

# Immunological Evidence Supporting the Use of Extracts From *Boehmeria jamaicensis* Urb for Treating the Common Cold and Sinus Infections

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## ABSTRACT

Mixed lymphocyte responses assays were conducted at 25.0 and 250.0 µg/mL of the crude ethanolic extract of *Boehmeria jamaicensis* Urb (coded as BJE) using peripheral lymphocytes obtained from individuals suffering from the common cold after four days of infection and from healthy individuals (without the common cold infection). At a concentration of 25 µg/mL, gamma interferon (IFN-γ) was increased by 24.03 fold and interleukin 4 (IL-4) by 1.71 fold for the cells obtained from individuals with the common cold (Group A). The extract suppressed IFN-γ by 8.3% while IL-4 was stimulated by 9.90 fold from peripheral lymphocytes obtained from healthy individuals (Group B). Gamma interferon was suppressed at 250 µg/mL while IL-4 was elevated by 1.86 fold for cells obtained from individuals suffering from the common cold (Group A). In conclusion, BJE could have implications for the treatment of the common cold.

# Evidencia Inmunológica que Fundamenta el uso de Extractos de *Boehmeria Jamaicensis* Urb Para el Tratamiento del Catarro Común y la Sinusitis

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## RESUMEN

Ensayos de reacción linfocitaria mixta fueron realizados a 25.0 y 250.0 µg/mL de extracto etanólico crudo de *Boehmeria jamaicensis* Urb (codificado como BJE), usando linfocitos periféricos obtenidos de individuos con catarro común luego de cuatro días de infección, y de individuos sanos (sin la infección del catarro común). Se halló que el interferón-gamma (IFN-γ) aumentó en 24.03 veces, y la interleucina 4 (IL-4) en 1.71 veces para las células obtenidas de individuos con catarro común, a 25 µg/mL. El extracto inhibió IFN-γ en un 8.3 % en tanto que el IL-4 fue estimulado en 9.90 veces a partir de los linfocitos periféricos obtenidos de individuos sanos. El gamma-interferón fue inhibido a 250 µg/mL, mientras que la IL-4 se elevó en 1.86 veces para las células obtenidas de individuos que sufren de catarro común.

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## INTRODUCTION

The common cold infection and sinusitis are two of the most frequent human illnesses and are responsible for substantial morbidity and economic losses (1). Several plant extracts

have been formulated for treating the common cold infection. These include Black Cherry (*Prunus serotina*), Echinacea (*Echinacea angustifolia* and *E purpurea*), Elderberry (*Sambucus nigra*), Ephedra (*Ephedra sinica*), Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) (2). Similarly, several plant species have been used in Jamaica for treating the common cold eg Aaron's Rod (*Gliricidia sepium* (Jacq) Kunth ex Griseb), Barsley (*Ocimum micranthum* L), Consumption weed (*Wedelia gracilis* LC Rich), Doctor Johnson (*Boehmeria jamaicensis* Urb), Fresh cut (*Justica pectoralis* Jacq Enum), Jackie's Saddle (*Peperomia amplexicaulis* (Sw) A Dietr), John Charles (*Hyptis verticillata* Jacq), Leaf-of-Life (*Bryophyllum pinnatum* (LAM) Oken), Susumber (*Solanum torvum* Sw) and White Sage (*Lantana camara* L) (3, 4). Based on the time taken to recover from the common

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cold and sinus infections by individuals using the plants listed above in the District of Windsor, St Elizabeth, Jamaica (Personal communication, 1987 – 2007) and research done in 1997 (5), it was indicated that *Boehmeria jamaicensis* was one of the most effective plants in treating the infections. Hence, this preliminary study to validate its mechanism on selected cytokines which are known to play important roles in recovery from the common cold and sinus infections. The common cold infection is associated with suppression of circulating levels of some cytokines *eg* gamma interferon (IFN- $\gamma$ ) and interleukins such as interleukin-4 (IL-4). Gamma interferon is considered an important tool in immunotherapy against viral pathogens and cancers, since the cytokine coordinates the cell mediated response through effects on signal transducer and activator transcript-1 (STAT-1). Signal transducer and activator transcript-1 (STAT-1) is a signal transduction pathway important in the tumoricidal and anti-microbial activation of cytokines from lymphocytes (6).

## MATERIALS AND METHODS

### Plant material

Leaves and stems of *Boehmeria jamaicensis* Urb with flowers were collected in February 2007 from the Nassau valley in the Parish of St Elizabeth, Jamaica. The plant was authenticated as *B jamaicensis* at the Herbarium at The University of the West Indies, Mona, Jamaica.

### Preparation of crude ethanolic extract of *Boehmeria jamaicensis* (coded as BJE)

Freshly collected leaves and stems of *B jamaicensis* weighing 300 g were cut into small pieces and then air dried for five days and milled into a powder that was extracted with 2.0 L of ethanol (99% purity) for eight days (four x 2). The resulting green ethanolic crude extract was then concentrated *in vacuo* using a rotary evaporator to yield a dark green oily residue. Stock solutions of 2.5 mg/mL and 25 mg/mL were produced in methanol for administration to the peripheral lymphocytes assays. The extracts were prepared at The University of the West Indies, Mona, Kingston, Jamaica.

### Mixed lymphocyte responses (MLR) assays for accessing the *in vitro* release of IFN- $\gamma$ and interleukin-4 (IL-4).

Mixed lymphocyte response assays were conducted using lymphocytes from human peripheral blood. Blood was withdrawn from individuals and categorized as follows: group A = blood from individuals infected with the common cold; Group B = blood from healthy individuals and group C = blood from healthy individuals, however the lymphocytes obtained from this category were irradiated with 2000 Rads of gamma irradiation for 10 minutes to serve as the 'stimulator' for activating the release of the cytokines.

### Preparation of blood samples for obtaining lymphocytes for MLR

Blood was layered over Ficoll histopaque and centrifuged at 1260 rpm for 45 minutes at 25°C. The buffered layer was then removed and cells washed with pure RPMI-1640 medium x 3 and lymphocytes prepared for culturing with the extract. *Lymphocytes culturing*: The responder cells (groups A and B) were stock at a density of  $2 \times 10^5$  cells/dL while the stimulators (group C) were cultured at  $4 \times 10^5$  cells/dL in pure RPMI-1640 medium. From each group of responder cells 1.0 mL of culture was added to 1.0 mL of stimulator cell culture. From both stock solutions of 2.5 mg/mL and 25 mg/mL a volume of 20  $\mu$ L was added to the 2.0 mL of pure RPMI-1640 medium containing the various groups of lymphocytes to give final concentrations of 25  $\mu$ g/mL and 250  $\mu$ g/mL. The control (positive) consisted of both responder and stimulator cells without the extract. This control represents cytokine production in situations where the lymphocytes are challenged by a "foreign" substance or pathogen which represents the stimulator cells. After five days of cell culture, the supernatants were harvested and stored at -70°C for ELISA analyses as stated by the manufacturer using a Titertek Multiskan<sup>(R)</sup> Mcc/340 Model (Flow Laboratories, USA). The levels of IFN- $\gamma$  and IL-4 were determined from established standard curves. All experiments were conducted in triplicates. There was ethical approval for the use of human blood (Morehouse School of Medicine).

## RESULTS

An analysis of the data presented in Tables 1 and 2 revealed that the common cold infection suppressed IFN- $\gamma$  by 98.7% after four days from the analysis done on groups A and B. Exposing lymphocytes harvested from individuals with the common cold infection to 25  $\mu$ g/mL of BJE increased IFN- $\gamma$  levels by 24.03 fold (Table 1) data compiled from group A to

Table 1: Effects of *Boehmeria jamaicensis* extract on gamma-interferon release in mixed lymphocytes response assays on blood obtained from individuals suffering from the common cold.

Doses $\mu$ g/mL	IFN- $\gamma$ levels pg/mL
25	187.5 $\pm$ 3.56
250	4.5 $\pm$ 0.50
Control	7.8 $\pm$ 0.40

2500  $\mu$ g/mL suppressed IFN- $\gamma$  below detectable limits.  
IL-4 was 4.4  $\pm$  0.20 pg/ml

which the extract was added. A concentration of 250  $\mu$ g/mL of BJE suppressed IFN- $\gamma$  to 4.5  $\pm$  0.50 pg/mL as compared to the control with a value of 7.8  $\pm$  0.40 pg/ml (Table 1). Dose of 2500  $\mu$ g/mL suppressed IFN- $\gamma$  below detectable limits (data not shown).

Table 3 revealed that *B. jamaicensis* elevated IL-4 to  $15.9 \pm 2.00$  pg/mL from cells harvested from individuals infected with the common cold virus compared to the control value of  $9.3 \pm 0.50$  pg/mL.

A comparative analysis of Tables 2 and 4 revealed that BJE at 25  $\mu$ g/mL stimulated the release of IL-4 in healthy individuals by 9.90 fold but not IFN- $\gamma$ .

Table 2: Effects of *Boehmeria jamaicensis* extract on gamma-interferon release in mixed lymphocytes response assays on blood obtained from healthy individuals.

Doses $\mu$ g/mL	IFN- $\gamma$ levels pg/mL
25	$187.5 \pm 3.56$
25	$550.0 \pm 8.24$
250	$23.2 \pm 2.10$
Control	$600.0 \pm 2.20$
2500 $\mu$ m/l suppressed IFN- $\gamma$ below detectable limits IL-4 was $4.4 \pm 0.20$ pg/ml	

Table 3: Effects of *Boehmeria jamaicensis* extract on IL-4 release in mixed lymphocytes response assays on blood obtained from individuals suffering from the common cold

Doses $\mu$ g/mL	IL-4 levels pg/mL
25	$15.9 \pm 2.00$
250	$15.6 \pm 3.50$
Control	$9.3 \pm 0.50$

Table 4: Effects of *Boehmeria jamaicensis* extract on IL-4 release in mixed lymphocytes response assays on blood obtained from healthy individuals.

Doses $\mu$ g/mL	IL-4 levels pg/mL
25	$48.5 \pm 3.82$
250	$45.8 \pm 1.50$
Control	$4.9 \pm 0.32$

## DISCUSSION

The ethanolic extract of BJE activated the release of both IFN- $\gamma$  and IL-4 from peripheral lymphocytes obtained from individuals suffering from the common cold infection. From this study, it is evident that BJE was more effective in up-regulating the release of IFN- $\gamma$ . Sethi *et al* (7) have demonstrated that IFN- $\gamma$  is critical in reducing rhinovirus titres in human. In addition, IFN- $\gamma$ , a known Th-1 (Type-1 or cell mediated) anti-viral lymphokine, is known to increase the levels of intercellular adhesion molecule-1 (ICAM-1) on uninfected cells while inducing a significant persistent down

regulation of ICAM-1 expression on human rhinovirus infected cells (7). These ICAM expressions are known to influence the progression of viral infections such as the common cold (7).

Linden *et al* (8) have reported that IL-4 can be suppressed below detectable limits in the nasal lavage (NAL) fluid of patients with the common cold. Thus, BJE if developed into a non-toxic product could be instrumental in stimulating IL-4 release in patients infected with the common cold. Interleukin-4 is known to potentiate IFN- $\gamma$  inducible nitric oxide synthase (iNOS) expression *via* STAT-1 activation in human respiratory epithelium (9). Thus, it would appear that IL-4 and IFN- $\gamma$  modulate the release of each other (9). In addition, it would appear that BJE could have other applications such as in the development of cancer vaccines since it activates the release of IL-4 which is one of the methods being developed for treating lung cancer (10).

From the dose responses obtained, it would appear that BJE should be evaluated at doses lower than 25  $\mu$ g/mL to see the effect on the release of both cytokines. This is under investigation in our laboratories along with the structural elucidation of the molecule(s) responsible for the activation/up-regulation of the cytokines.

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