

Scorpion venom cytolytic peptide Smp43 induces caspase-dependent apoptosis in ovarian carcinoma cell line OVCAR-3

Salwa I. Hussein, Mariam M. Gerges, Rana M. Al-Awadhi, Mohamed S. Nafie, Ismail M. Abdel-Nabi, Prashant P. Sharma & Mohamed A. Abdel-Rahman

To cite this article: Salwa I. Hussein, Mariam M. Gerges, Rana M. Al-Awadhi, Mohamed S. Nafie, Ismail M. Abdel-Nabi, Prashant P. Sharma & Mohamed A. Abdel-Rahman (2025) Scorpion venom cytolytic peptide Smp43 induces caspase-dependent apoptosis in ovarian carcinoma cell line OVCAR-3, Egyptian Journal of Basic and Applied Sciences, 12:1, 300-316, DOI: [10.1080/2314808X.2025.2555776](https://doi.org/10.1080/2314808X.2025.2555776)

To link to this article: <https://doi.org/10.1080/2314808X.2025.2555776>



© 2025 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



[View supplementary material](#)



Published online: 11 Sep 2025.



[Submit your article to this journal](#)



Article views: 67



[View related articles](#)



[View Crossmark data](#)

Scorpion venom cytolytic peptide Smp43 induces caspase-dependent apoptosis in ovarian carcinoma cell line OVCAR-3

Salwa I. Hussein^a, Mariam M. Gerges^a, Rana M. Al-Awadhi^b, Mohamed S. Nafe^c,
Ismail M. Abdel-Nabi^a, Prashant P. Sharma^d and Mohamed A. Abdel-Rahman^a

^aZoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt; ^bDepartment of Science, College of Basic Education, The Public Authority for Applied Education and Training (PAAET), Kuwait, Kuwait; ^cChemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt; ^dDepartment of Integrative Biology, University of Wisconsin-Madison, Madison, WI, USA

ABSTRACT

Ovarian cancer ranks as the sixth most prevalent type of gynecological cancer. Smp43, a cationic antimicrobial peptide isolated from the scorpion venom of *Scorpio maurus palmatus*, exhibits notable antibacterial, against both Gram-positive and Gram-negative bacteria, and antifungal activities. This study evaluates the anti-cancer efficacy of Smp43 and examines its effects on cell viability, cell cycle progression, apoptosis, necrosis, and oxidative stress in a human ovarian cancer cell line (OVCAR-3). Smp43 significantly reduced the viability of OVCAR-3 cells compared to the normal fibroblast cell line WI-38, with IC₅₀ values of 7.75 µg/mL and 29.50 µg/mL, respectively. The peptide effectively caused G1 phase cell cycle arrest and apoptosis in OVCAR-3 cells. It modulated apoptotic markers by downregulating the pro-survival marker Bcl-2 while upregulating the pro-apoptotic markers Bax, p53, caspase-3, caspase-8, and caspase-9. Additionally, Smp43 significantly increased DNA fragmentation in OVCAR-3 cells and decreased antioxidant parameters. These findings suggest that Smp43 possesses potential anti-ovarian carcinoma properties, exerting its effects through mechanisms involving apoptosis induction, necrosis, G1 cell cycle arrest, and inhibition of the antioxidant defense system.

ARTICLE HISTORY

Received 5 June 2025

Revised 30 July 2025

Accepted 27 August 2025

KEYWORDS

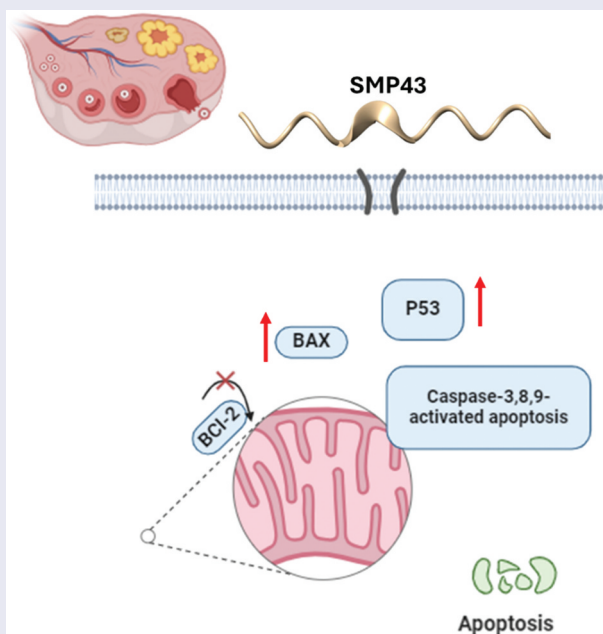
Apoptosis; necrosis; ovarian cancer; scorpion venom antimicrobial peptide; Smp43; *Scorpio maurus palmatus*

CONTACT Mohamed A. Abdel-Rahman mohamed_hassanain@science.suez.edu.eg Zoology Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt

Supplemental data for this article can be accessed online at <https://doi.org/10.1080/2314808X.2025.2555776>

© 2025 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.



Smp43, a scorpion venom-derived peptide, selectively induces apoptosis in ovarian cancer cells (OVCAR-3) by activating intrinsic and extrinsic pathways. Mechanistically, Smp43 upregulates pro-apoptotic Bax and p53, downregulates anti-apoptotic Bcl-2, and triggers caspase-3, -8, and -9 activation.

Introduction

Ovarian cancer is the sixth most prevalent type of gynecological cancer [1]. It is the second most lethal kind of cancer in women after breast cancer [2]. High death rates are associated with the late discovery of ovarian cancer, when the tumor has already metastasized to other organs, making early detection critical for providing efficient therapy [3]. Cancer patients treated with classical chemotherapy frequently have cumulative adverse effects and are more likely to develop treatment resistance [2]. Accordingly, it is critical to identify more effective therapeutic agents that can overcome these crucial side effects and drug resistance.

Natural products as anti-cancer agents are a viable treatment option due to their accessibility, selectivity, and low cytotoxicity [4]. Animal venoms and toxins have been known to humans for generations as a possible

bioresource and therapeutic aid through folk and traditional knowledge [5]. Venoms contain various bioactive components, including proteins and peptides that can work enzymatically or nonenzymatically, and these components show great promise in treating cancer and other disorders [6,7]. Venom components are selective, stable, and potent molecules, making them promising therapeutic options for treating various diseases, including cancer [8]. Scorpion venom is a natural biological resource with components that can cause death but also have therapeutic potential [9–11]. Peptides isolated from scorpion venom have been demonstrated to limit cancer cell proliferation by disrupting cell membranes, inhibiting voltage-dependent ion channels, or interfering with the cellular process by targeting specific cellular structures [12–14].

Antimicrobial peptides (AMPs) have a variety of functions, including immunomodulatory and

anti-cancer properties [15]. Cationic amphipathic peptides have the potential to be a selective and refractory source of developing novel anti-cancer drugs due to the higher levels of phosphatidylserine (negatively charged) on the surface of cancer cells compared to normal cells [16]. Smp43 is a cationic peptide isolated from the venom of the scorpion *Scorpio maurus palmatus* collected from Egypt [17]. Smp43 has been revealed to exhibit high antibacterial activity against Gram-positive and Gram-negative bacteria, as well as antifungal activity [18]. Smp43 also strongly inhibits tumor growth through various cellular and molecular pathways, including apoptosis, pyroptosis, autophagy, necrosis, and cell cycle arrest via mitochondrial malfunction and membrane rupture in leukemia cell lines myeloid KG1-a and lymphoid CCRF-CEM [19,20], human HepG2 [21], breast cancer cell lines MDA-MB-231 and MCF-7 [22] and non-small-cell lung cancer A549 cells [12]. In addition, Smp43 exhibits anti-tumor efficacy in the HepG2 xenograft mice model by reducing tumor size [21]. Previously, fast-scan atomic force microscopy (AFM) revealed that scorpion venom peptide Smp43 disrupts both prokaryotic and eukaryotic model lipid membranes through a common mechanism called DLD (diffusion-limited disruption). DLD involves elements of both the carpet model and pore formation [23]. The present work assessed the anti-cancer efficacy of Smp43 and investigated its impact on cell viability, cell cycle, apoptosis, necrosis, and oxidative stress in a human ovarian cancer cell line.

Materials and methods

Chemicals and reagents

Biowest (Maine et Loire, France) provided the RPMI-1640 Medium Liquid with L-glutamine, phosphate-buffered saline (PBS), and antibiotics (penicillin and streptomycin), while Seralab (UK) provided the fetal bovine serum (FBS). Trypsin was purchased from Sigma (USA),

while dimethyl sulfoxide (DMSO) was obtained from Amresco (USA). MTT is available from Lonza Verviers SPRL in Belgium. Annexin V-FITC stain kit was obtained from BioVision Research Products (CA 94,043, USA). Oxidative stress kits were obtained from Bio-diagnostics Company (El Motor St, Dokki, Egypt).

Scorpion venom peptide Smp43

The scorpion venom peptide Smp43 (GVWDWIKKTAGKIWNSEPVKALKSQALNAA-KNFVAEKIGATPS) [17,24] was synthesized using solid-phase chemistry. The synthesis was performed by the company Davids Biotechnologie GmbH (Deutschland, Germany) with a purity greater than 90% (the Raman analysis of Smp43 is provided as supplementary materials S1).

Culture of cell lines

The human ovarian cancer cell line OVCAR-3 and normal fibroblast cell line WI-38 were obtained from VACSERA (Egypt). OVCAR-3 was cultured in RPMI-1640 medium supplemented with L-glutamine, 10% FBS, and 1% antibiotic. The cells were incubated in 5% CO₂ in a humidified 37°C environment for growth. The culture was sub-cultured every 2–3 days and inspected for contamination using an inverted microscope.

Cytotoxicity of Smp43 using MTT assay

The MTT assay (3-[4, 5-methylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide) was used to assess the effect of Smp43 on the viability of cell lines [25]. WI-38 and OVCAR-3 cells were treated for 24 hours with different concentrations of Smp43 (100, 25, 6.25, 1.6, and 0.4 µg/mL) dissolved in phosphate-buffered saline (PBS). Following the measurement of cell count and vitality with trypan blue dye, cells (1×10^4 cells/well) were planted on a 96-well plate and left to adhere for 24 hours. The

following day, cells were incubated for 24 hours in a fresh medium containing the indicated concentrations of Smp43. After incubation, each well was treated with 10 μ L of MTT (5 mg/mL in PBS) before incubating the plates for 4 hours. Following the incubation, 100 μ L of DMSO was poured into each well, gently mixed using a pipette, and the plates were incubated for 10 minutes at room temperature to precipitate formazan crystals. The optical density at 540 nm was then evaluated using a Bio-Tek microplate reader to calculate the number of living cells. The absorbance at 720 nm, representing background levels in multiwell plates, was measured and subsequently deducted from the absorbance recorded at 540 nm. Staurosporine (Sigma), an apoptosis inducer, was used as a positive control.

The tests were carried out three times. The following formula was used to calculate the percentage of cell viability:

$$\% \text{cell viability} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

To determine the 50% inhibitory concentration (IC_{50}), GraphPad Prism software was used to plot the concentration–response curves for each dose.

Apoptosis assay (annexin V-FITC/PI staining)

Apoptosis was detected in cells using the Annexin V-FITC stain kit by flow cytometer (FACSCalibur, Becton-Dickinson, USA). OVCAR-3 cells were added to a 6-well culture plate (3×10^5 cells/well) and cultured at 37°C and 5% CO_2 for 24 hours. After this period, Smp43 (IC_{50} ; 7.75 μ g/ml) was added, and the cell culture was incubated for 24 hours before being harvested by trypsinization. PBS was used to wash the cells twice, followed by centrifugation. The pellet was suspended in 500 μ L of Annexin V binding buffer and stained with Annexin V-FITC and

propidium iodide following the manufacturer's protocol. For 5 minutes, cells were incubated in the dark at room temperature, and a flow cytometer was used to measure their mean fluorescence intensities. Annexin V⁻ and PI⁻ cells, annexin V⁻, PI⁺, annexin V⁺, PI⁻ and annexin V⁺, and PI⁺ were all reported as percentages of living cells, necrotic cells, early apoptotic cells, late apoptotic/dead cells, respectively [26]. After treatment with the Smp43 peptide, the percentage of apoptotic cells was measured and compared to that of untreated cells.

Cell cycle assay using flow cytometry

The cell cycle distribution of OVCAR-3 cells was checked using propidium iodide (PI) staining [27]. The cells were seeded in 6-well plates and cultured until the cell density reached 80%. After treatment with IC_{50} of Smp43 (7.75 μ g/ml), 1×10^6 cells were collected to analyze the cell cycle distribution and fixed in 70% ethanol at 4°C overnight. The cells were then labeled for 30 minutes with PI (1 mg/mL) in the presence of 1% RNase. A flow cytometer at 488 nm excitation and 630 nm emission wavelengths was used to determine the percentages of cells in the G0/G1, S, and G2/M stages.

Real time-PCR assay of apoptosis-related genes

OVCAR-3 cells (2×10^5 /well) seeded on 24-well culture plates were cultured for 24 hours. Then, control and treated (IC_{50} ; 7.75 μ g/ml of Smp43) cell cultures were incubated for 24 hours. Total RNA was isolated from cells using Trizol reagent (Qiagen RNA extraction) [28], and RT-PCR was performed using BioRad SYBR green PCR MMX according to the manufacturer's instructions for the *bax*, *Bcl-2*, *p53*, *caspase3*, *caspase8* and *caspase9* genes. The PCR primers (Table 1) to amplify products within target and control

Table 1. Summary of primer sequences for the genes of *Bax*, *Bcl-2*, *P53*, *caspase3*, *caspase8*, *caspase9* and β -actin used in RT-PCR assay.

Gene marker	Primer sequence
<i>BAX</i>	F: 5'-ATGGACGGGTCCGGGGAG-3' R: 5'-ATCCAGCCCAACAGCCGC-3'
<i>Bcl-2</i>	F: 5'-AAG CCG GCG ACGACT TCT-3' R: 5'-GGT GCC GGT TCA GGTACT CA-3'
<i>P53</i>	F: 5'-ATGTTTTGCCAACTGGCCAAG -3' R: 5'-TGAGCAGCGCTCATGGTG-3'
<i>Caspase-3</i>	F: 5'-TGTTTGTGTGCTTCTGAGCC -3' R: 5'-CACGCCATGTCATCATCAAC -3'
<i>Caspase8</i>	F: 5'-AGAAGAGGGTCATCCTGGGAGA-3' R: 5'-TCAGGACTTCCTCAAGGCTGC-3'
<i>Caspase9</i>	F: 5'-CATTTCATGGTGGAGGTGAAG-3' R: 5'-GGGAAGTGCAGGTGGCTG-3'
β -actin	F: 5'-ATC GTG GGG CGC CCC AGG CAC-3' R: 5'-CTC CTT AAT GTC ACG CAC GAT TTC-3'

sequences were designed by Rotor-Gene RT-PCR Software 1.7 (Build 87), Corbett Research, a Division of Corbett Life Science. The intensity of each gene was standardized based on its β -actin levels, and the relative standard curve approach was employed for analysis. The results were determined as the mean \pm standard error of the mean of triplicate experiments.

Detection of DNA fragmentation by agarose gel electrophoresis

Ovarian cancer cells were incubated at 37°C with IC₅₀ of Smp43 (7.75 μ g/ml) for 24 hours. To release DNA, the nuclei of the cells were ruptured using the lysis solution of 0.2% Triton X-100, 10 mM Tris, and 1 mM EDTA (pH 8.0). DNA was extracted from the supernatant using a 25:43:1 (v/v/v) equal volume of phenol, chloroform, and isoamyl alcohol. TE buffer was utilized to dissolve the DNA after ethanol precipitation and air-drying with 5 mM Tris-HCl (pH 8.0) and 20 mM EDTA containing RNase A (0.1 mg/mL; Sigma). Electrophoretically, the specimens were examined on a 1% agarose gel containing 0.1 μ g/mL ethidium bromide [29].

Western blot of cell proliferation/migration/invasion-related proteins

Western blot assay evaluated the expression of cell proliferation/migration/invasion-related proteins in treated and untreated cells [30]. Ovarian cancer cell lysates were prepared in RIPA buffer after being treated with Smp43 (IC₅₀; 7.75 μ g/ml) for 24 hours in 6-well plates, and the SDS-polyacrylamide gel was then loaded with protein samples and transferred to polyvinylidene fluoride (PVDF) membranes via electrophoresis after being separated. PVDF membranes were blocked using 5% milk in 1 \times TBS solution (tris-buffered saline) containing 0.05% (v/v) Tween[®] 20 for 4 hours at room temperature, followed by seven washings with 1 \times TBS and 1 \times TBST (tris-buffered saline with Tween[®] 20) alternatively. Membranes were then treated overnight at 4°C with primary antibody, followed by an hour-long treatment with secondary antibody at room temperature, and finally detected by ECL (enhanced chemiluminescence) reagents. The optical density of bands was captured by a CCD camera-based imager (Biorad), and the band intensities were then quantified by Image Lab (Biorad), and the relative protein expression levels were normalized to β -actin.

Oxidative stress biomarkers

OVCAR-3 cells (2×10^6 cells/mL in complete RPMI medium) were incubated with IC₅₀ (7.75 μ g/mL) of Smp43 venom peptide for 24 hours at 37°C. The cells were harvested by centrifugation at 3000 \times g, and then the supernatant was collected. Using a colorimetric method, the supernatants were used for the determination of glutathione reduced (GSH) form [31], catalase (CAT) [32]; nitric oxide (NO) [33]; and malondialdehyde (MDA) [34]. Every test was performed according to the manufacturer's guidelines of Bio-diagnostics Company (El Motor St, Dokki, Egypt).

Statistical analysis

GraphPad Prism 9.5.0 (GraphPad Software Inc., CA, USA) was used to do all the statistical analysis, and the data is presented as mean \pm standard error (SEM). The statistically significant difference was defined as $p \leq 0.05$, while high levels of significance were referred to as $**p \leq 0.01$ and $***p \leq 0.001$. An unpaired Student's T-test was performed to assess the significance of differences between the two groups (control and treated groups). One-way ANOVA was used to compare between more than two groups.

Results

Cytotoxicity of Smp43

The toxicity of Smp43 and staurosporine (0.40, 1.60, 6.25, 25, and 100 $\mu\text{g}/\text{mL}$) on the cancer cell line (OVCAR-3) and the non-cancer cell line (WI-38) was examined using MTT assay. The data obtained demonstrate that

Smp43 had a direct, dose-dependent cytotoxic effect on the examined cell lines (Figures 1 and 2). Smp43 exhibited IC_{50} values of 7.75 and 29.50 $\mu\text{g}/\text{mL}$ for OVCAR-3 and WI-38 cells, respectively, while Staurosporine IC_{50} values were 6.69 and 11.31 $\mu\text{g}/\text{mL}$ (Table 2).

Apoptotic and necrotic activities of Smp43

Apoptosis and necrosis were investigated using Annexin V-FITC/PI staining in both control and Smp43-treated OVCAR-3 cells ($\text{IC}_{50} = 7.75 \mu\text{g}/\text{mL}$, 48 h (Figure 3)). The early apoptotic proportion for Smp43-treated OVCAR-3 cells was 6.2%, while the control OVCAR-3 cells had a proportion of 6%. On the other hand, the proportion of late apoptotic OVCAR-3 cells treated with Smp43 was 46.4% as opposed to 10.1% in control OVCAR-3 cells. In untreated OVCAR-3 cells, necrotic events reached 10.9%, but in Smp43-treated OVCAR-3 cells, the rate dropped to 4.4% (Figure 3).

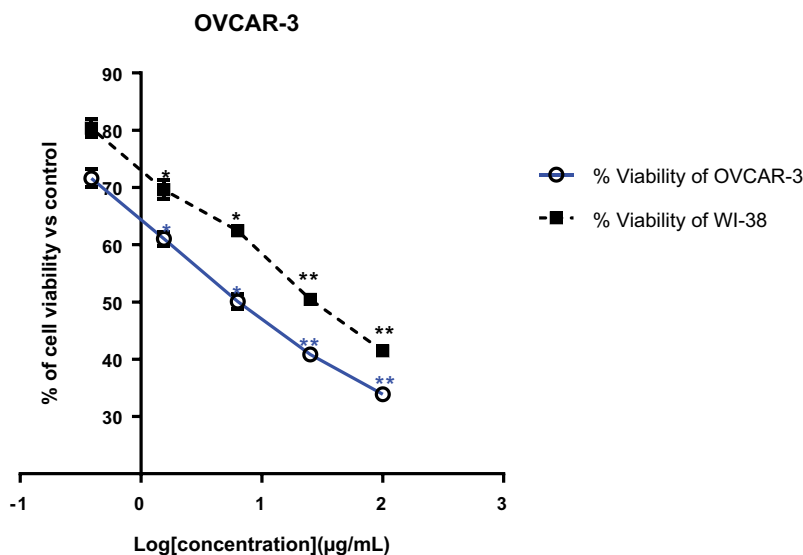


Figure 1. Percentage of cell viability of Smp43 (the $\text{IC}_{50} = 7.75 \mu\text{g}/\text{mL}$) against OVCAR-3. Percentages of cell viability were calculated compared to control $** (p \leq 0.001)$, and $* (p \leq 0.05)$ are significantly different between different concentrations using one-way ANOVA. Values are expressed as mean \pm SEM of three independent experiments.

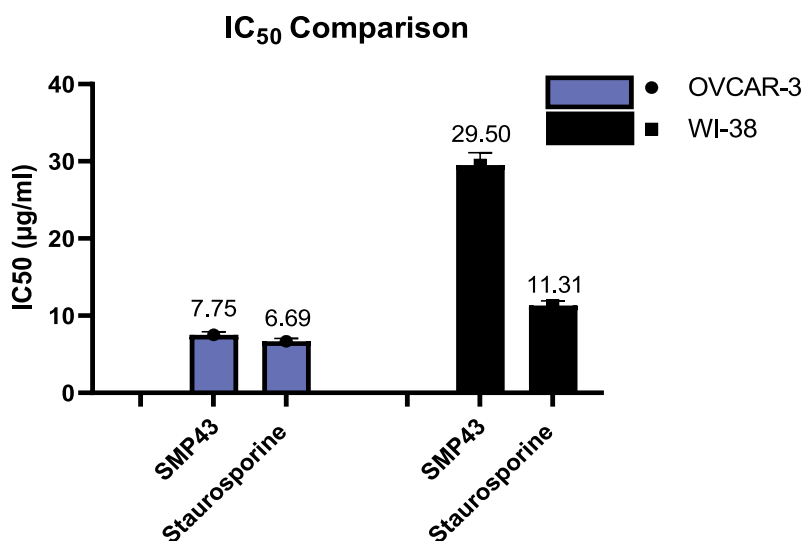


Figure 2. Percentage of cell viability of Smp43 on OVCAR-3 and WI-38 cell lines after using serial concentrations [100, 25, 6.25, 1.6, and 0.4 µg/mL]. IC₅₀ values were calculated using non-linear regression. Values are expressed as mean ± SEM of three independent experiments.

Table 2. IC₅₀ (µg/mL) of Smp43 and staurosporine on cancer cell line (OVCAR-3) and the non-cancer cell line (WI-38).

Peptide	IC ₅₀ ± SEM* (µg/mL)	
	Cancerous cell line OVCAR-3	Non-cancerous cell line WI-38
Smp43	7.75 ± 0.43	29.50 ± 1.62
Staurosporine	6.69 ± 0.37	11.31 ± 0.62

Values are expressed as mean ± SEM of three independent experiments.

These results revealed that the Smp43 peptide effectively induced apoptosis in OVCAR-3 cells, especially late apoptosis.

Effect of Smp43 on the cell cycle of OVCAR-3

OVCAR-3 cells were treated with IC₅₀ (7.75 µg/mL, 48 h of Smp43 to determine its effect on the cell cycle. Cell cycle progression was tracked by flow cytometry after being labeled with PI. Smp43 treatment increased the cells' population in the G1 phase from 31.5% to 51.7%, while it decreased the cell population at the S and G2/M phases from 13.7% and 45.5% to 6.3% and 25.1%, respectively (Figure 4). These results

revealed that Smp43 could prevent cell division in OVCAR-3 by blocking the cell cycle at the G1 phase.

RT-PCR analysis of apoptosis-related genes

Expression patterns of *Bax*, *Bcl-2*, *P53*, *Caspase-3*, *Caspase-8*, and *Caspase-9* were investigated by RT-PCR to determine their involvement in Smp43-induced cell death in OVCAR-3 cells. After 48 hours of treatment, the effect of Smp43 (IC₅₀ = 7.75 µg/mL) on apoptosis-related genes was assessed. Compared to β-actin, the internal control, the expression of *Bcl-2* (an anti-apoptotic signal) was significantly ($***p \leq 0.001$) down-regulated by 0.2-fold change in treated cell lines (Figure 5). In contrast, pro-apoptotic signals *Bax* ($***p \leq 0.001$), *p53*, *Caspase-3*, *Caspase-8*, and *Caspase-9* were significantly ($**p \leq 0.01$) up-regulated by 6.92, 5.81, 5.37, 5.77 and 2.76-fold change in treated cell lines, respectively, confirming apoptosis as the effective molecular target of this toxin.

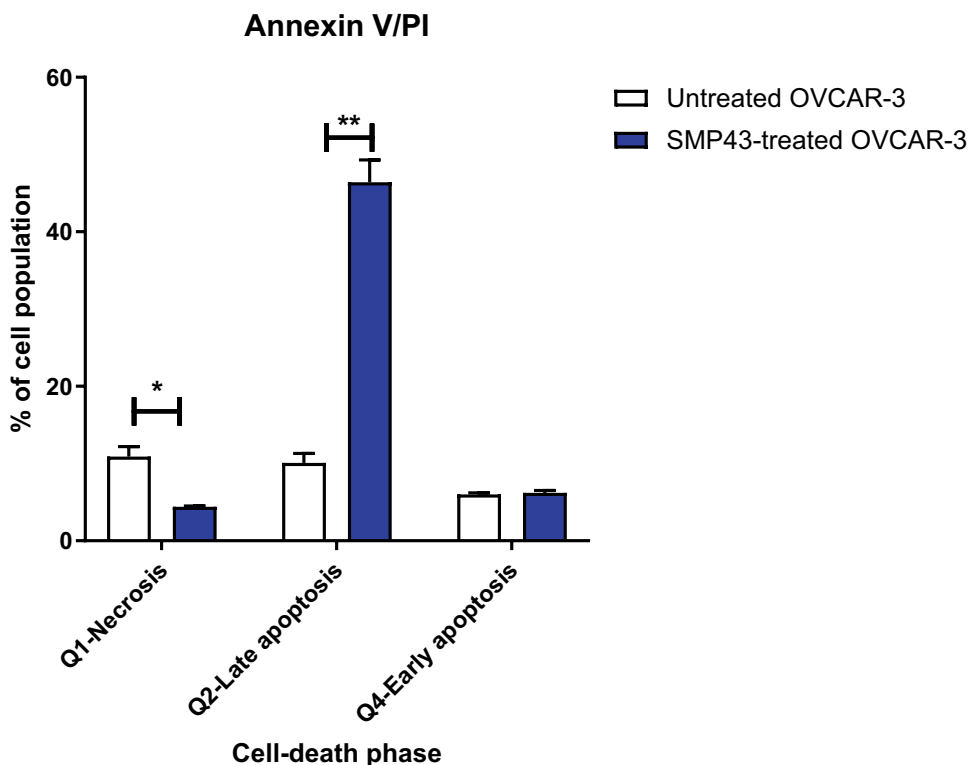


Figure 3. Annexin-V/Propidium iodide staining for apoptosis-necrosis assessment in both untreated and Smp43-treated OVCAR-3 cells ($IC_{50} = 7.75 \mu\text{g/mL}$, 48 h). 'Q1 (necrosis, AV-/PI+), Q2 (late apoptotic cells, AV+/PI+), Q3 (normal cells, AV-/PI-), Q4 (early apoptotic cells, AV+/PI)', ** ($p \leq 0.001$), and * ($p \leq 0.05$) significantly different between untreated and treated OVCAR-3 cells using unpaired t-test in GraphPad prism. Values are expressed as mean \pm SEM of two independent experiments.

Effect of Smp43 on DNA fragmentation

The obtained results from agarose electrophoresis (Figure 6) revealed that Smp43-induced DNA fragmentation in OVCAR-3 cells compared to untreated cells.

Western blotting analysis of apoptosis-related proteins

Additionally, Western blotting for Bcl-2, Bax, Caspase-3, Caspase-9, and p53 has been used to validate the apoptotic pathway of Smp43 ($IC_{50} = 7.75 \mu\text{g/mL}$, 24 h) at the protein expression level on OVCAR-3 cells. Treatment with Smp43 resulted in a considerable increase ($*p \leq 0.05$) in the band

intensity of Bax from 11.69% to 23.12%, Capases-3 from 13.96% to 28.96%, Capases-8 from 7.7% to 17.47%, and P53 from 15.56% to 27.28%, and a significant decrease ($*p \leq 0.05$) in the band intensity of Bcl-2 from 24.19% to 17.47% relative to β -actin as a housekeeping protein (Figure 7).

Effect of Smp43 on oxidative stress biomarkers

The status of oxidative stress biomarkers (oxidants and antioxidants) in both untreated and Smp43-treated OVCAR-3 cells is presented in Figure 8. Smp43 treatment caused a significant decrease in MDA level from 3.5 nmol/mL to 1.78 nmol/mL and NO level from 3.2 nmol/mL to 2.1

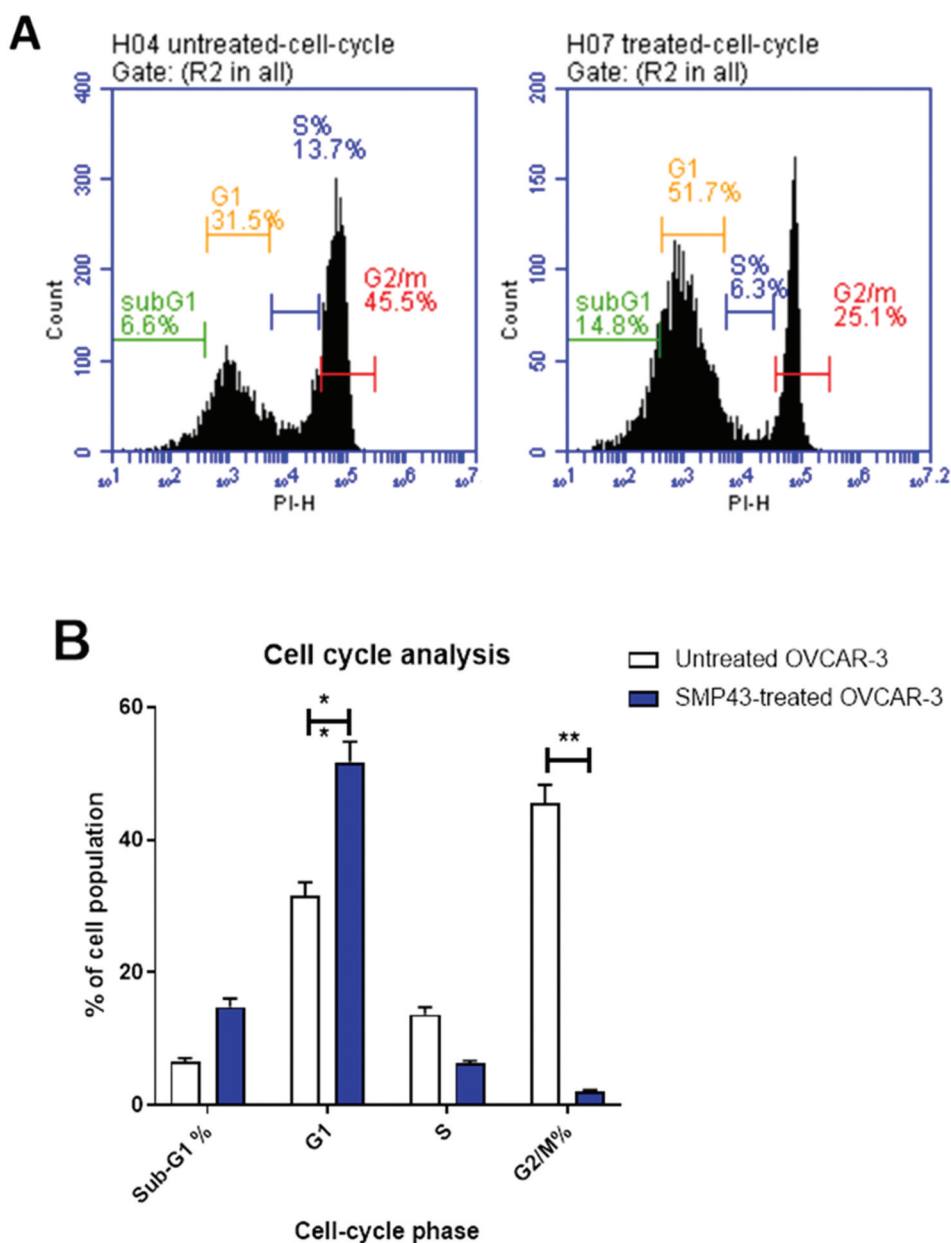


Figure 4. A: cytogram reflecting cell cycle distribution at each cell phase of OVCAR-3 cells in both untreated and Smp43-treated cells. B: bar representation for quantification of cell population at each cell cycle. ** ($p \leq 0.001$), and * ($p \leq 0.05$) significantly different between untreated and treated OVCAR-3 cells using unpaired t-test in graph pad prism. Values are expressed as mean \pm SEM of two independent experiments.

nmol/mL for the oxidant activity. Also, Smp43 treatment caused a significant decrease in SOD activity from 5.88 nmol/mL to 2.19 nmol/mL, CAT activity from 13.2 nmol/mL to 7.9 nmol/mL, and

GSH from 8.4 nmol/mL to 2.64 nmol/mL. So, Smp43 induced a significant decrease ($p \leq 0.05$) in both oxidants (MDA and NO) and antioxidant parameters (GSH, CAT, and SOD).

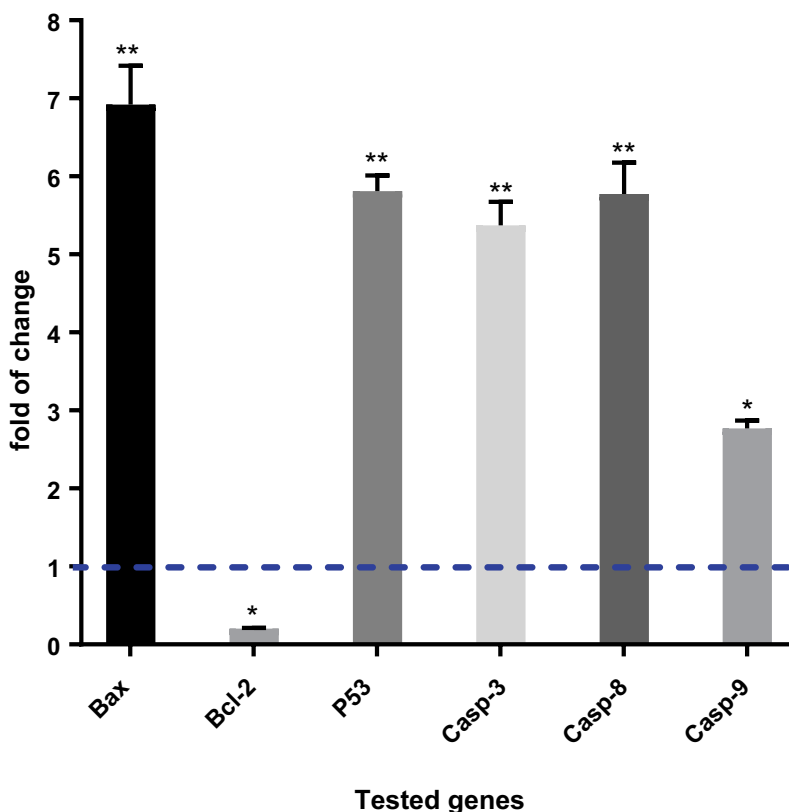


Figure 5. RT-PCR analysis of apoptosis-related genes in OVCAR-3 cells treated with Smp43 ($IC_{50} = 7.75 \mu\text{g/mL}$, 48 h). Fold-change = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ the difference between mean values of genes, CT values in the treated and control groups. Fold of change in the untreated control = 1." statistical significance was examined using student's t-test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). Values are expressed as mean \pm SEM of three independent experiments.

Discussion

The resistance of cancerous cells to chemotherapeutics has made treatment more difficult. Animal venoms have emerged as a novel anti-cancer therapy approach [8]. Smp43, a cationic antimicrobial peptide derived from the venom of *Scorpio maurus palmatus* (Abdel-Rahman et al., 2013) has been shown to inhibit the proliferation of several cancer cell lines [12,13]. The cytotoxicity of Smp43 was tested using an MTT assay on human ovarian cancer (OVCAR-3) and non-cancer (WI-38) cell lines after 24 hours of incubation. Our findings indicate that Smp43 effectively inhibits the growth of OVCAR-3 cells, with just a minimal impact on WI-38

cells. This suggests that Smp43 exhibits a strong preference or selectivity for targeting tumor cells [13,20,35]. It was found that Smp43 has strong anti-tumor effects against hepatoma HepG2, lung adenocarcinoma A549, and colorectal cancer HCT-116 cells through various mechanisms [13,35,36].

Besides membrane disruption, Smp43 has been shown to have an anti-tumor effect by targeting different cellular structures, interfering with intrinsic pathways, and altering apoptosis, autophagy, and cell cycle distribution [37]. Overall, the anti-cancer impact of Smp43 is linked to its ability to trigger apoptosis, pyroptosis, autophagy, and cell cycle arrest through

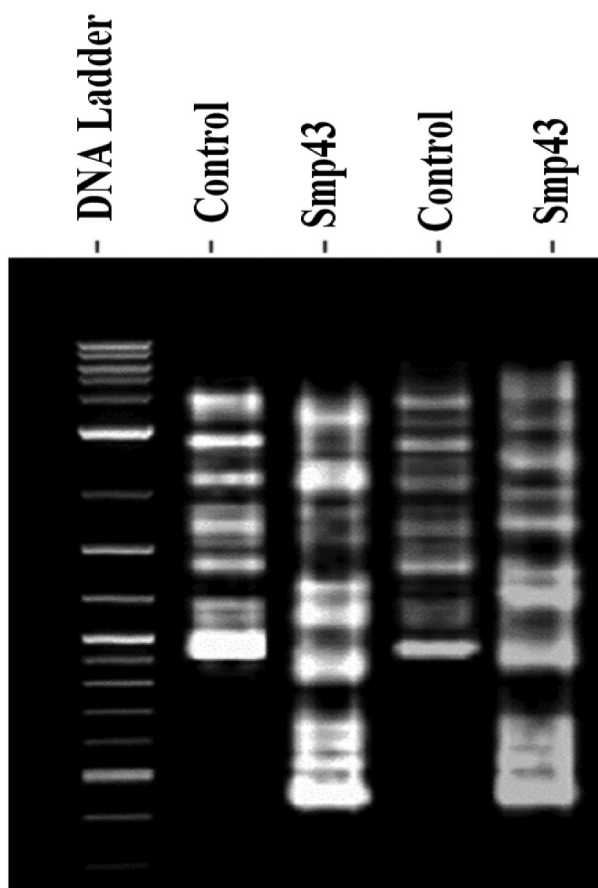


Figure 6. DNA fragmentation analysis in OVCAR-3 cells after 24 hours of treatment with the IC_{50} value ($7.75 \mu\text{g}/\text{mL}$) of Smp43 using agarose gel electrophoresis (1% agarose gel containing $0.1 \mu\text{g}/\text{mL}$ ethidium bromide). Results are expressed in duplets.

mitochondrial malfunction and the formation of reactive oxygen species (ROS) [13,19,36].

The present work used Annexin V-FITC/PI staining to examine apoptosis and necrosis induced by the scorpion venom peptide Smp43. It caused apoptosis in the ovarian cancer cell line OVCAR-3 by showing an increase in early and late apoptotic cells. Smp43 was found to have a greater effect on late apoptosis than early apoptosis in OVCAR-3 cell lines. At the concentration of IC_{50} , the proportion of OVCAR-3-treated cells that were apoptotic and necrotic was comparatively higher than that of untreated cells. Similarly, BmKn-2, an

antimicrobial peptide produced from scorpion venom, reduced proliferation and caused apoptosis in the canine mammary gland cancer cell lines CHMp-5b and CHMp-13a [38]. In addition, neoplamine-1 and -2 from *Tityus discrepans* scorpion venom triggered apoptosis in the human breast cancer cell line SKBR3 [39].

To investigate the effect of Smp43 on the cell cycle, OVCAR-3 cells were treated with the IC_{50} of the scorpion venom peptide. Smp43 significantly enriched the G1 state in OVCAR-3 cells, leading to G1 cell cycle arrest. Consistent with the obtained effect [12], it was found that Smp43 treatment inhibited the expression of

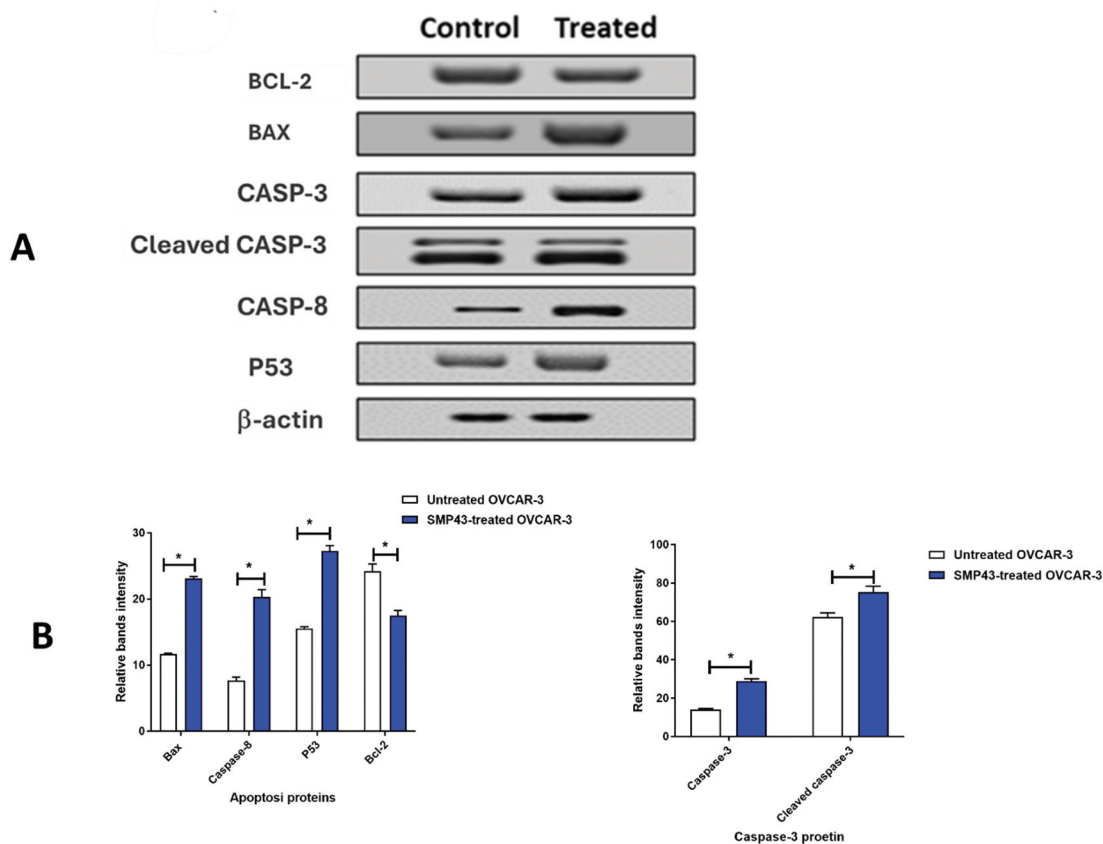


Figure 7. Western blot analysis for apoptotic proteins. (A) Western blot gels for the target proteins in control and treated groups, (B) bar charts for the target proteins relative to β -actin. Data are expressed as mean \pm SD of two independent trials and analyzed by unpaired t-test. P-value < 0.05 was statistically significant (*).

cyclin A, cyclin E, and CDK2, causing non-small-cell lung cancer A549 cells to accumulate in the S phase of the cell cycle.

The expression patterns of *Bax*, *Bcl-2*, *p53*, *caspase3*, *caspase8*, and *caspase9* were examined using RT-PCR to identify their role in OVCAR-3 cell death triggered by Smp43. Apoptotic genes play a significant role in the development and growth of cancer. The expression of *Bcl-2* was significantly down-regulated in OVCAR-3 cells treated with Smp43, while pro-apoptotic signals *bax*, *p53*, *caspase3*, *caspase8*, and *caspase9* were significantly up-regulated. Previous research on scorpion venom peptides found similar findings [12,13,40–42]. Moreover, DNA fragmentation

in OVCAR-3 cells was greatly exacerbated by Smp43. The same findings were obtained by other antimicrobial peptides (AMPs BMAP-27 and –28), which caused DNA fragmentation in various human leukemia cell lines [43]. The fragmentation of chromosomal DNA into small fragments of oligonucleosomal size is a crucial component of apoptosis [44].

Oxidative stress refers to a state when there is an unequal proportion between the generation of free radicals and reactive metabolites, known as oxidants or reactive oxygen species (ROS), and their removal by defensive mechanisms, also known as antioxidants [45]. Malondialdehyde (MDA) is a byproduct of the oxidation of polyunsaturated fatty acids. It has been utilized as a

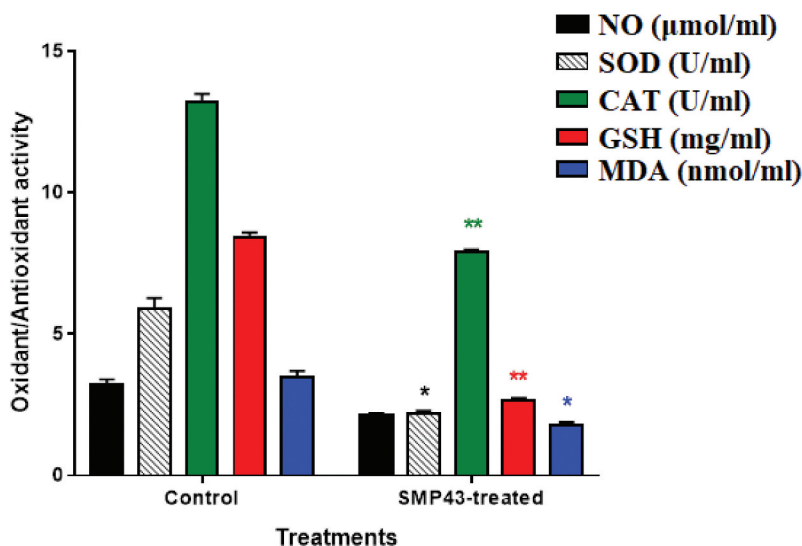


Figure 8. Oxidant and antioxidant activity in untreated and Smp43-treated OVCAR-3 cells ($IC_{50} = 7.75 \mu\text{g/mL}$, 48 h). Values are expressed as mean \pm SEM for three independent experimental runs. ** ($p \leq 0.001$), and * ($p \leq 0.05$) significantly different between untreated and treated OVCAR-3 cells using student's t-test in graph pad prism.

biomarker to quantify oxidative stress in diverse biological specimens in patients afflicted with a broad spectrum of disorders [46]. The formation of reactive oxygen species (ROS) can lead to the generation of reactive nitrogen species (RNS), including the extremely reactive peroxy nitrite anion. This anion is a potent oxidant that forms when O_2^- and nitric oxide (NO) combine [47]. Glutathione (GSH) is the predominant non-protein thiol found in mammalian tissues, existing in high quantities in the millimolar range. As a crucial antioxidant within cells, it functions as a regulator of the cellular redox state, safeguarding cells from harm caused by lipid peroxides, ROS, RNS, and xenobiotics [48]. Superoxide dismutase (SOD) and catalase are enzymes that safeguard cells against radical assault. Catalase catalyzes the decomposition of hydrogen peroxide, while SOD functions as an oxidoreductase to convert the superoxide anion into less harmful substances.

OVCAR-3 cells treated with Smp43 exhibited a notable reduction in oxidant parameters, including MDA and NO, as well as a significant decline in antioxidant parameters, such as GSH, CAT, and

SOD. The suppression of cancer cell proliferation may be attributed to the inhibition of oxidative and antioxidant markers. Tumor cells experience excessive cell division and produce a large amount of ROS. However, they have adapted to survive in an environment where the imbalance caused by oxidative stress pushes the redox balance away from a reduced state. Tumor cells achieve this by enhancing their antioxidant capacity to promote cell division driven by ROS while also avoiding ROS levels that would activate senescence, apoptosis, or ferroptosis [49].

In conclusion, the study reveals that the Smp43 peptide exhibits significant anticarcinoma properties against ovarian cancer cells (OVCAR-3, IC_{50} value of $7.75 \mu\text{g/mL}$). It induced apoptosis, particularly late apoptosis. Smp43 caused cell cycle arrest in the G1 phase, upregulated pro-apoptotic genes (*Bax*, *p53*, *caspase-3*, *caspase-8*, *caspase-9*), and downregulated the anti-apoptotic gene *Bcl-2*. It also induced DNA fragmentation and disrupted the redox balance by decreasing oxidant and antioxidant parameters. These findings suggest that Smp43 has potential as an anticarcinoma agent; further

research into its therapeutic applications and interactions with other anti-cancer drugs is needed.

Highlights

- Smp43 selectively inhibited ovarian cancer (OVCAR-3) cell viability with minimal toxicity to normal fibroblasts (WI-38), showing an IC₅₀ of 7.75 µg/mL vs. 29.50 µg/mL.
- Treatment with Smp43 induced significant late apoptosis and reduced necrosis in OVCAR-3 cells.
- Smp43 caused G1-phase cell cycle arrest in OVCAR-3 cells, impairing cell proliferation.
- Apoptosis induction was confirmed by upregulation of Bax, p53, caspase-3, -8, and -9, and down-regulation of Bcl-2 at both mRNA and protein levels.
- Smp43 significantly elevated DNA fragmentation and decreased antioxidant biomarkers, suggesting that oxidative stress is involved in cytotoxicity.

Disclosure statement


The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This paper was based upon work supported by the Science, Technology & Innovation Funding Authority (STDF; Egypt) under grant number 45890 awarded to Mohamed A. Abdel-Rahman and National Science Academy (USA) awarded to Prashant P. Sharma, U. S.-Egypt Science, and Technology Joint Fund.

ORCID

Mohamed S. Nafie  <http://orcid.org/0000-0003-4454-6390>

Mohamed A. Abdel-Rahman  <http://orcid.org/0000-0003-3862-5535>

Authors contribution

Salwa I. Hussein and Mariam M. Gerges: Investigation, Methodology, Data analysis, Data curation, original draft preparation. Rana M. Al-Awadhi: Data analysis, writing, review, and editing, resources. Mohamed S.

Nafie: Supervision, Validation, Software, Data curation, original draft preparation. Ismail M. Abdel-Nabi and Prashant P. Sharma: Supervision, writing, review, and editing. Mohamed A. Abdel-Rahman: Conceptualization, Supervision, Project administration, Resources, Validation, Data curation, Writing – review the final draft & editing. All authors have read and approved the final version of the manuscript.

Data availability statement

The data supporting this study's findings are available from the corresponding author, [Mohamed A. Abdel-Rahman], upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that generative artificial intelligence (AI) and AI-assisted technologies were not used in the writing process or any other process during the preparation of this manuscript.

Ethics statement

The Research Ethics Committee of the Faculty of Science, Suez Canal University, has approved the protocols of this work (Protocol No REC54/2020); no experimental animals have been used in this study.

References

- [1] Akbarzadeh M, Movassaghpour AA, Ghanbari H, et al. The potential therapeutic effect of melatonin on human ovarian cancer by inhibition of invasion and migration of cancer stem cells. *Sci Rep.* 2017;12/06 2017;7(1):17062. doi: [10.1038/s41598-017-16940-y](https://doi.org/10.1038/s41598-017-16940-y)
- [2] Yan YB, Tian Q, Zhang JF, et al. Antitumor effects and molecular mechanisms of action of natural products in ovarian cancer (review). *Oncol Lett.* 2020 Nov;20(5):1–1. doi: [10.3892/ol.2020.12001](https://doi.org/10.3892/ol.2020.12001)
- [3] Bernardo ADM, Thorsteinsdóttir S, Mummery CL. Advantages of the avian model for human ovarian cancer. *Mol And Clin Oncol.* 2015 Nov;3(6):1191–1198. doi: [10.3892/mco.2015.619](https://doi.org/10.3892/mco.2015.619)

- [4] Hashem S, Ali TA, Akhtar S, et al. Targeting cancer signaling pathways by natural products: exploring promising anti-cancer agents. *Biomed & Pharmacother.* 2022 [2022 Oct 1];150:113054. doi: [10.1016/j.biopha.2022.113054](https://doi.org/10.1016/j.biopha.2022.113054)
- [5] Gomes A, Bhattacharjee P, Mishra R, et al. Anticancer potential of animal venoms and toxins. *Indian J Exp Biol.* 2010 Feb;48(2):93–103.
- [6] Ejaz S, Hashmi FB, Malik WN, et al. Applications of venom proteins as potential anticancer agents. *Protein Pept Lett.* 2018;25(7):688–701. doi: [10.2174/0929866524666180614102104](https://doi.org/10.2174/0929866524666180614102104)
- [7] Abdel-Rahman MA, Cao Z, Abd El-Aziz TM, et al. Editorial: structural and functional venomomics as a powerful approach for drug discovery and development. *Front Pharmacol.* 2024-April-10 2024;15:2024. doi: [10.3389/fphar.2024.1405681](https://doi.org/10.3389/fphar.2024.1405681)
- [8] Chatterjee B. Animal venoms have potential to treat cancer. *Curr Top Med Chem.* 2018;18(30):2555–2566. doi: [10.2174/1568026619666181221120817](https://doi.org/10.2174/1568026619666181221120817)
- [9] Akef HM. Anticancer and antimicrobial activities of scorpion venoms and their peptides. *Toxin Rev.* 2019/01/02 2019;38(1):41–53. doi: [10.1080/15569543.2017.1414847](https://doi.org/10.1080/15569543.2017.1414847)
- [10] Baradaran M. Current status of peptide medications and the position of active therapeutic peptides with scorpion venom origin. *Jundishapur J Nat Pharm Prod.* 02/14 2023; 18(1). In Press. doi: [10.5812/jjnpp-134049](https://doi.org/10.5812/jjnpp-134049)
- [11] Pashmforoosh N, Baradaran M. Peptides with diverse functions from scorpion venom: a great opportunity for the treatment of a wide variety of diseases. *Iran Biomed J.* 2023 Mar 1;27(2):84–99. doi: [10.52547/ibj.3863](https://doi.org/10.52547/ibj.3863)
- [12] Deng Z, Gao Y, Nguyen T, et al. The potent antitumor activity of Smp43 against non-small-cell lung cancer A549 cells via inducing membranolysis and mitochondrial dysfunction. *Toxins (Basel).* 2023 May 19;15(5):347. doi: [10.3390/toxins15050347](https://doi.org/10.3390/toxins15050347)
- [13] Gerges MM, A-Rm A, Rt R, et al. Cytotoxic activity and mechanism of action of Smp43 scorpion peptide against colorectal cancer cell line HCT-116. *Toxin Rev.* 2024/07/02 2024;43(3):370–383. doi: [10.1080/15569543.2024.2344471](https://doi.org/10.1080/15569543.2024.2344471)
- [14] El-Qassas J, Abd El-Atti M, El-Badri N. Harnessing the potency of scorpion venom-derived proteins: applications in cancer therapy. *Bioresour And Bioprocess.* 2024 Oct 3;11(1):93. doi: [10.1186/s40643-024-00805-0](https://doi.org/10.1186/s40643-024-00805-0)
- [15] Kardani K, Bolhassani A. Antimicrobial/anticancer peptides: bioactive molecules and therapeutic agents. *Immunotherapy.* 2021 Jun;13(8):669–684. doi: [10.2217/imt-2020-0312](https://doi.org/10.2217/imt-2020-0312)
- [16] Deslouches B, Di YP. Antimicrobial peptides with selective antitumor mechanisms: prospect for anticancer applications. *Oncotarget.* 2017 Jul 11;8(28):46635–46651. doi: [10.18632/oncotarget.16743](https://doi.org/10.18632/oncotarget.16743)
- [17] Abdel-Rahman MA, Quintero-Hernandez V, Possani LD. Venom proteomic and venomous glands transcriptomic analysis of the Egyptian scorpion *Scorpio maurus palmatus* (Arachnida: scorpionidae). *Toxicon.* 2013 Nov;74:193–207. doi: [10.1016/j.toxicon.2013.08.064](https://doi.org/10.1016/j.toxicon.2013.08.064)
- [18] Harrison PL, Abdel-Rahman MA, Strong PN, et al. Characterisation of three alpha-helical antimicrobial peptides from the venom of *Scorpio maurus palmatus*. *Toxicon.* 2016 Jul;117:30–36. doi: [10.1016/j.toxicon.2016.03.014](https://doi.org/10.1016/j.toxicon.2016.03.014)
- [19] Elrayess RA, Mohallal ME, Mobarak YM, et al. Scorpion venom antimicrobial peptides induce caspase-1 dependant pyroptotic cell death. Article. *Front Pharmacol.* 2022 Jan;12:788874. doi: [10.3389/fphar.2021.788874](https://doi.org/10.3389/fphar.2021.788874)
- [20] Elrayess RA, Mohallal ME, El-Shahat YM, et al. Cytotoxic effects of Smp24 and Smp43 scorpion venom antimicrobial peptides on tumour and non-tumour cell lines. *Int J Pept Res Ther.* 2019;26(3):1409–1415. doi: [10.1007/s10989-019-09932-1](https://doi.org/10.1007/s10989-019-09932-1)
- [21] Chai J, Yang W, Gao Y, et al. Antitumor effects of scorpion peptide Smp43 through mitochondrial dysfunction and membrane disruption on hepatocellular carcinoma. *J Nat Prod.* 2021 Dec 24;84(12):3147–3160. doi: [10.1021/acs.jnatprod.1c00963](https://doi.org/10.1021/acs.jnatprod.1c00963)
- [22] Teleb WK, Tantawy MA, Xu X, et al. Cytotoxicity and molecular alterations induced by scorpion venom antimicrobial peptide Smp43 in breast cancer cell lines MDA-MB-231 and MCF-7. *Int J Pept Res Ther.* 2022/12/02 2022;29(1):8. doi: [10.1007/s10989-022-10474-2](https://doi.org/10.1007/s10989-022-10474-2)
- [23] Heath GR, Harrison PL, Strong PN, et al. Visualization of diffusion limited antimicrobial peptide attack on supported lipid membranes. *Soft Matter.* 2018;14(29):6146–6154. doi: [10.1039/C8SM00707A](https://doi.org/10.1039/C8SM00707A)
- [24] Abdel-Rahman MA, Harrison PL, Strong PN. Snapshots of scorpion venomomics. *J Arid Environ.* 2015/01/01 2015;112:170–176. doi: [10.1016/j.jaridenv.2014.01.007](https://doi.org/10.1016/j.jaridenv.2014.01.007)

- [25] Requena R, Vargas M, Chiralt A. Study of the potential synergistic antibacterial activity of essential oil components using the thiazolyl blue tetrazolium bromide (MTT) assay. *LWT*. 2019/03/01 **2019**;101:183–190. doi: [10.1016/j.lwt.2018.10.093](https://doi.org/10.1016/j.lwt.2018.10.093)
- [26] Rieger AM, Barreda DR. Accurate assessment of cell death by imaging flow cytometry. In: Barteneva N Vorobjev I, editors. *Imaging flow cytometry: methods and protocols*. (NY): Springer; **2016**. p. 209–220.
- [27] Rahmé R. Assaying cell cycle status using flow cytometry. In: Manfredi J, editor. *Cell cycle checkpoints: methods and protocols*. New York: Springer US; **2021**. p. 165–179.
- [28] Cao X, Zhao B. Quantitative real-time PCR to measure YAP/TAZ activity in human cells. In: Hergovich A, editor. *The hippo pathway: methods and protocols*. New York: Springer US; **2019**. p. 137–152.
- [29] Lee PY, Costumbrado J, Hsu CY, et al. Agarose gel electrophoresis for the separation of DNA fragments. *J Vis Exp*. **2012** Apr 20;62(62). doi: [10.3791/3923-v](https://doi.org/10.3791/3923-v)
- [30] Haniu H, Watanabe D, Kawashima Y, et al. Two-dimensional gel-based protein standardization verified by Western blot analysis. In: Kurien B Scofield R, editors. *Western blotting: methods and protocols*. (NY): Springer; **2015**. p. 473–479.
- [31] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med*. **1963** May;61:882–888.
- [32] Aebi H. Catalase in vitro. *Methods In Enzymol*. **1984**;13:121–126.
- [33] Montgomery H, Dymock JJA. The determination of nitrite in water: colorimetric method of nitric oxide assay. *Analyst*. **1961** June;86 (1023):414–416.
- [34] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal biochem*. 1979/06/01 **1979**;95(2):351–358. doi: [10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- [35] Nguyen T, Guo R, Chai J, et al. Smp24, a scorpion-venom peptide, exhibits potent antitumor effects against hepatoma HepG2 cells via multi-mechanisms in vivo and in vitro. *Toxins (Basel)*. **2022** Oct 21;14(10):717. doi: [10.3390/toxins14100717](https://doi.org/10.3390/toxins14100717)
- [36] Guo R, Chen X, Nguyen T, et al. The strong anti-tumor effect of Smp24 in lung adenocarcinoma A549 cells depends on its induction of mitochondrial dysfunctions and ROS accumulation. *Toxins (Basel)*. **2022**;14(9):590. doi: [10.3390/toxins14090590](https://doi.org/10.3390/toxins14090590)
- [37] Tornesello AL, Borrelli A, Buonaguro L, et al. Antimicrobial peptides as anticancer agents: functional properties and biological activities. *Molecules*. **2020** Jun 19;25(12):2850. doi: [10.3390/molecules25122850](https://doi.org/10.3390/molecules25122850)
- [38] Panja K, Buranapraditkun S, Roytrakul S, et al. Scorpion venom peptide effects on inhibiting proliferation and inducing apoptosis in canine mammary gland tumor cell lines. *Anim (Basel)*. **2021** Jul 16;11(7):2119. doi: [10.3390/ani11072119](https://doi.org/10.3390/ani11072119)
- [39] D'Suze G, Rosales A, Salazar V, et al. Apoptogenic peptides from Tityus discrepans scorpion venom acting against the SKBR3 breast cancer cell line. *Toxicon*. **2010** Dec;56(8):1497–1505. doi: [10.1016/j.toxicon.2010.09.008](https://doi.org/10.1016/j.toxicon.2010.09.008)
- [40] Almaaytah A, Albalas Q. Scorpion venom peptides with no disulfide bridges: a review. *Peptides*. 2014/01/01/ **2014**;51:35–45. doi: [10.1016/j.peptides.2013.10.021](https://doi.org/10.1016/j.peptides.2013.10.021)
- [41] Tong-Ngam P, Roytrakul S, Sritanaudomchai H. BmKn-2 scorpion venom peptide for killing oral cancer cells by apoptosis. *Asian Pac J Cancer Prev*. **2015**;16(7):2807–2811. doi: [10.7314/apjcp.2015.16.7.2807](https://doi.org/10.7314/apjcp.2015.16.7.2807)
- [42] Fawzy BS, Nafie MS, Ali IAI, et al. Scorpion venom peptide Smp24 revealed apoptotic and antiangiogenic activities in solid-Ehrlich carcinoma bearing mice. *Int J Pept Res Ther*. 2023/02/20 **2023**;29(2):29. doi: [10.1007/s10989-023-10494-6](https://doi.org/10.1007/s10989-023-10494-6)
- [43] Risso A, Zanetti M, Gennaro R. Cytotoxicity and apoptosis mediated by two peptides of innate immunity. *Cell Immunol*. **1998** Nov 1;189(2):107–115. doi: [10.1006/cimm.1998.1358](https://doi.org/10.1006/cimm.1998.1358)
- [44] Zhang JH, Xu M. Dna fragmentation in apoptosis. *Cell Res*. 2000/09/01 **2000**;10(3):205–211. doi: [10.1038/sj.cr.7290049](https://doi.org/10.1038/sj.cr.7290049)
- [45] Reuter S, Gupta SC, Chaturvedi MM, et al. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biol And Med*. **2010** Dec 1;49(11):1603–1616. doi: [10.1016/j.freeradbiomed.2010.09.006](https://doi.org/10.1016/j.freeradbiomed.2010.09.006)
- [46] Cordiano R, Di Gioacchino M, Mangifesta R, et al. Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: an update. *Molecules*. **2023** Aug 9;28(16):5979. doi: [10.3390/molecules28165979](https://doi.org/10.3390/molecules28165979)

- [47] Lubos E. Role of oxidative stress and nitric oxide in atherothrombosis. *Front BioSci.* 2008 May 1;8(13):5323–5344. doi: [10.2741/3084](https://doi.org/10.2741/3084)
- [48] Kennedy L, Sandhu JK, Harper ME, et al. Role of glutathione in cancer: from mechanisms to therapies. *Biomolecules.* 2020 Oct 9;10(10):1429. doi: [10.3390/biom10101429](https://doi.org/10.3390/biom10101429)
- [49] Dodson M, Castro-Portuguez R, Zhang DD. Nrf2 plays a critical role in mitigating lipid peroxidation and ferroptosis. *Redox Biol.* 2019 May;23:101107. doi: [10.1016/j.redox.2019.101107](https://doi.org/10.1016/j.redox.2019.101107)