[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

Original Article

A New Method for Determining Acute Toxicity in Animal Models

Enegide Chinedu*, David Arome, Fidelis Solomon Ameh

Department of Science Laboratory Technology (Physiology and Pharmacology Technology), University of Jos, Jos, Nigeria

ABSTRACT

Background: The intake of pharmacological substances by man has solely increased and this may be in the form of food, medicines and beverages, other industrial and household products. These substances are capable of eliciting chronic and acute toxicity, which may be mild or severe, depending upon their nature. Acute toxicity is defined as the unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours. The principal aim of this paper is to introduce a new method for testing toxicity, which if adopted, should produce more accurate and reproducible results using few animals. **Materials and Methods:** The proposed method is divided into three stages, with the outcome of each stage determining whether to terminate testing of proceed to the next stage. A confirmatory (confidence) test is used to validate the final test result. The method shows enormous advantages, which include the use of few animals, exploration of a wide range of doses, it is simple and inexpensive. **Conclusion:** Furthermore, accurate and reproducible result can be gotten through this method. We therefore recommend that the method should be considered for endorsement for the testing of acute toxicity by the regulatory bodies.

Key words: Acute toxicity, lethal dose$_{50}$, new method

INTRODUCTION

The amount of pharmacological substances and chemicals being used in the human community today, have increased to almost an innumerable amount.[1] These may be presented today in the form or as constituents of food substances, medicines, beverages, other industrial and household products. However, these chemicals or pharmacological substances may result in chronic toxicity in the living system when used over a long period of time or acute toxicity may also occur when large quantities capable of eliciting immediate toxic effect are used. These effects may be mild or severe, depending on the nature of the substance. Acute toxicity is defined as the unwanted effect (s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours. The unwanted (or adverse) effect is any effect that produces functional impairments in organs and/or biochemical lesions, which could alter the functioning of the organism in general or individual organs.[2] Studies of acute toxicity however tends to establish the dose-dependent unwanted (or adverse) effect (s), which may take place and this includes all information that is important in the assessment of acute toxicity including mortality. The assessment of the lethal dose (LD_{50}) (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent.[3] The toxicity assessment

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.121674

Address for correspondence: Mr. Enegide Chinedu, Department of Science Laboratory Technology (Physiology and Pharmacology Technology), University of Jos, Jos 23473, Nigeria. E-mail: Chinex.snow@gmail.com

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

of pharmacological agents is a very important procedure that is usually carried-out before they are allowed to enter the market for sale. Conversely, different methods have been developed and adopted for acute toxicity testing. However, most of these methods have their short-comings and is now important to develop a better method, which may require the use of fewer animals if possible. The aim of this paper is to introduce a new method for testing acute toxicity, which if adopted, should produce more accurate and reproducible results using few animals.

MATERIALS AND METHODS

Lorke's method

This method has two phases which are phases 1 and 2 respectively.

Phase 1

This phase requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behavior as well as if mortality will occur.

Phase 2

This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality.[4]

Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Karber's method

This method involves the administration of different doses of test substance to various groups, which has five animals each. The first group of animals is administered with the vehicle in which the test substance was dissolved or diluted in (e.g, water or normal saline). However, from the second group onward receives different doses of the test substance. The animals in each group receives specific doses, while increment in dose progresses from group to group (starting from group 2 which receives the lowest dose). The interval mean of a number of mortality recorded in each group and dose difference across the groups are key parameters in this method.[5] The LD_{50} is calculated using the arithmetical method of Karber, which is as follow:

$$LD_{50} = LD_{100} - \sum \left(\frac{a \times b}{n} \right)$$

Where, LD_{50} = Median lethal dose
LD_{100} = Least dose required to kill 100%
a = Dose difference
b = Mean mortality
n = Group population.

Up-and-down method

This method involves the sequential dosing of single animals with the test substance within a time interval of 48 hours. After the administration of the first dose, the next is determined by the outcome of the subsequent dose administered. If the animal survives the subsequent dose the dose is adjusted upward, but when mortality is recorded at subsequent dose, it is adjusted downward. The adjustment of dose either upward or downward is by a constant factor. Testing is terminated when the upper limit (2000-5000 mg/kg) have been reachedwithout mortality or when the LD_{50} have been established from the test.[6,7]

PROPOSED (NEW) METHOD

This method is divided into stages, with the outcome from each stage determine the next step to take (i.e, whether to terminate or proceed to the next stage).

Stage 1

This is the initial stage and it requires four animals. These animals are divided into four groups of one animal each. Then different doses of the test substance are administered to the different animals. The animals should be observed for 1 hours post-administration and then 10 minutes every 2 hours interval for 24 hours. The behavioral signs of toxicity and also mortality should be recorded. Where no mortality is recorded at this stage, the testing should proceed to stage 2.

Stage 2

This stage involves three animals, which are divided into three groups of one animal each. Different doses of the test substance (higher than those used in stage 1) are administer to the different animals and then observed for 1 hour after administration and periodically for 24 hours. Behavioral signs of toxicity should be noted and mortality as well. If no mortality occurred, testing should proceed to stage 3.

Stage 3

This stage also requires three animals which are distributed into three groups of one animal each. Various high doses of test substance (with 5000 mg/kg as the highest) are administered to the different animals. Observation is done for 1 hour after administration and

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

then 10 minutes every 2 hours for 24 hours. Behavioral toxicity signs and also mortality should be recorded. This is the final stage of testing and where no mortality is recorded at this stage, the LD_{50} of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety.

Confirmatory (or confidence) test

Where mortality was recorded at a given dose in any of the stages, a confirmatory test should be carried-out to actually validate that the test substance was the cause of such mortality.

This test simply involves the administration of the dose of test substance that caused mortality (or lowest dose that caused mortality in a situation that recorded more than one mortality) to two animals. Then observation should be done for 1 hour after administration and 10 minutes every 2 hours interval for 24 hours. Where at least a single animal from the two animals die, it should serve as a confirmation and validation of the test result.

Furthermore, if the main test did not show any mortality at 5000 mg/kg, a confirmatory test should also be carried-out. This can be done by administering 5000 mg/kg to two animals. Observation should be done for 1 hour after administration and 10 minutes every 2 hours interval for 24 hours. The recording of no mortality should be a confirmation of test result.

The confirmatory test can also be carried-out on a substance in which literature have clearly stated its LD_{50}.

Calculation

After the test procedure, the formula that should be employed in the calculation of the LD_{50} is shown below:

$$LD_{50} = \frac{[M_0 + M_1]}{2}$$

Where M_0 = Highest dose of test substance that gave no mortality,
M_1 = Lowest dose of test substance that gave mortality.

In carrying-out acute toxicity by this method, any of the two dose range in stage 1 as stated in Table 1 (which is 10, 100, 300 and 600 or 50, 200, 400 and 800) may be employed. The doses recommended for stage 2 and 3 are documented in the Table 1. A wide range of doses have been recommended for this method so as to ensure accuracy of result. This method has some advantageous difference over other methods and have been documented in Table 2. This includes that it is accurrate, involves few animals, expendicture is moderate, process is simple and involves less time duration.

Table 1: Recommended doses for the proposed (new) method

Stage	Recommended doses (mg/kg bdw)			
	Group 1	Group 2	Group 3	Group 4
1	10	100	300	600
	50	200	400	800
2	1000	1500	2000	
3	3000	4000	5000	

Table 2: Comparing the different methods[3]

Parameter	Lorke's method	Karber's method	Up and down method	Proposed (new) method
Result accuracy	Uncertain	Uncertain	Accurate	Accurate
No. of animals	Few	Much	Few	Few
Expenditure	Moderate	High	Moderate	Moderate
Simplicity of process	Simple	Seems complicated	Simple	Simple
Duration	Less	Less	May take more time	Less

CONCLUSION

The proposed method seems to be accurate, explores a wide range of doses and requires few animals to carry-out the entire process. This method is not expensive and therefore can be employed by both individuals and organizations. The method is also simple and requires less time. Since the method shows numerous advantages, we strongly suggest that it should be put through the various stages of validation and also considered for endorsement for the testing of acute toxicity by the regulatory.

REFERENCES

1. Sperling F. Introduction to toxicity evaluation session. Environ Health Perspect 1979;32:259.
2. Walum E. Acute oral toxicity. Environ Health Perspect 1998;106 Suppl 2:497-503.
3. Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose. Curr Sci 2007;93:917-20.
4. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;54:275-87.
5. Turner R. Acute toxicity: The determination of LD_{50}. In: Screening Methods in Pharmacology. New York: Academic Press; 1965. p. 61-3.
6. Bruce RD. An up-and-down procedure for acute toxicity testing. Fundam Appl Toxicol 1985;5:151-7.
7. Organization for Economic Co-operation and Development (OECD). Guideline for the testing of chemicals 423. Documentation on acute oral toxicity and acute class method, 2001. Available from: http://www.oecd.org. [Last accessed on 2013 Apr 25].

How to cite this article: Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int 2013;20:224-6.
Source of Support: Nil. **Conflict of Interest:** None declared.