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Short Communication

# EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *HELICIA NILAGIRICA* BEDD ON COTTON PELLET-INDUCED GRANULOMA IN RATS

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**ABSTRACT**

**Objective:** The present study was undertaken to screen the anti-inflammatory activity of *Helicia nilagirica* Bedd., an ethnomedicinal plant of Mizoram, India.

**Methods:** In this study, inflammation was induced by cotton pellet granuloma model (Sub-acute) using the method adopted by D'Arcy (1960). The anti-inflammatory effect of two doses of methanolic extract of *Helicia nilagirica* Bedd. was tested and diclofenac was used as a standard drug. The statistical analysis was carried out by One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests using GraphPad In Stat 3.0 software.

**Results:** This *in vivo* anti-inflammatory study shows that the plant extract at two different doses (250 mg/kg and 500 mg/kg) possess significant anti-inflammatory activity ($p<0.01$). The standard drug diclofenac (10 mg/kg) produces maximum activity by inhibiting the wet weight and dry weight of the cotton pellet, 37.45 % and 43.70 % respectively. Two different doses of the plant extract show significant reduction of wet weight and dry weight of cotton pellet at 15.30% and 17.67% respectively for 250 mg/kg and 21.98% and 23.35% for 500 mg/kg respectively.

**Conclusion:** The result of the study shows that the methanolic bark extract of *Helicia nilagirica* Bedd. possess anti-inflammatory activity.

**Keywords:** *Helicia nilagirica* Bedd, Anti-inflammatory, Diclofenac



Inflammation is defined as the local response of living tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues. Inflammation produces pain mediators like bradykinin, histamine, serotonin, prostaglandin, etc.[1]. Even though modern drugs are effective in the management of inflammation and associated conditions, but their use is often limited because of side effects [2].

In recent years, there is growing realization that apart from being safer, economical and easily available, herbs, phytochemicals and herbal products can influence the course of inflammatory diseases and may provide an amalgamation of nutritional substances, which help in restoring and maintaining wear and tear of tissues. Therefore, it would be rational to evaluate scientifically traditional medicines used for their potential use in inflammatory diseases [3].

The studies of the traditional system of medicines have resulted in the development of valuable compounds. *Helicia nilagirica* Bedd. is one of the most commonly used traditional medicine by the people of Mizoram. It is a medium sized tree belonging to the family Proteaceae. It is commonly known as Pasaltakaza in Mizoram, North East India. Traditionally, a decoction prepared by boiling the leaves or bark is used for various stomach ailments including peptic ulcers and indigestion. It is also used in scabies and other skin diseases [4].

Two compounds were isolated from the leaves of *Helicia nilagirica.* Compound 1 was elucidated as 1-O-3-D-glucopyranosyl-(2S, 3S, 4R, 8Z)-2-[(2'R)-2'-hydroxylignocenoyl-amino]-8-octadecene-1, 3, 4-triol. Compound 2 was an analogue of compound 1 [5]. Five compounds were isolated from the dichloromethane and n-butanol extracts of the seeds, identified as p-hydroxybenzaldehyde, p-hydroxybenzoic acid, gallic acid, helicide, 4-formylpymyl-O-beta-D-glucopyranoside [6]. There are very few studies reported on the plant *Helicia nilagirica* Bedd., the aim of this study is to evaluate its anti-inflammatory potential using cotton pellet-induced granuloma in rats.

Authentication of the plant was done at Botanical Survey of India, Kolkata (Voucher No. of the specimen: CHN/46/2013/Tech. II). The specimen was deposited at Regional Institute of Paramedical and Nursing Sciences, Aizawl. The powdered air-dried bark of *Helicia nilagirica* was extracted successively with petroleum ether and methanol using a Soxhlet apparatus. The methanolic extract was evaporated under vacuum in a rotary evaporator and used for the experiment.

Male Wistar rats (150-200 g) used in the study was obtained from the Animal House of Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Mizoram, India. They were housed in environmentally controlled conditions with free access to water and standard diet. The experimental protocols were approved by Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences, Dibrugarh University (Approval No: IAEC/DU/25 dtd. 21.11.12). The standard drug diclofenac was obtained from Novartis. The solvent for extraction, petroleum ether, and methanol was purchased from Sigma-Aldrich.

Acute oral toxicity was performed as per OECD Guideline 423. Two groups of animals were used for the study. Group I served as control and received distilled water. Group II received single oral dose of *Helicia nilagirica* (2000 mg/kg). The animals were observed for gross behavioural, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 d for any sign of delayed toxicity (OECD, 2001).

In this study, inflammation was induced by cotton pellet granuloma model (Sub-acute) using the method adopted by D'Arcy (1960). Under light ether anesthesia by using blunted forceps, a subcutaneous tunnel was made and sterilized cotton pellets (10±0.5 mg) were implanted in the axilla and groin region of the rat. After recovering from anaesthesia, the animals were treated orally with a standard drug, vehicle control (saline 10 ml/kg), standard drug and test drug were administered for seven consecutive days. On the eighth day, the animals were sacrificed by excessive anaesthesia and the cotton pellets were removed surgically. Pellets were separated from extraneous tissue and weighed immediately for wet weight and then dried at 60 °C until the weight become constant [7]. The

percentage inhibition of the wet weight and dry weight of the granuloma were calculated and compared.

The percent inhibition increase in the weight of the cotton pellets was calculated by:

$$\% \text{ Inhibition} = [Wc - Wt/Wc] \times 100$$

Where, $Wt$ is granulation weight in treated groups

$Wc$ is granulation weight in control group

The statistical analysis was carried out by One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests using GraphPad Instat 3.0 software; $p<0.01$ was taken to be statistically significant. The results are expressed as mean±SEM for six animals in each group.

The results (table 1) shows a marked protection in granuloma by *Helicia nilagirica* Bedd., which markedly reduced the dry weight and the wet weight of the cotton pellet at a dose of 250 mg/kg and 500 mg/kg when compared to control. The standard drug diclofenac produces maximum activity by inhibiting the wet weight and dry weight of the cotton pellet, 37.45 % and 43.70 % respectively. Two different doses of the plant extract show significant reduction of wet weight and dry weight of cotton pellet at 15.30% and 17.67% respectively for 250 mg/kg and 21.98% and 23.35% respectively for 500 mg/kg.

**Table 1: Shows the percentage inhibition in the dry weight and wet weight of cotton pellet in treated groups when compared with control group**

| Group | Mean wet weight of cotton (mg) | % Inhibition in wet weight | Mean dry weight of cotton (mg) | % Inhibition in dry weight |
|---|---|---|---|---|
| Vehicle control (10 ml/kg) | 119.65±2.83 | - | 33.47±0.91 | - |
| *H. nilagirica* (250 mg/kg) | 101.30±3.20** | 15.30 | 27.58±1.0** | 17.67 |
| *H. nilagirica* (500 mg/kg) | 93.33±2.12** | 21.98 | 25.61±0.63** | 23.35 |
| Diclofenac (10 mg/kg) | 74.80±2.96** | 37.45 | 18.0±0.51** | 43.70 |

Values are expressed as mean±SEM (n=6), **p < 0.01 when compared with control group

It was observed that the extract at dose 250 mg/kg and 500 mg/kg produced a significant anti-inflammatory activity by reducing the dry weight and wet weight granuloma but lower than the standard drug diclofenac. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation [8]. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in rats have been described. A transudative phase that occurs during first 3 h, an exudative phase occurring between 3-72 h after implanting the pellet and proliferative phase measured as the increase in dry weight of granuloma that occurs between 3 and 6 d after implantation [9]. The suppression of proliferative phase of sub-acute inflammation could result in a decrease in weight of granuloma formation [10]. It is well-known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide [11]. Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharides are indicators of the antiproliferative effect of NSAIDs [3].

## CONCLUSION

The present study demonstrated that the methanolic bark extract of *Helicia nilagirica Bedd.* has shown significant anti-inflammatory action in the sub-acute experimental model, comparable to diclofenac. Further studies may be directed to confirm its anti-inflammatory activity by using other models of anti-inflammatory studies and isolation of the actual bioactive ingredients that are responsible for the anti-inflammatory activity.

## CONFLICT OF INTERESTS

Declared none

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