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 LD50 and the acute toxicity of this formulation produced by Cytorex of Venezuela, S.A. The new formulation is a mixture of strong and weak acids, with a concentration of hydrofluoric acid (HF) referred by the supplier of 55 gr/liter. Hydrofluoric acid is the active compound in the acid-balanced mixture, further composed 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 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 0.83ml/Kg. The high doses produced apparently caustic lesions and the surviving animals did not present apparent macroscopic lesions at necropsy.

Keywords: Cytoreg®, Toxicity, medium lethal dose (LD50), Wistar rats

The experiment was carried out taking into account the regulations on the production and ethical use of the laboratory animals of the AVECAL (4) and the MPPCyT (5), and with the endorsement of the institutional ethics commission, registered with the N° CEBIOULA/013 (02/11/2010).

EXPERIMENTAL DESIGN

Experimental model

The amount of 118 female rats was used from the non-consanguineous Wistar line of 8 weeks of birth and with a weight comprised between 210 - 260 grams (average: 235 grams). 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.

The formulation used is 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.

Methodology

I. - The amount of 118 female rats was used from the non-consanguineous Wistar line of 8 weeks of birth and with a weight comprised between 210 - 260 grams (average: 235 grams). For the determination of the LD50, a group of 42 animals were utilized, divided in 6 groups of 7 animals each.

II. - Due to the lack of knowledge of effective and lethal doses of the compound, a preliminary experiment was carried out administering 2 ml., 1.5 ml., 1.0 ml., and 0.5 ml. of the compound, orally, to 4 experimental groups of 10 animals each, and a control group to which the amount of 2ml. of water was administered.

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

IV. - 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 is, 0.5 ml., 0.186 ml., 0.150 ml., 0.125 ml. and 0.1135 ml. to groups of 7 animals (N = 7) each, starting with a dose of 27.5 mg. (0.5 ml.), up to a non-lethal dose. The doses were also calculated normalized by Kg. of weight.

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 and intragastric, via esophageal tube; 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.

Results

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

Table 2 Arithmetic determination of the LD50 according to the method of Behrens and Kerber, using the administered dose.

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

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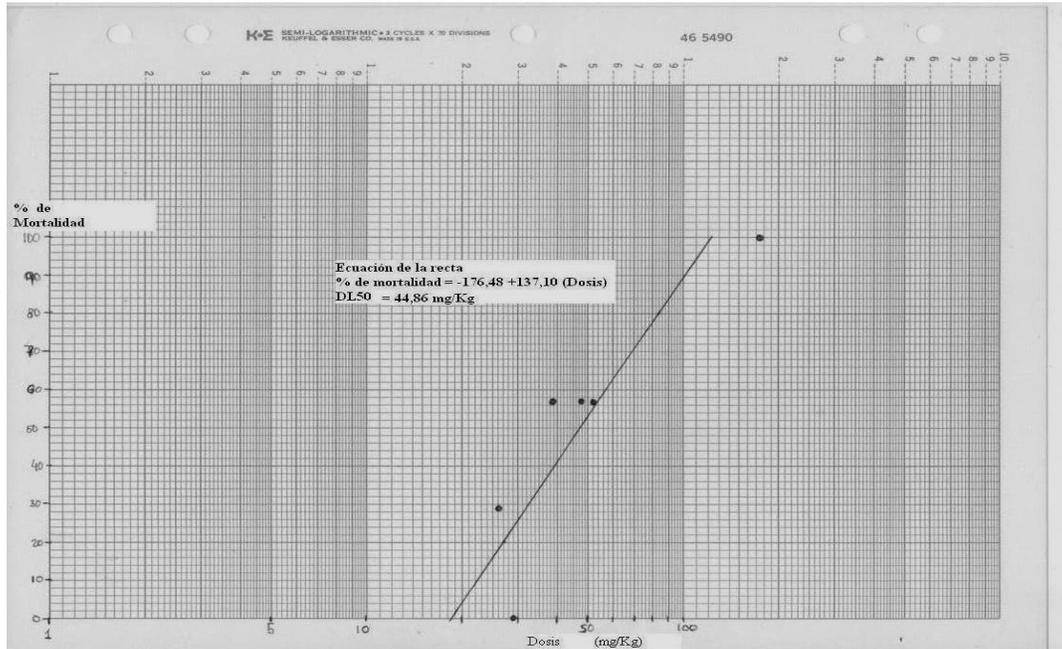
$$DL50^* = DL100^{**} - \frac{\sum (a) \times (b)}{N} = \frac{103,36}{7} = 27,5 - 14,76 = \textbf{12,74 mg}$$

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

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Graphical Method

Note of the translator: the original chart is in Spanish

Table 3. Comparison of calculation the LD50 and the LDminimum by different methods.

Method	DL50 (mg)	DL50 (mg/kg)	DL (ml)	DL (ml)/Kg	DL min mg/Kg	DL min ml/Kg
Behrens y Karber	12,75	53,57	0,23	1,08	-	-
Graphical	10,54	44,86	0,22	0,83	18,50	0,49

Discussion

In theory, the average lethal dose, or LD50, provides information on the amount of substance

necessary to have undesirable effects on humans (4); 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.

Note that the LD50 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). (3).

The determination of the lethality curve of the Cytoreg® compound shows us activity at relatively low doses; resulting in an average lethal dose (LD50) 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 55 grams per liter (55 mg/ml). A dose of 44.83 mg/Kg would suggest a dose in ml 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, that is, a dose of 0.0083 ml/ Kg, which for an individual of 70 Kg., the initial total dose of test substance to be administered would be 0.581 ml.

Conclusions

1.- Cytoreg® is a compound with potent activity.

2.- Animals that die early due to high doses present apparently caustic lesions. In the survivors at fifteen days the necropsies do not reveal apparent macroscopic lesions.

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, the estimated minimum lethal dose (LD$_{minimum}$) is suggested as the initial test dose to be used for chronic toxicity studies in rats.

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 and dosage.

BIBLIOGRAPHIC REFERENCES

1.-WHO (World Health Organization). Principles applicable to preclinical studies of the safety of medicines. Technical Reports Series. 1966.341.

2. Giráldez DA. And Romero VA. Chap.18. Experimental procedures in pharmacology and toxicology. 2000. [about25p] [Query: January 27. 2011].

Retrieved from http://minnie.uab.es/~veteri/0009/cap18pdf.

3.-AVECAL. Manual for the production and ethical use of laboratory animals (2008). Ministry of Popular Power for Science and Technology. Caracas Venezuela. MPPCyT

4.-Norms for the use of animals in research: in experimentation: in the laboratory. obtained in their habitats. studies of their pathologies and their natural behavior-Bioethics and Biosecurity Manual. 2008. Cap.3. pp: 33-35. Caracas Venezuela. 8.25.

5.-Díaz M. Bulus G. and Pica Y. Chapter 5. Statistical Methods for the Analysis of Toxicity Results. International Development Research Center. 2004. [Query: January 27. 2011]. [around11p] Retrieved from: http://www.idrc.ca/en/ev-84468-201-1-DO_TOPIC.html.

6.-Kumi-DiakaJ. Hassanhi M. Brown J. Merchant K. Garcia C, and Jimenez W. Cytoreg inhibits growth and proliferation of human adenocarcinoma cells via induction of apoptosis. 2006. Journal of Carcinogenesis 5: 1.