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ORIGINAL ARTICLES

# Evaluation of in-vitro bactericidal activity and anticancer effects of venom fractions of *Pseudocerastes persicus* snake

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**Abstract**: *Introduction: It was reported that snake venom contains a wide mixture of proteins and peptides with various toxicological and pharmacological effects. In recent years, the isolation of effective molecules from snake venom as a drug to treatment of some incurable diseases is considered by many biological research centers. This investigation aimed to determine electrophoretic and chromatographic patterns of Pseudocerastes persicus snake venom and in-vitro study of bactericidal and anti-cancer properties of its fractions in a human hepatocellular liver carcinoma cell line (HepG2).*

*Materials and methods: The fractions of venom were isolated and collected using preparative RP-HPLC. The Bactericidal activity of fractions in 20 µg/ml of protein concentration was investigated toward Gram-negative and Gram-positive bacteria. Antibacterial susceptibility tests were done using MTT and MIC assays. Furthermore, cell viability was measured by MTT reduction assay and confirmed with Neutral red uptake assay following exposure of HepG2 cancer cells to 20 and 40 µg/ml of protein concentrations of fractions having more antibacterial properties. Apoptotic effects were investigated using the comet assay.*

*Results: SDS-PAGE pattern of the crude venom revealed 10 major bands with molecular weight ranging from 13.2 to 99.25 kDa and the single-step separation of different fractions from the venom by HPLC resulted in isolation and collection of 11 fractions. The results of this study showed that two fractions of snake venom have significant antibacterial activity against Gram-positive as compared to Gram-negative bacteria. On the other hand, our findings indicated that one of them is not toxic to the HepG2 cells.*

*Discussion: We for the first time reported bactericidal and anticancer effects of isolated fractions of Persian horned viper venom. These properties make components of these fractions a favorable source for isolation of molecule(s) with antibacterial and antitumor activities.*

**Keywords**: *snake venom, Pseudocerastes persicus, antibacterial, cytotoxicity, cancer*

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## INTRODUCTION

One of the serious problems of the medical community in the treatment of infectious diseases caused by bacterial agents, many bacteria are resistant to common antibiotics. These bacteria are becoming a serious clinical problem around the world (Jami al Ahmadi et al., 2010). This has

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prompted pharmaceutical firms to explore new antibacterial drugs or optimize antibiotics to help them produce new and effective drugs. Nowadays poison of poisonous animals is considered by many researchers as one of the most important natural sources for the isolation and identification of antimicrobial molecules and it has been a great success.

Reports indicate that, despite that the oral and tooth smears of poisonous snakes in a wide range of pathogenic bacteria, there is a small incidence of bacterial infection in the bites of the injured patients due to snakebite (Talan et al., 1991). These observations have led the researchers to point out that antibacterial compounds are found in Snake (Deivy et al., 2005).

The first report on antibacterial activity in the venom poison the families of Alpidea and Vipride was published in 1948 and 1968 respectively (Ferreira, 1956). Also Skarnes in 1970, for the first time reported antimicrobial activity in some of the enzymes found in the venom of snakes (Aloof-Hirsch et al., 1968). Studies of Zey et al. in 2000 also led to the isolation of peptides from Naja atra, which in vitro inhibited the growth of resistant bacteria of Mycobacterium tuberculosis and showed inhibitory activity (Xie et al., 2003).

Studies show that molecular compositions and especially proteins present in the veniphil genus of the Viperidae variety are very diverse. Important compounds of these toxins include serine protease, metalloproteinase, phospholipase A2 (PLA2), C-type lectin, acetylcholinesterase, L-aminoacids, and detergents (Markland, 1997).

Recent studies have shown that many of these compounds have antimicrobial properties (Jami al Ahmadi et al., 2010; Stabeli et al., 2004; Lu et al., 2002).

In this study, the antibacterial effects of protein fractions isolated from Pseudocerastes persicus snake and the anti-cancer effects of fractions with antibacterial properties were studied.

## MATERIALS AND METHODS

### Venom preparation

To prepare the venom from the snake, the snake venom safety tips were placed directly into the venom collection jar, and the venom was briefly injected into the venous gland. The toxic solution was kept at minus 20 °C until lyophilization until use in the experiments.

### Microorganisms and cell lines used

Bacillus subtilis subsp. spizizenii a gram-positive and non-pathogenic bacteria (ATCC 6633), bacteria Escherichia coli a gram-negative bacteria (ATCC 25922), and pathogen Staphylococcus aureus a gram-positive bacteria (ATCC 25923) were prepared from the Persian Type Culture Collection (PTCC) of Iran and liver cancer cells (HepG2, C158) of Pasteur Institute of Tehran cell bank.

### Studying the crude venom electrophoresis pattern using SDS-PAGE method

To study the electrophoresis pattern of crude venom of Pseudocerastes persicus snake, 12-15% gel (Chellapandi and Jebakumar, 2008) was applied to each well of 5-10 microliters of toxic solution (containing 20 to 15 micrograms of protein) denatured with an appropriate volume of sample buffer was injected.

Standard protein was used to estimate the molecular weight of protein bands.

Electrophoresis first started at 50 V and after passing the dyeing gel through the concentrating gel, electrophoresis continued at 150V until the color of the tracing reached about 1 cm from the end of the gel cassette. After staining the gel with coma blue and removing excess dye from the gel surface, the estimated molecular weight of crude venom protein bands was determined.

### Isolation of crude venom fractions using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Fragile venom of Pseudocerastes persicus snake using RP-HPLC preparation with UV detector (220 nm absorbance), C18 SUPELCOSIC TM Pcc-18 (59185) 250mm×21.2mm, 12µm] column and Gradient Linear Application [buffer A (acetonitrile) 20% containing 0.1% TFA) and buffer B (80% acetonitrile containing 0.1% TFA) ] separated in 70 minutes. In the reverse phase method, a polar mobile and non-polar stationary phase are used and the material is separated by hydrophobicity (Steuten et al., 2007).

For the separation of crude venom fractions, 40 mg of dried powder in 20 ml of buffer (water). Containing 0.01% TFA containing 5% acetonitrile and dissolved for 2 hours at 4°C. After centrifugation, the insoluble material was removed from the suspension for 5 min at 10,000 rpm at 4°C. The supernatant was used for chromatographic patterning and fractions separation by HPLC. The corresponding fractions of each peak were collected manually in perfectly clean glassware free of any protein contamination and stored at -80°C after lyophilization. The retention time of each peak was recorded for each peak.

### Protein assay

For antibacterial and cytotoxicity tests, some of the lyophilized venoms were dissolved in sterile distilled water

twice. The amount of protein in the toxic solution was determined using Bradford (Bradford 1976) method and the protein-free fractions were excluded from further studies.

**Examination of antibacterial properties**

Culture media and antibiotics: MH (Mulberry Hinton) Company, Quelab, Canada, was used for bacterial culture. Tetracycline (Sigma, USA) at a concentration of 50 µg/ml was used to compare the effect of the venom with standard antibiotics.

**Evaluation of the antibacterial effect of fractions using MTT assay**

MTT assay is a colorimetric assay based on the reduction and breakdown of tetrazolium yellow crystals by the succinate dehydrogenase enzyme present in the cytoplasm and ultimately the formation of insoluble blue crystals. These crystals are soluble by the addition of DMSO (Hengwei et al., 2010). The bacterium was cultured in the Muller Hinton broth medium and then after 5 h when it reached the absorbance was equivalent to the absorbance of 0.5-McFarland (at 600 nm with an absorbance of 0.08 to 0.1) (Husniye et al., 2013). 5 µL per 96 plate sterile wells were cultivated. Fractions were added to wells at a concentration of 20 µg/ml and the final volume of each well was made up to 100 µL using a liquid culture medium (Muller Hinton Broth). In this experiment, tetracycline (50µg/ml) was used as a positive control, bacterial suspension and culture medium as a negative control, and non-bacterial culture medium as blank. The plate was incubated in the incubator for 23h at 37°C. Then 5µl MTT (5µg/ml concentration) was added to all wells. The plate was incubated at 37°C under dark conditions for one hour. Then 100 µL of DMSO was added to each well. After 2 hours of incubation in dark conditions, the absorbance of the wells at 595 nm was measured using a plate reader (Biotek, USA). The above experiment was performed 3 times and 3 wells (3 times repeated) were considered for each concentration. Bacterial viability after exposure to different concentrations of venom was calculated using the following formula (Husniye et al., 2013).

$$100 \times (a/b) = \text{Bacterial vital percent}$$

a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

**Evaluation of antibacterial effect of fractions using MIC assay**

In this study, we aimed to determine the inhibitory effects of crude venom on bacteria. The steps of the minimum inhibitory concentration test (Shebl et al., 2012) were similar to the MTT assay but after incubating the bacteria with different concentrations of the venom at 37 ° C for 24 hours without adding any biomarkers, the optical absorption of the wells at the wavelength of 605 nm was measured using a plate reader (Biotek, USA). The experiment was repeated 3 times and for each concentration 3 wells (3 times). The percent inhibitory effect of the standard venom and antibiotic effect was calculated using the following formula (Shebl et al., 2012).

$$100 \times (a/b)]\text{-}1[= \text{Inhibitory percentage of bacteria}$$

a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

**Anti-cancer properties of fractions**

*Cell culture*:

To eliminate possible contamination in the toxic solution required by cellular tests, 1% antibiotic-antimycotic (Invitrogen, USA) was added and used in experiments at 4°C overnight.

DMEM-F12 medium (Gibco, USA) containing 10% FBS (Fetal Bovin Serum) prepared from the company (Gibco, USA) was used for HepG2 cell culture. These cells were cultured in flasks containing 25-50 ml cube and stored at 37°C and 5% carbon dioxide. Replace the old culture medium every 2–3 days, and after providing the cell population required for each experiment using trypsin-EDTA prepared from Sigma-Aldrich, USA, isolated from the flask and after counted with neobars lam were used.

**Evaluation of cytotoxicity of fractions by MTT assay**

This procedure was performed according to the method of Zargan et al. (Zargan et al., 2011). For this test, 3×104 cells were cultured in 96 well plates containing 100 µl serum-free culture. After overnight incubation at 5% CO2, humidity about 80%, and 37°C, the old culture medium was discharged and the new serum-free medium containing different concentrations of 20 and 40 µg/ml of the fraction (With antibacterial properties) were added to the wells. The plate was incubated for 23 hours after incubation with the venom cell. Then 5 µL of MTT solution (5 mg/ml) was added to each well. After the formation of dark blue crystals (Formazone), the contents of each well were removed and after washing with PBS, 100 µL of DMSO was added. In this test, the culture medium was used as control and the cell culture medium as control. Optical absorption of wells at 570 nm was measured using a plate reader (Biotek, USA). The experiment was performed 3 times and 3 wells (3 times) for each concentration. The percentage of cell viability after exposure to different concentrations of venom was

calculated using the following formula (Shebl et al., 2012).

$$\% \text{ Viable cell} = (a/b) \times 100$$

a = The absorbance of treated cells - The absorbance of the blank

b = The absorbance of the control - The absorbance of the blank

**Evaluation of cytotoxicity of fractions by Neutral red uptake assay**

The steps of Neutral red uptake assay (Abdul et al., 2013) were similar to MTT assay but after incubating the cell adhered to the toxic solution for 23 h, one microliter of Neutral red solution (5 mg/ml) was replaced with MTT solution. Ml) was added to each well. The plate was then incubated under dark conditions at 37°C, with 5% $CO_2$ and about 80% moisture until red crystals (neutral red with cell lysosome surface). Then, the solution in each well was drained and after washing each well with PBS twice, 100 µl of fixative buffer (37% formaldehyde, 10% purchase). After one minute, this buffer was evacuated and 100 µL of the solvent buffer (0.5% acetic acid) was added. Plates were then incubated on a shaker for 20 minutes in dark conditions at laboratory temperature and optical absorption of wells at 540 nm was measured using a plate reader (Biotek, USA). The experiment was performed 3 times and 3 wells (3 times) for each concentration. Inhibitory percentages induced by different concentrations of venom on cell growth were calculated using the following formula.

$$100\times (a/b)]\text{-}1 \text{ [= Percentage of cell death}$$

a = The absorbance of treated cells - The absorbance of the blank

b = The absorbance of the control - The absorbance of the blank

**Evaluation of apoptosis induction by fractions using comet assay**

Comet assay or single-cell gel electrophoresis is one of the best tests for DNA fragmentation in the cell (Mir et al., 2013). For this experiment, 12×104 cells were cultured in a sterile 24-well plate containing 300 µl of serum-free medium. Plates were incubated overnight under 5% CO2, 80% humidity, and 37°C. In this test, the culture medium was used as blank and the cell culture medium was used as control. The primary culture medium was then added to each well of the test well, drained, and 300µL of a new medium (without serum) containing different concentrations of 20 and 40µg/ml of fractions (with antibacterial properties). The plate containing the cell was incubated for 24 hours at 37°C, 80% humidity, and 5% CO2. After the end of the mentioned time, cells in each well were separated by trypsin and collected in a 1.5ml tube. The microtubes containing the cells were centrifuged for 4 min at 4°C at 1500 rpm and the supernatant was discharged. The cells were washed with 400 µl of PBS (pH = 7.4) and washed after centrifugation with the supernatant and then added to 200 µl of PBS. The slides required for testing were coated with normal melting point agarose (NMA% 1), and the cell suspension with low melting point agarose (LMA% 1) was compared to one to one. Two mixtures were poured onto the slides, one slide was placed on each slide to create a cell layer, and all slides were lysed in the lysis buffer to lyse the cell and nucleus membranes. (Fresh 2.5M NaCl, 100mM EDTA, 10mM Tris, 0.2M NaOH and Triton X-100 1% pH=10) were cooled and cooled in the refrigerator for 16–18 h. After removing the sliding buffer, slides 20 the cells were washed for 2 min with electrophoresis buffer (300 mM NaOH, 1 mM EDTA, and pH> 13) and then incubated in cold electrophoresis buffer for 40 min and then incubated in the refrigerator. The electrophoresis was carried out in an electrophoretic tank containing buffer and electrophoresis was performed at 4 ° C for 45 minutes under 25 V and 300 mA current. Samples were placed in a neutral buffer (Tris 400mM, pH =7.5) for 10 min to neutralize the playing environment. For staining, cells were added to each slide with 100 µL of ethidium bromide solution at a concentration of 20µg/ml for 10 min at room temperature. The slides were then washed twice with distilled water for 10 minutes and studied by fluorescent microscopy. At least 100 cells of DNA were obtained for each sample and the results were statistically analyzed.

**Statistical analysis**

The results of each test were reported as Mean ± SD and the data were analyzed using software (GraphPad InStat). Different concentrations of venom were compared to the control group and compared to each other by one-way ANOVA and Tukey tests. $P<0.05$ was considered significant in all tests. Draw charts in the Microsoft Excel software (2013 version).

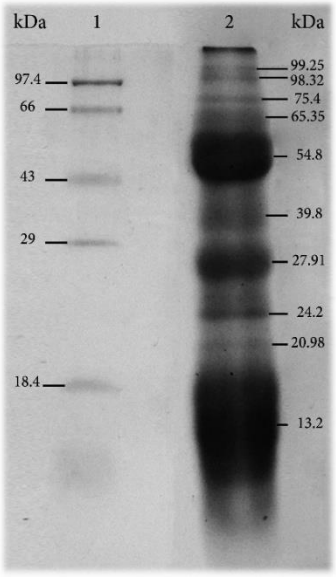
**RESULTS**

**Results of crude venom electrophoresis and chromatography results**

The molecular weights of the bands were determined using standard protein diagrams. SDS-PAGE (15% gel) results for 10 protein bands with molecular weight of 13.2, 19.6, 24.2, 27.91, 39.8, 54.8, 65.35, 75.4, 98.32 and 99.25 KDa Showed (Figure 1). The results of RP-HPLC separation of crude venom fractions showed 11 distinct peak peaks (Figure 2). The

fractions collected were named F1 to F11, respectively, at the time of departure from the column. The fractions corresponding to each peak were collected in perfectly clean and non-contaminated glass and stored at -80 ° C after lyophilization. The retention time of the product corresponding to each courier is given in Table 1.

**Figure 1:** SDS-PAGE pattern (15%) of venom of the Pseudocerastes persicus snake
Column 1: Standard Protein; Column 2: the venom of the Pseudocerastes persicus snake




Protein assay: The results showed that fractions 1, 2, and 11 lacked proteins that were excluded from subsequent studies (Table 2).

**Results of antibacterial tests**

*Results of antibacterial effect of fractions using MTT assay:*

The results showed that fractions 3, 4, 7, and 9 had no inhibitory effect on Escherichia coli. But fractions 5, 6, 8, and 10 showed antibacterial effects, respectively, with the bacterial viability percentage being 81.55, 81.2, 69.87, and 71.45 % respectively. Statistical analysis showed that recent fractions had a significant inhibitory effect on bacterial growth compared to the negative control (normal culture medium containing bacteria) but their mortality rate was not significant compared to tetracycline as a positive control (Figure 3).

**Figure 2**: Chromatogram of unicorn raw venom of the Pseudocerastes persicus snake based on the results of R-HPLC Fractions of raw venom of Pseudocerastes persicus snake using R-HPLC preparation with a UV detector (220 nm absorbance), C18 column, and linear gradient program (buffer A: acetonitrile 20% containing 0.1% TFA and buffer B: acetonitrile 80% containing 0.1% TFA percentage was separated. The fractions corresponding to each peak were manually collected in the non-contaminated glass and the retention time of each peak was also recorded. The fractions collected were named F1 to F11 at the time of exit from the column, respectively.

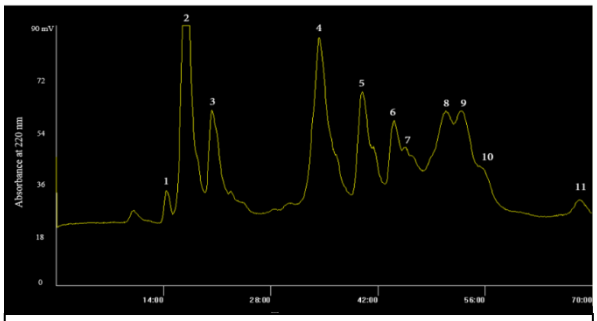



**Table 1**: Exit time of each component from column (in minutes)

| Start time and end of peak | Fractions | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 |
| Start | 13:50 | 15:36 | 19:22 | 31:14 | 38:27 | 52:51 | 45:01 | 47:50 | 51:59 | 54:56 | 67:06 |
| End | 15:01 | 19:22 | 24:35 | 38:27 | 42:51 | 45:01 | 47:50 | 51:59 | 54:56 | 59:18 | 69:78 |

**Table 2**: Protein content of fractions isolated from the venom

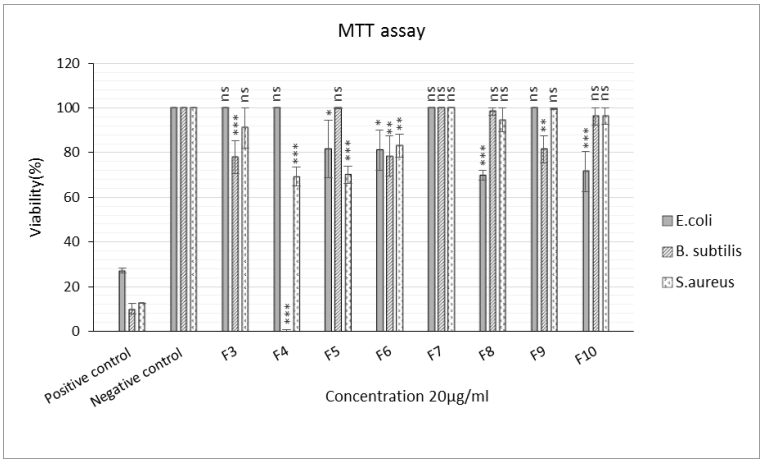
| Protein concentration (mg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fractions | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 |
| Protein concentration | 0 | 0.0075 | 1.55 | 11.77 | 10.77 | 6.29 | 1.93 | 12.85 | 11.35 | 4.5 | 0 |

Also, test results on Bacillus subtilis showed that its vital percentage in media containing fractions 3, 4, 5, 6, 7, 8, 9, and 10 were 78.41, 0.35, 99.91, 81.27, 100, 98.94, 81.15, and 97.49 % respectively. Fractions 3, 4, 6, and 9 had a significant inhibitory effect on the growth of this bacterium compared to negative control of growth inhibition, among which Fraction 4 had more inhibitory effect than standard antibiotic (tetracycline) (Figure 3).

The viable percentages of Staphylococcus aureus in media containing fractions 3, 4, 5, 6, 7, 8, 9 and 10 were 91.58, 69.23, 70.47, 81.38, 100, 96, 99.76, and 95.76 % respectively.

Statistical analysis of data showed that fractions 4, 5, and 6 had a significant inhibitory effect on bacterial growth compared to the negative control. The highest inhibitory effect of fractions isolated from crude venom belonged to fraction 4 with a 30.77% inhibitory effect, which was significantly less than that of tetracycline (Figure 3).

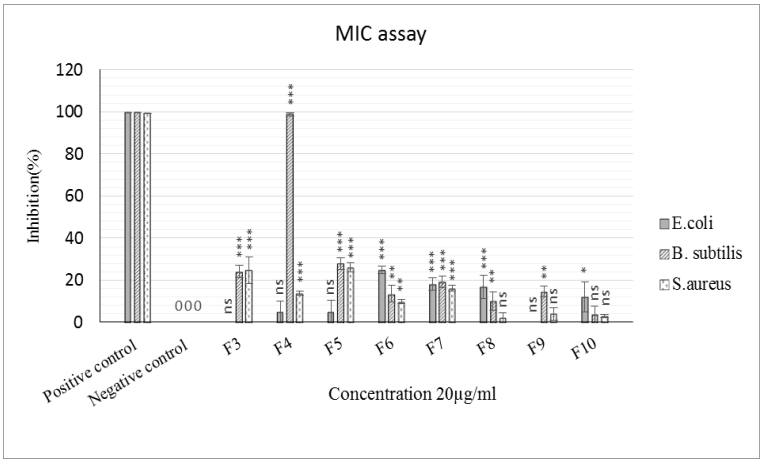
**Figure 3:** Critical percentages of Escherichia coli, Bacillus subtilis, and Staphylococcus aureus after exposure to 20 µg/ml protein concentration from different raw venom Pseudocerastes persicus snake fractions based on MTT assay. The effects of fractions were evaluated in comparison with the negative control group




*Results of antibacterial effect of fractions using Minimum Inhibitory Concentration Method (MIC assay)*

The inhibitory effects of the fractions isolated at 20 µg/ml on bacterial growth showed that fractions 3 and 9 did not affect Escherichia coli, but fractions 4, 5, 6, 7, 8, and 10, respectively 5.21, 3.48, 25.2, 17.71, 17.8 and 13.06 % had an inhibitory effect on bacterial growth. Statistical analysis showed that fraction 6 had more inhibitory effect than other fractions but this effect was not significant compared to standard antibiotics (Figure 4).

**Figure 4:** Percentage of Inhibitory Effect of Protein Fractions (20 µg/ml Concentration) of raw venom Pseudocerastes persicus snake on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus by MIC Test. The effects of fractions were evaluated in comparison with the negative control group




The results showed that fractions Nos. 3, 4, 5, 6, 7, 8, 9, and 10 in Bacillus subtilis were 24.06, 98.46, 28.77, 13.14, 17.88, 12.30, 13.76, and 2.61, respectively produced inhibitory effects. Statistical analysis showed that mortality caused by the effect of fractions 3, 4, 5, 6, 7, 8, and 9 on bacteria was significant compared to the negative control. It is noteworthy that the inhibitory effect of fraction 4 on the growth of this bacterium was much greater than that of the positive control (tetracycline), but this effect was not significant (Figure 4).

Also, fractions 3, 4, 5, 6, 7, 8, 9, and 10 were found to be effective on the growth of Staphylococcus aureus and were

25.46, 13.85, 25.88, 9.85, 15.88, 2.28, 4.74, and 3.03%, respectively. percent inhibitory effect on it. It is noteworthy that the inhibitory effect of fractions Nos. 3, 4, 5, 6, and 7 on this bacterium was significant compared to negative control but not significant compared to standard antibiotics (Figure 4).

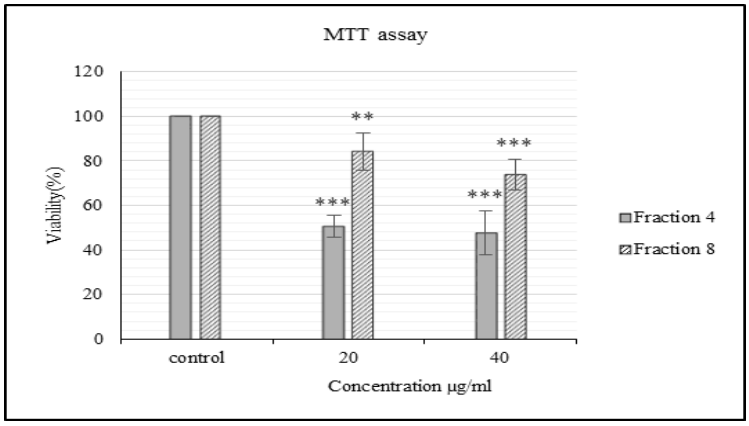
**Results of anti-cancer properties of fractions**

Summary of the results of the antibacterial effects of the fractions isolated from the crude venom of Pseudocerastes persicus snake and statistical analysis of data showed that fractions 4 and 8 had more inhibitory effects on the tested bacteria compared to the other fractions. Therefore, in this study, the anticancer effects and their cytotoxicity were considered.

**MTT assay for cytotoxicity test results**

Evaluation of cytotoxic effects of fractions 4 and 8 isolated from crude venom on the growth of liver cancer cells at two concentrations of 20 and 40 µg/ml showed that viable percentage of cells affected by fraction 4 at two concentrations compared to control, respectively, 51.55% and 50.62%. However, cell viability after exposure to fraction 8 increased at above concentrations and reached 82.29 and 74.81, respectively. Statistical analysis of data showed that the effects of fractions Nos. 4 and 8 on cell growth inhibition at two concentrations of 20 and 40 µg/ml were significant compared to control, but the inhibitory effects of the two fractions were not significant (Figure 5).

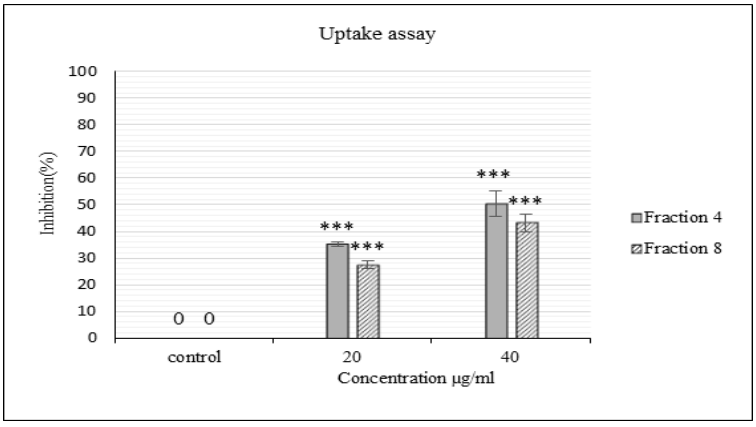
**Figure 5**: Vital percentages of liver cancer cells after 24 h exposure to 20 and 40 µg/ml protein concentrations of raw venom Pseudocerastes persicus snake fractions based on MTT assay. The effects of fractions were evaluated in comparison with the negative control group




**Evaluation of cytotoxicity of fractions by Neutral red uptake assay**

The results of the cytotoxicity study of fractions 4 and 8 using Neutral red assay showed that mortality from fraction 4 at two concentrations of 20 and 40 µg/ml in cells was 35.19 and 50.93, respectively. The percentages in the case of fraction 8 were 82.29 and 74.81 percent, respectively, which were significant compared to the control (Figure 6).

**Figure 6**: Mortality rate of liver cancer cells after 24 h exposure to different concentrations of raw venom Pseudocerastes persicus snake based on Neutral red uptake dye method. Mortality from the effect of each concentration was compared to the control group



**Results of fractions induction of apoptosis using comet assay**

The results of the comet assay showed that fraction 4 in two concentrations of 20 and 40 µg/ml had 39.31% and 38.97% and fraction 8 in the above two concentrations was 24.73% and 25.34% apoptotic, respectively. Created liver cancer cell that was significant compared to control (Figure 7–9).

**Figure 7**: Apoptosis rate induced by Pseudocerastes persicus snake Fragment Fraction No. 4 in Liver Cancer Cells Based on Comet Assay Test Results. The effect of each concentration was evaluated in comparison with the control

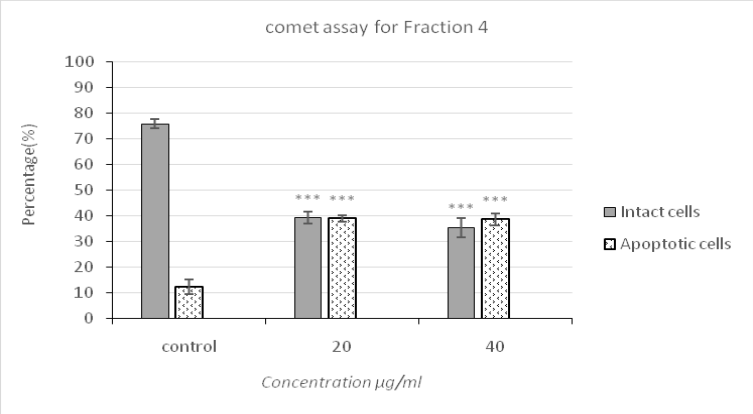
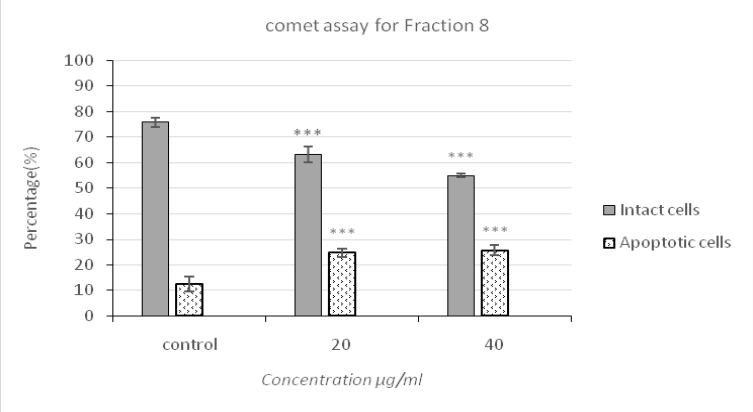



**Figure 8**: Apoptosis rate induced by a fraction of Pseudocerastes persicus snake number 8 in liver cancer cells based on Comet assay results. The effect of each concentration was evaluated in comparison with the control



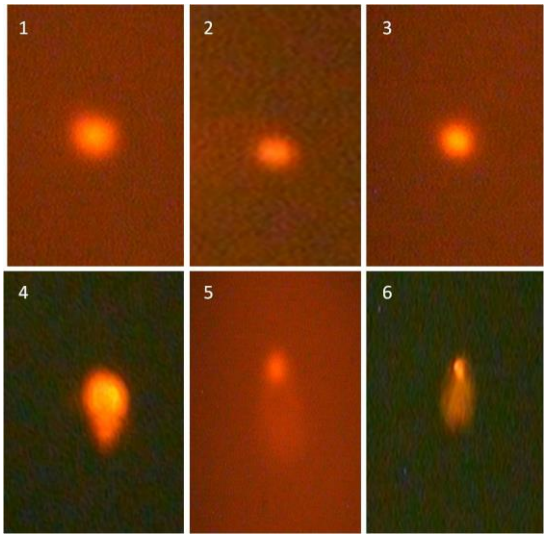




**Figure 9**: Morphological changes of DNA (40%) in liver cancer cells exposed to raw venom Pseudocerastes persicus snake fractions compared to control cells based on comet assay. [1-3: DNA image of healthy cells (40%), 4-6: DNA image of an apoptotic cell (40%)]

## DISCUSSION AND CONCLUSION

Snake venom is a complex of molecules with different biological activities, which can be of great interest in medical and biological research (Suntravat et al., 2010). It was first carried out by researchers over 40 years ago (Master and Rao, 1961). Since the proteins and peptides present in snake venom have a wide range of molecular size as well as biochemical properties, proteomic analysis can be very important in the identification and isolation of these compounds (Phizicky et al., 2003). In the present study, a combination of electrophoretic and chromatographic methods was used to investigate the protein profile of venom. The chromatographic pattern of the venomous snake venom was studied in 2004 by Mehriyar Amini Inst using Sephadex G-50 gel filtration and 6 fractions were isolated from the raw venom (Amininasab et al., 2004).

In this study, the RP-HPLC method was used to evaluate the toxicity of the chromatographic pattern. Finally, 11 fractions were isolated and collected. Protein assay results showed that only 8 fractions of the separated fractions contained protein. In addition, our findings showed 10 protein bands in the electrophoretic pattern (SDS-PAGE). The discrepancy in the results of the electrophoresis pattern with the chromatography pattern is probably because some proteins are close to each other and are collected under a fractionation during separation.

Previous studies have shown that the pattern of electrophoresis and chromatography of different snakes is different (Nawarak et al., 2003; Rioux et al., 1998; Fry et al., 2003; Mehdizadeh-Kashani et al., 2012; Bhaskar et al., 2011). In many of these studies, by separating different components of the venom, proteins and peptides were isolated. Have been shown to have excellent therapeutic properties that can have broad implications for medicine (Barker et al., 2000; Zhao et al., 2001; Marcinkiewicz et al., 2003; Shuting et al., 2004).

The alarming growth of infectious diseases caused by bacterial resistance has prompted more research in natural resources, including the venom of various toxic animals such as snakes to discover more effective and newer antibiotics.

Based on the results of this study, it was found that the raw venom of Pseudocerastes has antibacterial properties. Also, the results showed that the raw venom of this snake against high gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) has high inhibitory effects. And against Escherichia coli only the highest concentration used (400 µg/ml) has inhibitory effects. Therefore, in this part of the study, we tried to investigate the components of raw venom, isolation, and activity of each fraction against the mentioned bacteria. MTT redaction and MIC assay methods were used for this purpose.

The results showed that fraction 4 had the most inhibitor effects on the growth of gram-positive bacteria. The inhibitory effect of this fraction was 99% on Bacillus subtilis and 31% on Staphylococcus aureus. Fraction No. 8 with 30% inhibition had the most inhibitor effect on the growth of Escherichia coli.

These results are quite consistent with the data obtained from the study of the antibacterial effects of the raw snake venom listed on these bacteria. If these fractions were selected as antibiotics for use in animals or humans, their cytotoxicity on the cell should be assessed. Therefore, the cytotoxic effects of selected fractions (with antibacterial properties) were evaluated using MTT assay, Neutral red uptake assay, and Single cell gel electrophoresis (Comet assay) on liver cancer cell line (HepG2 cell line). This part of the study showed that fraction 8 induced the least toxicity in the cell. This property introduces this fraction as a suitable candidate for the isolation of effective molecules with antibacterial properties that have the least side effects on human cells. Fraction No. 4 also induced the greatest effect on cancer cells, which, according to Comet assay results, is most likely via induction of apoptosis. Therefore, this fraction is a suitable candidate for use in drug discovery. Will be anti-cancer. Previous studies on snake venom have also shown that their venom has anti-cancer properties (Yamileth et al., 2003).

In summary, it can be concluded that the results of the present study have the potential of fraction 8 for further study as an antibiotic and fraction 4 as an anti-cancer drug.

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