Name of Scholar : Ms. Shahnaz Akhtar

Name of Supervisor : Professor Arif Ali

Name of Co-Supervisor : Dr. V. M. Shingatgeri

Department : Department of Biotechnology

Title of Thesis :Prediction of Acute Toxicity (LD-50) of Drugs/Compounds by *In-Vitro* Methods

Abstract

Acute toxicity test is typically the first step in safety assessment of any compound. One of the major objectives of acute toxicity studies is hazard classification and labeling of chemicals using LD-50 values. A standard acute toxicity test to estimate LD-50 requires approximately 70-80 animals. With advent of animal activist's movements and with an aim to reduce the number of animals as per concept of 3R's, the efforts are going on world wide to use *in-vitro* methods for predicting acute toxicity. However, till date there is no *in-vitro* methods exist which can accurately predict acute oral toxicity for the purposes of hazard classification. Hence, the present study was conducted with the main objective to suggest and validate an *in-vitro* test for predicting *in-vivo* acute toxicity. Four *in-vitro* test system were used to evaluate the cytotoxicity (IC-50) of selected drugs/compounds by MTT assay. Total 198 experiments were conducted on 79 drugs/compounds using above-mentioned *in-vitro* systems and IC-50 values were calculated by linear and non-linear method. To evaluate and compare the productivity and correlation between the IC-50 values calculated by different methods and LD-50 values in rat and mice, separately, regression analysis was performed for various models.

Further, LD-50 values were predicted for drugs/compounds using the IC-50 values for different test systems using respective regression equations. These predicted LD-50 values were then used to categorize them into different categories as per the Global Harmonization System (GHS) of classification and labeling of chemicals. The predicted GHS categories were compared with the actual category of the compound as per their actual LD-50 values by the *in-vivo* test, to find what percentage of the compound was categorized correctly, as well as in ± 1 category.

After regression analysis, considering the coefficient of determination as well as based on the results of predictivity for GHS categorization, although all *in-vitro* systems showed

good predictivity, but IC-50 values generated either by linear method or non-linear method, using primary rat hepatocytes resulted in best correlation and predictivity. Among all the *in-vitro* test systems, weakest correlation and predicitvity was found with HepG2 Cell Line. There was no much difference in the correlation and predicitvity of IC-50 values for C2C12 and CHO cell line.

Considering the predictability and accuracy of all the *in-vitro* models, it was seen that correct category can be predicted for approximately 40-70 % of the drugs/compounds but prediction for ± 1 category was upto 100%, therefore these *in-vitro* methods can help to select the appropriate starting dose for any *in-vivo* acute toxicity method, which will help to reduce the number of animals used in the study.

Major Findings

Following were the major findings in the study:

- Log Millimole regression equations, were found the best model for LD-50 prediction.
- Primary rat Hepatocytes was better *in-vitro* system for prediction as compared to C2C12, CHO and HepG2 cell line.
- MTT assay showed better predictability and accuracy than neutral red uptake assay suggested by ICCVAM.
- Predictability and accuracy for rat LD-50 using MTT assay as per Registry of cytotoxicity (RC) rat only log millimole equation was similar to rat only combined log millimole equation.
- Predictability and accuracy for mice LD-50 was better by the mice only combined log millimole equation generated in the study than RC millimole equation.
- Regression equations can be used for the prediction of LD-50 of drugs/compounds, which have poor solubility if the IC-50 is extrapolated using linear regression.
- *In-vitro* method can help to select the appropriate starting dose for *in-vivo* acute toxicity studies as the accuracy for ± 1 category prediction was observed upto 100%, which will help to reduce the number of animals used in the study.
- Suggested regression equations to predict LD-50 using primary rat hepatocytes are:

Log LD-50 (mmol/kg)= 0.652 x Log IC-50 (mmol/l)+ 0.534 – MiceLog LD-50 (mmol/kg)= 0.680 x Log IC-50 (mmol/l)+ 0.636 -Rat