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Original Research Article

Anti-tumor activity of Iranian cobra snake (*Naja oxiana*) venom on lung cancer cell line

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ABSTRACT

Drug discovery from the venom of venomous animals is one of the marvelous and cost effective ways.Snake venom is one of the important sources of drug discovery. There are various active biomolecules in the snake venom. *N.oxiana* venom fractionated and cytotoxic effect of each fraction was examined by MTT assay. Antitumor activity of *N. oxiana* venom fractions were evaluated using TC-1 tumor bearing mouse. Results indicate for anti-tumor activity of some fractions of *N. oxiana* venom on TC-1 tumor proliferation and growth. Taken together, observed results indicate for potential of *N. oxiana* as drug discovery source for cancer therapy.

***Keywords*:** Snake venom, lung cancer, *Naja oxiana*

INTRODUCTION

Due to increasing rate of cancer, discovering drug that target cancer factors is very worthwhile .There are various mixture of biomolecules in snake venom [1]. It has been shown that some molecules in snake venom like Metalloprotease, Disintegrins, Phospholipases A_2, C-type lectines and L-amino acid oxidases have anti-angiogenesis activity [2-4].Vascular apoptosis inducing factor is other molecule in snake venom with anti-angiogenesis activity [2-4]. *Naja oxiana* also known as Caspian cobra is a member of *Elapidae* family and distributed in Central Asia. In Iran *Naja oxian* is found in

northeastern [5]. Some snake venom component showed anti-tumor activity on different type of tumor cells [6-10]. Cobra snake venom has been widely used in various researches for treatment of cancer. Jokhio R et al. in their study showed that Cobra snake venom resulted in reduction of level of tissue nucleic acid in human breast cancer [11] .In study of Feofanov AV et al. Cytotxins isolated from the venom of Cobra snake showed anti-cancer activity [12] Cysteine-rich secretory protein isolated from the venom of *Echis carinatus sochureki* showed antiangiogenesis activity on endothelial cells [13]. To the best of our knowledge there is no study describing anti-cancer activity of Iranian cobra snake venom on lung epithelial cancer cells. Therefore ,evaluation of anti-cancer activity of Iranian Cobra snake venom (*Naja oxiana*) on lung epithelial cancer cells was the main aim of our study.

MATERIALS AND METHODS

Sample preparation

Naja Oxiana crude venom was fractionated by Fast protein liquid chromatography and superdex 200 column (GE Healthcare Life Sciences) as previously described [14]. Concentration of each fraction was determined using Coomassie (Bradford) Protein Assay Kit (Thermo Fisher Scientific). Bovine serum albumin used as standard in concentration analysis. Optical absorbance of samples was measured at 595 nm using a 5000-CT Spectrophotometer.

Toxicity analysis

The TC-1 cells (C57Bl/6 mouse lung epithelial tumor cells) (ATCC: CRL-2785) was from National Cell Bank of Iran (Pasteur Institute of Iran) [15].The cells cultured in Roswell Park Memorial Institute 1640 (RPMI 1640, Gibco) medium and 10 % Fetal bovine serum (FBS, Gibco) as supplement. The cells incubated at 37^{o}C and 5 % CO_2 for 48 h and every two days the medium changed and replaced with fresh one. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to test toxicity of *Naja oxiana* venom on TC-1 cells. After confluency reached to 90 %, TC-1 cells trypsinized (Trypsin-EDTA (0.25 %), phenol red, Gibco) and counted. About 2.5 10^4 cells seeded on 96-well plate. Various concentration of *N.oxiana* crude venom and fractions (0-1000 µg/ml) with complete medium (RPMI 1640 and FBS 10 %) added to the wells and incubation carried out for 24 h at 37^{o}C and 5 % CO_2. Then, the medium removed and about 50 µl of MTT (5mg/ml in PBS) added to the wells and incubated for 4 h at same condition at dark. Formed dye dissolved in 100 µl of Dimethyl sulfoxide (DMSO,

SinaClon BioScience) and immediately the absorbance was measured at 570nm.

Animal study

Female C57BL/6 mouse (six weeks old) (Pasteur Institute of Iran) were used for anti-cancer activity of *N.oxiana* venom. All animal experiments approved by ethic committee of Pasteur Institute of Iran. About 10^6 TC-1 cells in 200 µl PBS were injected into shaved right flank of the mice (subcutaneous, S.C, rout of administration). Tumor size was measured after 2 weeks through following equation: Volume =Length × (Width) 2 × 0.52 [16]. The mouse TC-1 tumor models (tumor volume=150mm3) divided into ten groups (n=6). Injection of crude venom and fractions (50 µg in 200 µl PBS) were performed near to the tumor site through S.C route of administration. Injections were accomplished once a week for three weeks .One hundred microliter of PBS injected to control group.

Statistical analysis

GraphPad PRISM V.5.0 was used for Statistical analysis. Each group analyzed by Unpaired Student's t-test. The $p<0.05$ expressed as Statistical significant.

RESULTS

Cytotoxicity results

MTT results demonstrated cytotoxic effect of *N.oxiana* and fractions on TC-1 cells. IC_{50} of each fraction calculated using GraphPad Prism software. MTT results indicate dose and time-dependent effect of *N.oxiana* venom on TC-1 cells. The fraction numbers of 1, 6 and 7 showed the highest cytotoxicity on proliferation of TC-1 cells.

Animal study results

Sixty TC-1 tumor-bearing C57BL/6 mice randomly placed in ten groups. About 50 µg of *N.oxiana* crude venom and fractions were injected adjacent to the tumor once a week. Treatment study continued for three weeks and before each injection, the tumor volume was measured. The fraction numbers of 1, 6 and 7 showed the highest anti-tumor activity and tumor volume of mice receiving mentioned fractions increased slowly than other groups (Figure 1). Surprisingly, in animal group that injected by fraction 2 the tumor volume was higher than control group (PBS receiving group) at day 21.

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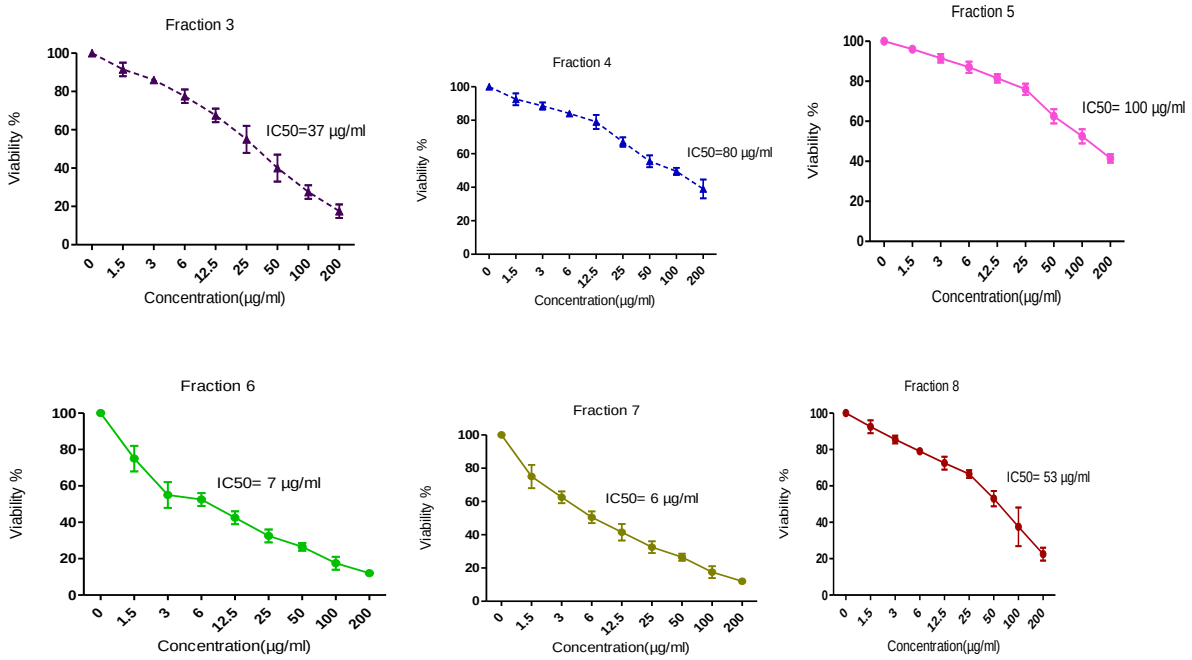

Figure 1. Cytotoxicity results: Various fractions of *N.oxiana* showed different cytotoxic effect on TC-1 cells. The graph indicates for mean of triplicate assay± SD.

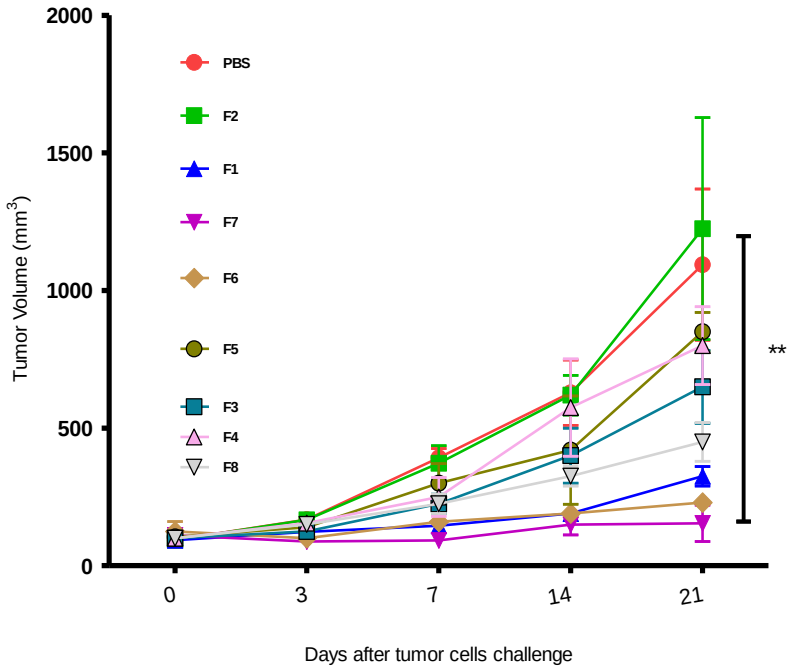

Figure 2. Animal study results: Treatment of TC-1 tumor bearing C57BL/6 with different fractions of *N.oxiana* reveled anti-tumor effect of fraction 1, 6 and 7. Error bar indicate for mean± SD .(**, P<0.01).

DISCUSSION

Lung cancer is one of the leading causes of mortality worldwide [17]. Because of importance of lung cancer around the world, it seems necessary to discover drug from natural sources like venom of venomous animals. In this study for the first time, we evaluated cytotoxic and anti-tumor activity of Iranian cobra snake venom on lung epithelial cancer cell line (TC-1 cell line, ATCC: CRL-2785). To this goal, *N.oxiana* venom fractionated by Fast protein liquid chromatography (FPLC) and superdex 200 (S-200) column. The crude venom as well as all eight collected fractions showed cytotoxic effect on TC-1 cells with different IC_{50}. The most inhibitory effect of *N.oxiana* venom on TC-1 cells were observed in case of crude venom, fraction number of 1, 6 and 7. However, cytotoxic effect of *N.oxiana* on TC-1 cells has been previously evaluated by MTT assay in our lab (unpublished data). Here we confirmed cytotoxic and anti-tumor effect of *N.oxiana* on TC-1 cells in *in vivo*. The TC-1 tumor-bearing C57BL/6 mouse was developed and anti-tumor activity of *N.oxiana* crude venom and fractions were studied. Animal study (*in vivo*) results confirmed MTT assay (*in vitro*) results. The fractions of 1, 6 and 7 showed highest inhibitory effect on TC-1 tumor growth. After 21 days of tumor challenge, tumor volume only reached to 250 mm^3 in group treated with fraction 1, 6 and 7. However, in group treated by PBS or fraction 2 the tumor volume reached to 1000 mm^3. Such observed effect in some fractions may relate to existence of venom growth factors that results in proliferation and growth of tumor cells [18,19].

CONCLUSION

Anti-tumor activity of Iranain *N.oxiana* on Tc-1 tumor cells was evaluated using TC-1 tumor bearing C57BL/6 mouse. *N.oxiana* venom significantly inhibited tumor proliferation and growth. The results indicate potential of *N.oxiana* venom as promising source for cancer therapy.

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