Effective alternative methods of LD$_{50}$ help to save number of experimental animals

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ABSTRACT

The most common test of acute toxicity is the LD$_{50}$ test. LD$_{50}$ means, if administered dose of drug to animal group, for experimental purpose for the estimation of therapeutic effectiveness kills 50% of animals, than it means that particular dose of drug is lethal dose 50 (LD$_{50}$). It was developed in 1920’s and called “classical LD50” involved 100 animals for 5 dose-groups, later in 1981 it was modified by the Organization for Economic Co-operation and Development (OECD) and reduced number upto 30 for 3 dose-groups. Methods to calculate LD50 values are - Litchfield and Wilcoxson, Reed-Muench, Miller-Tainter and Karber’s method. But all these methods require large number of animals. Factors which affect the results of LD$_{50}$ are- Species, Age, Sex, Amount of food, Social environment etc. LD$_{50}$ study has some Limitations and results may vary greatly. Due to excess of animal sacrifice we should go to alternative methods which minimum number of animals is required. 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$_{50}$. The OECD, the international governments’ advisory body abolished the requirement for the oral test in 2001. Three alternative methods and these are: Fixed Dose Procedure (FDP)-OECD TG 420, Acute Toxic Class method (ATC)-OECD TG 423, Up-and-Down Procedure (UDP)-OECD TG 425. These methods only consider signs of toxicity in place of death. Signs recorded during studies like; increased motor activity, anaesthesia, tremors, arching and rolling. Alternative methods save numbers experimental animals.

INTRODUCTION

Toxicity test examine toxic effects when a chemical is absorbed into the body, via mouth, skin, lungs. The most common test of acute (short-term) toxicity is the LD$_{50}$ test. Many different substances are tested in this way, including all drugs, agricultural chemicals, cleaners some
cosmetics and their ingredients. LD₅₀ means if we administer any dose of drug to animal group for experimental purpose for the estimation of therapeutic effectiveness of that drug, and if 50% of animal get died than it means that particular dose of drug is lethal dose 50 (LD₅₀). The smaller the LD₅₀ value, the more toxic is chemical. The opposite is also true: the larger the LD₅₀ value, the lower the toxicity. It was developed in 1920’s and called “classical LD₅₀” involved 100 animals for 5 dose-groups, later in 1981 it was modified by the Organization for Economic Cooperation and Development (OECD) and reduced number upto 30 for 3 dose-groups. In 1987 further reduced to 20 animals. Mice, rats, rabbits, guinea pigs, cats, dogs, fish, monkeys and birds are use for LD₅₀ study. The LD₅₀ values of a new drug are determined by various route of administration (intravenous, intraperitoneal, subcutaneous and oral). Results of LD₅₀ study may affected by some factors which are - Species, Age, Sex, Amount of food, Social environment, Route of exposure* (oral, dermal, inhalation) and Physical environment such as temperature and humidity.

*Route of exposure (example, some LD₅₀s for Dichlorvos, an insecticide commonly used in household pesticide strips):
- Oral LD₅₀ (rat): 56 mg/kg
- Dermal LD₅₀ (rat): 75 mg/kg
- Intraperitoneal LD₅₀: (rat) 15 mg/kg
- Inhalation LC₅₀ (rat): 1.7 ppm (15 mg/m³); 4-hour exposure

There are also some Limitations for LD₅₀ study like: The LD₅₀ gives a measure of the immediate or acute toxicity, results may vary greatly, LD₅₀ is not tested on humans, All relation to humans are only a guess. The LD₅₀ test is neither reliable nor useful, because the human lethal dose can't be predicted from animal studies.

FRAME (Fund for the Replacement of Animals in Medical Experiment) believes that the lethal dose test is unnecessarily cruel and scientifically invalid. The test involves giving groups of animal doses of a test substance until it kills half of them. Several countries, including the UK, have taken steps to ban the oral LD₅₀ and the OECD; the International governments’ advisory body abolished the requirement for the oral test in 2001.

To give an idea about alternative methods of LD₅₀ and how to reduce the use of animals in LD₅₀ study as much as possible.

**EXPERIMENTAL SECTION**

There are various methods to calculate LD₅₀ values; like the graphical method, arithmetical method and statistical approach. For research purpose, the most widely used method is Litchfield and Wilcoxon. For routine practical class work; Reed-Muench, Miller-Tainter and Karber’s method. For calculating LD₅₀ by any one method, find out the least tolerated (smallest) dose (100% mortality) and most tolerated (highest) dose (0% mortality) by hit and trial method. Once these two doses are determined, select at least 05 doses in between the least tolerated and most tolerated doses, and observe mortality due to these doses. Then apply correction factor to 0% and 100% mortality group [for 0% dead = 100 (0.25/n) and for 100% dead = 100x (n-0.25/n), where n = number of death]. The percentage mortality values are converted to probit values by reading the corresponding probit units from the probit table. Plot the probit value against log doses and read LD₅₀ value as the dose that corresponds to probit 5.
(1) **Arithmetical method of Karber 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.

\[
LD_{50} = \text{the apparent least dose lethal to all in a group} - \left(\frac{\sum a - b}{N}\right)
\]

Where \(N\) = number of animals in each group, \(a\) = dose difference and \(b\) = mean mortality.

(2) **Graphical method of Miller and Tainter**

The observed percentage mortality was converted into probit referring to the probit table. The values thus obtained were plotted against log dose. The LD$_{50}$ value and its standard error were determined from the graph, if the line was straight enough.

**Table 1: - Comparison of different methods:**

<table>
<thead>
<tr>
<th>Contents</th>
<th>Method Karber (^7)</th>
<th>Method of Miller and Tainter (^7)</th>
<th>Method of Lorke (^8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rodents used</td>
<td>More than necessary</td>
<td>More than necessary</td>
<td>Appropriate</td>
</tr>
<tr>
<td>Expenditure</td>
<td>High</td>
<td>High</td>
<td>Average</td>
</tr>
<tr>
<td>Accuracy of results</td>
<td>Inaccurate</td>
<td>Inaccurate</td>
<td>Doubtful</td>
</tr>
</tbody>
</table>

The deletion of the LD$_{50}$ test from the OECD guidelines was due to three alternative methods being adopted which all involve more humane treatment of the animals and use fewer animals than the LD$_{50}$ test. They record toxicity signs in place of death.\(^5\)

These three alternative tests are:

(1) **Fixed Dose Procedure** (FDP) — OECD TG 420.

This method does not use death as an end point, instead it uses the observation of clear signs of toxicity developed at one of a series of fixed dose levels to estimate the LD$_{50}$.\(^5\)

(2) **Acute Toxic Class** method (ATC) — OECD TG 423

This method does not use death as the only end points, it also uses signs of toxicity in its stepwise approach to estimating the LD$_{50}$.

*Principle:* - It is based on the Probit model.

*Procedure:* - The ATC method is a sequential testing procedure using only three animals of one sex per step. Depending on the mortality rate three but never more than six animals are used per dose level. This approach results in the reduction of numbers of animals used in comparison to the LD$_{50}$ test by 40–70%.\(^9\)

(3) **Up-and-Down Procedure** (UDP) — OECD TG 425

This method does still use death as an end point, but doses animals one at a time to see if the dose needs to be put up or down to achieve an estimate of the LD$_{50}$ therefore giving the minimum number of animals a lethal dose of the test substance.

In the up-and-down procedure, animals are dosed one at a time. If an animal survives, the dose for the next animal is increased; if it dies, the dose is decreased. Each animal is observed for 1 or 2 days before dosing the next animal. Surviving animals monitored for delayed death for a total of 7 days.\(^{10}\)

Signs recorded during acute toxicity studies:-

These are increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, tonic extension, lacrimation, Straub reaction, salivation, muscle spasm, writhing, hyperesthesia,
loss of righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis and analgesia.

RESULTS AND DISCUSSION

During our studies we have observed that available alternative methods are more humane than cruel traditional or classical methods. By following these classical methods we are only treating animals very cruelly and not getting fruitful results. Better results can be possible through alternative methods with less number of animals.

CONCLUSION

My all works showed that there is no need to use animals blindly, when there are some good alternatives are available. By applying the new alternative methods during Pharmacological work try to avoid animal sacrifice when without sacrifice proper Pharmacological responses are possible.

REFERENCES