Lactate dehydrogenase (LDH) enzyme assay in cytotoxicity testing and the interferences caused by Triphala

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Introduction

Measuring the activity of cytoplasmic Lactate dehydrogenase (LDH) enzyme released by damaged cells to the cell culture supernatant is a widely accepted method of evaluating the cytotoxicity of pharmacological compounds. The principle underlying relies on two hypotheses: the testing drug has zero interference with the enzyme activity, and the test drug itself has no UV absorbance ability. Triphala (TPL) is a renowned polyherbal formulation used in ayurvedic medicine. The objective of the study is to identify interferences caused by TPL in LDH assay

$$\%\text{Cytotoxicity} = \frac{\text{LDH Activity of the supernatant}}{\text{LDH activity in the supernatant + LDH activity of the cell lysate}} \times 100$$

Methodology.

LDH assay was conducted after incubating rhabdomyosarcoma cells (RD) with TPL (10-1000µg/ml) for 24 hours and the UV absorbance of same concentrations of TPL before and after incubating at 37°C for 24 hours, were measured. The Impact of TPL on LDH activity was determined using fresh human serum after incubating 15 minutes with a concentration series (100-750µg/ml) of TPL. Assays were done in technical triplicates. EC50 value was calculated considering the percentage cell viability and TPL concentration.

$$\%\text{Cell viability} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the negative control}} \times 100$$

Results

The LDH activity declined in both cell lysate and supernatant in a dose-dependent manner. TPL showed a concentration-dependent increment in UV absorbance (Figure 1) which inclined significantly after incubation ($p<0.05$). Negative control (without TPL) showed the highest enzyme activity compared to TPL treated serum (Figure 2). To minimize the interferences, percentage cell viability was determined by considering the enzyme activity in the cell lysates relative to the negative control, and the EC50 value was 871.0±57.9µg/mL.

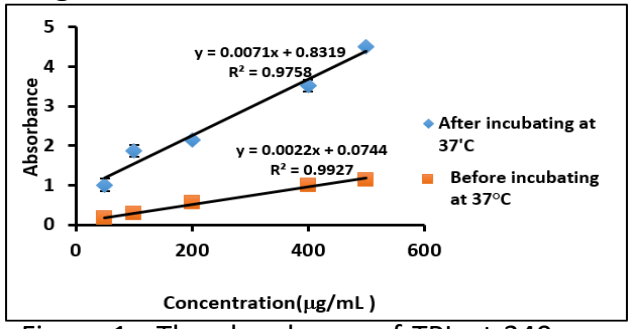

Figure 1: The absorbance of TPL at 340nm before and after incubating for 24hours at 37°C. The values are presented as Mean ± SD for three independent experiment

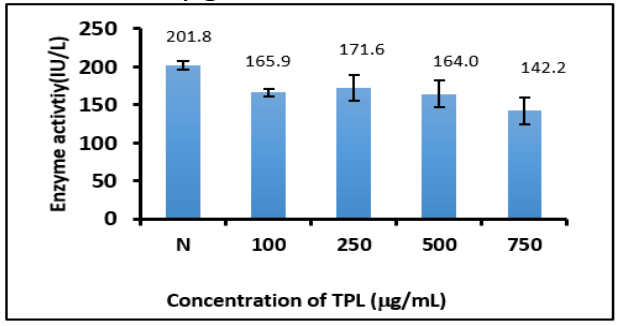

Figure 2: The Enzyme activity of the negative control (N) and blood samples, after incubating with TPL. The values are presented as Mean ± SD for three independent experiments.

Conclusions

TPL significantly affects the measurement of LDH activity due to its high UV absorbance and ability to inhibit LDH activity. Therefore, before conducting LDH assay, interference by the testing compound should be evaluated.