. The time spent by the rats in licking the injected paw as soon as the injection was given (early phase, 0-5 minutes post injection) and in the late phase (20-30 minutes post injection) was recorded. The mean time spent on licking the paw by each group was determined.

**5.2.3. Antipyretic study:** Brewer's Yeast Induced Hyperpyrexia: The test was carried out using the method described by [9]. The animals used for this study were fasted overnight before the experiment but water was made available *ad libitum*. The rats were randomly divided into four groups (A to D) containing five rats per group. Pyrexia was induced by subcutaneous injection of 20 % (w/v) brewer's yeast suspension (10 ml / kg) in the dorsum of the rats. 17hrs. After injection, the rectal temperature of each rat was measured, using a clinical thermometer. Only rats that showed an increase in temperature of at least  $0.7^\circ\text{C}$  were used for this study. The rats in group A to C were given (orally) 50, 100, and 200 mg / kg of the extract respectively while those in group D were given Paracetamol (150 mg / kg). The initial rectal temperature of the rats was measured and this served as the control. The temperatures were

subsequently measured at 60, 90 and 120 minutes post extract administration and the mean temperature of each group was recorded.

**5.2.4 Statistical analysis:** Values were expressed as mean ± standard error of mean (S.E.M). Statistical significance was determined by using student t-test; values with P < 0.05 compared with control were considered as significant.

### Results and Discussions

The analgesic and antipyretic properties of the Ethanolic extract of *Hamelia patens* leaves were investigated in this study using three laboratory models.

- The hot plate test,
- The formalin induced paw licking test and
- The brewer’s yeast induced pyrexia.

The models chosen for the analgesic test were carefully selected based on the advantages and the disadvantages of each of the models. The hot plate thermal test is a form of acute (phasic) test which is important in determining the fast type of pain. It is mainly sensitive to strong analgesics and causes limited tissue damage [8]. However, this model has a short coming, because it last for a short time and it is difficult to access modulatory mechanism that may be triggered by the stimulus itself [10]. The formalin test differ from the hot plate test in that it mimic human clinical pain condition in which the pain last for longer period of time and tonic in nature due to the inflammation accompanying the formalin injection [10]. It is sensitive to non-steroidal anti-inflammatory agents and other mild analgesic [8].

#### Hotplate anti-nociceptive test

The result from this study shows that oral administration of *Hamelia patens* leaf extract (50-200 mg / kg ) significantly (p < 0.05) increased the reaction time of the animals to thermal stimuli in a dose dependent manner from 2.2 + 0.3 second to 5.9 + 0.3 seconds [Table 1 ].

#### Brewer’s yeast induced pyrexia

In the brewer’s yeast induced hyperpyrexia model, artificial hyperthermia was induced by administration of exogenous pyrogens in the form of yeast. General reduction of the rectal

**Table1:** Effect of Ethanolic extracts of *Hamelia patens* leaves on hot plate test in rats.

Groups	Dose (mg / kg)	Reaction time (secs) a
Control	10 ml	2.2± 0.3
<i>Hamelia patens</i> leaf extract	50	2.7± 0.2
<i>Hamelia patens</i> leaf extract	100	3.4± 0.2*
<i>Hamelia patens</i> leaf extract	200	5.9± 0.3***
Diclofenac sodium	5	4.6± 0.7*

Each value is the mean + SEM of 5 rats\* P< 0.05, \*\*\*P < 0.001 compares with control; Student t-test.

#### Formalin induced paw licking test

In this model, oral doses (50-200 mg / kg) of the Ethanolic extract of *Hamelia patens* leaf extract inhibited both the early and late phases of the licking response. The licking time was reduced from 77.4 + 9.4 s to 33.6 + 4.4 second in the early phase and from 57.8 + 8.4 to 29.2 + 4.4 s in the late phase [Table 2].

**Table 2:** Effect of the ethanolic extract of *Hamelia patens* leaves on formalin-induced paw Licking test.

Group	Dose (mg / kg) Orally	Licking time (sec) a	
		Early phase	Late phase
Control (Saline)	-	77.4 ± 9.4	61.8 ± 7.58
<i>Hamelia patens</i> leaf extract	50	54.4 ± 3.2*	32.8 ± 4.3 *
<i>Hamelia patens</i> leaf extract	100	36.4 ± 2.8**	31.8 ± 7.0*
<i>Hamelia patens</i> leaf extract	200	33.6 ± 4.4**	29.2 ± 4.4*
Diclofenac sodium	5	40.4 ± 3.3*	37.5 ± 4.7 *

Each value is the mean ± S.E.M. of 5 rats.\* P < 0.05; \*\*p < 0.01, compared with control; student’s t-test.

temperature was observed 60 minutes, 90 minutes and 120 minutes after oral administration of the highest dose (200 mg / kg) of the extract. The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. The result from this study was given in [Table 3].

**Table 3:** Effect of Ethanolic extract of *Hamelia patens* leaf on yeast induced hyperpyrexia in rats.

Groups	Dose (mg / kg)	Pre-Drug Temp (°C)	Post Drug Temp (60min)	Post Drug Temp (90min)	Post Drug Temp (120min)
<i>Hamelia patens</i> leaf extract	50	38.4± 0.3	38.3± 0.3	38.2± 0.3	37.9± 0.3
<i>Hamelia patens</i> leaf extract	100	38.2± 0.4	37.8± 0.4	37.6± 0.5	37.0± 0.2*
<i>Hamelia patens</i> leaf extract	200	38.5± 0.3	37.6± 0.2	37.1± 0.1**	36.6± 0.3**
Paracetamol	150	38.5± 0.3	37.5± 0.4*	37.1± 0.4*	36.9± 0.4*

Each value is the mean ± S.E.M. of 5 rats. \*P < 0.05; \*\*p < 0.01 compared with control; student’s t-test.

The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. These flavonoids and alkaloids may act by blockage of the synthesis of prostaglandins E2 (a peripheral fever mediator) through the inhibition of prostaglandins synthesized by [11]. Therefore the extract could be mediating its analgesic and antipyretic effects like the non-steroidal anti-inflammatory drugs [12]. In the present study, administration of the extract led to significant increase in the latency to thermal (hot plate) stimulus and also a significant reduction in the licking time in both the first and second phase of formalin induced paw licking tests. The observed analgesic activities of *Hamelia patens* leaf extract may be due to mainly of active compounds including maruquine, isomaruquine, pteropodine, isopteropodine, palmirine, rubberize, seneciophylline and stigmast-4-ene-3, 6-dione [13].

Fire bush contains 17.5 percent crude protein and has in vitro digestibility of 61.6 percent [14]. The extract of the *H. patens* revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, quinines, saponins, steroids, coumarins, phytosterols and terpenoids. The plants are used in folk medicine against a range of ailments such as athlete's foot, skin lesions and rash, insect bites, nervous shock, inflammation, rheumatism, headache, asthma and dysentery. Scarlet bush is rich in active photochemicals including alkaloids and flavonoids.

It contains several of the same oxindole alkaloids as Cat's Claw (*Uncaria tomentosa*) including pteropodine and isopteropodine; both have been highly studied and even patented as effective immune stimulants. These two chemicals have also recently shown to have a positive modulating effect on brain neurotransmitters (called 5-HT<sub>2</sub> receptors) that are targets for drugs used in treating a variety of conditions including depression, anxiety, eating disorders, chronic pain conditions and obesity. Three new oxindole alkaloids have also been discovered in scarlet bush which have never been classified before; they've been named *Hamelia patens* alkaloid A, B and C. Scientists in India discovered that scarlet bush leaves contain small amounts (0.05%) of ephedrine - a stimulant alkaloid that has received some negative press of late. In addition, the aerial parts of the plant have been found to contain rosmarinic acid, a photochemical that has demonstrated immune modulating and antidepressant activity.

In the brewer's yeast induced hyperpyrexia model, artificial hyperthermia was induced by administration of exogenous pyrogenes in the form of yeast. General reduction of the rectal temperature was observed 60 minutes, 90 minutes and 120 minutes after oral administration of the highest dose (200 mg / kg) of the extract. The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. These flavonoids and alkaloids may act by blockage

of the synthesis of prostaglandins E2 (a peripheral fever mediator) through the inhibition of prostaglandin syntheses [11]. Therefore the extract could be mediating its analgesic and antipyretic effects like the non-steroidal anti-inflammatory drugs [12].

### Conclusion

In conclusion this study has confirmed that the analgesic and antipyretic activities of *Hamelia patens* leaf extract. It also shows that the analgesic activity is more pronounced than the antipyretic activity since lower doses of the extract produced analgesia while the lower doses fail to reduce pyrexia.

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