SUMMARY AND CONCLUSION

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In this study, one phospholipase A_2 enzyme (PLA) was isolated from the Sistrurus miliarius barbouri (S.M.B) crude venom (C.V). Kinetic studies, biological and toxicological properties were studied. The isolated enzyme was termed "PL-I".

Part I of this work was isolation and purification of PL-I. Two chromatographic steps were used. First step chromatography was fractionation of C.V by gel filtration on Sephadex G-100 and G-75. Different buffer systems with different hydrogen ion concentrations were used throughout the different chromatographic separations (six trials). Figures (3-8) and tables (5-10), which emphasized that C.V gave six peaks by gel filtration on Sephadex G-100, eluted with ammonium acetate buffer 0.02 M. Peak 4 and peak 5 showed the highest PLA activity. The second step of chromatography was refractionation of PLA from F_4 and F_5 by ion exchange chromatography using DEAE-Cellulose, then purity of PLA2 was checked using SDS-PAGE which showed to be pure.

Part II of this study was the biochemical characterization of the isolated enzyme. The molecular weight of PL-I estimated by SDS-PAGE was found to be 13,000 dalton with a k_m of 3.3 mg/L and their V_{max} was 1.43 mg/mL. The optimum temperature was found to be 45°C while the optimum pH was 7.0. Copper and magnesium ions enhanced the activity of enzyme, whereas calcium reduced it. The effect of some inhibitors on PLA2 activity was also studied and revealed that EDTA had an inhibitory effect, denoting that PLA2 is a metalloprotein, also iodoacetate showed inhibitory effect on PLA2 indicating that a thiol group was present at the catalytic site of enzyme activity.

Part III was in vitro studies of some biological and toxicological activities of the isolated enzyme compared with those of the C.V. It was found that PL-I had a slight anticoagulant activity while C.V had a potent anticoagulant one. On testing the hemolytic activity, it was found that PL-I showed a slight direct hemolytic activity and a very potent indirect hemolytic activity, while C.V showed a very weak direct and potent indirect hemolytic activity. On studying the effect of the isolated enzyme on platetet aggregation it was found that the isolated enzyme had an effect on platelet showing a 15% aggregation while the crude venom caused a 10% aggregation.

Part IV of this study was in vivo studies of the biological and toxicological activities of isolated enzyme in comparison with that of C.V through I.P injection of experimental albino mice. LD_{50} of C.V was 9.72 mg/kg while the PL-I was found to be non lethal to mice up to 30 mg/kg. The biochemical studies of some blood parameters (TP, AP, ALT, AST and BUN) revealed that both C.V and PL-I had no effect on liver and kidney functions. The histopathological studies showed that both PL-I and C.V had no hepatotoxic or nephrotoxic effects. Also no effect of both C.V and PL-I on the cardic muscles.

From the results obtained in the present study, it was concluded that the Sistrurus miliarius barbouri crude venom contained one phospholipase A2 enzyme termed (PL-I) which not lethal to mice up to 30 mg/kg and two steps chromatography were essential for its isolation in a pure form. It was also, concluded that C.V played a great role in the anticoagulant, indirect hemolysis and platelets effects, while PL-I had a slight anticoagulant, indirect hemolysis and platelet effects.