UNIVERSIDAD DE GUAYAQUIL

DEPARTMENT OF CHEMICAL SCIENCES

Ciudadela Universitaria ¨Dr. Salvador Allende¨

Telephone: 2293680, E-mail: fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT

CODE: 38/05

TITLE:
Establishment of the potential anti-inflammatory effect (via orally) of the product known as **Cumanda,** originating from NutraMedix Laboratories, LLC, Florida

OBJECTIVE:
To study the possible anti-inflammatory effect of CUMANDA, measured by edemas, induced by carragenin in the feet of laboratory mice.

BACKGROUND:
The method of inducing edemas by applying carragenine to the feet of mice is a classic model for the study of products with anti-inflammatory activity. The by-products of the metabolism of araquidonic acid via cecloxigenesis and the production of reactive species of oxygen are also involved. It is reported that there are four principle phases to this edema: an initial phase in which histamine and serotonin is released; a second phase measured by kinins; a third phase (about 5 hours) in which prostaglandins are liberated; and a fourth phase related to the local infiltration of neutrophils and their activiation. This model has recently been recommended as very useful for the evaluation of anti-oxidant products with anti-inflammatory properties and free radicals of oxygen inhibitors.

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As discussed in numerous international works, the pharmacological study of the above-mentioned effect is indispensable, and guarantees (within the margin of error associated with the technique) that the potential for producing anti-inflammatory effects in humans will be learned.

The basis of this work is the pharmacological effect as an anti-inflammatory, as described in international literature (1, 2).

TECHNICAL, SCIENTIFIC AND SOCIOECONOMIC BENEFITS:

The fact that this natural product possesses the above-explained effect represents a potential advantage in its use, given that it presents low toxicity. In addition the inflammatory processes involve different pathologies, from there the utility of this product.

VARIABLES TO MEASURE:

1. Weight of the feet
2. Percentage of Inflammation
3. Percentage of inhibition

PROCEDURES TO FOLLOW:

TEST MATERIALS:

Cumanda: The procedure followed was that described by CYTED (1996) and the Gerhard Voegel (1997).

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CHANGES IN THE CURRICULUM:
Changes did not take place in protocol proposed to the Unity of Quality Guarantee, whose number is referred to on page 1**.**

DATA FROM THE SAMPLE:
Organization soliciting services: NutraMedix Laboratories, LLC.
Person in charge of the Organization's application: Ing. Jose Icaza
Date of application: 7/15/05
Organization that carried out the work: University of Guayaquil, Department of Chemical Sciences.
Address: Ciudadela Universitaria "Dr. Salvador Allende"
Person in charge in the Executor Organization: Dr. Diadelis Remirez Figueredo
Storage: The product was stored at room temperature with controlled access.
Form of presentation of the product: amber glass drop bottle containing 30 milliliters
Storage: The product was maintained at room temperature before and during the experiment, and as indicated was protected from light and kept in a locked cabinet.

INFORMATION WITH RESPECT TO THE HANDLING:
No special handling instructions were needed.

COMPOSITION OF THE PRODUCT:
Cumanda bark extract
Mineral Water
Alcohol (20 – 25%)

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EXPERIMENTAL PROCEDURE:

INTRODUCTION:

This experiment was carried out with the intention of determining the possible anti-inflammatory effect of Cumanda, using oral application and employing the method of edema induced by carragenine as the inflammatory agent.

DOSAGE USED IN THE TEST:

In this study 15 mL of Cumanda per kilogram of body weight was administered according to the dosage recommended by the manufacturer.

PRINCIPAL TEST:

METHODS AND TECHNIQUES:

Study Material: **Cumanda**

Animal Model: A single rodent species (mouse) was utilized, with a minimum of 5 animals of a single sex in each group. In this case, male mice with an average weight within ± 20% (3), belonging to the Swiss line and coming from the Chemistry Department of the University of Guayaquil were appropriate and were utilized in the experiment.

The animals were maintained in quarantine conditions and were acclimated according to established procedures (4, 5), said period having a duration of five days minimum.

Access to the water and the food was "ad libitum.¨ (6, 7)

The animals were randomly distributed from within the different groups. (8)

Food was denied 4 hours before exposure to the test material.

The experiment lasted 6 days (5 of acclimation and 1 of test)

DEVELOPMENT OF THE METHOD:
The following four groups were constructed for the test:

TEST GROUPS	
1	**Flebogenous agent Carragenine 1%**
2	**Flebogenous agent Carragenine 1% + Ibuprofen (600mg/kg) orally**
3	**Carragenine 1% + Cumanda 15 ml/kg**
3	**Carragenine 1% + Celecoxib 200mg/kg**

The mice were denied food for four hours then weighed, after which began the experiment.

The irritant solution of 1% carragenine was dissolved in physiological saline and 0.1 ml was administered on the right sole. The saline alone was administered to the other foot as a negative control.

The composite solutions were administered orally one hour before the carragenine application.

Five hours after the application of carragenine, the animals are euthanized in a saturated ether atmosphere, and their feet are cut at the knee and weighed.

RESULTS CALCULATIONS:

Outcomes are rated by calculating the weight of each mouse's feet, both the treated and untreated.

Percentage of inflammation: % Inflammation = $\frac{T \times 100}{ST}$ -100

T = is the average of the weights of the treated feet (right) and ST is the average of the weights of the untreated feet.

Percentage of Inhibition is calculated as follows: % of inhibition = 100-(mean values of the treatment group/mean values of the control group) x 100

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DESCRIPTION OF THE DOSAGE, METHOD OF ADMINISTRATION AND DURATION OF THE TEST:

The test was achieved by following the method established by CYTED and using the dosage indicated for each mouse.

The Cumanda and the control medications Celecoxib and Ibuprofen were administered orally one hour before the application of the carragenine.

ANALITICAL RESULTS:

The results of the average value of the weights of the treated feet, the standard deviations, the percentage of inflammation and the percentage of inhibition are found in Table #1.

Table # 1: Anti-inflammatory effect of Cumanda on the edema induced by Carragenine.

Group	Weigh of feet (g) (Mean ± st. dev.)	% of inflammation	% of inhibition
Carragenine 1%	**0.3 ±0.6a**	**96.1**	**---**
Carragenine +Ibuprofen	**0.2 ±0.3b**	**42%**	**100%**
Carragenine + Cumanda	**0.21±1.0b**	**61%**	**88.2%**
Carragenine + Celecoxib	**0.22±0.6b**	**54%**	**78.5%**

As is demonstrated in Table # 1, Cumanda showed an anti-inflammatory effect similar to the conventional anti-inflammatories ibuprofen and celecoxib. The pharmaceutical celecoxib (inhibitor of ciclooxigenasis 2) showed a lesser anti-inflammatory effect than ibuprofen, a non-steroidal anti-inflammatory. It is important to note that Cumanda was capable of demonstrating this effect in the face of a potent inflammatory agent such as carragenine, which after 5 hours releases a series of mediators which are involved in the inflammatory response.

Other natural anti-oxidant products such as Vimang, obtained from the bark of the mango tree (9), and Phycocyanin pigment connected to a protein found in blue-green algae (10), have demonstrated similar or less percentages of inihibition, which speaks in favor of the product that is the object of this study. In addition, indomethecine, the pharmaceutical of favor in treating inflammation caused by carragenine, produces an inhibition of only 50%. This result sets standards for the investigation of the mechanism of action of this product as an anti-inflammatory and anti-oxidant.

CONCLUSIONS:

1. **Cumanda** demonstrated to have an anti-inflammatory effect similar to widely used conventional anti-inflamatories.

2. Ibuprofen and Celecoxib were also demonstrated to possess the effect for which they are sold.

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GENERAL CONCLUSIONS:

Cumanda demonstrated to have anti-inflammatory effect similar to ibuprofen and Celecoxib and can be used to reduce inflammation from inflammatory agents such as carragenine, as observed in animal testing and as appears in specialized literature.

PERSONNEL RESPONSIBLE FOR THE STUDY

Responsible Professional: **Signature:**

Dr. Diadelis Remirez Figueredo

Date: 07/18/05

BIBLIOGRAPHY:

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2. Drugs Discovery, Gerhard Voegel (1997).
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