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Anti-tumor potential of *Hottentotta zagrosensis* scorpion low molecular weight venom fractions: VEGF-mediated angiogenesis inhibition in Glioblastoma cells

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Abstract

Glioblastoma (GBM) is an aggressive and treatment-resistant brain tumor with poor clinical outcomes. In the recent years, natural bioactive compounds have attracted growing interest as sources of novel anticancer agents. Among these, Peptides derived scorpion venom (PESV) has emerged as a particularly promising reservoir of low-molecular-weight peptides with potent therapeutic potential. PESV from *Hottentotta zagrosensis* scorpion have attracted interest for their antitumor properties, particularly their ability to inhibit angiogenesis. This study examined the effects of PESV on angiogenesisin C6 glioblastoma cells. For this purpose, the venom from scorpion of *H. zagrosensis* was collected. The lyophilized whole venom was fractionated usingultra-filtration prior to load on SDS-PAGEand stained with silver. This process showed two protein bands, with the majority of molecular masses of 10kDa and a smaller band of 5kDa. The concentration of vascular endothelial growth factor (VEGF) in Glioblastoma cells significantly decreased following the treatment with Temozolomide (TMZ) compared to the control group. Similarly, exposure to 75 µg and 150 µg of peptides derived from *H. zagrosensis* venom (PESV) also resulted in a significant reduction in VEGF levels compared to the control. The reduction of VEGF levels in the PESV groups (75 and 150 µg/mL) is dose-dependent, and notably, the extent of VEGF reduction at the high PESV dose (150 µg/mL) is comparable to the effect of TMZ, indicating the strong anti-angiogenic potential of these peptides. In conclusion, this study provides robust preclinical evidence that peptide fractions extracted from the venom of *H. zagrosensis* scorpion exhibit significant suppression of angiogenesis indicating its promise as a potential therapeutic candidate for GBM.

Key words: *Hottentotta zagrosensis*, venom, ultra-filtration, SDS-PAGE, Khuzestan

Introduction

Glioblastoma multiforme (GBM) is the most prevalent and highly aggressive primary brain tumor in adults. Despite significant advances in surgical intervention, radiotherapy, and chemotherapeutic strategies, GBM continues to be associated with a grim prognosis, with a median survival of fewer than 15 months (Anjum et al, 2017).

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Angiogenesis is a defining pathological feature of GBM and is mediated through both hypoxia-dependent and hypoxia-independent mechanisms (Soumya et al, 2024). Under hypoxic conditions, the inactivation of prolyl hydroxylases leads to the stabilization and accumulation of hypoxia-inducible factor-1 alpha (HIF-1α), which in turn upregulates vascular endothelial growth factor (VEGF), a key mediator of neovascularization in tumors (Kararet al, 2011; Kaur et al,2005). The VEGF family comprises seven structurally related proteins, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF), alongside viral and venom-derived homologs such as VEGF-E (Orf-VEGF) and snake venom VEGF (svVEGF). These ligands exert their biological activity by binding to VEGF receptors (VEGFR-1, VEGFR-2, and VEGFR-3), tyrosine kinase receptors essential for vascular development and homeostasis (Holmes and Zachary, 2005). Among them, VEGF-A and its receptors VEGFR-1 and VEGFR-2 play pivotal roles in tumor-induced angiogenesis, with VEGFR-2 being recognized as the central driver of vascular proliferation (Karaman et al, 2022). Given its critical role, VEGF signaling has become a major target for therapeutic intervention in cancer (Ghalehbandi et al, 2023). In response to hypoxia, tumor cells enhance the secretion of VEGF and other proangiogenic factors, promoting neovascularization that supports tumor growth and invasion.

In the recent years, natural bioactive compounds have attracted growing interest as sources of novel anticancer agents. Among these, scorpion venom has emerged as a particularly promising reservoir of low-molecular-weight peptides with potent tumor-targeting potential (Pashmforoosh and Baradaran, 2022). These venom-derived peptides are distinguished by their structural stability, high cellular permeability, and selective affinity for cancer cells (Majc et al, 2022). Numerous studies have shown that scorpion venom can suppress tumor proliferation by inducing apoptosis and halting cell cycle progression (Al-Asmari et al, 2018). As a result, recent research has increasingly focused on isolating and characterizing peptides derived scorpion venom (PESV) as candidates for targeted cancer therapy. Notably, these peptides display selective cytotoxicity, effectively eliminating malignant cells while sparing healthy tissues (Nasr et al, 2023). Chlorotoxin, a well-characterized peptide originally identified by DeBin et al, (1993), has attracted considerable interest for its therapeutic potential, particularly in oncology. Subsequent research has revealed chlorotoxin-like peptides in a variety of scorpion species, many of which exhibit significant anticancer activity. Notably, a peptide isolated from *Rhopalurus junceus* demonstrated potent effects against glioblastoma multiforme (GBM), selectively binding to tumor cells and inhibiting their migration, viability, and angiogenesis (Lozano-Trujillo et al, 2021). Similarly, studies on Iranian scorpion species such as *Mesobuthus eupeus* (Baradaran et al, 2019) and *Hottentotta saulcyi* (Nosouhian et al, 2024) have shown broad antiproliferative effects across a range of cancer cell lines, including those derived from liver, breast, prostate, and brain malignancies. Notably, individual components of scorpion venom have demonstrated the ability to induce apoptosis, disrupt cell cycle progression, and further attributed to the suppression of angiogenesis via downregulation of VEGF (Duenas-Cuellar et al, 2023; Salabi et al, 2024).

Hottentotta zagrosensis, a black buthidae scorpion endemic to Iran's Zagros Mountain region including Fars and Khuzestan provinces, harbors a distinct array of venom peptides, though its therapeutic potential remains largely unexplored (Nasr et al, 2023; Salabi et al, 2024). *H. zagrosensis*, a medically relevant

scorpion species native to Iran, reaches a length of approximately 13 cm and is identified by its dark brown to black body and hairy metasoma and telson (Amiri et al, 2024). It is a frequent cause of envenomation in southwestern regions such as Ramhormoz, Ahvaz, Susangerd, and Bushehr (Bavani et al, 2021).

A recent transcriptomic analysis of the venom gland of *Hottentotta zagrosensis* by Salabi et al, (2024) identified peptide components (PESV) with promising antitumor potential. Bioinformatic predictions suggest that these peptides may induce apoptosis and suppress angiogenesis through downregulation of the VEGF signaling pathway, positioning them as novel candidates for anticancer therapy. However, the study primarily focused on lipolysis-activating peptides, and experimental evidence regarding the direct anticancer effects of *H. zagrosensis* venom on specific tumor cell lines remains limited.

In the present study, we investigated the antitumor properties of venom peptides isolated from *H. zagrosensis*, with particular emphasis on their anti-angiogenic effects against the C6 glioblastoma cell line.

Materials and Method

Scorpion Collection and Venom Extraction

H. zagrosensis specimens were collected nocturnally from the Khuzestan Province in the southwest of Iran, using ultraviolet (UV) light. A total of 100 scorpions were collected from the field between September and November and for venom extraction. Following capture, scorpions were transferred to the Razi Vaccine and Serum Research Institute for taxonomic identification (Vachon et al, 1974; Lamoral et al, 1979). Confirmed specimens underwent venom extraction via electrical stimulation of telson. Crude venom was immediately lyophilized and stored at –20 °C pending further analysis.

Peptide Purification and Fractionation

Fifty milligrams Lyophilized *H.zagrosensis* venom was dissolved in 10 mL of 0.1 M phosphate buffer (pH 7.2), incubated overnight at 4 °C for activation. The solution was centrifuged at 4000 rpm for 10 minutes at 4 °C to remove insoluble material. To isolate low-molecular-weight peptides, the second elution peak, corresponding to smaller molecules, was selected for ultrafiltration (Vivaspin, Sartorius, Germany) using 10 kDa molecular weight cutoff filters. The samples were centrifuged at 6000 rpm for 30 minutes at 4 °C, and the process was repeated to concentrate the target fraction. The purified peptides were lyophilized and stored at –20 °C for further analysis.

Protein Quantification via Bradford Assay

Protein concentration of whole venom and each fraction was determined using the Bradford assay (Bradford, 1976), which relies on the acid-induced binding of Coomassie Brilliant Blue G-250 dye to basic amino acid residues. This interaction shifts the dye's absorbance maximum from 465 nm to 595 nm. Absorbance at 595 nm was measured spectrophotometrically, and protein levels were calculated using a standard curve generated with bovine serum albumin (BSA).

Tricine-(SDS-PAGE) Electrophoresis

Low-molecular-weight proteins and peptides were separated using Tricine-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (18% resolving, 5% stacking gel). Gels were prepared from standard stock solutions of acrylamide/bis-acrylamide, Tris-HCl (pH 8.45), glycerol, APS, and TEMED, with polymerization initiated just prior to casting.Electrophoresis was performed using a discontinuous buffer system: the cathode buffer (0.1 M Tris, 0.1 M Tricine, 0.1% SDS) was used without pH adjustment and stored at 4 °C; the anode buffer (0.2 M Tris) was adjusted to pH 8.9. Protein extracts (1 µg/µL) were mixed with

4× loading buffer (Tris, SDS, glycerol, β-mercaptoethanol, bromophenol blue), heat-denatured at 95 °C for 5 minutes, and loaded (20 µL) per well along with molecular weight markers. Electrophoresis was initiated at 70 V and increased to 90 V once the dye front reached the resolving gel. Following electrophoresis, proteins were visualized via silver nitrate staining. Post-electrophoresis, gels were fixed in 5% glutaraldehyde (30 min), followed by 50% methanol, 12% acetic acid, and 0.5% formaldehyde (60 min). After ethanol washes (50% then 30%) and distilled water rinses, gels were sensitized in 0.025% sodium thiosulfate (1 min). Silver impregnation was carried out in 0.2% silver nitrate with 0.075% formaldehyde (20 min). Gels were then developed in 6% sodium carbonate, 0.0004% sodium thiosulfate, and 0.025% formaldehyde under low-light conditions, and development was halted upon band appearance. The final fixation was performed in 50% methanol with 12% acetic acid, and gels were stored in 1% acetic acid.

Cell cultures

C6 glioblastoma cells were obtained from the Pasteur Institute of Iran. Cell viability was assessed using trypan blue staining (Bioidea, Iran), and viable cells were counted manually. Cells were cultured in T25 flasks (Sanifico, South Korea) containing DMEM/Ham's F-12 medium (Bioidea, Iran) supplemented with 2.5% fetal bovine serum and 5% horse serum (Gibco® Life Technologies, USA), along with 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma, USA). Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2 using a PadidehNogene incubator (Iran). The medium was refreshed and cells were washed twice every 2 days to ensure optimal growth conditions. For the experimental use, cells were harvested by centrifugation at 1500 RPM for 10 minutes, the supernatant was discarded, and the pelleted cells were resuspended for further analysis.

Experimental design

C6 glioblastoma cells were seeded at a density of 1×10^6 cells per well in a 6-well plate containing basal medium and incubated at 37 °C with 5% CO_2 for 24 hours to reach approximately 70% confluency. After incubation, cells were divided into four groups: the Control group (untreated C6 cells), the TMZ group (cells treated with 1 µg/mL Temozolomide), the PESV 75 group (cells treated with 75 µg/mL of peptides derived from *Hottentotta zagrosensis* venom), and the PESV 150 group (cells treated with 150 µg/mL of PESV). All treatments were administered under identical incubation conditions, and cells were also harvested for subsequent molecular and functional analyses.

ELISA

VEGF levels in tissue lysates were quantified using a species-specific ELISA kit (BT Assay Technology Inc., China), following the manufacturer's protocol. The total protein concentrations were determined via the Bradford method, and VEGF expression was normalized to protein content and reported as pg/mg protein. Both intra- and inter-assay coefficients of variation were below 6%.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 8.0.2. (GraphPad Software, San Diego, CA, USA). Data were presented as mean ± SEM, and group differences were assessed by one-way ANOVA followed by Tukey's post hoc test. Significance levels were defined as $P<0.01$ (**), $P<0.001$ (***), and $P<0.0001$ (****).

Results

Venom fractionation

Lyophilized *H. zagrosensis* venom was dissolved in 10 mL of 0.1 M phosphate buffer. To isolate low-molecular-weight

peptides, the whole venom was fractionated using 10 kDa molecular weight cutoff ultra-filtration. According to SDS-PAGE, the molecular weight of crude venom proteins was estimatedbetween 5 and 160 kDa. The molecular weightof the fractionated venom proteins was about 10 to 5kDa with two detectable bands (Figure 1). We obtained a venom fraction with a protein concentration of 125 µg/mL following the ultrafiltration of the initial 1 mg/mL crude venom, using the Bradford assay for quantification.Lysozyme from chicken egg white (muramidase; catalog no. 5281, MERCK, Germany), with a molecular weight of 14.3 kDa electrophoresed alongside the filtered venom.

Figure 1: *H. zagrosensis* **venom separated by SDS-PAGE and stained in silver nitrate. Line 1: Ultra-filtration of venom fraction, line2: Lysozyme.**

The effect of PESV and TMZ on the levels of VEGF in GBM cells

In C6 glioblastoma cells seeded at a density of 1×10^6 cells per well, the concentration of VEGF in glioblastoma cells significantly decreased following treatment with Temozolomide (TMZ) compared to the control group (P<0.0001). Similarly, exposure to 75 µg and 150 µg of peptides derived from *H.zagrosensis* venom (PESV) also resulted in a significant reduction in VEGF levels compared to the control (P<0.01 and P<0.0001, respectively) (Figure 2).

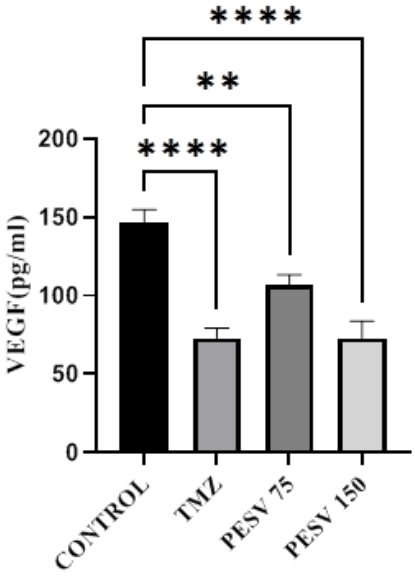

Figure 2: Quantification of VEGF concentrations in glioblastoma cells using ELISA. C6 glioblastoma cells were grouped into Control (untreated), TMZ (treated with 1 µg/mL Temozolomide), PESV 75 (treated with 75 µg/mL scorpion venom-derived peptides), and PESV 150 (treated with 150 µg/mL scorpion venom-derived peptides). Data are presented as mean ± standard deviation. Statistically significant differences versus the control group are indicated (P<0.01, ****P<0.0001).**

Discussion

Glioblastoma multiforme (GBM), despite multimodal interventions, remains one of the major challenges in the clinic and treatment of brain cancers, with a median survival rate of less than 15 months. This malignancy is characterized by aggressive growth, invasion into adjacent tissues, and significant resistance to conventional therapies (Pouyan et al, 2025; Wu et al, 2021). Simultaneously, pathological angiogenesis mediated by VEGF/VEGFR2 signaling creates the hypervascular microenvironment characteristic of GBM (Nowacka et al, 2025). These features highlight the urgent need for novel therapeutic strategies. In the recent years, the search for new antitumor agents has extended to investigating natural

compounds and resources, including scorpion venom (Raposo, 2017). Scorpion venom, particularly due to its content of bioactive low-molecular-weight peptides with selective toxicity against cancer cells, has emerged as a promising source (Xia et al, 2023).

The present study takes a step forward in this area by systematically investigating the antitumor potential of peptide fractions derived from the venom of *H. zagrosensis* in the C6 glioblastoma cell line, with a special focus on angiogenesis processes. The results of this research are presented in comparison with untreated control cells as well as with results obtained from treatment with temozolomide (TMZ), the standard drug, thereby providing a strong basis for assessing the therapeutic potential of these scorpion venom-derived peptides.

Angiogenesis is one of the fundamental factors in the progression and invasion of GBM, occurring within the hypoxic tumor environment and regulated by the VEGF signaling cascade (Ahir et al, 2020). Overexpression of VEGF and its receptors, is an indicator of new blood vessel formation within the tumor and is associated with poor prognosis in GBM. The findings of the present study show that both the group treated with TMZ and the groups treated with *H. zagrosensis* scorpion venom peptides (PESV) exhibit a significant decrease in VEGF protein levels in C6 glioma cells. Additionally, the reduction of VEGF levels by PESV was dose-dependent, with the higher concentration (150 µg/mL) producing a significantly greater effect than the lower one (75 µg/mL). Notably, the VEGF inhibition achieved by PESV at 150 µg/mL was comparable to that of the standard chemotherapeutic agent TMZ. This dose-dependent efficacy, alongside the potent activity rivaling TMZ, suggests that these *H. zagrosensis* peptides simultaneously target both hypoxia-dependent and hypoxia-independent VEGF production pathways. This multi-modal mechanism represents a potential therapeutic advantage over single-mechanism agents.

This finding is consistent with studies on *Mesobuthus eupeus* venom, which demonstrated 68% inhibition of VEGF in breast cancer via disruption of the NF-κB pathway (Keshavarz et al, 2021), although *H. zagrosensis* exhibits greater efficacy at higher concentrations.Crucially, the present study demonstrates a novel finding that is dose-dependent superiority in anti-angiogenic effects compared to TMZ. The selective toxicity of scorpion venom peptides toward malignant cells, coupled with their multitarget capacity to simultaneously intervene in multiple cancer hallmarks, makes these compounds promising candidates for new drug development. The differences between the results of the present research and the previous studies conducted on the venom of other scorpion species are likely due to differences in venom composition, cell model type, and treatment timing. For example, crude venom of *Hottentotta schach* can induce programmed cell death in breast cancer cells by activating caspase-3 and inducing DNA fragmentation (Dezianian et al, 2020). Such differences underline the need for more precise and comprehensive mechanistic investigations for each compound and model.

In conclusion, this study provides robust preclinical evidence that peptide fractions extracted from the venom of *H. zagrosensis* scorpion exhibit significant antitumor activity in the C6 glioblastoma cell line. These effects were observed in a key area where the angiogenesis was comparable to the standard drug temozolomide.

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Conflict of Interest

The authors declared no conflict of interest.

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References

Ahir, B. K, Engelhard, H. H., &Lakka S. S. (2020). Tumor development and angiogenesis in adult brain tumor: glioblastoma. *Molecular neurobiology*, 57(5), 2461-78.

Al-Asmari, A. K., Riyasdeen, A.,Islam, M. (2018). Scorpion venom causes apoptosis by increasing reactive oxygen species and cell cycle arrest in MDA-MB-231 and HCT-8 cancer cell lines. *Journal of evidence-based integrative medicine*, 23,2156587217751796.

Amiri, M., PrendinI, L., Hussen, F. S., Aliabadian, M., Siahsarvie, R., Mirshamsi, O. (2024). Integrative systematics of the widespread Middle Eastern buthid scorpion, Hottentotta saulcyi (Simon, 1880), reveals a new species in Iran. *Arthropod Systematics & Phylogeny*, 82,323-41.

Anjum, K., Shagufta, B. I., Abbas, S. Q., Patel, S., Khan, I., Shah, S. A. A. (2017). Current status and future therapeutic perspectives of glioblastoma multiforme (GBM) therapy: A review. *Biomedicine & Pharmacotherapy*,92,681-9.

Baradaran, M., Jalalim, A, Naderi, Soorki M., Jokar, M., Galehdari, H. (2019). Three New Scorpion Chloride Channel Toxins as Potential Anti-Cancer Drugs: Computational Prediction of The Interactions With Hmmp-2 by Docking and Steered Molecular Dynamics Simulations. *Iran J Pharm Res.*, 18(2),720-734.

Bavani, M. M., Saeedi, S., Saghafipour, A. (2021). Spatial distribution of medically important scorpions in Iran: A review article. *Shiraz E-Medical Journal*, 22(5).

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*,72, 248-54.

Duenas-Cuellar, R. A., Santana, C. J. C., Magalhaes, A. C. M., Pires, O. RJr., Fontes, W., Castro, M. S. (2020). Scorpion Toxins and Ion Channels: Potential Applications in Cancer Therapy. *Toxins (Basel)*, 12(5),326.

DeBin, J. A., Maggio, J. E., Strichartz, G. R. (1993). Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion. *Am J Physiol.*, 264(2 Pt 1), C361-9.

Ghalehbandi, S., Yuzugulen, J., Pranjol, M. Z. I., Pourgholami, M. H. (2023). The role of VEGF in cancer-induced angiogenesis and research progress of drugs targeting VEGF. *European journal of pharmacology*,949,175586.

Holmes, D. I., Zachary, I. (2005). The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. *Genome biology*,6,1-10.

Karaman, S., Paavonsalo, S., Heinolainen, K., Lackman, M. H., Ranta, A., Hemanthakumar, K. A.(2022). Interplay of vascular endothelial growth factor receptors in organ-specific vessel maintenance. *Journal of Experimental Medicine*,219(3).

Kaur, B., Khwaja, F. W., Severson, E. A., Matheny, S. L., Brat, D. J., Van Meir, E. G. (2005). Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. *Neuro-oncology*, 7(2), 134-53.

Keshavarz Alikhani, H., Zargan, J., Bidmeshkipour, A., Zamani, E., Mosavi, M., Heidari, A. (2021. Iranian Scorpion (Odontobuthus bidentatus) crude venom change the redox potential of MCF-7 breast cancer cell line and induce apoptosis. *Modern Medical Laboratory Journal*, 4(2), 28-35.

Lamoral, B.H. (1979). The scorpions of Namibia. Annals of the Natal Museum, 23: 497-784.Lozano-Trujillo LA, Garzon-Perdomo DK, Vargas AC, de Los Reyes LM, Avila-Rodriguez MF, Gay OT, et al. Cytotoxic effects of blue scorpion venom (Rhopalurusjunceus) in a glioblastoma cell line model. *Current Pharmaceutical Biotechnology*, 22(5),636-45.

Lozano-Trujillo, L. A., Garzon-Perdomo, D. K., Vargas, A. C. R., de Los Reyes, L. M., Avila-Rodriguez, M. F., Gay, O. T. G., Turner, L. F. (2021). Cytotoxic Effects of Blue Scorpion Venom (Rhopalurus junceus) in a Glioblastoma Cell Line Model. *Curr Pharm Biotechnol.*, 22(5), 636-645.

Majc, B., Novak, M., Lah, T. T., Krizaj, I. (2022). Bioactive peptides from venoms against glioma progression. *Frontiers in oncology*,12,965882.

Nasr, S., Borges, A., Sahyoun, C., Nasr, R., Roufayel, R., Legros, C. (2023). Scorpion venom as a source of antimicrobial peptides: overview of biomolecule separation, analysis and characterization methods. *Antibiotics*, 12(9), 1380.

Nosouhian, M., Rastegari, A. A., Shahanipour, K., Ahadi, A. M., Sajjadieh, M.S. (2024). Anticancer potentiality of Hottentottasaulcyi scorpion curd venom against breast cancer: an in vitro and in vivo study. *Scientific Reports*,14(1), 24607.

Nowacka, A., Sniegocki, M., Smuczynski, W., Bozilow, D., Ziolkowska, E. (2025). Angiogenesis in glioblastoma-treatment approaches. *Cells*, 14(6), 407.

Pashmforoosh, N., Baradaran, M. (2023). Peptides with Diverse Functions from Scorpion Venom: A Great Opportunity for the Treatment of a Wide Variety of Diseases. *Iran Biomed J.*, 27(2 & 3), 84-99.

Pouyan, A., Ghorbanlo, M., Eslami, M., Jahanshahi, M., Ziaei, E., Salami, A. (2025). Glioblastoma multiforme: insights into pathogenesis, key signaling pathways, and therapeutic strategies. *Molecular cancer*, 24(1),58.

Raposo, C. (2017). Scorpion and spider venoms in cancer treatment: state of the art, challenges, and perspectives. *Journal of clinical and translational research*, 3(2),233.

Salabi, F., Jafari, H., Mahdavinia, M., Azadnasab, R., Shariati, S., Baghal, M. L., Tebianian, M., Baradaran, M. (2024). First transcriptome analysis of the venom glands of the scorpion *Hottentotta zagrosensis* (Scorpions: Buthidae) with focus on venom lipolysis activating peptides. *Front Pharmacol.*, 15,1464648.

Salabi, F., Nemati, M., Lari Baghal, M. (2024) Fractionation and enzymes activity measurement of the scorpion *Androctinus crassicoda* (Scorpionida: Buthidae) venom. *Iranian Veterinary Journal*, 20(1): 109-120.

Soumya, S. J., Aryam K. R., Abhinand, C. S., Sunitha, P., Athira, A. P., Nair, A. S. (2024). Multi-target and natural product-based multi-drug approach for anti-VEGF resistance in glioblastoma. *Exploration of Drug Science*, 2(5),567-82.

Xia, Z., He, D., Wu, Y., Kwok, H. F., Cao, Z. (2023). Scorpion venom peptides: Molecular diversity, structural characteristics, and therapeutic use from channelopathies to viral infections and cancers. *Pharmacological research*, 197,106978.

Vachon, M. (1974). Etude des caracteresutilises pour classer les familles et les genres de Scorpions (Arachnides). 1. La trichobothriotaxie en arachnologie. Sigles trichobothriaux et types de trichobothriotaxie chez les scorpions. *Bulletin du Museum National dHistoireNaturelle*, 3, 857-958.

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خاصیت ضد توموری فراکسیون‌های با وزن ملکولی پائین زهر عقرب *Hottentotta zagrosensis*: مهار رگ‌زایی با واسطه VEGF در سلول‌های گلیوبلاستوما

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چکیده

گلیوبلاستوما (GBM) یک تومور مغزی تهاجمی و مقاوم به درمان با نتایج بالینی ضعیف است. در سال‌های اخیر، ترکیبات زیست فعال طبیعی به عنوان منابعی به عنوان عوامل ضد سرطان جدید، توجه فزاینده‌ای را به خود جلب کرده‌اند. در میان این ترکیبات، زهر عقرب مشتق شده از پپتیدها (PESV) به عنوان یک منبع نویدبخش از پپتیدهای با وزن مولکولی کم و با پتانسیل درمانی قوی ظهور کرده است. پپتیدهای PESV به دست آمده از عقرب H. zagrosensis به دلیل خواص ضد توموری، به ویژه توانایی آن‌ها در مهار رگ‌زایی، مورد توجه قرار گرفته است. این مطالعه اثرات PESV بر رگ‌زایی در سلول‌های گلیوبلاستومای C6 را بررسی کرد. برای این منظور، زهر عقرب H. zagrosensis جمع‌آوری ولیوفیلیزه شد و قبل از بارگذاری بر روی SDS-PAGE تریسین با استفاده از اولترافیلتراسیون تجزیه و فراکسیون‌های آن با نقره رنگ‌آمیزی شدند. این فرآیند دو باند پروتئینی عمده شامل یک باند با جرم مولکولی ۱۰ کیلو دالتون و یک باند کوچک‌تر ۵ کیلو دالتونی نشان داد. غلظت فاکتور رشد اندوتلیال عروقی (VEGF) در سلول‌های گلیوبلاستوما پس از درمان با تموزولومید (TMZ) در مقایسه با گروه کنترل به طور قابل توجهی کاهش یافت. به طور مشابه، قرار گرفتن در معرض ۷۵ میکروگرم و ۱۵۰ میکروگرم پپتیدهای PESV مشتق شده از زهر H. zagrosensis نیز منجر به کاهش قابل توجهی در سطح VEGF در مقایسه با گروه کنترل شد. کاهش سطح VEGF در گروه‌های ۷۵ و ۱۵۰ میکروگرم PESV وابسته به دوز است که به طور قابل توجهی، میزان کاهش VEGF به ویژه در دوز بالا (۱۵۰ میلی‌گرم در میلی‌لیتر) با اثر TMZ قابل مقایسه است، که این نشان دهنده پتانسیل قوی ضد رگ‌زایی پپتیدهای PESV است. در نتیجه، این مطالعه شواهد پیش‌بالینی قوی ارایه می‌دهد که بخش‌های پپتیدی استخراج شده از زهر عقرب H. zagrosensis سرکوب قابل توجهی از رگ‌زایی را نشان می‌دهند که این خود نویدبخش بودن آن را به عنوان یک کاندیدای درمانی بالقوه برای گلیوبلاستوما نشان می‌دهد.

کلمات کلیدی: *Hottentotta zagrosensis*، زهر، اولترافیلتراسیون، SDS-PAGE، خوزستان

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