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The diverse ways to determine experimental dose in animals

Abstract

Acute toxicity is manifested in terms of effects which occur after a single administration or a relatively brief exposure to a substance or mixture. The evidence for acute toxicity is usually obtained from animal testing so acute toxicity is usually characterised in terms of lethality and exposure times used in experimental protocols. Mainly two hazardous classes for acute toxicity are reported that are known as "Acute toxicity" and "STOT-SE (Specific Target Organ Toxicity-Single Exposure)". This classification is based upon the evident lethality which is commonly reported as LD_{50}/LC_{50} value. STOT-SE should be considered where there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality. Once the LD_{50}/LC_{50} value is determined, then acute toxicity estimate (ATE) could be decided therefore accurate measurement of LD_{50} is cardinal to understand the dose responses in animal experimentations. Current review entails a diverse method for determination of acute toxicity along with their merits and demerits. Also, it unfolds why three alternative methods i.e., fixed dose procedure (FDP), acute toxicity class (ATC) method and up and down method revealed a wide acceptance from scientific community even though the classical methods were present.

Keywords: LD_{50}, acute toxicity, chronic toxicity, Karber's method, $LC_{50,}$ ATE

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Introduction

Tests of toxicity are used to determine hazardous effects of a particular chemical which are is absorbed in our body by various routes such as through oral, cutaneous, inhalation and circulation. Acute (short-term) toxicity testing commonly done by LD_{50} test. Several drugs, agricultural chemicals, cleaners, some cosmetics and their ingredients are tested through this method.[1] LD_{50} means that any dose of drug or chemical which is given to animal group for the estimation of medical effectiveness of that drug, and it gets 50% of animal's death, then it means that particular dose of drug is lethal dose 50 (LD_{50}). The hazzardicity is related inversely proportional to LD_{50} values of a drug means: less LD_{50} value, there will be more toxic effects and opposite is also true: the less toxicity at higher LD_{50} value. Initially it was developed in 1920's and known as "classical LD_{50}" in which involved 5 dose-groups were made for 100 animals but after some modifications in 1981 by the Organization for Economic Cooperation and Development (OECD) the experimental design has been changed as 30 animals for 3 dose-groups. In 1987, further number of experimental animals was reduced to 20 animals for LD_{50} determination.[2]

There are different types of toxicity classes based on their exposure duration viz acute toxicity (14 Days), sub-acute (repeated doses) toxicity (28 Days), sub-chronic toxicity (3 Months), chronic toxicity (6 Months to 2 Years) and special toxicity (Carcinogenicity). Prior approval by Institutional Animal Ethical Committee (IAEC) was required before conducting any kind of toxicity testing in animals or collecting any cell lines and a satisfactory protocol should be necessary by the local governing body of animal experimentation.

There are two basic values are important for toxicity determination first one is LD_{50} stands dose required for 50% mortality of test animals' population. It is an index determination of medicine and poison's virulence. Lower the LD_{50} dose, the more toxic the pesticide. The other one is LC_{50} value means concentrations of the chemical in air for 50% death of experimental animals during the observation period. Other durations of exposure (versus the traditional 4hours) may apply depending on specific laws. Higher LD_{50} value indicates the less toxic nature of substance whereas less LD_{50} or LC_{50} value reflects the higher toxicity.

The mission of Organization of Economic Cooperation and Development (OECD) is to promote policies that can serve the good socioeconomic health of people all over the world. It aims to work with governments to understand what drives economic, social and environmental change.[3] It sets international standards on a wide range of things, from agriculture and tax to the safety of the chemicals.[4] OECD strongly objected on the number of animals which were used in experiments of toxicity testing and mentioned it as a cruelty to have a large group for toxicity determination. So, in the perspectives of above-mentioned points, current review explains the classical methods which were globally used for toxicity determination namely, Karber's method, Miller and Tainter method and Lorke's[5] method etc. The emphasis is given upon three alternative methods which are widely accepted for acute toxicity determination and their merits.

Acute toxicity vs chronic toxicity

When a drug is administered at different dose levels that is it could be a single dose or in multiple doses for 24 h in two mammalian species (one non-rodent) it shows some short-term adverse effects, that are determined by acute toxicity. This type o toxicity only gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent (Figure1 & Table 1).[6]

Major acute toxicity symptoms

Acute toxicity is characterized by headache, nausea, vomiting, diarrhea, altered respiration,weight loss, muscle spasm, salvation, convulsion, loss of righting reflex, tremor lacrimation and somnolence (Table 2).

Design of acute toxicity

It was developed in 1920's and name was "classical LD_{50}" in which 100 animals were taken for 5 dose-groups and later in 1981

some modifications were done by the Organization for Economic Co-operation and Development (OECD) and reduced number up to 30 animals for 3 dose-groups.

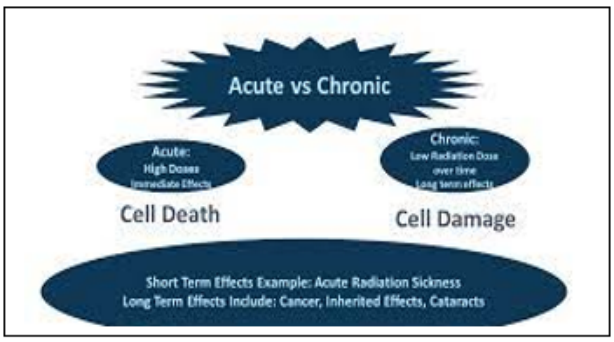

Figure 1 A comparison of acute toxicity with chronic toxicity.

Table 1 A comparison of different traits of acute and chronic toxicity

S.No.	Parameter	Attributes for short term and long-term exposure	
		Acute toxicity	Chronic toxicity
1.	Onset	Acute	Insidious
2.	Specificity	Nonspecific	Specific (where immune response is activated)
3.	Inflammatory Cells	Neutrophils, Macrophages	Lymphocytes, plasma cells, macrophages, fibroblasts
4.	Vascular changes	Active vasodilation, increased permeability	New vessel formation (Granulation tissue)
5.	Fluid Exudation and Edema	Present	Absent
6.	Cardinal clinical Signs	Present	Absent
7.	Tissue Necrosis	Generally absent If present (Supportive and necrotizing inflammation)	Continuous
8.	Fibrosis (Collagen Deposition)	Present	Absent
9.	Operative host Responses	Plasma factors: complement, immunoglobulins, properdin, etc; Neutrophils, nonimmune phagocytosis	Immune response, Phagocytosis, repair
10.	Systemic Manifestations	Fever, often high	Low-grade fever, weight loss, Anaemia
11.	Changes in Peripheral Blood	Neutrophil leucocytosis: (in viral infections)	Frequently none; variable leukocyte changes, increased plasma immunoglobulin

Table 2 List of toxicity categories along with their specific LD_{50} values and signal notation

Toxicity category	Oral LD_{50}(mg/kg)	Signal word	Approx. adult lethal dose(oral)
I	0-50	Danger/Poison	Few drops to 1 teaspoon
II	50-500	Warning	1 teaspoon to 1 ounce
III	500-5,000	Caution	1 ounce to 1 pound
IV	>5,000	Caution	More than 1 pound

Methods to calculate LD50 values are - Litchfield and Wilcoxson, Reed-Muench, Miller-Tainter and Karber's method. But large number of animals were required in all these methods and various factors like species, Age, Sex, Amount of food, social environment etc. affects the results of LD_{50}. This type of toxicity evaluation method has some Limitations and results may vary greatly.

We should go to some other alternative methods in which minimum number of animals is required during toxicity testing methods to avoid sacrifice animals in excess. FRAME (Fund for the Replacement of Animals in Medical Experiment) believes that the lethal dose test is unnecessarily cruel and scientifically invalid. Several countries, including the UK, have taken steps to ban the oral LD_{50}.[6] The OECD, the international governments' advisory body abolished the requirement for the oral test in 2001. Three alternative methods are used now namely Fixed Dose Procedure (FDP), Acute Toxic Class method (ATC), and Up-and-Down Procedure (UDP). In these methods, instead of animal death, only signs of toxicity were tested and recorded during studies like; increased motor activity, anaesthesia, tremors, arching and rolling. Alternative methods save numbers experimental animals.

A 14 days study

These studies include at least two species, one is rodent (mice/rat) and another is non-rodent (usually rabbit). Dose administration is orally and parenterally and groups of both sexes are treated by Various dose levels. Dose selection is such that causes less than 50% but not 0% and more than 50% but not 100% mortality. The main advantages of this study are as following:

- Reproducible procedure.
- Animal have less suffering.
- Moderately toxic doses carry it in and it is expected that lethal can be avoided.
- Few animals are used.

LD_{50} varies according to route

Route of exposure affects the dose of LD_{50}. For example, some LD_{50} values are shown below along with their administration routes for dichlorvos which is a pesticide commonly used in household strips for eradication of pests:

Oral LD_{50} of dichlorvos in rat: 56 mg/kg Dermal LD_{50} of dichlorvos in rat: 75 mg/kg Intraperitoneal LD_{50} of dichlorvos in rat: 15 mg/kg Inhalation LC_{50} of dichlorvos in rat: 1.7 ppm (15 mg/m3) in 4-hours exposure

Methods for the determination of LD_{50}

Several methods have been used since a long period of time for ascertaining the LD_{50} values which is crucial for each particular

compound in therapeutics. Few methods were developed earlier and used at a global scale but later on due to their practices of applying a large number of animals majority of them were discarded by scientific community and thus new alternative methods developed. All the methods try to find the least tolerated dose and most tolerated dose by hit and trial method. Once these two doses are determined, at least 5 doses are selected between them and a mortality rate is observed due to these doses. The percentage mortality values are converted to probit values by reading the corresponding probit units from the probit table. Finally, the probit values are plotted against log doses and LD_{50} value is read that corresponds to probit. Following are the few important methods which are of great significance in ascertaining LD_{50}:

Karber's methodMiller and Tainter methodLorke's method

Alternative methods

Fixed dose procedure (FDP)Acute toxic class method (ATC)UP and down Procedure (UDP)

Karber's Method (Arithmetic method): The sum of the product was divided by the number of animals in a group and the resulting quotient was subtracted from the least lethal dose in order to obtain LD_{50} value.

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

Where, LD_{50} = Median lethal doseLD_{100} = Least dose required to kill 100%a = Dose differenceb = Mean mortalityn = Group population.

Graphical method of Miller-Tainter: The Miller-Tainter method is the standard use in getting LD_{50}.

The dose is plotted against the probit value. Based on the graph, the LD_{50} will be estimated.

The experiment demonstrates the determination of LD_{50} of neostigmine on the experimental animals and its comparison to the standard LD_{50} of neostigmine.

Neostigmine (0.1 mg/kg,0.2 mg /kg,0.4 mg/kg, 0.8 mg/kg, 1.6 mg/kg) i.p.

Toxicological information

Oral LD_{50} Mouse:7500mg/kg IV LD_{50} Mouse:0.3mg/kg

SC LD_{50} Mouse:0.54mg/kg IM LD_{50} Mouse:0.395mg/kg

IV LD_{50} Rat:0.315mg/kg SC LD_{50} Rat: 0.445mg/kg

IM LD_{50} Rat:0.423mg/kg

Lorke's method: The method given by Lorke comprises of an initial examination in which a total of nine subjects are allocated into three groups. Each group of three animals obtains a different dose of the study compound. This process has two stages which are known as 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. Then the LD_{50} is calculated by the formula:D_0 = Highest dose that gave no mortality,D_{100} = Lowest dose that produced mortality

Limitations

Some limitations are noticed for LD_{50} analysis such as, the LD_{50} can provide a measure of the instantaneous, immediate or acute toxicity, but the results may fluctuate greatly. LD_{50} cannot be tested on humans so all data reported in relation to humans is only a conjecture. The LD_{50} test is neither steadfast nor expedient, because of the fact that human lethal dose can't be anticipated from animal investigations.[8,9]

Alternative methods

Fixed dose procedure (FDP): This method was given by British Toxicology Society in 1992. It is a process which can evaluate a constituent's acute oral toxicity.[10,11] When compared to the earlier LD_{50} test which was given in 1927, this method gives alike results while using lesser animals and instigating less pain and suffering.[12] 1992 this test was proposed as an alternative to the LD_{50} test by the Organisation for Economic Co-operation and Development under OECD Test Guideline 420.[13–15] This method does not use death as an end point, instead an observation of well-defined insignias of 5 toxicity are developed at one of the series of fixed dose levels to evaluation the LD_{50}.

Acute toxic class method (ATC): The ATC method is a substitute to the LD_{50} trial, when we intend to decline the number of experimental animals under test. The use of considerably scarcer number of animals is actually needed for the classification of substances proposed by the German Federal Health Authority, and it provides alternatives to the LD_{50} test for grading substances by their acute oral toxicity confirmed by animal tryouts and biometric determination[12] in a national German[13] and international cooperative study.[16] The benefit of this method is that death is not used here as an end point, rather it uses symbols of toxicity in stepwise manner for determining the LD_{50}. The principle of ATC method is that it is based on the Probit model.

The ATC method is a serial assay method which uses only three animals of one sex in each stage. Based on the mortality rate usually three animals but never farther than six animals are used per dose level. This methodology consequents in the decrease of numbers of animals utilized in comparison to 9, by 40–70%.[17]

Up and down procedure: In this unique method, experimental animals are administered with the test compound one at a time and survivability is checked. If it is found that an animal endures tested concentration of compound, the quantity for the next experiment is amplified. Whereas if the animal dies, the dose amount is usually declined for the next animal. The duration of observation for each animal is generally between 1 to 2 days before treating the next animal. Dose enduring animals are continuously monitored for delayed death for a total 10 of 7 day.[18]

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Conflicts of interests

The authors declared no conflicts of interest.

References

1. Turner R. Acute toxicity: The determination of LD50. *In Screening Methods in Pharmacology*. New York: Academic Press;1965:pp.300.
2. Botham PA. *Toxicology in Vitro*. 2004;18:227–230.
3. Nikfer S, Farshchi A, R Visser R. Organization for Economic Cooperation and Development (OECD). *Encyclopedia of toxicology*. 2014; 3:704-710**.**
4. Miller GC, Brady PM, Murta TL. Emergency response and preparedness. *Encyclopedia of toxicology*. 2014;3:321–331.
5. Lorke Dr. A new approach to practical acute toxicity testing**.** *Arch Toxicol*. 1983;54(4):275–87.
6. Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose. *Curr Sci.* 2007; 93(7):917–20.
7. Maheshwari DG, Shaikh NK. An overview on toxicity testing method. *Int J Pharm Technol.* 2016;8(2):3834–3849.
8. Deora Paramveer S et al. Effective alternative methods of LD50 help to save number of experimental animals. *J Chem Pharm Res*. 2010;2(6):450–453.
9. Walum E. Acute oral toxicity. *Environmental Health Perspectives.* 1998;106:497–503.
10. Stallard N, Whitehead A. Reducing animal numbers in the fixed-dose procedure. Human & Experimental Toxicology. 1995;14(4):315–323.
11. Van den *Heuvel MJ, Clark DG, Fielder RJ, et al. The international validation of a fixed-dose procedure as an alternative to the classical LD50 test. Food and Chemical Toxicology.* 1990;28(7):469–482.
12. Stallard N, Whitehead A, Ridgway P. Statistical evaluation of the revised fixed-dose procedure". *Human & Experimental Toxicology*. 2002;21(4):183–196.
13. Diener W, Siccha L, Mischke U, et al. The biometric evaluation of acute toxic class method (oral). *Arch Toxicol*. 1994;68:596–910.
14. Diener W, Mischke U, Kayser D, et al. The biometric evaluation of the OECD modiWed version of the acute toxic class method (oral). *Arch Toxicol.* 1995;69(10):729–734.
15. Diener W, Siccha L, Mischke U, et al. The biometric evaluation of the acute-toxic-class method (oral). *Arch Toxicol*. 1994;68(10):599–610.
16. Schlede E, Mischke U, Roll R, et al. A national validation study of the acute toxic class method- An alternative to the LD50 test. *Archives of Toxicology.* 1992;66(7):455–470.
17. Schlede E, Genschow E, Spielmann H, et al. Oral acute toxic class method: A successful alternative to the oral LD50 test. *Regulatory Toxicology and Pharmacology*. 2005;42(1):15–23.
18. Paramveer DS, Chanchal MK, Paresh M, et al. Effective alternative methods of LD50 help to save number of experimental animals*. J Chem Pharm Res*. 2010;2(6):450–453.