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Original Research Article

Anticancer activity of *H. lepturus* venom and its hemolytic fraction (heminecrolysin)

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ABSTRACT

It is observed that some venom may have an anticancer effect. *Hemiscorpius lepturus* is a most dangerous scorpion in Iran. Heminecrolysin is the most toxic fraction of *Hemiscorpius lepturus* venom and it is responsible for hemolytic effects of the venom. In this research, we purified heminecrolysin by FPLC, and HPLC methods and used MTT assay in prostatic cancer cell line (PC-3 cells). We observed that *H.lepturus* venom and its fraction (heminecrolysin) had anticancer effects on prostatic cancer cell line (PC-3) and inhibited cell growth.

Keywords: *H.lepturus*, venom, heminecrolysin, prostate cancer, anticancer

INTRODUCTION

Prostate cancer is a threatening life conditions and many individuals are suffering from it [1]. Current therapeutic approaches for prostate cancer includes local treatments, such as surgery or radiation therapy in the early stage of the cancer and systemic treatments such as androgen deprivation therapy (ADT) and chemotherapy in

the metastatic stages. Provenge is a novel candidate to kill prostate cancer cells [2]. However, No certain cure has been found for the disease yet [3, 4].

Hemiscorpius lepturus, belongs to the Scorpionidae family, is the most deadly scorpion in Iran. It causes pathological manifestations like haemolysis, renal failure, necrotic ulcers, mental health problems and in some cases even death [1, 2].

In the last few years, there has been a great attention to the curative potential of the natural venoms and has opened up a new therapeutic field called venom therapy [5]. Several publications have been appeared in recent years documenting medicinal efficacy of bee venom in the treatment of diseases such as arthritis, asthma and, fibrosis. Wasp and bee venom also demonstrated as a therapeutic agent for the treatment of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Epilepsy, Multiple Sclerosis [3-10].

Research shows that some venom may have an anticancer effect on cancer cell lines for example bee venom shows an anticancer effect [6].

There is no report for anticancer efficacy of *H.lepturus* venom, yet. Regarding to the importance of venom therapeutic fractions, in this research, we examined the anticancer effect of *H.lepturus* total venom and its toxic fraction (heminecrolysin) on prostate cancer cell line.

MATERIALS AND METHODS

Venom preparation

H.lepturus scorpions were hunted in Khuzestan province. The crude venom is extracted by WHO standard protocol [7]. Water extraction of the venom was done [8]. The concentration of the purified venom was measured by spectrophotometer according to the Bradford method [9].

Heminecrolysin purification

The purification process of the hemolytic fraction of *H.lepturus* (heminecrolysin) was performed [8]. In summary, the total venom was fractionated into seven fractions by FPLC apparatus (AKTA purifier system, GE Healthcare Bio-Sciences) on a Sephadex G-50 superfine column (1.6×100 cm; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). The buffer was ammonium acetate buffer (20 mM, pH =8.5). Collected fractions were analyzed by SDS-PAGE under reducing conditions and visualized after Coomassie blue staining. Then the most hemolytic fraction was further fractionalized by HPLC using C18 reverse-phase column (C18, 250×4.6 mm). The HPLC conditions were 0.1% trifluoroacetic acid (TFA) in water as A Solution, and 0.1% TFA in acetonitrile as B solution under a 5–60% linear gradient of B solution (flow rate of 1 ml/min for 50 min). The eletions were

collected, and analyzed under reducing conditions by 15% SDS-PAGE gel. The fifth peak (P5) at 36 min showed hemolytic activity and considered as heminecrolysin. The heminecrolysin concentration was measured at 595 nm according to Bradford assay (Epoch; BioTek).

Cell line culture

Prostate cancer cell line (PC-3) was purchased from the National Cell Bank of Iran (Pasteur institute of Iran, Tehran). The cell line was cultured in DMEM medium (Gibco RL, Grand Island, NY) supplemented with 10% fetal bovine serum (10 %FBS). The cell line growth conditions were: 5% CO2, and 37 °C. For assay, all of the cells were reached to 85% confluency.

MTT assay

The cytotoxic activity of *H.lepturus* total venom, and heminecrolysin weredetermined by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay [10]. Briefly, 1×10^4 PC-3cells were cultured in 200 µl of medium and incubated in the 96 microplates at 37 °C, 5% CO2. After 4 hours, different volumes (5, 25, 50, 80, 100 µl of *H.lepturus* venom (0.06 mg/ml), and heminecrolysin (0.06 mg/ml) were added to the wells. PBS was used as negative control. The mixtures were incubated at 37°C, 5% CO2 for 72 hours. 30 µl of MTT solution was added to the wells and incubated for 4 hours at 37°C. Then 100 µl of DMSO was added to achieve the formazan crystals. Optical Density was measured at a wavelength of 575 nm.

RESULTS

The venom of *H.lepturus* was extracted by water, and characterized by SDS-PAGE gel (Fig. 1).The heminecrolysin fraction of *H.lepturus* was successfully purified by FPLC, and HPLC methods (Fig. 2 and Fig. 3). SDS-PAGE of the fifth peak (P5) on 15% reducing gel showed a 33 kDa band (Fig. 4). This peak also had hemolytic effects on mice *in vivo*. So, the P5 was called heminecrolysin.

The total venom of *H.lepturus*, and its fraction (heminecrolysin) were exhibited a cytotoxic activity on the PC-3 cells in a dose-dependent manner (Fig. 5). Data showed that heminecrolysin has more potent anticancer activity than total venom of *H.lepturus*. The results showed that the anticancer potency of heminecrolysin fraction was more significant than *H.lepturus* total venom at $p<0.05$ by T-Test.

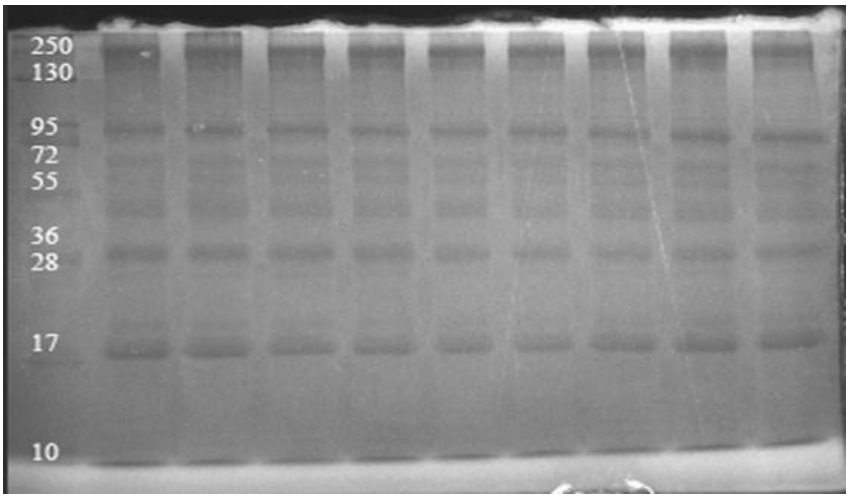

Figure. 1. Characterization of H. lepturus venom by SDS-PAGE under reverse staining using imidazole and zinc salts. The 33 kDa bands show the hemolytic fraction of H.lepturus.

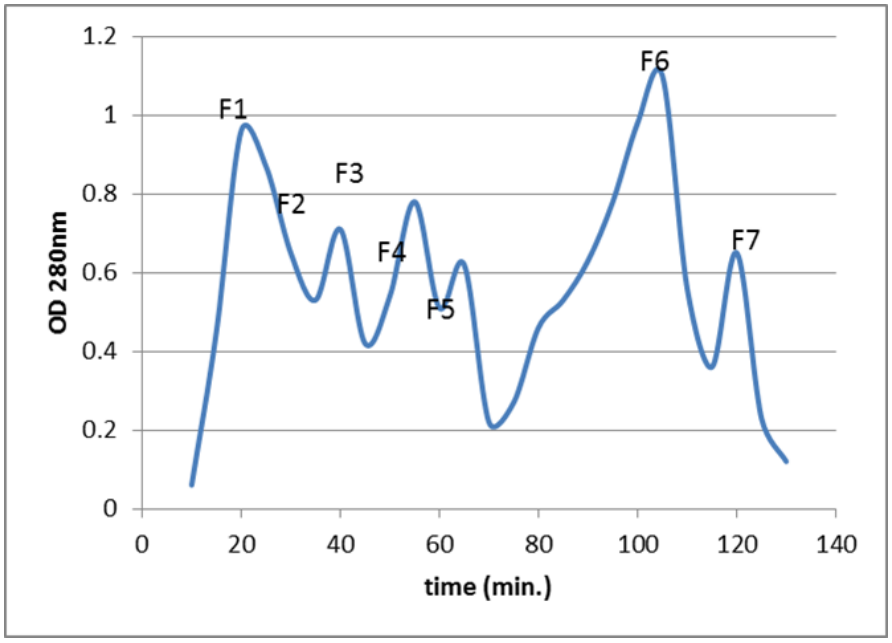

Figure. 2. Gel filtration of H.lepturus venom: Seven fractions obtained from H. lepturus total venom onto a Sephadex G-50 superfine column. (1.6×100 cm).

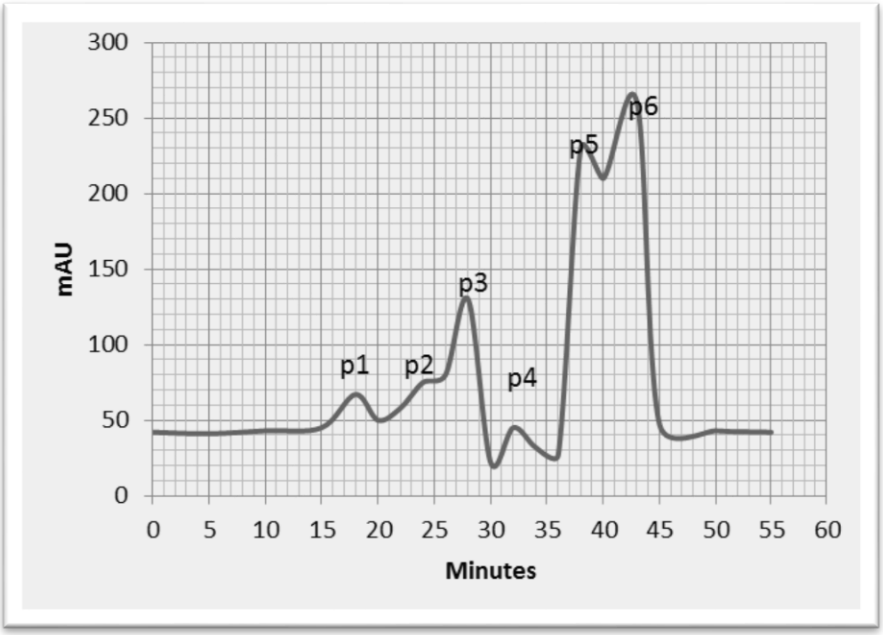

Figure. 3. HPLC chromatogram of H. lepturus hemolytic fraction (F2), the fifth peak (P5) isolated at 36 min, was considered as heminecrolysin.

Figure. 4. Lane 1: DNA ladder, Lane 2: the fifth peak (P5) of HPLC analysis. The 33 kDa hemolytic band called heminecrolysin.

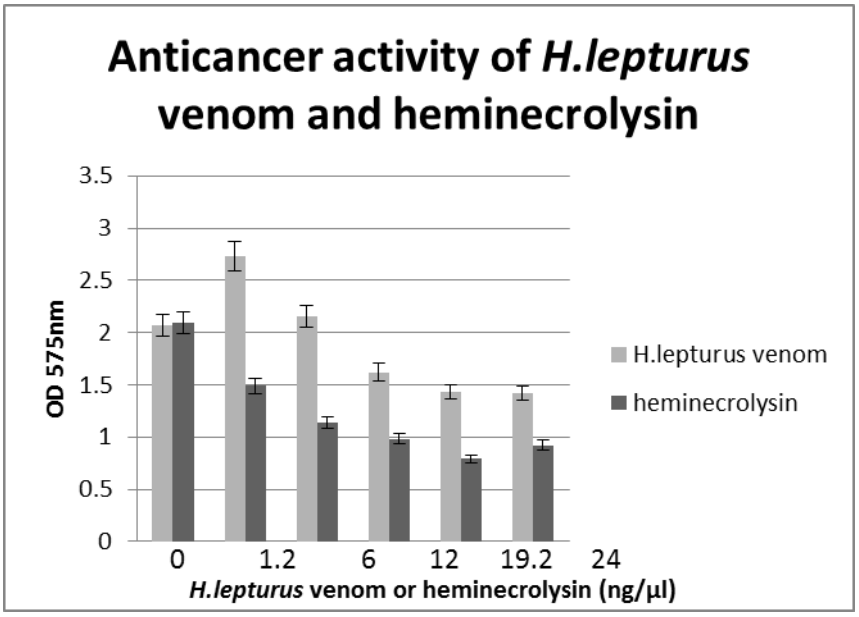

Figure. 5. Anticancer activity of H.lepturus venom, and heminecrolysin

DISCUSSION

Prostate cancer is a very dangerous disease and no obvious treatment exists yet [1]. Attempts for discovery of new anticancer strategies have been made up to now [3].

The toxic components of some creatures are considered as new therapeutic agents for cancer treatment [11, 12]. Also scorpion venoms are considered as an important source for anticancer drug discovery [13].

Hemiscorpius lepturus is the most toxic scorpion in Iran. It causes many problems such as renal failure and even death. The major toxic fraction of *H.lepturus* venom called heminecrolysin, a 33 kDa protein. Heminecrolysin is responsible for hemolytic activity of the total venom [14, 15]. In this study, we investigated anticancer activity of *H.lepturus* total venom and heminecrolysin. The data of this experiment confirmed the anticancer activity of *H.lepturus* total venom and its cytotoxic fraction (heminecrolysin) on prostatic cancer (PC-3) cell lines. -Similar studies showed the anticancer activity of bee venom and the molecular mechanism of bee venom peptides on cancer cells [6, 16]. Some studies reported anticancer potential of snake venom [11]. Many researches showed decreasing viability on PC-3 cancer cell lines by Vipera lebetina venom

(VLAIP) [17]. Snake venom toxin (SVT) decreased PC-3 Cell line proliferation and also showed an anti-apoptotic effect [18]. Mauriporin, a non-disulfide bridged peptide from the Moroccan scorpion, inhibited growth of PC-3 cancer cell line in a dose-dependent manner [19]. Venom extracted from Walterinnesia aegyptia (WEV) or with a silica nanoparticles (WEV+NP) decreased the viability on PC-3cells by MTT assay [20]. This study has opened up a new issue to find new therapeutic agents for prostate cancer from the venom of *H.lepturus*.

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