Determination of medium lethal dose (LD50) and acute toxicity of formulation Cytoreg®, an ionic mixture of strong and weak acids.

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ABSTRACT

A series of toxicological and pharmacological tests were started in the bioterium of the University of Los Andes, in order to evaluate a new formulation for therapeutic purposes. The present work reports the results found regarding the LD_{50} and the acute toxicity of this formulation produced by Cytorex of Venezuela, S.A. The new formulation supplied in liquid form, containing a variety of acid atoms whose conjugated bases have different electrical charges. It constitutes an ion transport complex of positively charged cations and negatively charged anions in high concentrations that help regulate mitochondrial and cellular metabolism and utilizes the inorganic fluoride ion (HF) as the main active compound, as referred to by the supplier. All the concentrations described in this work are referred to hydrofluoric acid (HF), which is the active compound of the formulation. Conventional pharmacological methods were used and it was found that the average lethal dose in rats resulted in 44.83 mg/Kg, and of 0.82ml/Kg. At the concentration of minimum dose of 0,49 mL/Kg of weight, the surviving animals did not present apparent macroscopic lesions at necropsy.

Keywords: Cytoreg®, Toxicity, medium lethal dose (LD_{50}), Wistar rats.

1 INTRODUCTION

In screening drugs, determination of DL_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance.

The evaluation of the Toxicity of a substance is a very - very important aspect of any biochemical or pharmacological experiment. The toxicity of a substance can be measured in many ways. Generally it is estimated on the test animal, rats, mice, cat and other animal models (WHO 1966; Kumar and Trivedi, 2016).

A new formulation for therapeutic purposes (Kumi-Dark et al., 2006), is a mixture of strong and weak acids, with a concentration of hydrofluoric acid (HF) referred by the supplier of 55 g/liter. Hydrofluoric acid is the active compound in the acid-balanced mixture and in this assays was evaluated the medium lethal dose (LD50) and acute toxicity in the experimental model rat BIOULA:Wistar.

2 ETHICAL AVAL

The experiment was carried out taking into account the regulations on the production and ethical use of the laboratory animals of the Laboratory Animals Science Venezuelan Association (AVECAL 2008) and the Guidelines of MPPCyT (2013), and with the endorsement of the institutional ethics commission, registered with the Nº CEBIOULA/013 (02/11/2010).

3 EXPERIMENTAL DESIGN

Animal Model: Female rats were used from the non-consanguineous Wistar line of 8 weeks of birth and with a weight comprised between 210 - 260 g (average: 235g). The animals were housed in cubicles under sanitary barriers of sterilization of inputs and maintenance, with 12 hours of light and 12 hours of darkness, and with food and drink ad-libitum.

Therapeutics formulation: Cytoreg®, which is composed of strong and weak acids, with a concentration of hydrofluoric acid (HF), referred by the supplier of 55 g/liter. Hydrofluoric acid is the active compound in the acid-balanced mixture further compounded by 10% sulfuric acid (H_2SO_4), 10% hydrochloric acid (HCL), 3% phosphoric acid (H_3PO_4), 0.3% oxalic acid ($C_2H_2O_4$) and 0.3% citric acid ($C_6H_8O_7$). All the concentrations referred to in this work are represented by those of hydrofluoric acid.

3.1 METHODOLOGY

The amount of 45 female rats was used from the non-consanguineous Wistar line of 8 weeks of birth and with a weight comprised between 210 - 260 g (average: 235 g).

For the determination of the LD_{50}, a group of 10 animals were utilized, divided in 5 groups of 2 animals each. Due to the lack of knowledge of effective and lethal doses of the compound, a preliminary experiment called "up and down method" using two animals (Randhawa, 2009). The rats was carried out administering 2 mL, 1.5 mL, 1.0 mL, and 0.5 mL of the compound orally, and a control group to which the amount of 2mL of water, for determinate of approximate LD_{50} by pilot study the so called.

III. - After these preliminary groups, mortality was observed in all doses of the agent. In the control group, there was no mortality.

IV. – To determine minimum dose and toxicity acute (Colerangle 2013). Dilutions with water were prepared in equal parts, in order to administer decreasing amounts, starting from the lowest dose that killed 100% of the animals, that was 0.5 mL (27.5 mg), as: 0,25 mL (13.75 mg), 0.186 mL (10.23 mg), 0.150 mL (8.25 mg), 0.125 mL (6.88 mg) and 0.1135 mL (6.24 mg), the doses were also calculated normalized by Kg of weight and administer to groups of 7 animals (N = 7) each.

V. - The animals were observed daily for 15 days, registering the current symptoms and mortality in each group. The survivors, at day 15, were slaughtered.

VI. - All doses were administered as single doses intragastric with esophageal cannule; this route of administration was selected because it is one of the proposals by the inventor laboratory of the agent, as a route of administration to persons, and following indication of OECD (2001).

4 RESULTS

The Table 1, report of the mortality resulting from the administration of Cytoreg® in the experimental groups, in the determination lethal dose 50. In this can observed that to the concentration of 27.50mg is the lethal dosis and in the 1.43 mg there is not death of the animals.

Table 1. Mortality resulting from the administration* of Cytoreg® in the experimental groups

Doses mg (mg/Kg.)	**Doses ml (ml/Kg.)**	**Deaths**	**% Deaths**
6.25 (1.43)	0.1135 (0.48)	0/7	0.00
6.87 (1.61)	0.125 (0.53)	2/7	28.57
8.25 (1.93)	0.150 (0.63)	4/7	57.14
10.25 (2.40)	0.186 (0.79)	4/7	57.14
13.75 (3.23)	0.25 (1.05)	4/7	57.14
27.50 (6.46)	0.50 (2.13)	7/7	100

*Doses calculated in mg and ml/Kg

The Table 2 and Figure 1, present results related to the DL_{50} calculation by both arithmetic and graphical methods. The arithmetic calculation was according Behrens and Kerber methods, here it can

be seen that DL_{50} was to concentration of 12.74 mg and the graphical methods present 10.54 mg, and in milliliters 0.23 and 0.22 mL respectively (Table 3).

Table 2. Determination of the LD_{50} according to arithmetic and Behrens and Kerber methods (Chinedu et al., 2013).

Lot N°	1	2	3	4	5	6
Doses (mg)	6.25	6.875	8.25	10.25	13.75	27.5
No. of animals	7	7	7	7	7	7
No. of deaths	0	2	4	4	4	7
Differences between doses (a)	0	0.63	1.37	2.0	3.75	13.75
Median difference between deaths (b)	0	1	3	4	4	5
(a) X (b)	0	0.63	4.11	8.0	15	75.62

Dose (mg):

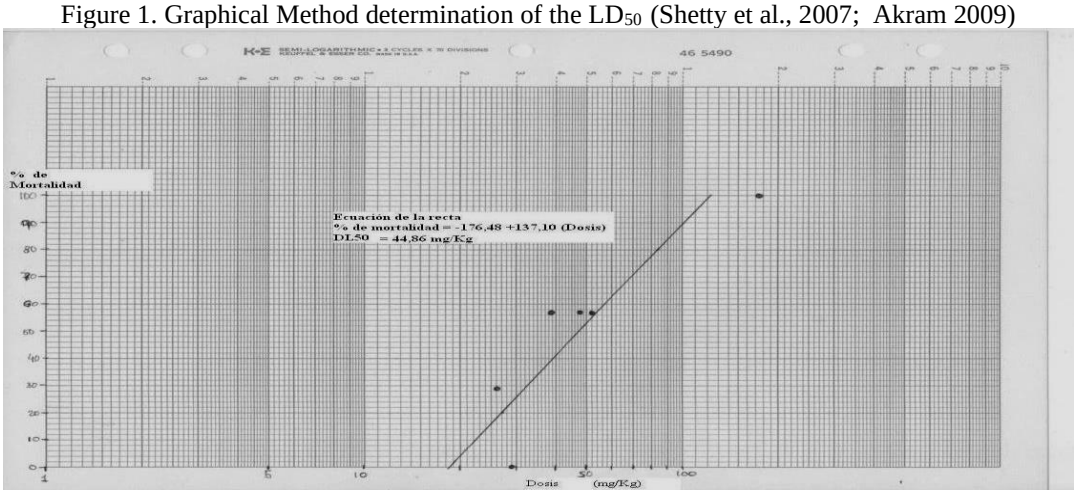
$DL50^{*} = DL100^{**} - \frac{\sum (a) \times (b)}{N} = \frac{103,36}{7} = 27,5 - 14,76 =$ **12,74 mg**

DL50* = Doses capable of killing 50% of animals in a lot

DL100** = Doses capable of killing 100% of animals in a lot

N = number of animals per lot

Figure 1. Graphical Method determination of the LD_{50} (Shetty et al., 2007; Akram 2009)



Note of the translator: the original chart is in Spanish

The Table 3 presents the comparison of the results between methods, but in the graphical methods it can be seen the minimum lethal dose was in mg/Kg of 18.50 and 0.49 mL/Kg, respectively.

Table 3. Comparison of calculation the LD_{50} and the LD_m by different methods.

Method	DL_{50} mg	DL_{50} mg/kg	DL ml	DL ml/Kg	DL_m mg/Kg	DL_m ml/Kg
Behrens y Karber	12.4	53.57	0.23	1.08	-	-
Graphical	10.54	44.86	0.22	0.83	18.50	0.49

5 DISCUSSION

In theory, the average lethal dose, or LD_{50} provides information on the amount of substance necessary to have undesirable effects on humans; there are many ways to determine toxicity, and although biochemical, physiological, reproductive and behavioral effects are very useful, the most commonly used indicator is the death of the test organism (Giráldez et al., 2000).

Note that the LD_{50} measures the fatal dose but no other serious non-lethal side effects or unwanted effects that need to be verbalized (that the patient can refer), (WHO, 1966).

The determination of the lethality curve of the Cytoreg® compound shows us activity at relatively low doses; resulting in an average lethal dose (LD_{50}) in rats of 44.83 mg/Kg obtained graphically, and in milliliters it turned out to be 0.83 mL/Kg., taking into account that the referred concentration of the active is 55g per liter (55 mg/mL). A dose of 44.83 mg/Kg would suggest a dose in milliliters of 0.81 mL/Kg. The small difference that results may be due to experimental error. Although the results in animals cannot be extrapolated to the human, following general principles of clinical pharmacology, the dose to initiate in patients should be 100 times lower than the average lethal dose observed in animals, in this case that is a dose of 0.83 mL/Kg, which for an individual of 70 Kg, the initial total dose of test substance to be administered would be 0.581 mL.

At the concentration of minimum dose of 0.49 mL/Kg of weight, the surviving animals did not present alterations in the consumption of food and water, the weight of animals presented an increase relation to their aged and they did not present apparent macroscopic lesions at necropsy.

6 CONCLUSIONS

1. Cytoreg® is a compound with potent activity.
2. In the survivors animals at fifteen days the necropsies do not reveal apparent macroscopic lesions with down doses.
3. By the graphic method, the average lethal dose in rats resulted in 44.86 mg / Kg, and of 0.83 mL / Kg, and the estimated minimum lethal dose is 18.50 mg / Kg, and of 0.49 mL / Kg.
4. Theoretically an initial dose of test for chronic toxicity studies in rats, the estimated minimum lethal dosage, would be suggested.
5. More research would be required in other animal species such as rabbits and dogs, as well as chronic toxicity studies to determine the effect of this dosage.

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