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An in vitro study on Hemiscorpiuslepturus (scorpionida: Hemiscorpiidae) venom cytotoxicity effects on K562 cells.

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ABSTRACT

Objective of this study was to evaluate the capacity of *Hemiscorpiuslepturus* venom on K562 cell lines which had been derived from human chronic myeloid leukemia (CML). After calculating the concentration of protein venom by Bradford method, the cells were treated with *H.lepturus* venom using an increasing rate of concentrations during a 24 hour incubation period. Inhibition of CML growth was assessed by MTT assay. IC50 was determined about 14µg/ml.
Keywords: *Hemiscorpiuslepturus,* Venom, MTT, IC50, K562 cells

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INTRODUCTION

Cancer is considered as an ancient health problem of mankind being. It is also believed that cancer has been being before the first humans walked on the Earth and it is a major worldwide health problem. World Health Organization (WHO) reports that approximately 14 million new cases and 8.2 million cancer-related deaths in 2012 and it is expected annual cancer cases will rise to 22 million within the next two decades.[1, 2]

In Iran, cancer is the third cause of death after cardiovascular disease and trauma.[3]

There have been being different methods to cure the cancer with different degrees of successfully and failure to treat the disease.

Evaluation of therapeutic course from cancer is very unsteady. The earliest modern treatment of cancer referred to surgery approaches which continue so far. Hormone therapy ideas advanced in 19th century. In 1896 X-ray cause remarkable developments in world of science and shortly begin the use of this for diagnostic and treatment of cancer. As soon it was discovered that radiation could cause cancer as well as cure it. In the continue of establishment the many hypothesis for cancer therapy such as chemotherapy method was used but it has very side effects.[4]

Following those methods, drug discovery has been developed, advanced and taken to cure cancers. According to development of the disease and its control methods using natural products have been concerned to remove the cancers. In this way , searching for therapeutic purpose made attention to animal venoms.[5]

Using of animal venoms is recently to be interested by scientists to inhibit the cancerous cells. Scorpions are one of these animals which their venoms are employed to remove the cancer cells. Scorpions are producing a different type of venom which is composed of 50–100 different toxic polypeptides.[6]

Many active principles produced by scorpion venoms including inhibitor protein synthesis and inducing apoptosis have been employed in the development of new drugs for the treatments of diseases including cancers. Scorpion toxins are a promising approach to fight cancer, since they have shown both *in vitro* and *in vivo* effects on cancer cells, as well as in phase I and phase II clinical trials.[6]

A study by Fu*et. al* (2007) showed that toxin (rBmKCTa) from *Buthusmartensii*Karschin a concentration-dependent manner had specific toxicity effects against glioma cells, and IC50 value was approximately 0.28µM but the IC50 value for normal astrocytes was significantly determined as 8 µM against glioma cells.[7]

In a research project by Song (2012), isolated toxin of BmK from crude scorpion venom of *Buthusmartensii* Karsch showed anti-proliferative effects on the THP-1 (human acute monocytic leukemia) cell line. The IC50 value was calculated as 29 µg/ml for inhibiting of THP-1.[8]

More specially, in the article published by Gupta *et al* (2007) it has been reported that the venom extracted from *Heterometrusbengalensis,* Indian black scorpion, could inhibit growth of U937 and K562cell lines with IC50 values of 41.5 µg/ml and 88.3 µg/ml , respectively. This venom presented anti-proliferative and apoptogenic efficacies against those human leukemic cell lines.[9]

Khodadadi et al (2012) in a comparative study have indicated that *H. lepturus* venom had toxicity effect on K562 cell line in low concentrations in comparison to *Androctonus crassicauda* and *Mesobuthus*eupeus.[10]

Hemiscorpiouslepturus which is known as the most dangerous scorpion in the middle east including Iran. This scorpion species which has been found throughout Iran, Iraq, Pakistan, and Yemen is present in Khuzestan as w Province of Iran.[11]

*Hemiscorpiouslepturus*is well known for its potent cytotoxic venom that can cause cutaneous necrosis and severe systemic pathology that may lead to death. Venom from *H. lepturus*causes severe and fatal

haemolysis, secondary renal failure, deep and necrotic ulcers, ankylosis of the joints, psychological problems, and death in man.[12, 13]

However, there are many research paper regarding negative effects of this species against human body but the positive effects of *H.lepturus* against diseases such as cancer is not well documented. There is rare research paper to discuss anti-cancer effects of this species, however, there are some papers regarding different effects of the other scorpion species against cancer cells.

Therefore, the current study was carried out to find in vitro cytotoxicity effects of *H. lepturus* venom regarding inhibition of K562 cell line.

MATERIALS AND METHODS

Scorpion venom preparation:

Hemiscorpiouslepturus scorpions were collected from Bagh Malek County in the sw of Iran using UV lights during nights. They were transported to the Ahvaz Jundishapur University of Medical Sciences (AJUMS) lab animal breeding centre. Then, their venoms were milked using electrical stimulation of their telsons (6-10V, 500 mA). The extracted venom was kept under -40 degree C. Then the venom solution was dissolved in distilled water and mucous were separated by centrifugation at 20,000 g and 4°C for 30 minutes in the department of AJUMS immunology

Bradford assay was used to measure of protein concentration. In briefly serial dilutions were prepared from BSA, then absorbance measured at 595 nm by spectrophotometer to calculate the protein concentration of *H.Lepturus* venom.

Cell cultures

K562 cell line was purchased from Pasteur Institute, Tehran, Iran. RPMI1640 was purchased from Biosera, UK. FBS and penicillin were purchased from Gibco, USA. DMSO and MTT assay kit were purchased from sigma, USA.

K562 cell line is composed of undifferentiated blast cells and is derived from a CML patient in blast crisis.K562 cells were cultured in RPMI 1640 and supplemented with 10% heat inactivated fetal bovine serum, penicillin(100 units/ml),streptomycin 100µg/ml and incubated at 37◦ C for 24h in a humidified atmosphere containing 5% CO2 inside a CO2 incubator.

Cell viability analysis using *MTT assay* and cytotoxicity of scorpion venom

The MTT assay is a colorimetric assay for assessing cytostatic activity (loss of cell viability), proliferation and cell metabolic activity. About 10^6cells/ml of K562 cell line were seeded in a 96 well tissue culture plate and increasing concentrations of *H.Lepturus* venom from1 to 18µg/ml for 24 h. After incubation time, 3[4-dimethylthiazol-2-yl]-2-5-diphenyl tetrazolium bromide (MTT) assay was performed. Briefly, both control and treated cells were incubated for 4 h. with 10 µL MTT Reagent until purple colored form azan precipitate was visible and dissolved in dimethyl sulfoxide(DMSO).

The absorbance was measured at 570nm in a micro-plate reader. Cytotoxicity of *H.lepturus* venom on K562 cells was performed as mean percentage ± SD of three replicates and Control values were set at 0%.The percentage of each inhibition concentration was calculated by using this formula:[14]

$$\%\ inhibition = 1 - \left(\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} * 100\right)$$

Finally data were analyzed for IC50 value, which is half maximal inhibitory concentration, using the program Probit Analysis (Prof. Hsin Chi software) and Excel 2010. Also an ANOVA was performed to confirm results of probit analysis.

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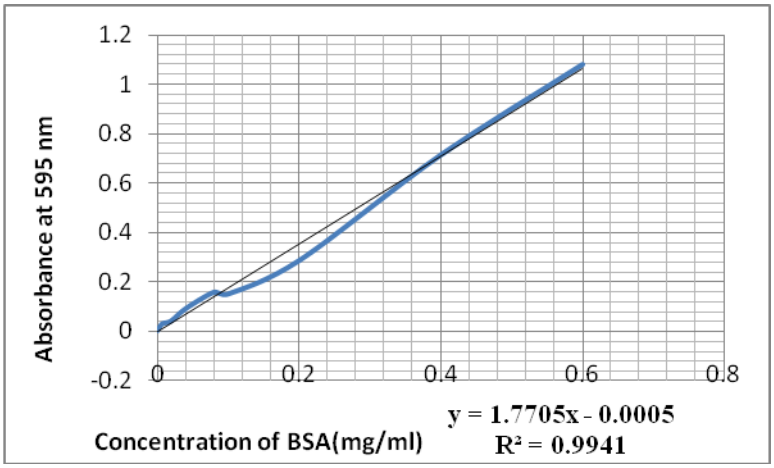
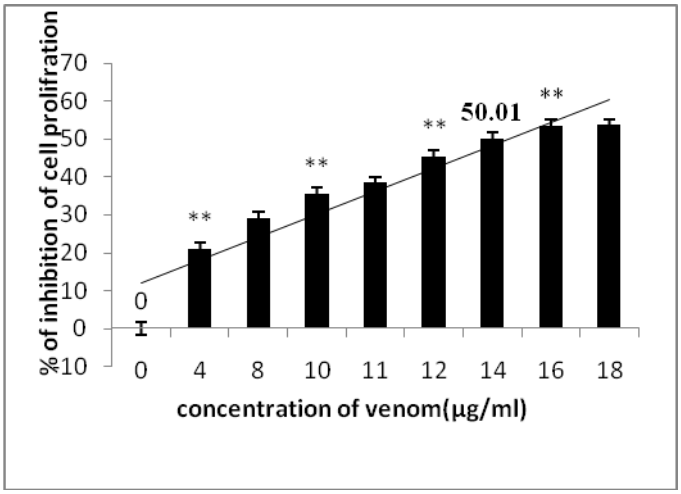
RESULTS

The results of current study are performed in the graphs (I) and (II) and table (I).



Graph (1): This Graph showed the concentration of BSA (mg/ml) against absorbance at 595 nm that used to calculate the amount of protein in*H.lepturus* venom and dilution factor of venom was considered.

Cytotoxic effect of*H.lepturus* venom on K562 cells (Graph 2)



Graph (2): Cytotoxic effect of *H. lepturus* crude venom on K562 cells after 24-hours exposure to different venom concentrations. Cell proliferation inhibitory was determined by MTT assay. P<004 (One way ANOVA test). **significant difference

Table 1: Cytotoxic effect of *Hemiscorpiuslepturus* venom on K562 cells by probit analysis

Number	log(Dose)	% of inhibition of proliferation	probit	Lower	Upper

of trial				probit	probit
1	0.60	0.21	4.19	3.85	4.32
2	0.90	0.29	4.44**	4.45	4.66
3	1	0.35	4.62	4.61	4.80
4	1.04	0.38	4.70**	4.68	4.86
5	1.07	0.45	4.88	4.73	4.93
6	1.14	0.50	5.00**	4.82	5.05
7	1.20	0.53	5.08	4.89	5.16

** Significant difference

Protein concentration of *H.Lepturus*venom

The amount of protein concentration in *H.Lepturus*crude venom was examined by the result from Bradford assay (Graph1). The OD have been read in venom sample is 0.626 that affected by dilution factor calculated 3.35 mg/ml.

The MTT assay results for *H.lepturus* venom against K562 Cell Viability

The obtained result of current study showed that viability of K562 cells were decreased by using *H.lepturus*venom. It means that viability of K562 cells were decreased when concentration of the venom was increased in comparison to untreated control cells. This was a dose dependent trend. Also increasing of growth inhibitions was in concentration-dependent manner of this venom (Graph 2).

The IC_{50} value was calculated which to measure the effect of *H. lepturus* scorpion venom on cell viability in K562 cell lines. Graph (II) shows the IC_{50} value of under our experimental conditions.

The MTT assay result showed that the IC50 value was 14µg/ml after 24h incubation of K562 cells with scorpion venom (Graph2). In the other words 14µg/ml [(5(4.82-5.05 as probit transformation)] of this venom could kill the 50% of K562 cell proliferation in our conditions(inhibition percentage). The results that come from using ANOVA followed by LSD POST-HOC TEST and probit analysis confirmed the concentration dependent due to increased inhibition of proliferation (decreased viability of K562 cells) using the scorpion venom in the Graph (2).

The results of probit analysis**,** Table 1, confirmed the dose dependent trend [(obtained 50% mortality of 5 (4.822-5.053)] K562 cells.

DISCUSSIONS

In this study ability of *H. lepturus* venom to change the viability and cytotoxic effects on human CML (K562) was examined. The results showed that the venom was cytotoxic for these cells. Protein concentration of *H. lepturus* was determined about 3.35 mg/ml.

The study of Haddad *et al* (2015) showed the protein concentration of *H. lepturus* venom was 5mg/ml. The different result of protein concentration between two studies has come from different race of used *H. lepturus*, lyophilization method of the venom and different biochemical composition and pharmacology at different times.[15,16]
These differences are also performed regarding calculation of IC_{50}s. The reasons are reflected in the different species and time of treatment.

Furthermore, the type of cells is significantly correlated to IC_{50} value for each venom. *Buthusmartensii Karsch* venom´IC_{50} values were 0.28 µM and 8 µM against glioma cells and normal astrocytes, respectively.[7]

Heterometrusbengalensis Koch scorpion IC_{50} value was obtained as 88.3mg/ml after 48h against K562 cells. The calculated IC_{50} of *H.lepturus* in the present study was 14µg/ml on K562 cell .It means IC_{50} of

H.lepturus venom had a better performance against K562 cells than *B. martensii* against the same cells in the study of Gupta et al.[9]

The MTT analysis the cytotoxic effect of venomous Levantine viper, *Macroviperalebetinalebetina* crude venom on L929 cells was estimated as the IC50 value at 0.62 ±0.18 and 1.62 ± 0.25 µg/mL during 2 and 48hr incubation period, respectively. The IC50 value was after 48 hours treatment while the calculated value was after 2h.[17]

As we see the IC_{50} of Levantine viper venom is very lower than it in the *H.lepturus* venom, because the viper is a vertebrate animal and the other one is an invertebrate animal. This fact, being different species of *Hemiscorpius* genus or different sub species of *H.lepturus* in Khuzestan Province, is a reason regarding in obtained results of different studies. [18, 19]

The results of Khodadai*et al* (2012) in an invitro study showed that, unlike the venoms from *Androctonuscrassicauda and Mesobuthus. eupeus*, the venom from *H. lepturus* produced dose-dependent lysis of human RBCs and showed phospholipase activity.This confirms our results in inhibition of K562 cell in a dose dependent trend.[10]

The venom components can be fractioned and characters of each to be demonstrated by the value of each component. Different fractions have been isolated of *H. lepturus* venom. Two of them are Hemitoxin and ICD-85.The first potassium channel blocker that isolated from *H. lepturus* venom was Hemitoxin which contained only 0.1% of the venom proteins, but it was able to bloke potassium channel with IC50 value about 2 to 16 nM.[20]

Voltage-gated K+ channels (VGPCs) performed a proliferation property of cells. This character may be contributed in cell invasion and metastatic process. It has been found the fractions that are blockers of K+ channel, have antiproliferative effects on human breast cancer cells.[21]

Another fraction which is called ICD-85 is with the character to inhibit the growth of various cancer cells. It has been determined in the study of Koohi et al that this fraction is responsible for inducing of apoptosis in breast cancer cells. Both characters of two fractions in inhibiting of cancer cells confirm our results in inhibiting of K562 cells.[22]

CONCLUSION

Results of the current study suggest that H.lepturusvenom increase inhibition of growth activity of K562. According to results of the other studies and the current study it is worthy to do more venom fractioning studies due to H.lepturus which is hoped to be a step to *drug discovery* from natural toxins to treat the cancers.

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