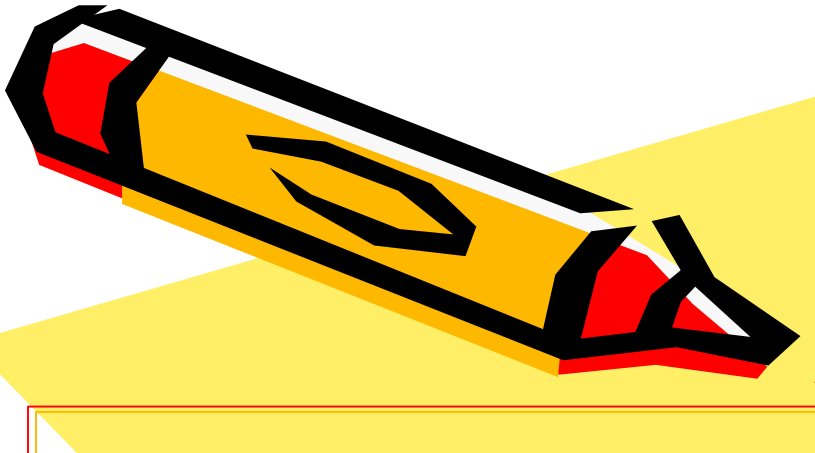
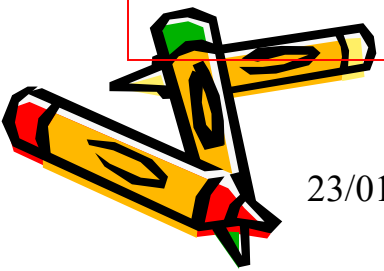


College of Pharmacy/Al-Mustansiriyah University
Department of Pharmacology & Toxicology
General Toxicology Lab. for 4th Year Students
Lab.no.1: Determination of LD50

By: Dr. KASSIM AL-SAEDI

HISTORICAL BACKGROUND

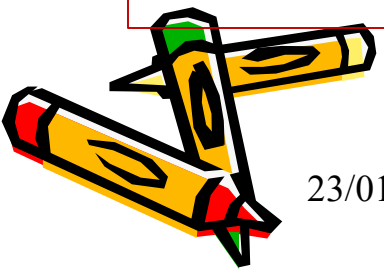
- Originally developed in 1927 by J.W. Trevan the LD50 test was used to determine the potency of **digitalis extracts**, **insulin**, and **diphtheria antitoxin**. Scientists soon developed other methods for determining potency but the LD50 catch on as a "scientific" measure of toxicity..??. The ease of performing an LD50, as well as the urgent need for getting concrete numbers quickly, has made results of the test a standard in toxicology studies. Governments also liked the numerical results that the LD50's provided and quickly mandated (authorized) the test for assessing the toxic effects of products ranging from drugs , pesticides to industrial solvents.



Definition

LD 50: IS STAND FOR MEDIAN LETHAL DOSE

- An LD50: represents the individual dose of drug (**Xenobiotic**) **usually per kg (or gm for small animals) of body weight** required to kill **50 percent** of a population of test animals. It is an index determination of drugs and poison's virulence. The lower the LD50 dose, the more toxic the drug (xenobiotic).



Toxicological Significance

- *It is the most common test of acute toxicity assessment ;* Before a product on new drug formulation is released to the market, its potential safety to the users is evaluated by a series of biological tests on lab. animals. Each animals given a single dose of the tested materials. usually three or more dose levels are tested.

❑ **Related terms**

Median effective dose or ED50: This is the dose (mg/kg), which produces a desired response in 50 per cent of test population.

Therapeutic index: It is an approximate assessment of the safety of the drug. It is the ratio of the median lethal dose and the median effective dose. Also called as therapeutic window or safety.

Therapeutic index (T. I) = LD50/ED50

The larger the therapeutic index, the safer is the drug. Penicillin has a very high therapeutic index, while it is much smaller for the digitalis preparation

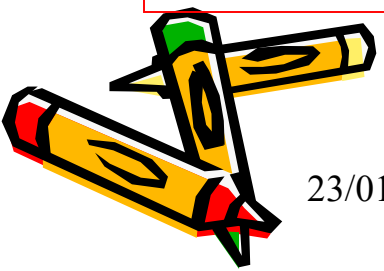
The types of exposures are

1-**Acute**, which is exposure to a chemical for **24 hours or less**.

2-**Chronic**, which is exposure to a chemical for **more than 3 months**.

3-**Sub-acute**, which is exposure to a chemical for **1 month or less**.

4-**Sub-chronic**, which is exposure to a chemical **between 1 to 3 months**.



Objective (aim)

To determine the LD50 of a drug • (Neostigmine) in a given species (mouse).

Experimental method

- **Thompson and Weil Method:**

There are many methods in determining LD50 such as Bliss, sequential method, grouping method ect. The method used in this experiment is the one described by Thompson and Weil.

Experimental animals

- mice

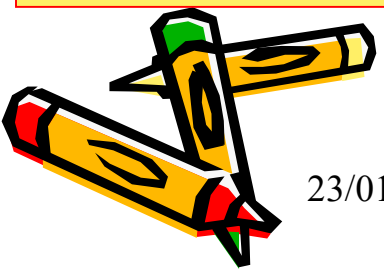
➢ Display the videos concerning mouse restraint technique ,then the i.p injection in mouse.

Toxicant (Drug): Physostigmine

- **Physostigmine**, is an alkaloid (tertiary amine) isolated from seeds of the plant Physostigma venenosum from West Africa. Physostigmine is used in ophthalmology, in eye drops (in form of physostigmine sulfate), in combination with pilocarpine, for the lowering of intraocular pressure, in glaucoma.
- Physostigmine is also used as an antidote at the poisoning by compounds with anticholinergic effect (e.g. atropine, scopolamine and imipramine) and tricyclic anti-depressants, as well as at the poisoning with anti-cholinergic organophosphates

- **Pharmacological mechanisms**

➢ Physostigmine is used as antidote in organophospate poisoning (e.g. poisoning with parathion, malathion and dichlorvos) because it binds to the enzyme acetylcholinesterase (AChE) reversibly and preserves the enzyme from irreversible phosphorylation by organophosphates. Physostigmine serves as alternate substrate for AChE.



Mechanism of intoxication

- Acetylcholine (ACh) plays an important role as a neurotransmitter in the CNS and in the parasympathetic nervous system (PNS). At the high concentrations of ACh, neuromuscular transmission may be blocked and the adverse effects can occur.

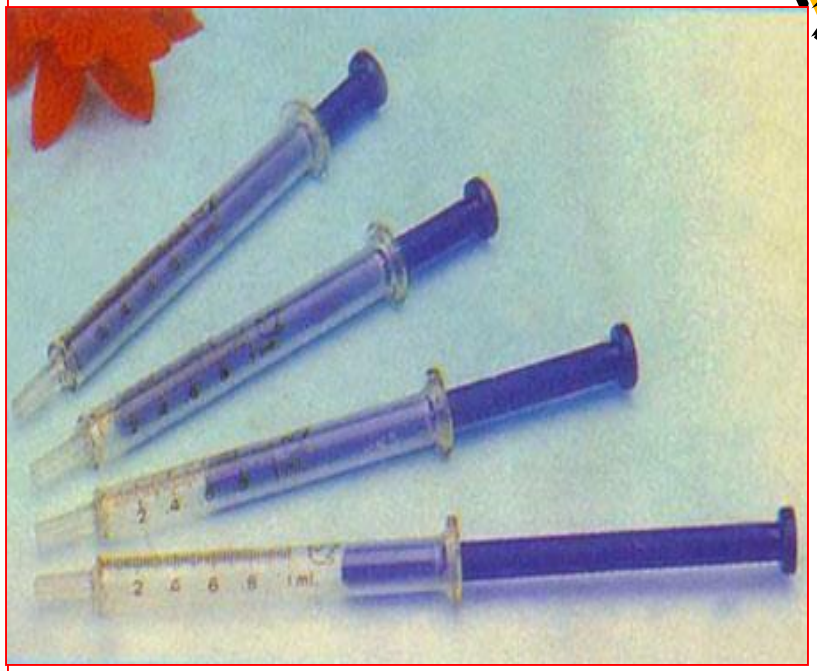
-In similarity with other anti-cholinergic agents, physostigmine is an inhibitor of the enzyme AChE, which catalyzes hydrolysis of ACh into choline and acetic acid.

- The following acute toxic effects can be produced:
- a) stimulation of muscarinic receptor responses at autonomic organs; muscarinic effects include nausea, vomiting, abdominal pain, diarrhea, increased salivation, perspiration and tearing, blurred vision (miosis), respiratory tract secretions, bradycardia and atrio-ventricular block;
- b) nicotinic receptor stimulation, followed by muscle twitching, weakness and paralysis;
- c) stimulation of cholinergic receptor sites in the CNS, following in severe cases by CNS depression, convulsions, coma, and death.

Target organs: CNS and PNS.

Experimental apparatus

- mice cage, animal's equi-armbalance, 1ml injection syringe, and electronic calculator



Experimental procedure & calculations

the method used in this experiment is the one described by Thompson and Weil. It involves the use of moving averages, and is calculated by the use of logarithms and antilog to the following formula:

Log M = Log Da + d (*f+1*) , K= 3& n= 6

Where*

M= estimated LD50

D= dosage

Da= lowest dose level

d= Log of constant ratio between the doses (i.e. log of geometric factor)

f= the function of (r) used in the calculation of an LD50.See the table listed in for calculation of LD50.

n= number of animals used for each dose level.

K= number of doses -1

r- Vales: the number of death for each dose levels.

6*f* = the function for the LD50,this is used in calculation the confidence limits.

Log confidence limits (95%)= Log M$\pm$2d (6*f*).

Confidence limits (95%) = it is the tang which when the experiment, is repeated 100 times, 95% time out of 100, the LD50 value will be within this range.

- Weils table for calculation of LD50

Where n= 6 k=3

r-values	*f*	*6f*	r-values	*f*	*6f*
0, 0, 3, 6	1.000 000	0.22361	1, 1, 4, 6	0.060000	0.3226
0, 0, 4, 6	0.83333	0.21082	1, 1, 5, 6	0.400000	0.30724
0, 0, 5, 6	0.66666	0.16667	1, 2, 5, 6	0.20000	0.36000
0, 0, 6, 6	0.50000	0.0000	1, 2, 6, 6	0.0000	0.26833
0, 1, 2, 6	1.00000	0.26874	1, 3, 3, 6	0.40000	0.39799
0, 1, 3, 6	0.83333	0.27889	1, 3, 4, 6	0.20000	0.42000
0, 1, 4, 6	0.66667	0.26874	1, 1, 4, 6	0.0000	0.36878
0, 3, 6, 6	*0.00000*	0.22361	2, 0, 4, 6	*0.75000*	*0.32566*
1, 1, 2, 6	*1.00000*	*0.32249*	2, 0, 5, 6	*0.50000*	*0.29580*
1, 1, 3, 6	*0.80000*	*0.33706*	2, 0, 6, 6	*0.25000*	*0.23717*
0, 2, 4, 6	*0.50000*	*0.29814*	2, 0, 3, 6	*1.0000*	*0.33541*

Example:

Group of six rats were administered single oral dose of drug X at **four** successive dose levels; namely **1.00 mg/kg, 1.2 mg/kg,1.44 mg/kg, and 1.77 mg/kg**. Death occurring in each group were **0, 2, 4, and 6**. Calculate the LD50 and confidence limits of the product X.

Answer:

Log M = Log Da + d (*f+1*) , K= 3& n= 6

= 0.00 + Da+ 0.0792(0.50000+1)

= 0.0792(1.5000)

Log M = 0.1188

Anti log or (LD50) = 1.314 mg/kg

Log confidence limits (95%) = log M $\pm$ 2d (*6f*)

= 0.1188+ 2×0.792×0.29814

= 0.1188 + 0.047

The log of the upper limit = 0.1658

Antilog of the upper limit = 1.465

The log of the lower limit =0.1188-0.047

= 0.0718

Antilog of the lower limit = 1.18

There for the 95% confidence limits are = 1.18 - 1.465 mg/kg


ok: