ABSTRACT

Scorpion venom is composed of water, mucus, enzymes, neurotoxins, low molecular weight peptides and proteins having wide range of biological activities. Low molecular weight peptides or toxins constitute more than a third of all the peptides present in the scorpion venom. They are potent and highly selective ligands for voltage gated sodium and potassium ion channels. Therefore, they are considered interesting compounds for the development of novel drugs. In the present study, two common buthid scorpions i.e. *Hottentota tamulus* (Fabricus, 1798) and *Androctonus finitimus* (Pocock, 1897) were maintained in the laboratory for venom recovery. The effect of diet on venom production was recorded by offering different prey items (i.e. grasshopper nymphs, grasshopper adults, house crickets, moths and house flies) to the scorpions. Scorpions were reared throughout the year to observe the effect of temperature on venom production. Quantity and quality of venom extracted from scorpions by manual and electrical methods were compared. Venom was subjected to high performance liquid chromatography (HPLC) for characterization of its components from which dominant fractions (prominent by peaks in HPLC chromatogram) were manually collected and dried in a concentrator. Selected fractions were further analyzed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to obtain mass finger print data. Furthermore, anti-proliferative and apoptogenic effects of scorpion venom were determined using HeLa cell lines. Results showed that grasshopper nymphs and adults were the best diet for the scorpions to get maximum yield of venom as compared to other prey types (i.e. house crickets, house flies and moths). Production of venom and activity of scorpions was found to be associated with temperature. During winter season, venom recovery was comparatively low as compared to the hottest part of year; when venom milking and activity of scorpions both were increased. Electrical method of venom extraction yielded good quality and higher quantity of venom as compared to manual method. During MALDI analysis, majority of components ranging from 3000-8000 m/z in molecular masses were detected in the venom fractions of both scorpion species. These were corresponding to short chain (3000-4500 m/z) and long chain (6000-8000 m/z) toxins that target potassium (K^+) and sodium (Na^+) ion channels respectively. In the

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay, significant decrease in the cell viability in all venom treated groups was observed as compared to the control after 24 hours. Apoptotic analysis of cells was determined using the acridine orange (AO)/ethidium bromide (EB) fluorescence staining. No significant apoptosis was observed in the control group whereas shrinkage of cells was seen in treated group (1/2 IC_{50}) after 24 hours. Apoptotic cells were marked by irregular shape or granular green AO nuclear staining. With increasing concentration of venom (2 IC_{50}), necrosis of cells was detected with orange red EB nuclear staining. Scorpion venom induced both apoptosis and necrosis of cells depending upon the concentration of venom. At lower concentration (250 ug/ml), *H. tamulus* venom caused apoptosis whereas at higher concentration (1000 ug/ml) it caused necrosis of cells. Similarly, *A. finitimus* venom caused apoptosis and necrosis of cells at lower (100 ug/ml) and higher concentration (500 ug/ml). Scorpion venom of both species displayed remarkable apoptogenic, cytotoxic and anti-proliferative effects on HeLa cells.