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Physicochemical Properties and Toxicity Studies on a Lectin from the Bulb of Dioscorea bulbifera

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Abstract : In this study, a lectin from the bulb of Dioscorea bulbifera was purified, characterised, and its acute and sub-acute toxicity was investigated with a view to evaluate its toxic effects in mice. The protein from the bulb was extracted by homogenising 50 g of the bulb in 500 ml of phosphate buffered saline (0.025 M) of pH 7.2, stirred for 3 hr, and centrifuged at the speed of 3000 rpm. Blood group and sugar specificity assays of the crude extract were determined. The lectin was purified in a two-step procedure- gel filtration on Sephadex G-75 and affinity chromatography on Sepharose 4-B arabinose. The degree of purity of the purified lectin was ascertained by SDS-polyacrylamide gel electrophoresis. Detection of covalently bound carbohydrate was carried out with Periodic Acid-Schiffs (PAS) reagent staining technique. Effects of temperature, pH, and EDTA on the lectin were carried out using standard methods. This was followed by acute toxicity studies via oral and subcutaneous routes using mice. The animals were monitored for mortality and signs of toxicity. The sub-acute toxicity studies were carried out using rats. Different concentrations of the lectin were administered twice daily for 5 days via the subcutaneous route. The animals were sacrificed on the sixth day; blood samples and liver tissues were collected. Biochemical assays (determination of total protein, direct bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), catalase (CAT), and superoxide dismutase (SOD)) were carried out on the serum and liver homogenates. The collected organs (heart, liver, kidney, and spleen) were subjected to histopathological analysis. The results showed that lectin from the bulbs of Dioscorea bulbifera agglutinated non-specifically the erythrocytes of the human ABO system as well as rabbit erythrocytes. The haemagglutinating activity was strongly inhibited by arabinose and dulcitol with minimum inhibitory concentrations of 0.781 and 6.25, respectively. The lectin was purified to homogeneity with native and subunit molecular weights of 56,273 and 29,373 Daltons, respectively. The lectin was thermostable up to 30 0C and lost 25 %, 33.3 %, and 100 % of its heamagglutinating activity at 40°C, 50°C, and 60°C, respectively. The lectin was maximally active at pH 4 and 5 but lost its total activity at pH eight, while EDTA (10 mM) had no effect on its haemagglutinating activity. PAS reagent staining showed that the lectin was not a glycoprotein. The sub-acute studies on rats showed elevated levels of ALT, AST, serum bilirubin, total protein in serum and liver homogenates suggesting damage to liver and spleen. The study concluded that the aerial bulb of D. bulbifera lectin was non-specific in its heamagglutinating activity and dimeric in its structure. The lectin shared some physicochemical characteristics with lectins from other Dioscorecea species and was moderately toxic to the liver and spleen of treated animals.

Keywords : Dioscorea bulbifera, heamagglutinin, lectin, toxicity
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