

Cytotoxic Effect of Purified and Crude Hyaluronidase Enzyme on Hep G2 Cell Line

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Abstract : Hyaluronidase enzyme was purified from the clinical isolate *Staphylococcus aureus* in three purification steps, first by precipitation with 90% saturated ammonium sulfate, ion exchange chromatography on DEAE-Cellulose, and gel filtration chromatography throughout Sephacryl S-300. Specific activity of the purified enzyme was reached 930 U/mg protein with 7.4 folds of purification and 46.5% recovery. The enzyme has an average molecular weight of about 69 kDa, with an optimum pH of enzyme activity and stability at pH 7, also the optimum temperature for activity was 37°C. The enzyme was stable with full activity at a temperature ranged between 30-40 °C. Metal ions showed variable inhibitory degree with the strongest effect for Fe³⁺, however, the chelating and reducing agents had no or little effects. Cytotoxic studies for purified and crude hyaluronidase against cancer cell Hep G2 type at different enzyme concentrations and exposure times showed that the inhibition effect of both crude and purified enzyme increased by increasing the enzyme concentration with no change was observed at 24hr, while at 48 and 72 hrs the same inhibition rate were observed for purified enzyme and differ for the crude filtrate.

Keywords : hyaluronidase, *S. aureus*, metal ions, cytotoxicity

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