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ORIGINAL

Evaluating the anticancer activity of protein crude extract from *Californicus Conus*

Evaluación de la actividad anticancerígena del extracto crudo proteico de Californicus Conus

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Abstract

Aim and methods: In this study, venom proteins of cone snail (Californicus Conus) were isolated by protein precipitation. This acquired crude extract was assessed by SDS-PAGE. Biological activities (cell toxicity and gene expression) was evaluated on normal (HEK) and cancerous (HT-29) cell lines.

Results: Electrophoresis evaluation showed that the weight range of proteins was <3kDa->10kDa. Thus, this extract contain peptides and large proteins. The extract showed a dose-dependent anticancer and cell toxicity activities against HT-29 cancerous and HEK normal cell lines. However, the anticancer activity was higher than normal cell toxicity. The extract had no toxicity on normal cells in concentrations of 0.5, 1, 2, and 4 µg/ml.

Conclusions: Based on results, venom proteins of cone snail contains a suitable anticancer agents. Therefore, further study of this protein isolate is suggested in future studies.

Keywords: *Californicus Conus*, Anticancer, Toxicity, Venom, Extract.

Resumen

Objetivo y metodología. En este estudio, las proteínas del veneno del caracol cono (Californicus Conus) se aislaron por precipitación de proteínas. El extracto crudo obtenido se evaluó mediante SDS-PAGE. Se evaluaron las actividades biológicas (toxicidad celular y expresión génica) en líneas celulares normales (HEK) y cancerosas (HT-29).

Resultados. La evaluación por electroforesis mostró que el rango de peso de las proteínas era <3kDa->10kDa. Así pues, este extracto contiene péptidos y proteínas de gran tamaño. El extracto mostró una actividad anticancerígena y de toxicidad celular dependiente de la dosis contra las líneas celulares HT-29 cancerosas y HEK normales. Sin embargo, la actividad anticancerígena fue superior a la toxicidad de las células normales. El extracto no tuvo toxicidad sobre las células normales en concentraciones de 0,5, 1, 2 y 4 µg/ml.

Conclusiones. Según los resultados, las proteínas del veneno del caracol cono contienen agentes anticancerígenos adecuados. Por lo tanto, se sugiere seguir estudiando este aislado proteico en estudios futuros.

Palabras clave: *Californicus Conus*, Anticancerígeno, Toxicidad, Veneno, Extracto.

Introduction

The marine gastropods known as cone snails (*Conus*) constitute an unusually species-rich group of venomous predators, one of the largest single genera (~700 species) of living marine invertebrates. They are usually classified into three groups depending on their feeding habits: worm hunters (vermivorous), mollusk hunters (molluscivorous), and fish hunters (piscivorous)[1,2]. The venom of marine gastropods belonging to the genus Conus has yielded numerous structurally and functionally diverse peptidic components. The increase variety of bioactive agents identified in cone snail venoms is the product of the variety of molecular adaptations taken by Conus species in evolving neuroactive molecules to suit their diverse biological purposes[3]. These compounds, called conotoxins, are synthesized in the venom gland of the snails[4,5]. The other aspect of cone snails that has attracted human interest is that they can be deadly to humans like *C. geographus*[6,7], which has caused more than thirty human fatalities[8]. It has been estimated that approximately 100,000 different agents can potentially be expressed in the venoms of the entire current *Conus genus*[9,10]. To date, several conotoxins have already demonstrated potential therapeutic effects in preclinical or clinical trials[11-15]. An early focus of conotoxin discovery has been to identify and characterize novel pain modulators, which was the logical first step considering that conotoxins often target ion channels involved in pain signaling. Having said that, in recent years scientists are characterizing these conotoxins for other therapeutic properties —including antioxidant (xx), cytotoxicity (xx) and anticancer properties— which are promising agents in pharmacology. Only few studies have reported the anticancer effects of these conotoxins (xxx). In addition, many *Conus* species which have therapeutic potential are unknown, for example, *Conus. Pennaceus*. This species is one of the rare species in the world that live in the harsh ecosystem of Persian Gulf. Thus, the aim of present study was to Evaluating the anticancer activity of protein crude extract from *Californicus Conus*.

Materials and Methods

Collection of Specimen

Live specimens of *C.penneus* were collected from the Persian Gulf, Qeshm Island, Iran. Samples then were frozen at -20ºC. Then transported to the laboratory within the liquid Nitrogen container.

Venom Extraction

Frozen cone snails were smashed with hammer and the venomous apparatuses (venom bulbs and venom ducts) separated from the specimens immediately. Venom ducts cut into smaller pieces (fragmented) and mixed with cold homogenization solution (40% ACN+60% PBS+0.1%TFA) and centrifuged for 20 min at 12000 rpm (2 times). The supernatants were collected and preserved at -80 0C for further studies.

Bicinchoninic Acid (BCA) Assay

BCA assay was used for protein determinations using the Pierce BCA Protein Assay Kit (ThermoFisher) in 96-well-plate format. The assay mixture contained 200 µL of the reagent (solution A + B) and 10 µL of sample containing either fractionated conopeptides or BSA standard as well as different potential interfering reagents. Absorbance was read at 562 nm using 9 nm bandwidth using a Biotech plate reader.

SDS-PAGE

The chromatographic fractions were analyzed by 15% polyacrylamide non-reducing SDS-PAGE gel with 29:1 of acrylamide:bisacrylamide ratio . Each sample (10 µL) was mixed with an equal volume of Laemmli SDS-sample buffer (62.5 mM Tris-HCl pH 6.8, 2.0% SDS, 10% glycerol, 0.005% bromophenol blue) and boiled for 5 min using a digital dry bath (Labnet, USA), and then cooled on ice. The 15% separation or resolving gel was prepared with a acrylamide:bisacrylamide solution at a ratio of 29:1 in 250 mM Tris-HCl, pH 8.8, 5% glycerol, and 5% stacking gel in 189 mM Tris-HCl, pH 8.8. Both gel components contained 0.1% SDS. The wells were rinsed with Tricine running buffer (25 mM Tricine, 400 mM glycine, 0.1% SDS) using a syringe with a 24G 1 inch needle (Terumo, Tokyo, Japan) before loading. Electrophoresis was carried out at 30 V per gel for 3 h until the dye had run to the edge of the gel. Protein profile was visualized by silver staining.

Cytotoxicity Assay

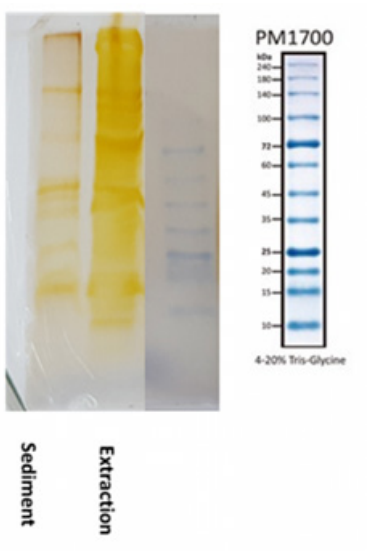
Human colone cancer cells (HT-29) and humane embryoinic kidney (HEK-293) were obtained from the Pasteur Institue, Tehran, Iran . Mentioned cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin-streptomycin at 370C in 5 % CO2 and 95% air. In vitro experiments were done at ~70% cell confluence. Cells were subcultivated using trypsin-EDTA (0.05% trypsin) in 96-well plates (Corning, USA) at a density of 30000 cells/well in 100 µL complete medium. The toxicity of venom peptides isolated from C. Penneceus was evaluated against HT-29 and HEK-239 as normal cell line using an MTT assay. The assay was carried out using 96 microtiter plates with various concentrations (0.5, 1, 2, 4, 8, 16, 32, 64 µg/µl) of each conopeptide fractions with three replicate wells. The MTT staining method was performed. After incubation period (24h), the absorbance of the formazan was read at the wavelength of 570 nm using a Biotek Microplate reader.

Results

The electrophoresis pattern of acquired extract was shown in **figure 1**. According to this figure, there are

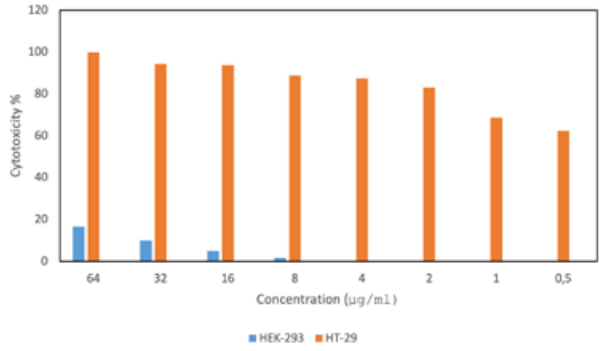
various peptides and proteins in this extract with the weight range of <3kDa to >10kDa.

Figure 1: Electrophoretic pattern of crude extract obtained from cone snail (*Californicus Conus*).



Based on BCA assay, the acquired crud extract had 54.31 mg/ml of proteins. After treatment of cancerous and normal cells with different concentrations of extract (0.5, 1, 2, 4, 8, 16, 32, and 64 µg/ml), the highest toxicity belonged to concentration of 64 µg/ml (**Figure 2**). Figure 2 showed that the extract had no toxicity on normal cells in concentrations of 0.5, 1, 2, and 4 µg/ml. On the other hand, all concentrations showed highest toxicity on cancerous cells than normal cells.

Figure 2: Comparison of the toxicity effect of protein extracts from cone snail (*Californicus Conus*) on cancer and normal cells.



Discussion

Colon cancer is the main cause of cancer-related deaths worldwide and is one of the most common malignant tumors[16]. This cancer accounts for one third of all malignant tumors in the world and is the fourth most common cause of death. This disease occurs due to the uncontrolled growth of cells that can invade other body tissues (metastasis) or multiply in those tissues[17]. Despite major advances in current treatment strategies, the survival rate is still not satisfactory[18]. Therefore, it is necessary to develop new therapeutic methods for cancer treatment. Nature has always been an attractive source for drug design research[19]. Natural sources have the potential to help discover new promising anticancer agents with low side effects[20]. Proteins and peptides may be interesting anticancer agents because they act specifically against their targets to induce apoptosis[21,22]. Different marine species are known as a potential source of compounds with different biological effects[23]. Proteins and peptides of marine origin are one of the most important of these compounds[24]. These compounds have shown various beneficial effects, including their anti-cancer effect[25]. One of the animals with potential biological agents is the cone snail of the genus Conus. Cone snails are a family of marine mollusks with approximately 700 species. It is estimated that there are more than 50,000 compounds in the venom of cone snails, but less than 0.1% have been functionally characterized. Many of these compounds target different types of ion channels[26]. However, there are only a few reports that have studied the cytotoxic effects of Conus cone snail venom on eukaryotic cells[27,28]. We examined the anticancer activity of protein crude extract from *Californicus Conus*. According to results, this extracts has potent anticancer activity on Human colone cancer cells (HT-29). Our data showed that protein crude extract from *Californicus Conus* can be considered as potent anticancer agent for treatment of colon cancer. Damsio et al. showed that the compound isolated from snake venom (*Bothrops Jararacussa*) has a lethal effect on HT-29 colon cancer cells and causes apoptosis in cancer cells through increased expression of caspase 8, BAX and other related proteins to apoptosis[29]. Fezai et al. indicated that the crude venom of lesser weaver fish causes cell cycle arrest and activation of apoptosis in HCT116 clone cancer cells[30]. Abdel-Rahman et al showed that *C. vexillum* cone venom has cytotoxic potential in EAC tumor cells by inducing oxidative stress-mediated mechanisms[31]. Magdy et al. showed thta the cytotoxic activity of Conus flavidus crude venom was confirmed through apoptotic cell death in HepG2 liver cancer cells[32]. Salimi et al showed that crude textile cone venom is selectively cytotoxic to U87MG human glioma cells, causing activation of caspase-3 and induction of cell apoptosis through mitochondrial signaling[33]. So, anticancer activity of our extract can also be related to the mentioned reasons.

Conclusion

Based on results, venom proteins of cone snail contains a suitable anticancer agents. Therefore, further study of this protein isolate is suggested in future studies.

Competing interests

All authors declare no competing interest.

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