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Estimation of Median Lethal Dose of Copper Sulphate and Potassium Dichromate in Albino Rats

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Abstract: Present study has been undertaken to calculate the median lethal dose of two trace heavy metals, copper and chromium against female albino rats. Salts of copper and chromium namely copper sulphate and potassium dichromate respectively were used to estimate the median lethal dose of experimental compounds under study (vide supra). This study was conducted on female albino rats selected from an inbreed colony. Experimental female albino rats were initially divided into two groups, one corresponding to copper and other for chromium salts respectively. These groups were further divided according to the dosages. Survival and mortality number was observed following 96 hours of intoxication. LD_{50} was calculated statistically by log-dose/probit regression line method and came out to be 269.00 and 77.00 mg/kg b.wt. for copper and chromium respectively. Perhaps there must have been formation of comparatively more toxic by-products inside the body following intoxication of chromium salt compared to copper salt, responsible for the differences in LD_{50}.
Keywords: LD_{50}, copper, chromium, female albino rat, mortality, log-dose.

I. INTRODUCTION

Exposure to heavy metals can be an outcome of various environmental as well as anthropogenic activates. Among heavy metals, some are considerably toxic to various organisms residing on this planet. On contrary, some of the heavy metals at very small amounts are very important for continuity of many metabolic activities inside the body and thus life as a whole. Such heavy metals are commonly called trace heavy metals (Mohammed *et al.*, 2014; Gamakaranage, 2018, Tiwari, 2007).

Still, when present in higher amounts, these so considered important trace heavy metals can cause considerable toxicological manifestations in various living organism upto different levels. Copper and chromium are two trace heavy metals having broad applications. These two have central role in bringing about many metabolic pathways related to macromeolecules inside the mammalian body. In addition they are also incorporated in household, industry and agriculture related products (Sinkovic *et al.*, 2008; Elshazly *et al.*, 2016; Tiwari *et al.*, 2019).

Their presence in variety of consumer products and environmental transformation into other related forms, points out towards the possibility of their biomagnification as well as biotrasformation, thus can cause considerable threat to the life of many organisms residing in different habitats(Tiwari and Saxena, 2017;Tiwari *et al.*, 2019).

It is with the reason that median lethal dose estimation of copper and chromium has been undertaken in the present study to have a preliminary idea of their toxicity.

II. MATERIAL AND METHODS

A. Experimental Animal

Present study was conducted on seventy female albino rats, *Rattus norvegicus*, eight weeks age, selected from an inbred colony and weighing 110 ± 20 gm. These experimental animals were well acclimatised for two weeks to laboratory conditions and provided standard rat pellet feed and water *ad libitum*. Thenafter, these acclimatised albino rats were randomly divided into two main groups initially containing equal i.e. thirty five rats each. Each group was further sub-divided into five sub-groups comprised of seven rats corresponding to different doses of copper sulphate and potassium dichromate respectively. The experimentation was approved by the Ethical committee of Dr. B. R. Ambedkar University, Agra, India.

B. Experimental Compounds

Commercial formulations of copper and chromium namely copper sulphate and potassium dichromate were obtained from Sigma chemicals Ltd., Mumbai. Their LD_{50} was calculated by log-dose regression line method (Finney, 1971).

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III. DOSE ADMINISTRATION AND DETERMINATION OF LD50

A. Copper Sulpahte

Standard solution of experimental test compound, copper sulphate, was prepared by dissolving in distilled water. Different doses quantitatively, 100, 200, 300, 400 and 500 mg/kg b.wt. were administrated to animals corresponding to sub-group 1-5. This dose was administrated orally through gavage tube. Following this for 96 hours, mortality and survival number of rats were recorded for each dose. The numerical data so obtained, was statistically analyzed by log dose/probit regression line method (Finey, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for LD_{50} determination (Table 1).

B. Potassium Dichromate

Standard solution of second experimental test compound potassium dichromate was prepared by dissolving in distilled water. Different doses *viz.* 25, 50, 75 100 and 125 mg/kg b.wt. of potassium dichromate were administrated orally to albino rats of respective sub-groups (5-10). The mortality and survival number of rats were recorded for each dose after 96 hours. The data was analyzed statistically by log dose / probit regression line method (Finey, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for LD_{50} determination (Table 1).

IV. RESULTS

In the different experimental sets, experimental rats were treated with different doses of different concentrations of copper sulphate and potassium dichromate, for the estimation of LD_{50}. Survival number and percentage for each dose have been noted after 96 hours which decreased with increasing dose of copper sulphate and potassium dichromate respectively(Table 1). LD_{50} has been calculated (Tables-II-IV) by log dose/probit regression line method (Finney, 1971). The test doses were then converted to their logarithm. The empirical probit values, equivalent to percentage mortality were then obtained from the table (Finney, 1971) and plotted against log dose on graph paper. The provisional lines was drawn fitting to the expected values were read for the values of log dose (X).

Working probits

Y = Y0 + kp

Where, p = percentage mortality

Y0 and k are two factors.

The weighting coefficients for each point were also obtained from the table. The weight has been calculated by multiplying each coefficient by number of rats used (Table II-III). The value of 'b' is obtained by the formula

$$b = \left(\sum wxy - \overline{X}\sum wy\right) / \left(\sum wx^2 - \overline{X}\sum wx\right)$$

The values of Y can be obtained by regression equation (Fig.1-3)

$$Y = \overline{Y} + b(X - \overline{X})$$

From the equation values of X, equivalent to Y and $\overline{Y}$ were estimated, and the calculated values of LD_{50} thus been obtained (Tables-II-IV, Fig. 1-3). Variance V was calculated followed by 95% confidence fiducial limits as below:

$$\text{Variance (V)} = \frac{1}{b^2}\left(\frac{1}{\sum w} + \frac{(X-\overline{X})^2}{\Sigma wx^2 - \frac{(\sum wx)^2}{\sum w}}\right)$$

By the following formula 95% confidence fiducial limits have been obtained (Table v).

m1 = m + 196 V

m2 = m-1.96 V

Applying all these calculation, LD_{50} for copper sulpahate and potassium dichromate came out to be 269.00 and 77.00 mg/Kg b.wt. Respectively.

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V. DISCUSSION

Copper and chromium are among important heavy trace metals, having vital role in various body metabolic functions (Tiwari and Saxena, 2017; Tiwari *et al.*, 2019).

Median lethal dose (LD_{50}) determination is an important initial indicator of the toxic potential of any compound. It is also helpful in framing precautionary guidelines in case of domestic intoxications. Both copper and chromium are used indiscriminately as well as thrown here and there by the general population, therefore evaluation of LD_{50} becomes essential for better evaluation of toxicity characteristic of copper and chromium (Bhushan and Saxena, 2013; Saxena and Bhushan, 2013; Tiwari at al., 2019).

In the present investigation the chromium has been found to be more toxic then copper based on the LD_{50} values. Trace heavy metals are an integral part of metabolism. Altered levels of these metals inside the body of an organism can cause deleterious consequences. Copper is present in the environment in two forms viz. Cu^{+} and Cu^{2+} where as chromium exists in the various forms from Cr(II-VI), with stable tetravalent and hexavalent forms. Both these trace heavy metals as a consequence of biotransformation, are involved with the formation of reactive oxygen species (ROS) within the body of intoxicated rats. However, various stable forms of chromium perhaps have given rise to more toxic reactive oxygen species and other realated intermediates. These by-products may have affected various tissues, their membranes, macromolecules, variety of other metabolic reactions of the body, ultimately causing death of concerned animal more in chromium than copper (Witmer *et al.*, 1994; Gaggelli *et al.*, 2002; Tiwari, 2007; Tiwari and Saxena; 2017; Tiwari *et al.*, 2019).

VI. CONCLUSION

This preliminary study shows that both these trace heavy metals can be toxic to non-target organisms at higher levels, so need to be used and need not to be thrown in open environment.

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Table I

Percentage survival of *Rattus norvegicus* after Copper sulpahte and Potassium Dichromate intoxication

S.No.	Number of rats in each set	ExposureDuration (in hours)	Dose (mg/kg b.wt.)		Survival number		Survival percentage	
			Copper sulphate	Potassium dichromate	Copper sulphate	Potassium dichromate	Copper sulphate	Potassium dichromate
1.	7	96	100	25	7	7	100	100
2.	7	96	200	50	5	5	71.43	71.43
3.	7	96	300	75	3	4	42.86	57.14
4.	7	96	400	100	2	2	28.57	28.57
5.	7	96	500	125	0	0	0	0

Table- II

Determination of LD_{50} by log-dose/probit regression analysis after oral intoxication of different doses of Copper sulphate into *Rattus norvegicus*

S.No.	Dose in mg/kg b.wt.	No. of rats 'n'	Mortality Number	Mortality (%)	Log dose 'X'	Empirical Probit	Expected Probit 'Y'	Working Probit 'y'	Weighing coefficient 'N'	Weight W=n×N	WX	Wy	Wxy	WX^2	Wy^2
1.	100	7	0	0	2.00	--	--	--	--	--	--	--	--	--	--
2.	200	7	2	28.57	2.30	4.45	4.41	4.44	0.558	3.91	8.98	17.37	39.95	20.66	77.24
3.	300	7	4	57.14	2.47	5.18	5.04	5.17	0.637	4.46	11.01	23.08	56.99	27.20	119.41
4.	400	7	5	71.43	2.60	5.55	5.50	5.55	0.581	4.07	10.57	22.58	58.72	27.49	125.41
5.	500	7	7	100.00	2.69	0.0	5.81	6.53	0.503	3.52	9.47	22.99	61.86	25.48	150.18
										∑W= 15.95	∑WX= 40.04	∑Wy= 86.03	$\sum WX^2$= 217.52	$\sum WX^2$= 100.84	$\sum Wy^2$= 472.25

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Table- III

Determination of LD_{50} by log-dose/probit Regression analysis after oral intoxication of different doses of Potassium dichromate into *Rattus norvegicus*

S.No.	Dose in mg/kg b.wt.	No. Of rats 'n'	Mortality Number	Mortality (%)	Log dose 'X'	Empirical Probit	Expected Probit 'Y'	Working Probit 'y'	Weighing coefficient 'N'	Weight W=n×N	WX	Wy	Wxy	WX^2	Wy^2
1.	100	7	0	0	1.39	---	---	---	---	---	---	---	---	---	---
2.	200	7	2	28.57	1.69	4.45	4.32	4.45	3.724	6.29	16.59	27.99	47.30	73.88	124.56
3.	400	7	3	42.86	1.87	4.80	4.80	4.79	4.389	8.21	21.01	39.33	73.54	100.62	188.37
4.	800	7	5	71.43	2.00	5.55	5.33	5.54	4.312	8.62	23.89	47.75	95.51	132.39	264.56
5.	1600	7	7	100.00	2.10	0.0	5.71	6.48	3.724	7.82	24.11	50.67	106.41	156.13	328.36
										ΣW= 16.15	ΣWX= 30.95	ΣWy= 165.74	ΣWxy= 322.76	$ΣWX^2$ = 463.02	$ΣWy^2$= 905.85

Table-IV

Toxicity evaluation of Copper sulphate and Potassium dichromate for *Rattus norvegicus* specifying fiducial limits

Experimental compound	Regression Equation	LD_{50} (in mg/kg b.wt.)	Variance	Fiducial limits
Copper sulpahte	Y=5.39+4.89(x-2.51)	269.00	0.002	m_1= (+)2.4317 m_2= (-)2.4277
Potassium dichromate	Y=5.30+1.92(x-4.80)	77.00	0.002	m_1= (+)1.8554 m_2= (-)1.8514

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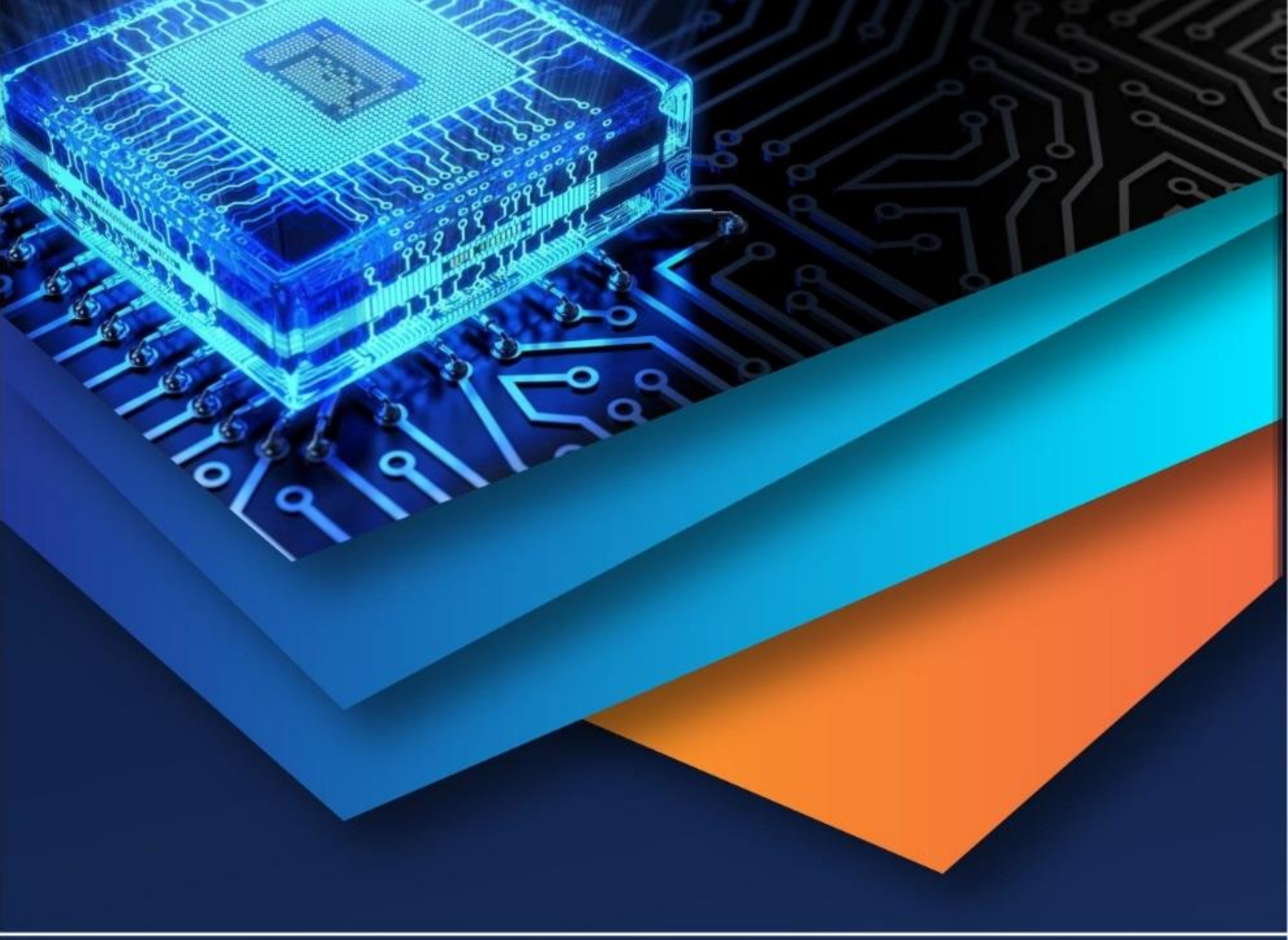

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