

Heteroctenus junceus* SCORPION VENOM INDUCES *IN VIVO* APOPTOSIS OF F3II TUMOR CELLS AND INCREASES THE ANTINEOPLASTIC EFFECT OF CISPLATIN *IN VITRO

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ABSTRACT

Heteroctenus junceus scorpion venom has been frequently used in traditional medicine as an anti-inflammatory, analgesic, and antitumor agent. This venom has been shown to reduce tumor volume in mice carrying a tumor induced by the F3II metastatic tumor cell line. However, the type of cell death that the venom promotes *in vivo*, as well as its ability to potentiate the antineoplastic effects of cytostatic agents used in clinical practice, remains unknown. To determine the ability of *H. junceus* scorpion venom to induce apoptosis *in vivo* and to evaluate the effect of combined scorpion venom and chemotherapy treatment in the F3II mouse tumor model. *In vivo* apoptosis was assessed by immunohistochemistry using the commercial kit ApopTag in tumors extracted from mice treated orally with *H. junceus* scorpion venom. In addition, F3II murine tumor cells were treated with a simultaneous combination of *H. junceus* scorpion venom with cisplatin or cyclophosphamide. *In vitro*, the combination index and dose reduction index were determined. Oral treatment with 12.5 mg/kg ($p < 0.01$) and 25 mg/kg ($p < 0.001$) of venom, compared to the negative control group (0 mg/kg of venom), induced a statistically significant increase in the number of TUNEL-positive cells. Therefore, the same doses of venom that exhibit an antitumor effect in the F3II model are capable of inducing DNA fragmentation in a ladder pattern. The combination of venom with chemotherapy *in vitro* synergistically increased its cytotoxicity, depending on its concentration and the type of cytostatic agent. The scorpion venom of *H. junceus* increases DNA fragmentation *in vivo*, suggesting that it may inhibit the resistance to apoptosis of tumor cells. The venom synergistically enhances the antineoplastic effect of cisplatin in the murine F3II breast tumor cell line.

Keywords: Apoptosis, *Heteroctenus junceus*, scorpion venom, synergism.

INTRODUCTION

Cancer is one of the most impactful diseases in both developed and developing countries and is among the leading causes of death worldwide (Siegel *et al.*, 2023). In both sexes, the five most common types of cancer are breast, lung, colorectal, prostate, and stomach, while the type of cancer that causes the highest mortality is lung cancer (Sung *et al.*, 2021). Conventional cancer treatment includes three basic methods: surgery, radiotherapy, and chemotherapy (García-Fernández *et al.*, 2021). In recent years, significant advances have been made in stem cell therapy, immunotherapy, targeted therapies, ablation therapies, nanoparticles, and natural antioxidants (Debela *et al.*, 2021).

Chemotherapy is one of the most widely used strategies in the clinical management of cancer patients (Stout and Wagner, 2019). However, all available therapeutic alternatives have limitations due to the progressive resistance that tumor cells acquire to this treatment and the toxicity it causes in non-tumor cells (Pezzani *et al.*, 2019). For this reason, due to the sensitivity of the disease and its cost in terms of human lives, research aimed at finding treatment options that contribute to both the prevention and elimination of cancer occupies a very broad area in the field of research (Pan *et al.*, 2019).

In particular, scorpion venoms are widely used in traditional medicine for their antitumor potential (Mikaelian *et al.*, 2020; Rajput *et al.*, 2021). The venom of the scorpion *H. junceus* Herbst 1800, formerly known as *Rhopalurus junceus* (Esposito *et al.*, 2017), is a species endemic to Cuba that belongs to the Buthidae family and has anti-inflammatory, analgesic, and antitumor

properties (Díaz-García *et al.*, 2013; Casamayor-Laime *et al.*, 2020). This venom exerts a selective cytotoxic effect against tumors of epithelial origin, without affecting non-tumor cells (Díaz-García *et al.*, 2013). A previous study related to the antineoplastic effect of the venom suggested the possible induction of apoptosis in cervical cancer cell lines, HeLa (Díaz-García *et al.*, 2013), and metastatic breast cancer, MDA-MB-231 (Díaz-García *et al.*, 2017), because treatment with the venom increased the expression of proapoptotic genes. However, its ability to induce cell death by apoptosis *in vivo* is unknown.

On the other hand, one of the novel strategies to counteract the emergence of resistance has been the combination of drugs. Numerous studies related to natural products in combination with conventional chemotherapeutic agents have shown that it increases the effectiveness of treatment and achieves a reduction in the side effects caused by anticancer drugs (Pezzani *et al.*, 2019; Hackman *et al.*, 2020; Zhang *et al.*, 2020; Vladu *et al.*, 2022). In this sense, it would be interesting to understand the ability of *H. junceus* scorpion venom to enhance the effect of conventional anticancer agents in F3II tumor cells.

MATERIALS AND METHODS

Cell line and culture conditions

The mouse F3II mammary adenocarcinoma (metastatic from a spontaneous tumor in BALB/c mice) was donated by the Center of Molecular Immunology (Havana, Cuba). The cells were grown and maintained in DMEM (Sigma, USA) supplemented with 10% heat-inactivated fetal bovine serum purchased from Hyclone, 2 mM L-glutamine, and 80 µg/mL gentamicin

(Sigma, USA). Cells were routinely passaged using 0.25% trypsin (Sigma, USA) containing 0.02% EDTA (Sigma, USA). The cells were incubated in a 5% CO₂ atmosphere at 37°C.

Animals

Adult male BALB/c mice (18–20 g) with ages between 8-12 weeks were used. The animals were obtained from the National Center for Laboratory Animal Breeding (CENPALAB, Havana, Cuba). They were housed in plastic cages under standard conditions; food and water were administered *ad libitum*. The experimental procedure using animals was approved by the Institutional Committee for Care and Use of Laboratory Animals (Protocol 2013/3), performed in accordance with the EU Directive 2010/63/EU for animal experiments and considered the recommendations of the Guide for the Welfare and Use of Animals in Cancer Research (Workman *et al.*, 2010).

Evaluation of apoptosis in tumors from animals treated with the *Heteroctenus junceus* scorpion venom harboring breast carcinoma induced with the F3II cell line

The TUNEL (TdT-mediated-dUtp Nick End Labeling) assay was performed using the commercial ApopTag[®] Plus Peroxidase in Situ Apoptosis kit (Sigma, USA), following the manufacturer's instructions. Breast carcinoma samples induced with the F3II cell line were used from BALB/c mice treated orally with 6.25 mg/kg, 12.5 mg/kg, and 25 mg/kg of *H. junceus* scorpion venom.

Twenty-four hours after the last administration, three mice were randomly selected from each experimental group and euthanized by cervical dislocation. Tumors

were removed, fixed in 10% buffered formalin, and embedded in paraffin. Three histological sections of 5 µm each were made and deparaffinized by treatment with xylene (100%), rehydration with decreasing strength ethanol (100%, 90%, 80%, and 70%), and phosphate-buffered saline (PBS). The deparaffinized and rehydrated sections were incubated with proteinase K (20 µg/mL) for 15 min at room temperature and washed four times with distilled water (2 min each). Subsequently, they were treated with 3% H₂O₂ (Sigma, USA) for 5 min and washed twice with PBS (5 min each).

The equilibration buffer was then added to the histological sections for 30 min, the excess was removed and the preparations were incubated with the enzyme terminal deoxynucleotidyl DNA transferase at 37°C for 1 h in a humid chamber at room temperature in the dark. The reaction was stopped with the addition of the stop solution for 30 min at 37°C and subsequently washed three times for 5 min with PBS. The slides were then incubated with the anti-digoxigenin-peroxidase conjugate for 30 min at room temperature in a humid chamber. They were then washed four times with PBS (5 min each) and incubated with the diaminobenzidine chromogenic solution for 3 min.

Subsequently, they were counterstained with methyl green for 10 s and washed three times with distilled water and 100% butanol. They were passed three times through xylene (2 min each) and the slide was ready for analysis by adding the coverslip with a Permount solution (Merck, Germany). Simultaneously, positive and negative controls were processed, consisting of thymus from BALB/c mice as a positive control and the negative control consisting of the omission of the deoxynucleotidyl

DNA transferase replaced by equilibration buffer. The sections were observed with a 40× lens and 10× eyepiece (400× magnification) using a brightfield microscope (Olympus IX-71, Japan) and 10 fields per slide were photographed using a DP-72 camera (Olympus, Japan) attached to the microscope. Finally, the presence of TUNEL-positive cells with fragmented DNA (brown nuclei) and viable cells (nuclei contrasted in green) was determined in 10 fields. The average number of TUNEL-positive cells per field was determined for each experimental group, and the results were expressed as TUNEL-positive cells/10 and their standard deviation (SD).

Evaluation *in vitro* of the combination of *Heteroctenus junceus* scorpion venom with conventional cytostatics in F3II tumor lines

F3II cells were detached by treatment with a trypsin-EDTA solution (0.25%) and 10^4 cells/well were added. The assays were performed in 96-well flat-bottom polystyrene cell culture plates. Fifty microliters of the cell line were added to each well and incubated in a 5% CO₂ atmosphere at 37°C for 24 hours. Subsequently, 50 µL of the treatment was added as a monotherapy of the *H. junceus* scorpion venom (0.063-1.0 mg/mL). In the individual treatment with cytostatics, the following cytostatics were used: cisplatin (CDDP) ($0.45-3.61 \times 10^{-3}$ mg/mL) and cyclophosphamide (CTX) ($9.35-74.8 \times 10^{-3}$ mg/mL). A fixed concentration of venom was combined simultaneously with CDDP/CTX (mg/mL): *H. junceus* (0.5) + CDDP ($0.45-3.61 \times 10^{-3}$), *H. junceus* (0.5) + CTX ($9.35-74.8 \times 10^{-3}$). Also, different concentrations of venom were combined with CDDP at a constant and non-constant

ratio (mg/mL): *H. junceus* (0.063-0.5) + CDDP ($0.45-3.61 \times 10^{-3}$). A group of cells that were cultured in the absence of treatment was used, which represents 100% growth.

To determine the effect on cell viability of the different treatments, the MTT assay was used, and the % of viable cells was determined and graphed. Briefly, after 72 h of treatment, 10 µL of sterile MTT (5 mg/mL) was added. The supernatant was removed, 150 µL DMSO was added to each well and the absorbance was measured in a microplate reader at 570 nm. Wells with cells and without venom were used as controls and the experiment was repeated three times. Cell viability was expressed as the % viability, using the formula: %viability = $A_{570-630\text{nm}}$ of treated cells / $A_{570-630\text{nm}}$ of control cells $\times 100\%$.

To analyze the type of interaction between *H. junceus* scorpion venom and conventional cytostatics, the combination index (CI) of each treatment was determined using the Calcsyn program (version 2.1, Biosoft, USA). The CI was calculated in relation to the fraction of cells affected (Fa) for each treatment, $Fa = (100 - \% \text{ viable cells}) / 100$. A CI < 1 indicates synergism, a CI = 1 or very close to 1 indicates an additive effect, and a CI > 1 indicates antagonism (Chou, 2006; Chou, 2010). The dose reduction index (DRI) of the venom and the cytostatics was automatically determined using the Calcsyn program. A DRI > 1 and < 1 indicate favorable and unfavorable dose reductions, respectively. For DRI = 1 there is no dose reduction (Chou, 2006; Chou, 2010).

RESULTS

Evaluation of the effect of *Heteroctenus junceus* scorpion venom on promoting

apoptotic cell death in BALB/c mice bearing a tumor induced by the F3II tumor line

To evaluate the venom's ability to induce apoptosis in tumor cells, an *in vivo* study was performed to identify DNA fragments *in situ*. Figures 1A-D show immunohistochemical staining images of the F3II tumor extracted 24 h after the last administration to the animals with different doses of venom. Apoptotic nuclei can be visualized in brown

and are indicated by arrows. The highest number of stained cells was observed for the 12.5 and 25 mg/kg venom doses (Figures 1A-D). In Figure 1E, the mean $\pm$ SD of the number of TUNEL-positive cells is represented, where a statistically significant increase is observed between treatment with 12.5 mg/kg ($p < 0.01$) and 25 mg/kg ($p < 0.001$) of the venom compared to the negative control group (0 mg/kg of *H. junceus* scorpion venom).

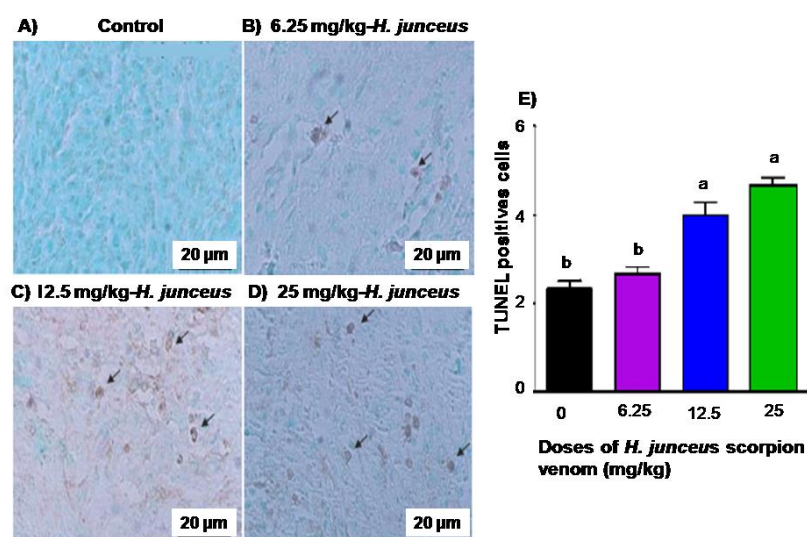


Figure 1. ApoptTag labeling of tumors from mice treated with *H. junceus* scorpion venom. F3II tumors extracted from BALB/c mice 24 h after the last oral administration of (A) untreated control (saline, 0 mg/kg of venom), (B) 6.25 mg/kg, (C) 12.5 mg/kg, and (D) 25 mg/kg of *H. junceus* scorpion venom were processed and DNA fragmentation was detected by the TUNEL technique through the ApoptTag assay. Apoptotic cells are visualized as brown-stained nuclei and are indicated by arrows. The graph shows the mean $\pm$ SD of the number of TUNEL-positive cells in 10 fields (E). Different letters indicate statistically significant differences, according to the post hoc Tukey test ($p < 0.05$).

Evaluation of the combination of *Heteroctenus junceus* scorpion venom with conventional cytostatics *in vitro*

Figure 2 shows the mean and SD values corresponding to the percentage of viable F3II cells obtained from the combination for 72 h at a fixed concentration of 0.5 mg/mL of *H. junceus* scorpion venom with varying

concentrations of CDDP/CTX. Individual treatment with CDDP compared to untreated cells significantly decreased the viability of the F3II tumor line at the following concentrations: 0.45 μ g/mL ($p < 0.01$), 0.9 μ g/mL ($p < 0.01$), 1.81 μ g/mL ($p < 0.001$), and 3.61 μ g/mL ($p < 0.001$). Statistically significant differences ($p < 0.001$) were also obtained between the percentages of viable

cells induced by the combination of venom with CDDP compared to untreated cells for all concentrations tested.

There were statistically significant differences ($p < 0.05$) between individual treatment with CDDP and its combination with *H. junceus* scorpion venom at 3.61 $\mu\text{g/mL}$ of CDDP (Figure 2A). On the other

hand, individual treatment with CTX compared to untreated cells significantly decreased the viability of the F3II tumor line ($p < 0.05$) to 74.8 $\mu\text{g/mL}$. No statistically significant differences ($p > 0.05$) were observed between the percentages of viable cells of CTX monotherapy compared to its combination with venom (Figure 2B).

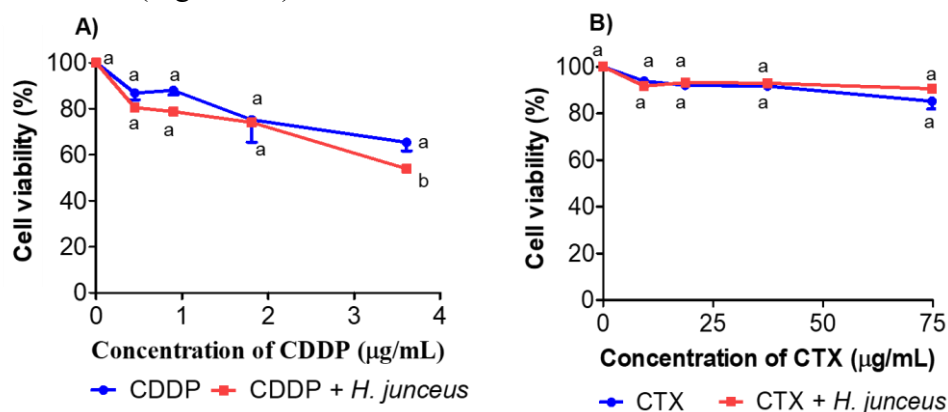


Figure 2. Effect on F3II viability of the combination of *H. junceus* scorpion venom with CDDP and CTX at a non-constant ratio. (A) The graph represents the mean $\pm$ SD of the percentage of viable cells in F3II cells treated for 72 h with CDDP and the combination of a fixed concentration of 0.5 mg/mL of *H. junceus* scorpion venom with 0.45, 0.9, 1.8, and 3.6 $\mu\text{g/mL}$ of CDDP. (B) Representation of the mean $\pm$ SD of the percentage of viable cells in the treated cells and the combination of a fixed concentration of 0.5 mg/mL of *H. junceus* scorpion venom with 9.35, 18.7, 37.4, and 74.8 $\mu\text{g/mL}$ of CTX. Three independent experiments with 3 replicates each were performed. Different letters indicate statistically significant differences, according to the Bonferroni post hoc test ($p < 0.05$).

A CI less than, greater than, and equal to 1 corresponds to a synergistic, antagonistic, and additive drug interaction, respectively. In Table 1, synergistic combinations are shaded in yellow and antagonistic combinations in green. Synergism was observed in the combination of venom with CDDP in three of the four combinations. At 0.9 $\mu\text{g/mL}$ of CDDP, the synergism was moderate and the antagonism was slight at 1.81 $\mu\text{g/mL}$ of CDDP. In the combination with CTX, under our experimental conditions, the CI obtained was greater than 1, with values indicative of antagonism,

ranging from moderate to strong. In addition, Table 1 shows favorable dose reductions ($\text{DRI} > 1$) and gray shading for the cytostatics CDDP and CTX. The DRI values for the combination treatment of CDDP with *H. junceus* scorpion venom indicate that the combination can reduce CDDP concentrations by 1.23- to 2.54-fold, achieving the same effect as monotherapy. For the combinations with CTX, except for 9.35 $\mu\text{g/mL}$ of CTX, the RDI was less than 1, suggesting that reducing the drug dose is not beneficial.

Table 1. Combination index and dose reduction index of the combination of *H. junceus* scorpion venom for 0.5 mg/mL with varying concentrations of CDDP or CTX in the F3II tumor model.

<i>H. junceus</i> (mg/mL)	CDDP (μg/mL)				CTX (μg/mL)			
	0.45	0.9	1.81	3.61	9.35	18.7	37.4	74.8
0.5	Combination index (CI)							
	0.55	0.82	1.13	0.82	3.03	3.56	4.41	3.34
	Dose reduction index (DRI)							
	2.54	1.42	0.94	1.23	1.32	0.71	0.4	0.42

Synergistic interactions are shaded in yellow, antagonistic interactions are in green. A favorable dose reduction index (>1) is indicated in blue, and an unfavorable dose reduction index (<1) is indicated in gray. CDDP: cisplatin; CTX: cyclophosphamide. Interpretation of the combination index: 0.3-0.7: synergism; 0.7-0.85: moderate synergism; 1.45-3.3: antagonism; 3.3-10: strong antagonism, as described by Chou (2010).

Once it was determined that the most favorable results between the combination of venom at a fixed concentration with variable concentrations of CDDP or CTX in the F3II tumor line were obtained with the combination with CDDP, the effect on cell viability of the combination at variable concentrations of venom with CDDP was evaluated at a constant and non-constant ratio. Figure 3 shows that in the F3II mouse tumor line, the combined treatment of *H. junceus* scorpion venom with CDDP was

more effective than individual treatment with CDDP. There were significant differences ($p < 0.001$) between the individual treatment of CDDP with respect to its combination with *H. junceus* scorpion venom, at a constant ratio, for all evaluated concentrations of the conventional cytostatic (Figure 3A). Also, statistically significant differences ($p < 0.001$) were observed for all combinations at a non-constant ratio of venom with CDDP (Figure 3B).

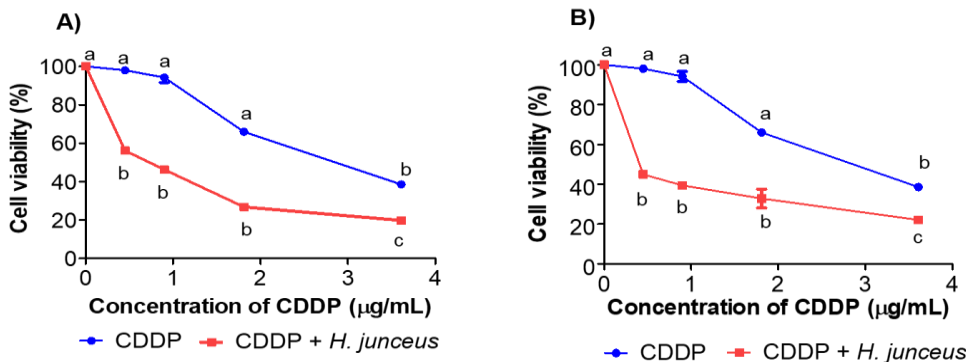


Figure 3. Effect of the combination of *H. junceus* scorpion venom and cisplatin on F3II viability. (A) The $\pm$ SD of the percentage of viable cells corresponding to the combination at constant ratios (mg/mL) of 0.06 *H. junceus* + 0.45 CDDP, 0.11 *H. junceus* + 0.9 CDDP, 0.23 *H. junceus* + 1.81 CDDP, and 0.45 *H. junceus* + 3.61 CDDP. (B) The $\pm$ SD of the percentage of viable cells corresponding to the combination at non-constant ratios (mg/mL) of 0.06 *H. junceus* + 3.61 CDDP, 0.11 *H. junceus* + 1.81 CDDP, 0.23 *H. junceus* + 0.9 CDDP, and 0.45 *H. junceus* + 0.45 CDDP. Different letters indicate statistically significant differences, according to Dunn's post hoc test ($p < 0.05$).

In Table 2, the synergistic combinations are shown in yellow. Synergism was observed in all combinations because a CI less than 1 was obtained. Specifically, strong synergism was observed at the lowest concentration of venom and CDDP evaluated (0.063 *H. junceus* + 0.45 CDDP) because the CI reached a value less than 0.3. Furthermore, in Table 2, a DRI > 1 is shown in blue for all combinations. The most favorable value for achieving dose reduction was 8.77 times in

the combination with the lowest concentration of CDDP and the highest concentration of venom (0.5 *H. junceus* + 0.45 CDDP) evaluated under our experimental conditions. The DRI values for the combined treatment of CDDP with the *H. junceus* scorpion venom indicate that, with this combination, the concentration of CDDP can be reduced by a factor of 1.96 to 8.77 to achieve the same effect as monotherapy.

Table 2. Combination index and dose reduction index for the combination of variable concentrations of *H. junceus* scorpion venom and CDDP at constant and non-constant ratios in the F3II tumor cell line.

<i>H. junceus</i> (mg/mL)	CDDP			
0.063	0.45 (µg/mL)	3.61 (µg/mL)	0.45 (µg/mL)	3.61 (µg/mL)
	0.25	0.54	6.68	1.96
0.125	0.9 (µg/mL)	1.81 (µg/mL)	0.9 (µg/mL)	1.81 (µg/mL)
	0.36	0.44	4.54	2.89
0.25	1.81 (µg/mL)	0.9 (µg/mL)	1.81 (µg/mL)	0.9 (µg/mL)
	0.44	0.44	6.47	4.97
0.5	3.61 (µg/mL)	0.45 (µg/mL)	3.61 (µg/mL)	0.45 (µg/mL)
	0.68	0.66	2.20	8.77

Synergistic interactions are shaded in yellow, and the favorable CDDP dose reduction index (>1) is shaded in blue. Interpretation of the combination index: 0.1-0.3: strong synergism; 0.3-0.7: synergism, as described by Chou (2010).

DISCUSSION

The extensive heterogeneity among cancer cells poses a challenge for the development of new drugs. In this regard, it is recommended that one of the most effective strategies is to search for compounds that act on some of the distinctive characteristics of cancer (Zhang *et al.*, 2020; Gourmet *et al.*, 2024). These include signaling for sustained proliferation based primarily on the evasion of apoptosis (Dembic, 2020; Hanahan, 2022). Therefore, drugs that can induce apoptotic cell death in cancer cells, either directly or indirectly, will target one of the

relevant metabolic pathways in cancer. Apoptosis is a highly regulated process with well-defined biochemical characteristics and represents one of the main types of cell death induced in cancer treatment (Singh *et al.*, 2022). Previous studies with the *H. junceus* scorpion venom suggest its ability to induce apoptotic cell death *in vitro* (Díaz-García *et al.*, 2013; Díaz-García *et al.*, 2017). Also, the ability to induce apoptosis of tumor cells has been suggested as a mechanism of action of other scorpion venoms (Bernardes-Oliveira *et al.*, 2019; Desales-Salazar *et al.*, 2020; Mikaelian *et al.*, 2020). However, the ability of venom to induce this type of cell death *in*

vivo had not been evaluated until now, so this hypothesis was corroborated using the TUNEL assay. This methodology allows the staining of cells undergoing apoptosis, even those in very early stages of chromatin margination (Kari *et al.*, 2022). In this regard, it was shown that oral administration of *H. junceus* scorpion venom increased the number of TUNEL-positive cells per field.

There are many techniques available for the detection of apoptosis based on morphological, biochemical, molecular biological, and immunological aspects (Guo *et al.*, 2021). If the objective is to locate apoptotic cells (as is the case in the present study), a TUNEL method or immunofluorescence staining is recommended. Doses of 12.5 and 25 mg/kg of venom significantly increased the number of apoptotic cells compared to the negative control group (saline solution). These doses induced a significant reduction in tumor volume. The induction of apoptosis *in vivo* coincides with that observed in *Hemiscorpius lepturus* scorpion venom, although in the mentioned study, the CT26 colon tumor line was evaluated and the expression levels of *Bax*, *Bcl-2*, *Trp53*, and *Casp3* in the tumor tissue were determined by RT-PCR (Moradi *et al.*, 2019). Furthermore, it has been shown that other venoms, such as that of the *Leiurus quinquestriatus* scorpion induce apoptosis *in vivo*, promoting the suppression of tumor growth of Ehrlich's ascites carcinoma through an increase in the expression of the apoptotic genes *Bax* and *Casp3* and a decrease in the antiapoptotic gene *Bcl-2* (Salama *et al.*, 2023).

Natural products have been primarily used in combination with conventional chemotherapeutic agents, which increases the effectiveness of cancer treatment and achieves a reduction in the side effects

caused by antineoplastic agents (Pezzani *et al.*, 2019; Hackman *et al.*, 2020; Zhang *et al.*, 2020; Vladu *et al.*, 2022). An additional advantage of the combination with natural products is the possibility of reducing the concentration of the conventional drug, which makes it possible to reduce its potential toxic effect on non-tumor cells and prevents chemoresistance, which often leads to the interruption and ineffectiveness of antitumor therapy (Eslami *et al.*, 2024). To date, the type of drug interaction of the combination of *H. junceus* scorpion venom with conventional cytostatics in F3II tumor cells is unknown.

The combination of a fixed concentration of *H. junceus* scorpion venom with different concentrations of CDDP significantly decreased F3II viability to a greater extent than monotherapy *in vitro*. Furthermore, in three of the four combinations of venom and CDDP evaluated, a combination index (CI) indicative of a synergistic interaction between the drugs was obtained. However, in the combination of venom and CTX, no statistically significant differences were observed between the percentage of viable cells between the individual and combined treatments. These results led to CIs indicative of moderate to strong antagonism at all concentrations evaluated.

A study was also conducted combining variable concentrations of venom and CDDP in the F3II model. The results obtained were very positive. For all concentrations evaluated, a synergistic effect and a favorable reduction in the CDDP dose of up to 8.77 times were observed, which can reduce the adverse effects of conventional therapy. The result obtained on the combination at variable concentrations, synergistic and not antagonistic interactions,

is obtained as could be observed in the combination of a fixed concentration of the natural product, coinciding with that reported by Wu *et al.* (2023) with snake venom. Furthermore, the results may vary, depending on the tumor line used in the study.

The mechanism of action of combining scorpion venoms with conventional cytostatics has not been elucidated, so multiple mechanisms are likely involved due to the variety of compounds present in scorpion venoms. However, *H. junceus* scorpion venom has been shown to induce apoptotic cell death in HeLa (Díaz-García *et al.*, 2013) and MDA-MB-231 (Díaz-García *et al.*, 2017) by upregulating the pro-apoptotic genes *Trp53* and *Bax*, as well as downregulating the anti-apoptotic gene *Bcl-2*. Typically, decreased BCL-2 expression has been associated with enhanced chemosensitivity to agents such as DOX (Singh *et al.*, 2022). Authors such as Khazaei *et al.* (2017) have suggested that decreasing the expression of BCL-2 by the natural extract *Allium atrovioleaceum* improves drug sensitivity by modulating the apoptotic signal transduction pathway.

CONCLUSIONS

Heteroctenus junceus scorpion venom increases DNA fragmentation *in vivo*, suggesting that it may inhibit apoptosis resistance in F3II metastatic tumor cells. Furthermore, combined treatment with *H. junceus* scorpion venom and CDDP in F3II may synergistically enhance its antitumor effect *in vitro* and *in vivo*, potentially representing an effective therapeutic strategy in cancer patients, potentially reducing the adverse effects and chemoresistance of conventional therapy.

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CONFLICT OF INTEREST

The authors declared no conflict of interest with respect to the research, authorship, and/or publication of this article.

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