Original Articles
Toxicology International, *May-Aug 2015 / Vol-22 / Issue-2, 83-87*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2015/v22/i2/137667

An Approach to Acute, Subacute, Subchronic, and Chronic Toxicity Assessment in Animal Models

Enegide Chinedu[1*], David Arome[2], Fidelis Solomon Ameh[2] and Dabum Luka Jacob[3]

[1]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria; chinex.snow@gmail.com
[2]Department of Science Laboratory Technology (Physiology and Pharmacology Technology), University of Jos, Plateau State, Nigeria
[3]Department of Human Physiology, Faculty of Medical Sciences, University of Jos, Plateau State, Nigeria

Abstract

The safety of pharmacological/chemical agents and food additives has become the main and longest-standing toxicology issue of public interest. Exposure of the public to pharmacological/chemical agents without proper knowledge about their toxicity has ended up in several notable tragedies. Although toxicity profiling of many chemical/pharmacological agents has increased, there are still not enough information on the toxicity of some of the currently used agents. The aim of this paper is to unveil a simple step-wise method by which toxicity test from acute to the chronic level can be carried out in animal models. The procedure involves the usage of two different strains of the test animals at all levels of the test. The method also adopts continuity from the previous level at the next level of assessment. This ensures that same conditions are maintained at all levels of assessment, and therefore, guaranty consistency, accuracy, and reproducibility of the results gotten from the assessment. The method addresses some issues (such as cost and time) which have hindered toxicity assessment of some agents. It can be effectively adopted in developing countries due to its cost-effective nature. It can also serve as a guide for obtaining appreciable toxicity information of traditional medicines.

Keywords: Acute Toxicity, Animal Models, Chronic Toxicity, Method

Received: 25-07-2015 **Revised:** 19-08-2015 **Accepted:** 22-08-2015

1. Introduction

Man has always sort for ways to improve his health quality, eradicate, and prevent disease conditions that always attack him. This has led to the discovery and development of many pharmacological/chemical agents which are currently used for diagnosis, treatment, and preventive purposes. These pharmacological/chemical agents have been developed from synthetic, semi-synthetic, and natural (e.g., plant) materials. Although these agents have played a remarkable role in improving man's health quality, they are not all totally safe as they possess the potential for causing harm.[1]This has led to the inclusion of toxicity test for pharmacological/chemical agents and food additives being developed for use in man. This is with the purpose of ensuring that these agents are relatively safe at a therapeutic dose (or intended dose for use) before they could be used as medicines (and for other purposes). The safety of pharmacological/chemical agents and food additives has become the main and longest-standing toxicology issue of public interest. Risks associated with lack of safety of these agents are likely to affect a large percentage of population.[2] Exposure of the public to pharmacological/chemical agents without proper knowledge about their toxicity has ended up in several notable tragedies (e.g., the thalidomide disaster and diethylene glycol tragedy).[3,4] There are now strict government requirements in most countries for safety

* *Author for correspondence*

testing of pharmacological/chemical agents and food additives before they can be marketed.[5] This is applicable for pharmacological agents, food additives, cosmetics, pesticides, and other industrial chemicals. This therefore shows that the toxicity profile of a pharmacological/chemical agent is as important as the information on its beneficial use. There are various levels of toxicity test, however, acute, subacute, subchronic, and chronic toxicities are the most commonly assessed. Although toxicity profiling of various pharmacological/chemical agents has increased, there are still not enough information on the toxicity of some of the currently used agents.[6] This is very common with locally developed agents (like herbal medicines). One of the reasons for this is the nonscientific claim that they have zero-toxicity. However, irrespective of the source, all pharmacological/chemical agents have a potential for causing toxicity except otherwise proven in the laboratory. The aim of this paper is to unveil a simple step-wise method by which toxicity test from acute to the chronic level can be carried out in animal models. The procedure involves the usage of two different strains of the test animals at all levels of the test.

2. Materials and Methods

2.1 Acute Toxicity Test

The method developed for this level is that proposed by the authors,[7] but, critical modifications were made to it. This test is divided into stages, and the result from each stage determines whether to terminate or proceed to the next stage.

Stage 1

This stage requires four animals (consisting of two different strains). These animals are divided into four groups of one animal each. Animals in Groups 1 and 3 are of the same strain, while those in Groups 2 and 4 are also of the same strain.

Different doses of the test substance as shown in Table 1 are administered to the animals. The animals are kept under intense observation for an hour after administration and then 10 min at 2 h interval for 24 h. The behavioral signs of toxicity and also mortality are recorded. Where no mortality is recorded at this stage, the assessment proceeds to Stage 2.

Stage 2

This stage involves three animals (consisting of two different strains). The animals are divided into three groups of one animal each. Animals in Groups 1 and 3 are of the same strain while that in Group 2 of a different strain. Higher doses of the test substance [Table 1] are administered to the animals and then carefully observed for 1 h after administration and periodically for 24 h. Behavioral signs of toxicity including mortality are recorded. If no mortality is recorded, assessment proceeds to Stage 3.

Stage 3

This stage involves three animals (consisting of two different strains) which are assigned to three different groups of one animal each. Animals in Groups 1 and 3 are of the same strain while that in Group 2 of a different strain. Higher doses of the test substance as shown in Table 1 are administered to the animals. Careful observation is performed for 1 h of post-administration and then 10 min at 2 h intervals for 24 h. Behavioral signs of toxicity and also mortality are recorded.

Table 1. Recommended doses for the acute toxicity test

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

2.2 Confirmatory (or Confidence) Test

The confirmatory test is carried out as a confirmation of the test result. Where mortality was recorded, the test involves the administration of the lethal dose (or lowest lethal dose in a situation that recorded more than one mortality) to two animals (consisting of two different strains). Observation is done for 1 h of post-administration and 10 min at 2 h interval for 24 h. If an animal (irrespective of the strain) from the two animals die, it serves 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 is also done. This involves administering 5000 mg/kg to two animals (consisting of two different strains). Observation is done for 1 h after administration and 10 min every 2 h interval

for 24 h. The recording of no mortality is a confirmation of test result. The confirmatory test is also recommended for a substance of which literature have clearly stated its LD_{50}.

Calculation

The formula for calculation the LD_{50} is expressed below:

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

M_0 = Highest dose of test substance without mortality.
M_1 = Lowest dose of test substance that gave mortality.

2.3 Subacute Toxicity Test

The subacute toxicity test is a continuation of the acute toxicity test. It mainly involves the continued observation of the test animals used for acute toxicity for a longer period. All animals administered the test substance (irrespective of dose administered) during the acute toxicity test and did not produce mortality are kept under further careful observation for 14 days. Signs of toxicity and mortality are recorded if any is observed (just as during acute toxicity test). Where mortality is observed within the 14 days of observation, the dose that gave mortality (or lowest lethal dose, in a situation that recorded more than one death) is administered to two animals (consisting of two different strains) and observed for 14 days. The observation of a single mortality of any of the animals within the period of observation is a confirmation of its subacute toxicity at that dose.

Calculation

The formula for calculating the subacute lethal dose is expressed as:

$$\text{Lethal Dose}_{\text{subacute}} = \frac{[M_0 + M_1]}{2}$$

M_0 = Highest dose of test substance without mortality after the subacute test.

M_1 = Lowest dose of test substance that gave mortality after the subacute test.

2.4 Subchronic Toxicity

Subchronic toxicity assessment requires information gotten from both the acute and subacute toxicity test levels. This will help determine the test doses to be used at this level. At least, three dose levels are recommended for this test. The test doses can be determined using the following formula:

$$\text{Dose} = \frac{M_0}{[1 + k]}$$

M_0 = Highest dose of test substance without mortality after the subacute test.

k = positive real numbers starting from 0 with an increment number of 1 (i.e., k = 0, k = 1 and k = 2 for three respective dose levels).

Ten animals (consisting of equal numbers of two different strains) are required for each Group. A control group of 10 (consisting of equal numbers of two different strains) is also required. The test doses are administered to the animals in the respective groups for a period of 30 days while the control group animals are administered with the vehicle. Observation of all animals is done on daily basis throughout the period of study. Signs of toxicity and also mortality are recorded. The mortality of two animals from the same strain in a particular group indicates toxicity at that dose. At any time mortality is observed, the vital organs (kidney, lung, liver, heart, spleen, small intestine, stomach and duodenum and adrenal gland) of such animal are isolated immediately for histological examination. At the end of the period of administration, four animals (two from each strain) from each group (including the control group) are sacrificed 6 h after the last administration. The vital organs (kidney, lung, liver, heart, spleen, small intestine, stomach and duodenum, and adrenal gland) are isolated for histological examination. The histology of the test groups organs is compared with control. Where there is mortality in a test group within the period of assessment, the dead animal(s) is counted as among sacrificed. The histology is compared with control. Signs of organ tissue damage if observed are recorded.

Calculation

The formula for calculating the subchronic lethal dose is expressed as:

$$\text{Lethal Dose}_{\text{subchronic}} = \frac{[M_0 + M_1]}{2}$$

M_0 = Highest dose of test substance without mortality after the subchronic test.

M_1 = Lowest dose of test substance that gave mortality after the subchronic test.

2.5 Chronic Toxicity

Chronic toxicity assessment can only be carried out for test doses that passed the subchronic test. It begins 24 h after administration of the last subchronic doses. Six animals each (consisting of three animals per strain) should be alive from both control and passing dose groups at the end of the subchronic procedure (including histological assessment). Administration of the test doses to the animals in the respective groups is continued for a period of 62 days (making a total of 92 days of administration), while the control group animals still receive the vehicle. All animals in the respective groups are carefully observed daily throughout the period of study. Weight taking across the groups is also done at 2 weeks interval. Mortality and other signs of toxicity are recorded. At any time mortality is observed, the vital organs (kidney, lung, liver, heart, spleen, small intestine, stomach and duodenum and adrenal gland) of such animal(s) should be isolated immediately for histological examination. The date (with respect to the test) of such mortality is recorded also. The mortality of two animals from the same strain in a particular group shows toxicity at that dose. At the end of the period of administration, all the animals from test groups control are sacrificed 6 h after the last administration. The vital organs (kidney, lung, liver, heart, spleen, small intestine, stomach and duodenum, and adrenal gland) are isolated for histological examination. The histology of the test groups organs is compared with control. Where there is mortality in a test group within the period of assessment, the animal is counted among the sacrificed, and the histology is compared with control. Signs of organ tissue damage are recorded.

Calculation

The formula for calculating the chronic lethal dose is expressed as:

$$\text{Lethal Dose}_{\text{chronic}} = \frac{[M_0 + M_1]}{2}$$

M_0 = Highest dose of test substance without mortality after the chronic test.

M_1 = Lowest dose of test substance that gave mortality after the chronic test.

3. Results and Discussion

Toxicity assessment is as important as discovering and developing new agents for man's use. Although some attention has been given to the issue of toxicity testing of pharmacological/chemical agents, more efforts still need to be put into it. At present, there are still a number of pharmacological/chemical agents with little or no (or even inaccurate) toxicity information about them.[8,9] This is because some of the manufacturers of such agents claim that their products are totally safe since they were gotten from natural sources (e.g., herbs)[10] and therefore, do not require any toxicity assessment. Others are of the opinion that the processes currently available for toxicity testing are tedious and very expensive to perform.[11] However, procedures outlined in this paper are quite simple, cost-effective, and requires less time.

This method employs the use of two strains of animals at every level of the test. The main reason for this is to identify any adverse effect or toxicity that may not occur in one strain but may be present in the other due to some genetic variation (this occurs in humans within different and even the same population). The method also adopts continuity from the previous level at the next level of assessment. This ensures that same conditions are maintained at all levels of assessment, and therefore, guaranty consistency, accuracy, and reproducibility of the results gotten from the assessment. It also has an advantage of using fewer animals in totality (total number of animals used at all levels) and less time (total time to complete all levels of assessment) when compared with other methods. It is therefore not only cost-effective but time effective also. The application of this method is limitless as it can be employed for all routes of administration (with only slight modification where necessary). The method is applicable to almost all pharmacological/chemical agents (including drugs, agricultural and industrial chemicals, as well as food additives).

With these advantages, this method therefore addresses some issues (such as cost and time) which have hindered toxicity assessment of some agents. This method can be effectively adopted in developing countries due to its cost-effective nature. It can also serve as a guide for obtaining appreciable toxicity information of traditional medicines.

4. Conclusion

The method is simple, cost-effective, and requires less time. It explores a wide range of doses and has the capability

of producing accurate, reliable, and reproducible results. The use of two different strains also ensures that some signs of toxicity that may not be detected when using a single strain of animal for assessment are unmasked. This therefore indicates that the method is an ideal method for assessing toxicity in animal models. We therefore encourage its adoption by individuals, organizations, and agencies for toxicity assessment of various agents.

5. References

1. Laurence DR, Bennett PN, Brown MJ. Clinical Pharmacology. 8th ed New York: Churchill Livingstone. 1997; 3 pp.
2. Gad SC. Drug Safety Evaluation. New York: John Wiley and Sons, Inc. 2002; 30 pp.
3. Kim JH, Scialli AR. Thalidomide: The tragedy of birth defects and the effective treatment of disease. Toxicol Sci. 2011; 122:1–6.
4. National Research Council. Science, Medicine, and Animals: A Circle of Discovery. Washington, DC: National Academies Press. 2014.
5. Bhardwaj S, Gupta D. Study of acute, sub acute and chronic toxicity test. Int J Adv Res Pharm Bio Sci. 2012; 2:103–29.
6. Zurlo J. No animals harmed: Toward a paradigm shift in toxicity testing. Hastings Cent Rep. 2012; 42(1):S23–6.
7. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20:224–6.
8. Winston D, Maimes S. Adaptogens: Herbs for Strength, Stamina and Stress Relief. Rochester, Vermont: Healing Arts Press. 2007.
9. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evid Based Complement Alternat Med. 2006; 3:433–9.
10. Allison DB, Fontaine KR, Heshka S, Mentore JL, Heymsfield SB. Alternative treatments for weight loss: A critical review. Crit Rev Food Sci Nutr. 2001; 41:1–28.
11. New England Anti-Vivisection Society (NEAVS). Product Development Drug Testing. Available from: http://www.neavs.org/research/testing [Last accessed on 2015 Jul 20].