ACUTE TOXICITY EVALUATION OF SIX MEDICINAL PLANTS USING THREE ALTERNATIVE METHODS

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Summary

The implantation of new experimental procedures or methods requieres a standardization process to compare its results and conclude about its use feasibility. OECD, for known reazons abolished the Acute Toxicity classical test for three alternative methods: Acute Toxic Classification (ATC), Up and Down Procedure (UDP) and Fix Dose Procedure (FDP). On the other hand it has been strategicaly decided to promote the use of pharmaceutical forms derived from plants such as tilo (*Justicia pectoralis*), guava (*Psidium guajava*), oregano (*Plethranctus amboinicus*), caña santa (*Cymbopogum cytratus*), aucaliptus (*Ecaliptus citriodora*) and majagua (*Hibiscus elastus*). Materials and Methods: the animals were dosed orally (2000 mg/kg BW, on total solids basis), according its body weight. The plants were collected according to the regulations indicated and aequous extracts were done. The animals were observed systematicaly during the first 24 hours and daily until 14 days. Body weight was controlled on days 1, 7 and 14. After that all the animasls were sacrified previous clinical inspection and later on anatomopathologiocal studies were executed. Results: No mortality neither other toxic symptoms were observed, only in the case of J pectoralis light sedation which is atributed to the medicinal effect of this plant. No body wight neither anatomopathological lession were also seen. Conclusions: the uniformity results of each experiment for the extracts studied indicates its reproducibility and validity confirming that the LD50 of those extracts is above 2000 mg/kg BW.

Keywords: acute toxicity, medicinal plants, alternative methods.

Introduction

The implantation and execution of new methods and/or experimental procedures, independently of the control of the slant variables, require a standardization process for which the methods are applied using individual or diverse substances to compare its results to conclude that its systematic use in the particular conditions of the laboratory is feasible. The increasing pressure exerted by protectionistic groups together with the scientific and technological advances nowdays have made possible the Alternative Methods to the Experimentation Animal development, based on the principle of 3R (Refinement, Reduction and Replacemen) (1).

In December of 2002 was abolished by the OECD, the execution of the Classic Test for the determination of Acute Toxicity and Lethal Dose 50 (LD50), which was replaced by three alternative methods whose common objective is to reduce the number of used animals and to diminish its suffering (2,3), these methods include the test of Acute Toxic Classification (ATC) or method of the Classes of Toxicity (4,5), Up and Down Procedure (UDP) (2) and Fixed Dose Procedure (FDP) (6).

Single dose toxicity studies are included within the first barrier battery of essential tests in the substance evaluation. They allow to obtain data on the intrinsic toxicity of the product, prediction of damage on species and in target and non target organs, to determine the most susceptible species, for the design and selection of doses for further repeated dose studies, to offer information to clinicians to predict, diagnose and prescribe treatments in case of acute sobrexpositions (poisoning). From the regulatory point of view, the data of these studies is essential for the classification, labeled and transportation of chemical (7).

WHO has stated that by 2020 world-wide population will be of 7,500 million people, and that 75% will live in developing countries, consuming 15% of total marketed medicines which strengthens the use of natural products derived from plants by its accessibility and low cost. Considering these and the popular roots of the use of medicinal plants by our population the Cuban Ministery of Health decided to promote the development of pharmaceutical forms derived from medicinal plants, among them are tilo (*Justicia pectoralis*), guayaba (*Psidium guajava*), oregano (*Plethranctus amboinicus*), caña santa (*Cymbopogun citratus*), eucalyptus (*Eucaliptus citriodora*) and majagua (*Hibiscus elastus*) those that are of ample popular use by properties like sedatives, antidiarreic, and so on.

ATC and UDP are a reproducible alternative studies of toxicity, validated national and internationally that use a considerably smaller amount of animals with respect to the classic test to determine acute toxicity of a substance, evaluating it in a similar way

(2,4,5). FDP is based on proving a series of progressive doses (5, 50, 300 and 2000 mg/Kg) to discern the classification of the toxicity of substances in categories.

The selected dose must be nonlethal, nonpainful and nonestressing, but it must cause evident toxicity. The collected data are adapted for the estimation of the risk, as a comparative reference and for labeling and classification of products. (6).
The objectives of this paper are: to validate the proposed alternative methods in the particular conditions of our laboratory and to determine the acute toxicity of the plants in study according to the accuracy of the obtained results.

Materials and Methods

For the UDP study the animals were administered by oral rout (intragastric cannula 16 G) with a unique dose of the studied extracts. The volume of administration was determined according to the body weight of animals considering that each one would receive a 2000 mg/kg BW limit dose. The food was retired the previous afternoon (16-18 hours). Originally it is considered that it must be dosed an animal every 48 hours although by investigator´s criterion and based on the scientific evidence it can be varied this period of time taking into account also the traditional use of these extracts by the population without toxicity reports, so it was decided to dose the five animals the same day (2). In the case of ATC the food was retired for the same period of time and animals were dosed, beginning with females and later, according to mortality, the males (4,5).

FDP included two experimental groups of females SD rats: group I (Treated) administered with the watery extract and group II (Control vehicle) receiveing the vehicle of this extract. The animals were observed systematically during the 24 hours after the application and the administration of the following dose was not necessary depending on the results obtained in the dose of 2000mg/Kg BW, following the scheme of the test method. In case of appearing mortality in 2 or more animals it would be necessary an inferior dose (300mg/kg) not appearing such situation, a total of 5 animals by dose level were completed, in each experimental group. (6).

The three experiments have some common aspects as detailed:
Scheme of sample preparation: the plants were collected according to the established norms for that and the extracts were made in the Provincial Laboratory of Medicinal Plants. Later they were rotoevaporated to eliminate the ethanol and to obtain an effective concentration for the toxicological test, refering to preparation of the doses and administration the test substances: they were administered in constant volume varying the concentration of the solution.

Observations: the animals (8,9) were systematicaly observed during the first 30 min, periodically during the first 24 h, with special attention during the first 4 h and daily until the 14 days of the experiment. The observations were directed to the determination of: death, time of occurrence of the death, signs and symptoms of toxicity in addition to its beginning and duration, changes in the skin, mucous membranes and eyes, also, in the respiratory, circulatory and nervous systems and in the somatomotor activity and conduct, with special attention to the potential occurrence of tremor, convulsions, salivation, diarrea, lethargy, somnolency and comma. The body weight was controlled days 1, 7 and 14 of the experiment

Metaevaluations: on day 14 all the animals were sacrificed, previously a rigorous clinical inspection of the animals (skin, mucous, masses and osteomioarticular system) was done and later macroscopic anatomopathological studies were carried out.

Results

In our days it is considered that to regulatory effects it is not necessary to define with a precise value the LD50 of a compound, that is practically sufficient to locate this dose in a rank, in this case it was used a limit dose of 2000 mg/kg BW not observing neither mortality nor other indicative symptom of toxicity, only in the case of tilo (*Justicia pectoralis*), in the three experiments light sedation was observed which is attributed to the sedative effect of this plant not considering it a toxic symptom. On the other hand the body weight, as a toxicity indicator, behaved within the parameters established for the growing curve of the species and line in(8) and the macrocospic anatomopathological studies did not show any alteration in the analyzed organs.

Discussion

The congruency of results of each experiment for each one of the 6 extracts in study indicates its reproducibility and on the other hand the coincidence of results of each extract in the different tests shows the validity of the experiments, coinciding with the studies of Diener (5), reason why is assumed that it is feasible to introduce these three alternative tests systematicaly in our laboratory according to the the principles of “3R” (1,10), implying in first place the care and the responsibility with respect to the animals and the obvious diminution of costs as much by acquisition and manteinance of animals, by reagents and other materials. The validity of the experiments allows affirming that the LD50 of the watery extracts of the plants in study is over 2000 mg/kg BW, being considered nontoxic these substances under our conditions of study.

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