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Research article

Acute Effect of Copper on *Puntius javanicus* Survival and a Current Opinion for Future Biomarker Development

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ABSTRACT

Puntius javanicus experimental groups were exposed with to different concentration of copper (II) sulfate for 96 hours. Their mortality was recorded to determine LC_{50} value of copper concentration based on arithmetic, logarithmic and probit graphic analyses. The results obtained from these three mathematical analyses were 11.37±0.58, 11.01±0.73 and 10.68 mg/L, respectively. From the present study, we suggested that in the future, the range of 0 to 5.0 mg/L can be used to study the effect of copper concentration on fish activity at biochemical and physiological levels. Based on probit analysis, this maximum range is lower than LC_{10} value i.e. 6.11 mg/L. Therefore, it can be positively hypothesised that there would be no mortality occur except for several symptoms of adverse effects beyond of 5.0 mg/L treatment.

INTRODUCTION

Copper (Cu) is a vital component needed for maintaining various metabolism as well as to regulate a number of copper-containing enzymes. Cu deficiencies may cause hematological and neurological disorder but this case are very rare because it is easily recovered by consuming food or feed that contains traces amount of copper [1,2]. However, intake of Cu exceeded its normal internal body concentration can be toxic, including the inhibition of several biochemical activities, increasing ROS production lead to induction of programme cell death, followed by alteration at the physiological level which at the end may cause mortality [3,4]. Cu contamination in soil, ground water and water bodies has been studied [5,6]. Most of heavy industries and agricultural sectors ultilised Cu as a trace element of in their products such as alloy, electrical component, fertilizer and pesticide. Unfortunately, its over production and application may accidently leaching it out into groundwater flow or directly to the water bodies. This process will slowly concentrate Cu in the water bodies which consisting of a number of aquatic organisms. Since this element is unable to be degraded chemically, biologically and physically, it will bioaccumulate in the body of various aquatic organisms. Thus, this can affect the food chain and then a continuous transfer to the other level of consumer until to the final consumer especially human.

Puntius javanicus (Lampam jawa) was selected in this present study as this fish has a commercial value, widely distributed in almost all types of freshwater in Malaysia, it is an alternative food source for native tribe, and the most important one is that this species is highly exposed to various toxicant present in aquatic environment. Furthermore, *P. javanicus* is potentially to be used as an alternative biomarker for environmental contaminant especially Cu. In light of the danger of Cu and great potential of *P. javanicus* as a fish model, this study was carried out to test the accute effect of Cu on *P. javanicus* survival. This study was also aimed to explore P. javanicus potential in future development of biomarker for Cu and other environmental contaminant.

MATERIALS AND METHODS

Copper (II) sulfate concentrations of 2.0, 4.0, 6.0, 8.0, 10, 13, 15 and 20 mg/L. Fish mortality were recorded until at the end of 96 hours of treatment. LC50 value of Cu concentration was calculated based on arithmetic, logarithmic and probit analysis as referred to methods of Karber, [7], Bliss, [8-11], and Finney, [12], respectively. Calculation was carried out using Microsoft Excel software for arithmetic and logarithmic analyses, whereas for probit analysis, it was done using Biostat professional version 9.

RESULTS AND DISCUSSION

96 hours LC_{50} value of Cu concentration

The number of fish mortality was recorded (Table 1). LC_{50} value of copper concentration was obtained from arithmetic graphic method with the average value of 11.37±0.58 mg/L (Figure 1). Boyd, [13] mentioned that the estimation of the toxicant concentration that killed half of the test organisms can be done base on arithmetic method by plotting the percentage of survival on semi-log paper versus the toxicant concentration, and the LC_{50} value is calculated graphically by interpolation of 50% survival to concentration intersection point.

Table 1: 96 hour cumulative toxicity test results represents percentage (%) of fish survival.

	mg/L							
No. of replicate	2	4	6	8	10	13	15	20
1	100	100	100	77.78	66.67	22.22	0	0
2	100	100	100	100	77.78	44.44	0	0
3	100	100	100	88.89	66.67	33.33	22.22	0

Logarithmic analysis was performed which the interpolation of the curve obtained by drawing a horizontal line from the 50% of mortality point to the logarithmic concentration-response which the intersection point is LC_{50} value [14], and this study showed the value at the average of 11.01±0.73 mg/L (Figure 2). Logarithmic method is considered as the best method because the concentration used are base on logarithmic scale which narrowing down the big scale factor of number of replicates [15] especially for LC_{50} value determination. However, LC_{50} values in both methods of analysis showed no significant different ($p<0.05$). Previous studies done for LC_{50} determination are based on arithmetic and logarithmic analyses as reported by Guise et al., [16], Ishikawa et al., [17], Yusuf et al., [18] and Koakoski et al., [19].

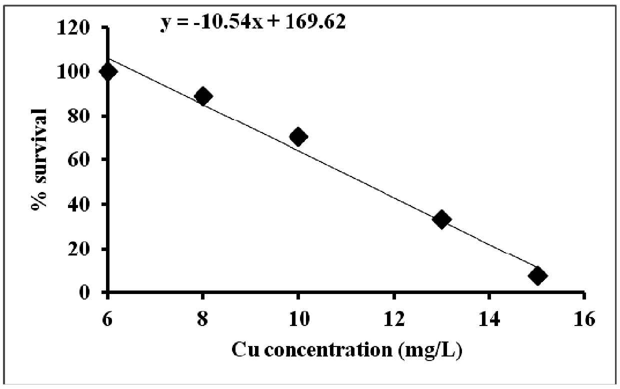

Figure 1: Arithmetic graphic analysis on percentage (%) survival of *P. javanicus* after 96 hour incubation with various cu concentrations.

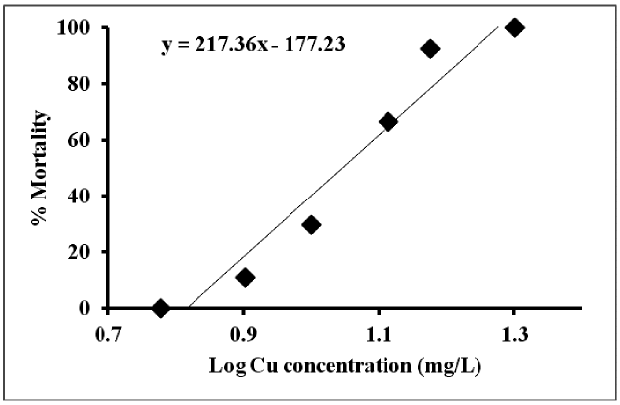

Figure 2: Logarithmic analysis on percentage (%) *p. javanicus* mortality after 96 hour incubation with various Cu concentrations in logarithmic scale.

Figure 3a and 3b show the graphs were generated based on probit analysis using Biostat professional version 9 software. Table 2a and 2b show the details which two types of probit analyses were generated based on Finney method [Lognormal Distribution] and Least squares [Normal Distribution] method. The calculated LC_{50} for Cu from these two methods were of 10.37±0.67 and 10.89±1.0 mg/L, respectively. Both LC_{50} values displayed no significant difference ($p>0.05$) with the average of 10.68 mg/L. Reish and Oshida, [15] mentioned that the application of probit analysis for LC_{50} value determination is done by minimizing the extreme values associated with maximizing the middle percentage survival. Furthermore, the analysis is run by also excluding the zero and 100% point. Probit analysis is known as the most precise method for LC_{50} value determination in various test organisms such as bacteria [20], fish [21,22], mice [23] and insect [24].

Table 2a: Complete data for LC_{50} Cu concentration determination using probit analysis based on Finney method [Lognormal Distribution] generated from Biostat professional version 9 software.

Alpha Value (for confidence interval) 0.05							
Log10[dose (stimulus)]	Actual percent (%)	Probit percent (%)	N	R	E(R)	Difference	Chi-Square
0.30103	0.027778	5.63e-13	9	0.25	5.06e-12	0.25	1.23e+10
0.60206	0.027778	1.91e-05	9	0.25	0.000172	0.249828	363.6387
0.778151	0.027778	0.008999	9	0.25	0.080987	0.169013	0.352713
0.90309	0.222222	0.130938	9	2	1.178446	0.821554	0.572747
1	0.333333	0.437516	9	3	3.937643	-0.93764	0.223274
1.113943	0.777778	0.835712	9	7	7.521405	-0.52141	0.036145
1.176091	0.972222	9.45e-01	9	8.75	8.50e+00	0.247562	0.007208
1.30103	0.972222	9.98e-01	9	8.75	8.979657	-0.22966	0.005874

Chi-square	1.23e+10
Degrees of freedom	6
P-level	0

Dose (Stimulus) Percentile					
Percentile	Probit (y)	Log10[dose (stimulus)]	Standard error	Dose (stimulus)	Standard error
1	2.673215	0.782058	0.075949	6.054218	1.064161
5	3.354789	0.850527	0.057961	7.088047	0.948775
10	3.718271	0.887041	0.048957	7.709759	0.870951
16	4.005578	0.915903	0.042361	8.239534	0.804958
20	4.158543	0.931269	0.039126	8.536287	0.770083
25	4.325811	0.948072	0.035889	8.873034	0.734073
30	4.475998	0.963159	0.033322	9.186698	0.705559
40	4.747067	0.99039	0.029775	9.781153	0.671119
50	5	1.015799	0.028108	10.37048	0.671669
60	5.252933	1.041208	0.028321	10.99531	0.717532
70	5.524002	1.068438	0.030584	11.7068	0.82509
75	5.674189	1.083526	0.032601	12.12064	0.910712
80	5.841457	1.100329	0.035353	12.59879	1.026709
84	5.994422	1.115695	0.038241	13.05254	1.15079
90	6.281729	1.144557	0.044371	13.94944	1.427654

Table 2a (Continued): Complete data for LC_{50} Cu concentration determination using probit analysis based on Finney method [Lognormal Distribution] generated from Biostat professional version 9 software.

95	6.645211	1.181071	0.053009	15.17299	1.856584
99	7.326785	1.24954	0.070648	17.76395	2.902485

regression statistics			
LC_{50}	10.37048	LC_{50} Standard Error	0.671669
LC_{50} LCL	9.081668	LC_{50} UCL	11.70445
Log10[LC50]	1.015799	Standard error	0.028108
Beta	9.954553	Intercept	-5.11182
Beta standard error	2.295707		

Table 2b: Complete data for LC_{50} Cu concentration including LC10 determination using probit analysis based on Least squares [Normal Distribution] method generated from Biostat professional version 9 software.

Dose (stimulus)	Actual percent (%)	n	Probit (Y)	Weight (Z)
2	0.027778	9	3.085069	1.170139
4	0.027778	9	3.085069	1.170139
6	0.027778	9	3.085069	1.170139
8	0.222222	9	4.235519	3.971038
10	0.333333	9	4.569708	4.569708
13	0.777778	9	5.764481	3.971038
15	0.972222	9	6.914931	1
20	0.972222	9	6.914931	1

Regression statistics			
LC_{50}	10.88959	LC_{50} Standard Error	1.015241
LC_{50} LCL	8.802737	LC_{50} UCL	12.97645
Beta	0.26808	Intercept	2.080719
Beta standard error	0.056603		
LC_{10}	6.108448		
LC100	16.48494		

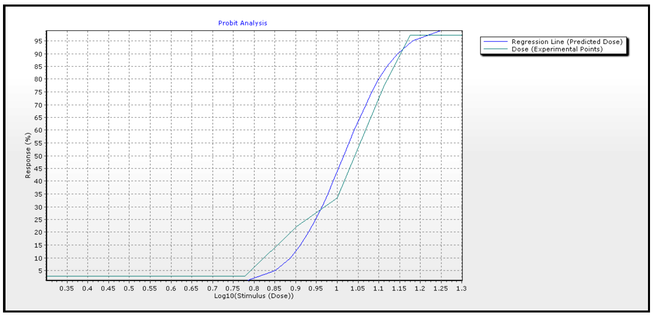

Figure 3a: Screenshot of probit analysis based on Finney method [lognormal distribution] generated by Biostat professional version 9 software.

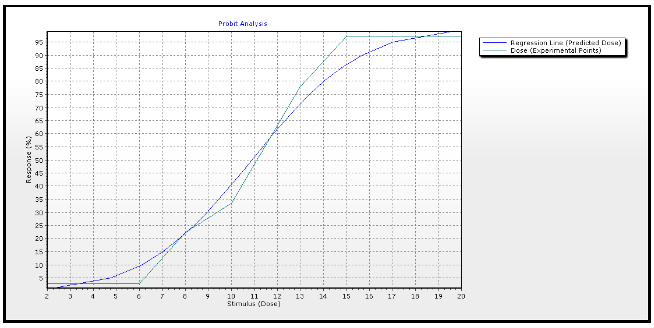

Figure 3b: Screenshot of probit analysis based on least squares [normal distribution] method generated by BIostat professional version 9 software.

Expected biological effects at the lower than LC_{10} of copper concentration.

The concentration that caused 10% fish mortality (LC_{10}) according to probit analysis was determined at the value of 6.11 mg/L. Thus, for the future study, the range of 0 to 5.0 mg/L will be selected which can be hypothesised that zero mortality (except death signs) at the highest concentration of Cu treatment. Expected biological effects were displayed in Figure 4. At the starting point of treatment, biochemical reaction will take place as evident by upregulation of defensive related protein such as metalloenzyme and the increased of antioxidant activities. At the beginning of toxic effect, several biochemicals were upregulated for mantaining hemeotasis mechanism which has been mark as grey region, and at the same time affected protein will be downregulated due to inhibition or degradation. Until at the certain maximum level, adverse effect of biochemical reaction lead to the cellular alteration caused by imbalance of protein recovery and toxic effect associated with the induction of programe cell death. The death of cell is associated with the alteration of fish behaviour such as swimming performance, avoidance behaviour and food intake. Beyond 5.0 mg/L of Cu concentration treatment, our expectation is more toward the induction of fish mortality. Jeram et al., [21] has mentioned that fish is the most sensitive biomarker for environmental assessment. Currently, we are conducting a study for the effect of copper on morphology (ultrastructure), proteome and cholinesterase activity of *P. javanicus* liver. Liver was selected due to its function in toxicant neutralisation and detoxification. Moreover, at the elevated level of a certain toxicant, it can cause adverse effect to this organ followed by negative effect to the whole biological system of *P. javanicus*. Thus, *P. javanicus* liver was selected for future analysis in development of biomarker tool for environmental risk assessment.

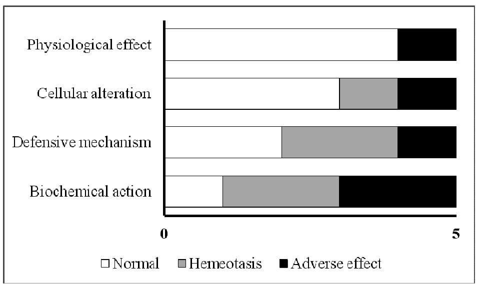

Figure 4: Expected different stages of biological effect of cu concentration ranging from 0 to 5.0 mg/l on *P. javanicus* survival.

CONCLUSION

LC_{50} value of Cu concentration on P. javanicus survival is determined in this study based on arithmetic, logarithmic and probit graphic analyses. Concentrations of Cu from 0 to 5.0 mg/L are determined as range of concentration just before LC_{10} that caused no signs of mortality or mortality of the fish. *P. javanicus* is proven as a potential alternative biomarker for assessment of Cu contamination in the water.

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