CHAPTER 4

ACUTE TOXICITY STUDIES

4.1. Introduction

Toxicology is the science that deals with the adverse effect of chemicals on living organisms. Toxicity tests are focused at discerning the compilations arising from the therapeutic use of the drug. Special attention should be paid to the solvent and dispersing agents as the toxic effect may arise from these sources. Animal care during the period of the toxicity tests is also of paramount importance.

Traditional use of herbal drugs may broadly be divided into three categories as follows

- Those are well known and have been widely used for many years.
- Those are not well known in the country but for which international experience is available.
- That represent a new compound hitherto not evaluated as to its safety and efficacy.

The first category consists chiefly of foodstuff product (s), which have been in use for a long time as traditional herbal remedies, and the requirements are limited. In general, it seems unnecessary to require the proof of safety of these products. For the second category, views concerning the type of documents required to be presented may differ from country to country. So it is necessary that varieties of requirements will be elaborated for these products covering anything from reference in scientific literature confirming that the product is safe. To satisfy the demands for limited or shortened toxicological testing of these products, an investigation must be carried out on toxicity profile. The third group, where the authority is faced with a product not previously screened for its toxicological properties, toxicity studies of those product must have to be undertaken.

The index of the acute toxicity is LD_{50} (median lethal dose at which 50 percent of the population dies), which should not be regarded as a biological constant, since different results are observed on different sets of tests or when the investigations are carried out in different laboratories. This has been indicated very clearly in multicentric study carried out in the European community with five substances [1, 2]. Historically, the LD_{50} was determined with high degree of precision and was used to compare toxicities of

compounds relative to their therapeutic doses. It is now realized that high degree of precision may not be necessary to compare toxicities [3]. Therefore, the median lethal dose is now estimated from the smallest number of animals possible [4].

The median lethal dose or LD_{50}

This is the dose (mg/kg body weight), which would be expected to kill one-half of an unlimited population of the same species and strain. The median effective dose or ED_{50} is the dose (mg/kg body weight), which produces a desired therapeutic action in 50 percent of the test population [5]. In this present study lethal dose (LD_{50}) of the methanol extracts of *Colebrookea oppositifolia* leaves and *Heracleum nepalense* roots has been determined by oral as well as intraperitoneal route of administration on mice.

4.2. Materials and Methods

4.2.1. Plant materials

Methanol extracts of *Colebrookea oppositifolia* leaves and *Heracleum nepalense* roots (described in **Chapter 3**) were used as test drug in these experiments and propylene glycol was used as control vehicle.

4.2.2. Animals

Swiss albino mice of either sex weighing between 18-20 g were used for the study. The mice were housed in standard stainless steel cages having solid bottom in well-ventilated animal room. Sawdust was spread on the bottom of the cage to absorb urine and moisture from feces. Noisy atmosphere was avoided as much as possible for healthy living condition of mice, as mice are very sensitive to noise. The mice were fed with standard pellet diet of the following composition:

Wheat flour - 63%
Casein - 15%
Sucrose - 10%
Groundnut oil - 05%
Salt mixture - 04%

Shark liver oil - 02%

Vitamin mixture - 01%

Water was given *ad libitum*

4.2.3. Methods of evaluation

Ten groups of animals were used taking ten animals in each group. Different doses of methanol extract of *Colebrookea oppositifolia* leaf and *Heracleum nepalense* root suspended in propylene glycol were administered orally to nine groups of animals. One group of animal was treated as control and was fed with propylene glycol. The method of Lorke [6] was followed to determine the acute toxicity study of the extracts. The method was repeated with ten groups of animals separately following intraperitoneal route of administration. The animals were kept under observation in open field condition for 72 hrs after the administration of extracts of *Colebrookea oppositifolia* leaf and *Heracleum nepalense* root in both of the routes as mentioned earlier, and the number of deaths and signs of clinical toxicity were recorded. The median lethal dose (MLD) and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon [7].

4.3. Results

The results of the acute toxicity studies of leaf have been presented in Table 4.1 and 4.2. The MLD of methanol extract of *Colebrookea oppositifolia* leaf was found to be 3.0 g/kg body weight and 4.5 g/kg body weight in intraperitoneal and oral route respectively. On the other hand the MLD of the methanol extract of *Heracleum nepalense* root was to be 4.5 g/kg body weight and more than 5.5 g/kg body weight in intraperitoneal and oral route respectively. These results have been presented in Table 4.3 & 4.4.

Table 4.1. Determination of LD_{50} of methanol extract of *C.oppositifolia* leaf after intraperitoneal administration in mice.

Group	Dose (mg/kg)	Log dose (X)	No of animals used	No of animals dead	Dead (%)	Corrected dead (%)	Probit (y)	X^2	Xy	Y
1	250	2.39	10	0	0	2	2.95	5.71	7.05	2.88
2	500	2.69	10	1	10	10	3.72	7.23	10.00	3.56
3	1000	3.00	10	2	20	20	4.16	9.00	12.48	4.27
4	2000	3.30	10	4	40	30	4.75	10.89	15.67	4.95
5	3000	3.47	10	5	50	50	5.00	12.04	17.35	5.34
6	4000	3.60	10	6	60	60	5.25	12.96	18.90	5.63
7	4500	3.65	10	7	70	70	5.52	13.32	20.14	5.75
8	5000	3.69	10	9	90	90	5.84	13.61	21.54	5.84
9	5500	3.74	10	10	100	98	7.05	13.98	26.36	5.95

$\Sigma X = 29.52$ $\Sigma y = 44.24$ $\Sigma X^2 = 98.74$ $\Sigma Xy = 149.49$

$x = 3.28$ $\bar{y} = 4.91$

$$b = \frac{\Sigma Xy - (\Sigma X. \Sigma y) / n}{\Sigma X^2 - \Sigma X^2/n} = \frac{149.49 - (29.52 \times 44.24) / 9}{98.74 - (29.52)^2 / 9} = \frac{149.49 - 145.10}{98.74 - 96.82} = 2.28.$$

Linear regression equation

$Y = \bar{y} + b(X - x)$, where x, $\bar{y}$ are the mean values of X and y, b is known as the regression coefficient

$Y = 4.91 + 2.28 (X - 3.28)$

Corrected formula: for 0% dead = $100 \times 0.25 / n$; for 100% dead = $100 (n - 0.25) / n$, where n is the number of animals in each group.

Table 4.2. Determination of LD_{50} of methanol extract of *C.oppositifolia* leaf after oral administration in mice.

Group	Dose (mg/kg)	Log dose (X)	No of animals used	No of animals dead	Dead (%)	Corrected dead (%)	Probit (y)	X^2	Xy	Y
1	500	2.69	10	0	0	2	2.95	7.23	7.93	2.67
2	1000	3.00	10	1	10	10	3.72	9.00	11.16	3.57
3	2000	3.30	10	2	20	20	4.16	10.89	13.72	4.45
4	3000	3.47	10	3	30	30	4.48	12.04	15.54	4.94
5	4000	3.60	10	4	40	40	4.75	12.96	17.10	5.32
6	4500	3.65	10	5	50	50	5.00	13.32	18.25	5.47
7	5000	3.69	10	7	70	70	5.52	13.61	20.36	5.58
8	5500	3.74	10	8	80	80	5.84	13.98	21.84	5.73
9	6000	3.77	10	10	100	98	7.05	14.21	26.57	5.81

$\Sigma X = 30.91$ $\Sigma y = 43.47$ $\Sigma X^2 = 107.24$ $\Sigma Xy = 152.47$

$x = 3.43$ $\tilde{y} = 4.83$

$$b = \frac{\Sigma Xy - (\Sigma X.\ \Sigma y)/n}{\Sigma X^2 - \Sigma X^2/n} = \frac{152.47 - (30.91 \times 43.47)/9}{107.24 - (30.91)^2/9} = \frac{152.47 - 149.29}{107.24 - 106.15} = 2.91$$

Linear regression equation

$Y = \tilde{y} + b(X - x)$. where x, $\tilde{y}$ are the mean values of X and y. b is known as the regression coefficient

$Y = 4.83 + 2.91(X - 3.43)$

Corrected formula: for 0% dead = $100 \times 0.25 / n$: for 100% dead = $100 (n - 0.25) / n$. where n is the number of animals in each group.

Table 4.3 Determination of LD_{50} of methanol extract of *H.nepalense* root after intraperitoneal administration in mice.

Group	Dose (mg/kg)	Log dose (X)	No of animals used	No of animals dead	Dead (%)	Corrected dead (%)	Probit (y)	X^2	Xy	Y
1	250	2.39	10	0	0	2	2.95	5.71	7.05	2.39
2	500	2.69	10	0	0	2	2.95	7.23	7.93	3.11
3	1000	3.00	10	1	10	10	3.72	9.00	11.16	3.86
4	2000	3.30	10	2	20	20	4.48	10.89	13.72	4.58
5	3000	3.47	10	3	30	30	4.75	12.04	15.54	4.99
6	4000	3.60	10	4	40	40	5.00	12.96	17.10	5.31
7	4500	3.65	10	5	50	50	5.52	13.32	18.25	5.43
8	5000	3.69	10	8	80	80	5.84	13.61	21.54	5.52
9	5500	3.74	10	10	100	98	7.05	13.98	26.36	5.64

$\Sigma X = 29.53$ $\Sigma y = 40.90$ $\Sigma X^2 = 98.74$ $\Sigma Xy = 138.65$

$x = 3.28$ $\bar{y} = 4.54$

$$b = \frac{\Sigma Xy - (\Sigma X . \Sigma y)/n}{\Sigma X^2 - \Sigma X^2/n} = \frac{138.65 - (29.53 \times 40.90)/9}{98.74 - (29.53)^2/9} = \frac{138.65 - 134.19}{98.74 - 96.89} = 2.41$$

Linear regression equation

$Y = \bar{y} + b(X - x)$, where x, $\bar{y}$ are the mean values of X and y, b is known as the regression coefficient

$Y = 4.54 + 2.41(X - 3.28)$

Corrected formula: for 0% dead = $100 \times 0.25 / n$; for 100% dead = $100 (n - 0.25) / n$, where n is the number of animals in each group.

Table 4.4 Determination of LD_{50} of methanol extract of *H.nepalense* root after oral administration in mice.

Group	Dose (mg/kg)	Log dose (X)	No of animals used	No of animals dead	Dead (%)	Corrected dead (%)	Probit (y)	X^2	Xy	Y
1	500	2.69	10	0	0	2	2.95	7.23	7.93	3.02
2	1000	3.00	10	0	0	2	2.95	9.00	8.85	3.64
3	2000	3.30	10	1	10	10	3.72	10.89	12.27	4.24
4	3000	3.47	10	2	20	20	4.16	12.04	14.43	4.58
5	4000	3.60	10	3	30	30	4.48	12.96	14.97	4.84
6	5000	3.69	10	4	40	40	4.75	13.61	17.52	5.02
7	5500	3.74	10	5	50	50	5.00	13.98	18.70	5.12
8	6000	3.77	10	8	80	80	5.84	14.21	22.00	5.18
9	6500	3.81	10	10	100	98	7.05	14.51	26.86	5.26

$\Sigma X = 31.07$ $\Sigma y = 40.90$ $\Sigma X^2 = 108.43$ $\Sigma Xy = 143.53$

$x = 3.45$ $\tilde{y} = 4.54$

$$b = \frac{\Sigma Xy - (\Sigma X.\ \Sigma y)/n}{\Sigma X^2 - \Sigma X^2/n} = \frac{143.53 - (31.07 \times 40.90)/9}{108.43 - (30.07)^2/9} = \frac{143.53 - 141.19}{108.43 - 107.26} = 2.00$$

Linear regression equation

$Y = \tilde{y} + b(X - x)$, where x, $\tilde{y}$ are the mean values of X and y, b is known as the regression coefficient

$Y = 4.54 + 2.00(X - 3.45)$

Corrected formula: for 0% dead = $100 \times 0.25 / n$; for 100% dead = $100 (n - 0.25) / n$, where n is the number of animals in each group.

References

1. Hunter, W.J., Ling, W. and Recht, R. (1979). Intercomparision study on the determination of single administration toxicity in rats. *J Assoc Anal Chem* 62, 864-873.
2. Kingk, W. (1979). Commission of the European communities, Industrial Health and safety; quality assurance of toxicological data; Proceeding of the international colloquium Luxembourg Report Eur 7270, pp.89.
3. Malmford, T. and Teiling, A. (1983). LD_{50}; its value for the pharmaceutical industry in safety evaluation of drugs. *Acta Pharmacol and Toxicol* 52 (Suppl 2), 229.
4. Katzung, B.G. (1998). Basic and Clinical Pharmacology, 7th ed., Appleton & Lange, Stamford. Connecticut, U.S.A. pp. 62-67.
5. Satoskar, R.S. and Bhandakar, S.D. (1978). Pharmacology and pharmacotherapeutics in; dose response relationship, 6th ed, Popular Prakashana, Bombay, India.
6. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch Toxicol* 54, 275-284.
7. Litchfield, J.T. and Wilcoxon, F. (1949). A simplified method of evaluating dose effect experiments. *J Pharmacol and Therpeu* 96, 99-113.