LETTER TO EDITOR

CALCULATION OF LD_{50} VALUES FROM THE METHOD OF MILLER AND TAINTER, 1944

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Dear Editor,

Acute toxicity of a drug can be determined by the calculation of LD_{50}, i.e., the dose that will kill 50% of animals of a particular species. Recently, we reported the LD_{50} of thymoquinone, an active principle of *Nigella sativa*, in mice and rats by the method described by Miller and Tainter, 1944.[1,2] Since then some post-graduate students and investigators have asked us to explain the details of calculation of LD_{50} by this method, as the original article, being very old, is not easily available in the literature. We also faced problem in the calculations, however, we found some details for the calculation of LD_{50} in the Fundamentals of Experimental Pharmacology by Ghosh[3], which also is not easy to obtain in many parts of the world. Therefore, few lines are written, on behalf of the other authors, to elaborate the calculation of LD_{50} by the method of Miller and Tainter for the benefit of young researchers.

Estimation of the dose range and percentage of mortality

An approximate LD_{50} can be initially determined as a pilot study by a so called 'staircase method' using a small number of animals (2 each dose) and increasing the doses of the drug. Five doses can be chosen for determination of LD_{50} starting from no death to 100% mortality. In our study for estimation of LD_{50} of thymoquinone, 5 doses were given intraperitoneally to 5 groups of rats, 10 in each group (Table-1).

The animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms. After 24 hours, the number of deceased rats was counted in each group and percentage of mortality calculated.

Table-1: Results of the lethal doses of thymoquinone for the determination of LD_{50} after intraperitoneal injection in rats (n=10)

Group	Dose (mg/kg)	Log dose	% Dead	*Corrected %	Probits
1	25	1.4	0	2.5	3.04
2	50	1.7	40	40	4.75
3	75	1.88	70	70	5.52
4	100	2	90	90	6.28
5	150	2.18	100	97.5	6.96

*Corrected % Formula for 0 and 100 is given in the text.

Conversion of percentage mortalities to probits and calculation of LD_{50}

The percentage of animals that had died at each dose level is then transformed to probit (Table-2).

Table-2: Transformation of percentage mortalities to probits

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

The percentage dead for 0 and 100 are corrected before the determination of probits as under:

Corrected % Formula for 0 and 100% mortality[3]:

For 0% dead: 100(0.25/n)

For 100% dead: 100(n-0.25/n)

The probit values are plotted against log-doses and then the dose corresponding to probit 5, i.e., 50%, is found out (**Figure-1**). In the present case the Log LD_{50} is 1.76 and LD_{50}= 57.54 mg/kg.

Calculation of Standard Error (SE) of LD_{50}

The SE of LD_{50} can be calculated from the following formula:[3]

$$\text{Approx. SE of } LD_{50} = \frac{(\text{Log } LD_{84} - \text{Log } LD_{16})}{\sqrt{2N}} \quad \ldots (a)$$

where N is number of animals in each group.

The probits of 84 and 16 from Table-1 are 5.99 and 4.01 (Approx. 6 and 4), respectively. The log-LD values for the probits 6 and 4 are obtained from the line on the graph in Figure-1, which in the present case are 1.96 and 1.58 and their antilog are 91.2 and 38.02. Using these values in formula (a) the SE of LD_{50} is 11.9. Therefore, LD_{50} of thymoquinone when given intraperitoneally is 57.54±11.9, with 95% confidence interval of 45.64–69.44.

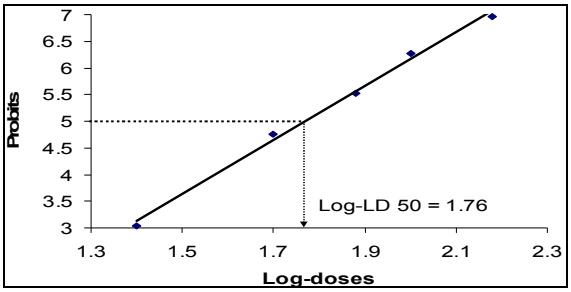

Figure-1: Plot of log-doses versus probits from Table-1 for calculation of LD_{50} of thymoquinone administered intraperitoneally.

REFERENCES

1. Al-Ali A, Alkhawajah A, Randhawa MA, Shaikh NA. Oral and intraperitoneal LD50 of thymoquinone, an active principle of *Nigella sativa*, in mice and rats**.** J Ayub Med Coll Abbotabad 2008;20(2):25–7.
2. Miller LC, Tainter ML. Estimation of LD50 and its error by means of log-probit graph paper. Proc Soc Exp Bio Med 1944;57:261.
3. Ghosh MN. In Statistical Analysis, Fundamentals of Experimental Pharmacology, 2nd ed, Scientific Book Agency Calcutta, 1984. pp187–9.

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