



Investigación original

## Antipyretic effect of the aqueous extract obtained from leaves of *Neurolaena lobata* (Asteraceae) on a pyretic model induced by brewer's yeast.

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### Resumen

*Neurolaena lobata* es un arbusto propio de las zonas tropicales, que se puede encontrar principalmente en el Caribe y en América Central. De acuerdo con diversas encuestas de TRAMIL, *N. lobata* se utiliza para la preparación de muchos remedios caseros en diversas culturas indígenas de la zona, para tratar enfermedades que presentan fiebre. Basado en esto, el objetivo principal de este trabajo fue la determinación de la posible actividad anti-pirética de los extractos acuosos de las hojas de *N. lobata*. Se utilizaron ratas Wistar (n=40), las cuales fueron divididas en 4 grupos de 10 animales cada uno. En tres de los grupos se indujo fiebre por medio de la administración de levadura de cerveza (como agente pirético). La idea del estudio fue comparar el efecto antipirético del extracto acuoso de las hojas de *N. lobata* con el del acetaminofén, así como también comparar ambos efectos con el producido por el control de agua destilada (placebo). Los tres grupos que recibieron levadura de cerveza experimentaron el efecto pirético esperado ( $p < 0.05$ ), mientras que el grupo al que no se le suministró la levadura,

no mostró ningún efecto. El grupo A recibió agua destilada como placebo (2 ml/animal), el grupo B recibió acetaminofén como tratamiento antipirético (grupo control positivo; 1000 mg/kg), el grupo C recibió el extracto acuoso de las hojas de *N. lobata* (solución experimental; 2000 mg/kg) y el grupo D no recibió ningún tratamiento (control negativo). El grupo A no mostró ningún efecto antipirético, mientras que los grupos B y C mostraron un efecto antipirético estadísticamente significativo ( $p < 0.05$ ) y en ambos grupos el efecto se observó a los 30 minutos después de la administración. En conclusión, en este estudio se mostró que las hojas de *N. lobata* en solución acuosa tienen actividad antipirética, por lo que el uso tradicional es validado en ratas. Este extracto puede ser de utilidad parcial como remedio para controlar la fiebre en ausencia de otros medicamentos disponibles.

**Palabras claves:** *Neurolaena lobata*, antipirético, fiebre, levadura de cerveza, gavilana

### **Abstract**

*Neurolaena lobata* is a bush native of the tropics, that can be found mainly in the Caribbean and Central American areas. Based on TRAMIL surveys, *N. lobata* leaves are used for the preparation of many remedies among the indigenous cultures, to treat several illnesses associated with fever. Based on that, the main objective of this study was to determine whether the aqueous extract of *N. lobata* leaves has antipyretic activity on an experimental animal system. We used Wistar rats ( $n=40$ ), which were divided into 4 groups, and fever was induced in 3 of the groups with brewer's yeast. The idea behind the study was to compare the antipyretic effect obtained with acetaminophen and the aqueous extract of *N. lobata* leaves, and both of them with a control of distilled water (placebo). All three groups receiving brewer's yeast experienced a pyretic statistically significant effect ( $p < 0.05$ ), while the negative control that did not receive the yeast challenge, showed no effect. Group A received distilled water as a placebo (2 ml/animal), group B received acetaminophen as an antipyretic treatment (1000 mg/kg), group C received the aqueous extract of *N. lobata* leaves as the experimental solution (2000 mg/kg) and Group D received no treatment (negative control). Group A shown no antipyretic effect, while groups B and C showed an effect of up to 2.9 °C ( $p < 0.05$ ). Treatment with acetaminophen and *N. lobata* extract showed an antipyretic effect within 30 minutes. In conclusion, we showed that the aqueous extract of *N. lobata* leaves has an antipyretic effect, therefore the traditional use is validated in rats. This extract could be partially helpful as a remedy to control fever in the absence of other available drugs.

**Key words:** *Neurolaena lobata*, antipyretic, fever, brewer's yeast, gavilana

## Introduction

*Neurolaena lobata* is a bush which can grow up to four meters high, with elongated leaves ranging from 5 to 24 cm long. *N. lobata* is a native to the tropics and can be found mainly in the Caribbean and Central America. Depending on the country, it is called by several different names: "gavilana" in Costa Rica, "jackass bitter" in Antigua and Barbados, "salvia" in Cuba, and "mano de lagarto" in Honduras. Based on information collected through TRAMIL surveys, *N. lobata* has been used to treat several diseases including diabetes, stomach aches, and the common cold, among others, and some illnesses that present fever [1,2].

Extracts from the leaves and stems of *N. lobata* have been shown to contain different germacranolides, including neurolenin B [3]. Also up to twelve different flavonoides and some by-products of thymol have been isolated from this plant [4]. Regarding to its toxicity, doses ranging from 1 to 5 g/kg of, both leaves and the whole plant aqueous extract, have been administered to mice without showing obvious adverse effects [2].

In the last few years, several different scientific studies have validated some of the popular uses of *N. lobata*. Recently, it was shown that an ethanol extract of *N. lobata* leaves had an important filaricidal effect when given at concentrations ranging from 125 to 1000

mg/ml. This extract has been applied in vitro and in vivo against *Brugi pahangi*, and its effect has been shown to be dose dependent [5].

Studies have also shown that *N. lobata* has an antiprotozoal effect. An study showed that an ethanol extract of *N. lobata* leaves was capable of inhibiting the growth of *Leishmania mexicana*, *Trypanozoma cruzi* and *Tricomonas vaginalis* through the effects of its germacranolides 1, 2 and 3 [6].

Other studies have proven that *N. lobata* has a neutralizing effect on the hemorrhaging properties of the *Bothrops atrox* venom on mice. An alcoholic extract was used for this purpose and a local intradermic application was proven to be more efficient than an oral or intravenous application [7].

Studies done in rats, using a hydroalcoholic extract of *N. lobata* leaves, have shown that *N. lobata* has a protective effect on the gastric mucosa, reducing the incidence of gastric ulcers by up to 57% during stress tests [8]. The same authors had previously shown that a *N. lobata* hydroalcoholic extract of the leaves had analgesic and anti-inflammatory effects on rats [9].

Based on the fact that most of the diseases for which *N. lobata* is used, present fever, we decided to investigate whether the aqueous extract of the leaves of this plant has effective antipyretic activity.

## Material

**Plants:** *N. lobata* was collected in August, 2005 in Fortuna, San Carlos, Alajuela province (Costa Rica) by biologist and taxonomist Alonso Quesada (1501). A sample of this

material was placed in the National Herbarium of the National Museum of Costa Rica, with the reference number CR-0241340.

**Aqueous extract:** 4 kg of fresh leaves were homogenized in 7.5 liters of distilled water and heated to 80° C for 30 min. The extract was filtered through a gauze and lyophilized, yielding 120 g of material. With this sample, an aqueous solution of 200 mg/ml was prepared.

**Animals:** Forty male Wistar rats were obtained from Laboratorio de Ensayos Biológicos, Universidad de Costa Rica. The average weight of the animals was 180.8±13.3 g. Standard laboratory procedures were followed for their care.

## Methods

The following solutions were used: 20% brewer's yeast aqueous solution [10], aqueous extract of *N. lobata* leaves (200 mg/ml), and 100 mg/ml acetaminophen (Calox International) [11].

Animals were divided into four groups by randomization and the groups were labeled A, B, C or D. Basal rectal temperatures were measured using OMRON digital thermometers (model MC-120). Before the experiment, animals were left overnight with water ad libitum. The next day basal temperatures were measured and animals from groups A, B and C received a subcutaneous injection of brewer's yeast solution at a dose of 4 g/kg of body weight. Rectal temperatures were

measured in all groups, 17 and 18 hours after brewer's yeast injection according to previous validation assays done with Wistar rats, to assure pyretic activity (data not shown). After the 18<sup>th</sup> hour of temperature measurement, the following solutions were given orally: Group A: Distilled water, 2 ml/animal (placebo); Group B: acetaminophen, 1000 mg/kg (antipyretic positive control); Group C: *N. lobata* leaves extract, 2000 mg/kg (experimental solution); Group D: no treatment (negative control).

Rectal temperatures were then measured 30, 60 and 90 minutes after the treatment was applied, to determine antipyretic activity. Data was collected and analyzed (ANOVA,  $p < 0.05$ ).

## Results

Basal temperature of groups A (water), B (acetaminophen), C (plant extract) and D (no treatment), were 36.88±0.35 °C, 36.79±0.54 °C, 36.81±0.46 °C and 36.82±0.27 °C, respectively. There was no statistically significant differences among the groups. After injecting groups A, B and C with brewer's yeast, each group received its corresponding treatment to test the potential antipyretic effect at 30, 60 and 90 minutes. We observed no

effect with water, but both acetaminophen and *N. lobata* had a clear antipyretic effect. There were no changes in the untreated group (Table 2). A, B and C with brewer's yeast, it was observed a sustained pyretic effect at 17 and 18 hours post-injection, representing a statistically significant difference compared to their basal temperatures ( $p < 0.05$ ), but no significant difference among the groups (Table 1).

At the time-response graph, it can be observed the pyretic effect of brewer's yeast and the antipyretic activities

induced by acetaminophen and *N. lobata* aqueous extract (Figure 1).

Table # 1. Basal temperatures and pyretic effect induced by brewer's yeast on Wistar rats

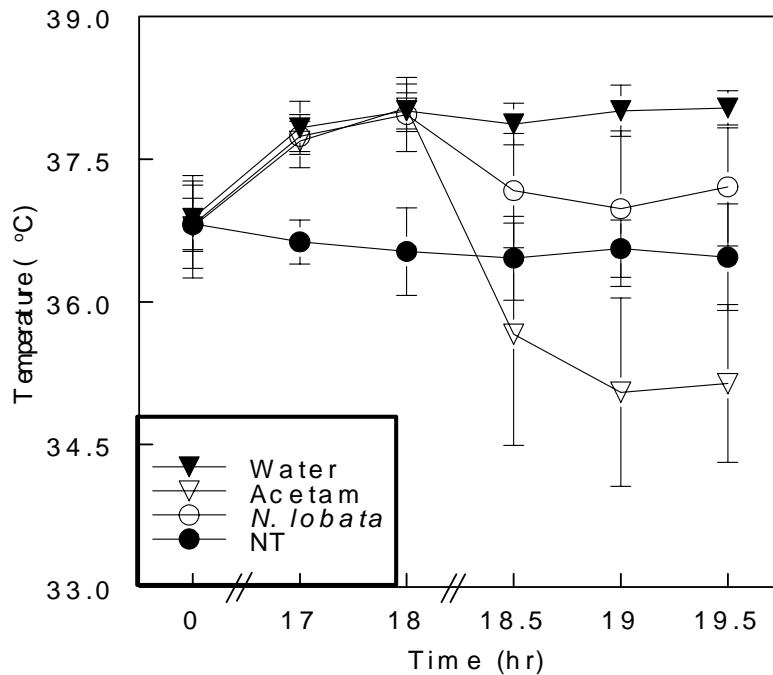
	Basal	17 hr post B.Y.	18 hr post B.Y.
<i>Distilled water</i>	36.88 ±0.35 °C	37.83 ±0.28°C *	38.01 ±0.19 °C *
<i>Acetaminophen</i>	36.79 ±0.54 °C	37.69 ±0.28 °C *	38.04 ±0.25 °C *
<i>N. lobata extract</i>	36.81 ±0.46 °C	37.74 ±0.16 °C *	37.97 ±0.39 °C *
<i>No treatment</i> <sup>1</sup>	36.82 ±0.27 °C	36.63 ±0.23 °C	36.53 ±0.46 °C

B.Y.= Brewer's yeast. <sup>1</sup> = Group D did not receive brewer's yeast. \* = statistically significant increase compared to basal temperature (p<0.05).

Table # 2. Antipyretic effect of *N. lobata* extract treatment on Wistar rats

<i>Treatment</i>	18 hr post B.Y.	30 min post Tx.	60 min post Tx.	90 min post Tx.
<i>Distilled water</i>	38.01±0.19°C	37.87± 0.22 °C	38.01 ± 0.27 °C	38.04 ± 0.18 °C
<i>Acetaminophen</i>	38.04±0.25°C	35.66 ± 1.17 °C*	35.05 ± 0.99 °C*	35.14 ± 0.83 °C*
<i>N. lobata extract</i>	37.97±0.39°C	37.17 ± 0.60 °C*	36.98 ± 0.82 °C*	37.21 ± 0.62 °C*
<i>No treatment</i> <sup>1</sup>	36.53±0.46°C	36.46 ± 0.44 °C	36.56 ± 0.30 °C	36.47 ± 0.56 °C

B.Y.= Brewer's yeast. Tx. = Treatment. <sup>1</sup> = Group D did not receive brewer's yeast. \* = statistically significant decrease compared to temperatures at 18 hrs post B.Y. administration (p<0.05).



**Figure # 1.** Time course of the pyretic effect induced by brewer's yeast and antipyretic activity of different treatments (time is not at exact scale to illustrate the effect more clearly). NT= no treatment.

## Discussion

In this study, pyrexia was induced with brewer's yeast because it is one of the easiest and more reproducible methods reported in the literature [10]. Based on a previous validation test with 20 Wistar rats, we decided to use the 18<sup>th</sup> hour as the time point to test the antipyretic effect of our extract, because it is the point at which the pyretic effect has stabilized. Also, the 18<sup>th</sup> hour point is the one reported in several other previous studies [10]. As was expected, groups A, B and C, which received brewer's yeast,

showed a pyretic effect average of 1.12 °C, which was statistically significant ( $p < 0.05$ ). Group D did not receive any treatment, and its average temperature did not vary significantly during the course of the study.

Group A, which received distilled water, was used as a controlled placebo and, as expected, no changes in temperature were observed in this group. Group B, which received acetaminophen, exhibited a maximum decrease in temperature of up to 2.99 °C. This

antipyretic effect was evident 30 minutes after administration and was statistically significant ( $p < 0.05$ ). The experimental group, group C, received *N. lobata* leaves extract. This group had an antipyretic effect evident also 30 minutes after administration with a maximum antipyretic effect of 0.81 °C.

As it has been described before [12], isoflavones have antipyretic effects. Kerr and collaborators [4] had isolated 12 different flavonoides from *N. lobata* and it is suggested that these could be some of the components responsible for *N. lobatas'* antipyretic effect. In a similar, but more detailed study [13], it was

shown that *Pueraria lobatas'* isoflavones were responsible for the antipyretic effect induced by this plant, but an antipyretic effect of other germacranolides has not been demonstrated [14].

This study validated the antipyretic effect attributed to *N. lobata*. We suggest that, in situations where there is no immediate access to medical care, or where traditional and indigenous herbal medicine is the mainstream treatment of disease, *N. lobata* may play an effective role in relieving the pyretic symptoms of some diseases.

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