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: 35/05

TITLE:

Determination of the possible Hepatoprotector potential of the product **Burbur Detox** originating from NutraMedix LLC Laboratories, Jupiter, Florida, United States.

OBJETIVES:

Study the possible Hepatoprotector effect of the medication known as **Burbur Detox**, measured by the variations experienced by the Pyruvate and Oxaloacetic transaminase enzymes when a hepatotoxic agent is administered to laboratory animals.

BACKGROUND:

Burbur Detox will be used by humans, which means that it is vitally important to carry out first-level studies conducted to guarantee its quality.

Medications can have beneficial effects, as is thought to be the case for Burbur Detox. Thus it is important to demonstrate that it displays a hepatoprotector effect in the models established for measuring this effect.

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This study intends to demonstrate whether the administration of Burbur Detox to laboratory animals is capable of preventing hepatic damage that hepatotoxic agents can cause, as measured by variations in Oxaloacetic and Pyruvate Transaminases.

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 Hepatoprotector effects in humans will be learned.

The pharmacological effect such as Hepatoprotector is described in the international literature, from which our work was extracted (1, 2).

TECHNICAL, SCIENTIFIC AND SOCIOECONOMIC BENEFITS:

The demonstration that this product has the mentioned effect is important due to its potential as a new, plant-based medication, with its associated low toxicity. This was demonstrated by us in a previous work, allowing us to enter the product as a new medication in the appropriate Register.

VARIABLES TO MEASURE:

Determinations of blood biochemistry:

1. Glutamate Pyruvate Transaminase (GPT)
2. Glutamic-Oxaloacetic Transaminase (GOT)

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PROCEDURES TO FOLLOW:

TEST MATERIALS: Burbur Detox, the procedure described by CYTED (1996) and the Gerhard Voegel (1997) was followed.

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: Jose Icaza
Date of application: 4/20/05
Person in charge in the Executor Organization: MSc. Gastón Garcia Simón.
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.
Organization that carried out the work: University of Guayaquil, Department of Chemical Sciences.
Address: Ciudadela Universitaria "Dr. Salvador Allende"
Form of presentation of the product: amber glass drop bottle containing 30 milliliters.

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

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COMPOSITION OF THE PRODUCT:
Burbur leaf extract
Mineral Water
Ethanol 20 – 25%

EXPERIMENTAL PROCEDURE:

INTRODUCTION:
This experiment was carried out with the intention of determining the possible Hepatoprotector effect of Burbur Detox with the method that utilizes variations in the GOT and GPT enzymes.

PRINCIPAL TEST:

METHODS AND TECHNIQUES:

Test Materials: Burbur Detox
The hepatoprotector effect of **Burbur Detox** was determined by means of an acute test.

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 Wister 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).

DOSAGE USED IN THE TEST:
A dosage of 0.3 mL per 200 grams of mouse body weight was used.

EXPERIMENTAL PROCEDURE:
After 18 hours of fasting the animals were sedated using a 40 mg/kg dosage of sodium pentothal. Blood was taken from the ocular plexus by means of a hematocrit capillary to obtain plasma, thus allowing a determination of basal levels of the enzymes that are the subject of the study, GPT and GOT.

Following this, the specified dose of N-Acetyl-cysteine was administered orally along with Burbur Detox in the dosage specified above.

One hour later acetaminophen was administered in the predetermined dosage, and finally the N-Acetyl-cysteine and Burbur Detox dosages were repeated according to the groupings.

24 hours after the last administration the animals were re-sedated to allow a second blood extraction with the intent of studying the behavior of the enzymes (GOT and GPT).

The animals were then euthanized using procedures to avoid pain and suffering, in accordance to the principles of the ethical treatment of laboratory animals. In our case a saturated atmosphere of ether was used.

METHOD DEVELOPMENT:
The test was conducted using 4 groups, as follows:

Table # 1. TEST GROUPS

GROUP	TREATMENT
I. Control	**No Treatment**
II. Acetaminophen	**Acetaminophen was administered orally in a dosage of 600 mg/kg of body weight (bw) in a volume of 10 mL/kg.**
III. Positive Control	**N-Acetyl-cysteine was administered orally in a dosage of 600 mg/kg of bw, in a volume of 10 mL/kg of bw, plus Acetaminophen, administered using the same method, dosage and volume.**
IV. Burbur Detox	**Burbur Detox was administered orally in a volume of 1.5 mL/kg of bw, plus Acetaminophen in a dosage of 600 mg/kg, using the same method and volume.**

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RESULTS CALCULATIONS:
The mean and standard deviation for each group was obtained, and later the normal population distribution was determined in order to apply the Student Newman Keuls means test ($p<0.05$).

DESCRIPTION OF THE DOSAGE, METHOD OF ADMINISTRATION AND DURATION OF THE TEST:
The administered volume of acetaminophen and N-Acetyl-cysteine was 10 mL/kg of body weight, and 1.5 mL/kg for Burbur Detox.

Medication was administered orally through an intragastric canula.

The dosage of acetaminophen and N-Acetyl-cysteine was 600 mg/kg.

ANALITICAL RESULTS:
In the table one may observe the mean and standard deviation for the four test groups, as well as the statistical comparison of the results of the aforementioned procedures.

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Table # 2. DETERMINATION OF THE ENZYMES GPT Y GOT (U/L)

GROUP	Values of the Glutamate Pyruvate Transaminase (GPT) and Glutamic-Oxaloacetic Transaminase (GOT) (U/L)			
	GOT		GPT	
	0 HOURS	24 HOURS	0 HOURS	24 HOURS
I. Control	154.0 ± 18.4 a	151 ± 14.7 b	42.2 ± 3.6 d	44.4 ± 4.7 e
II. Acetaminophen	149.4 ± 12.5 a	319.0 ± 17.0 c	48.2 ± 12.44 d	369.0 ± 77.18 f
III. Positive Control	146.2 ± 16.1 a	151.2 ± 21.6 b	54.0 ± 14.9 d	51.6 ± 7.4 e
IV. Burbur Detox	176.6 ± 34.8 a	200.5 ± 85.5 b	54.8 ± 6.8 d	89.5 ± 48.8 e

a, b, c, d: Statistical significance ($p<0.05$).

As can be appreciated from the table, three groups did not differ from each other: Burbur, N-Acetyl-cysteine, and those that received no treatment. This in spite of the fact that, as may be observed, the values obtained from the Burbur group were high. However, they are below Acetaminophen, which is logical if one takes into account that a product (Acetaminophen) that causes liver damage is being administered to the animals. No matter how much protection there is, there will always be damage, reflected in elevated transaminases. But it does differ from the positive control that received N-Acetyl-cysteine, that is, the antidote to Acetaminophen.

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CONCLUSIONS:

Burbur Detox was capable of protecting the liver when acetaminophen, a hepatoxic agent, was administered, in specific concentrations, in laboratory tests using mice.

GENERAL CONCLUSIONS:

Burbur Detox was demonstrated to possess a HEPATO-PROTECTOR effect at the indicated dosage in the established model for this type of study.

PERSONNEL RESPONSIBLE FOR THE STUDY:

Responsible Professional: MSc. Gastón García Simón.

Signature: 

Date: 05/27/05

BIBLIOGRAPHY:

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