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Original Research Article

Antiproliferative effect of the Red Sea cone snail, *Conus geographus*

Najla Ali Alburae[1]*, Afrah Eltayeb Mohammed[2]
[1]Department of Biology, Faculty of Science, King Abdulaziz University, PO Box 80203, Jeddah 21589, [2]Biology Department, College of Science, Princess Nourah bint Abdulrahman University, PO Box 84428, Riyadh 11671, Saudi Arabia

For correspondence: **Email: nalbourai@kau.edu.sa; **Tel.:** +966-50-33710116*

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Abstract

Purpose: *To investigate the antiproliferative effect of the Red Sea cone snail, Conus geographus, against 4 MCF-7 (breast), MDA-MB-231 (epithelial human breast), HepG2 (hepatocellular) and SKOV-3 (ovarian) cancer cell lines.*
Methods: *Extraction of Red Sea cone snail sample with a mixture of CH_2Cl_2 and CH_3OH (1:1, v/v) yielded 0.55 g of a green viscous material. The cytotoxic effects of the organic extract against the cancer cell lines were determined using cell proliferation (MTT) assay, and the half-maximal concentration (IC_{50}) values measured. The effect of the crude extract on the cell cycle of the HepG-2 was determined by flow cytometry.*
Results: *The extract produced significant inhibitory effects against SKOV-3, MDA-MB-231, MCF-7 and HepG2, with IC_{50} values of 22.7 ± 2.2, 68.7 ± 6.2, 47 ± 4.2 and 19 ± 2.1 µg/mL, respectively. Cell cycle analysis revealed that the extract enhanced accumulation of HepG2 cells in the Go/G1 phase, at a level of 23.4 and 24.1 % at IC_{50} (19 µg/mL) and ½ IC_{50} (9.5 µg/mL), respectively, when compared to the untreated cells.*
Conclusion: *These results indicate that C. geographus extract exhibits potent cytotoxic effect against HepG2 cells via a mechanism involving G0/G1 cell cycle arrest. Thus, C. geographus is a potential source of a new anti-cancer agent.*

Keywords: *Conus geographus, Marine invertebrate, HepG2, Antiproliferation*

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INTRODUCTION

High biological diversity and harsh environmental conditions are the main features of marine ecosystems, and they reflect different archetypes of natural compounds, when compared to those originating from terrestrial organisms. Indeed, several new compounds of marine origin are discovered every year [1, 2]. Many of these natural products have already been considered in clinical trials with regard to their antitumor activities. Marine organisms offer many challenges and great opportunities for drug discovery, particularly anticancer agents [3-6].

Conus is a widely distributed genus in tropical and sub-tropical areas. It includes more than eight hundred identified species, which are

characterized by their specialized feeding behavior in which they overcome their prey by injection of potent neurotoxic and paralytic venoms [7].

In 2014, the Saudi Cancer Registry reported a total of 15,807 diagnosed cancer cases, with breast cancer placed first (15.9%), followed by colorectal cancer (11.5 %), thyroid cancer (8.5 %), NHL (6.4%), leukemia (5.9 %), liver cancer (4.0%) and lung cancer (3.9 %) [8].

The present study was carried out to investigate the cytotoxicity of the cone snail *C. geographus* from Saudi territorial waters against SKOV-3, MCF-7, MDA-MB-231 and HepG2 cancer cell lines.

EXPERIMENTAL

Materials

Conus geographus was collected from Rabigh area (21° 29′ 31″ N 39° 11′ 24″ E), Saudi Arabia. A voucher specimen was kept in the Faculty of Marine Science King Abdulaziz University, Saudi Arabia.

Cell lines and reagents

The ovarian cancer (SKOV-3), breast cancer [MCF-7 (ER-positive) and epithelial human breast cancer (MDA-MB-231 and HepG2) cell lines were obtained from ATCC, USA. All cells were cultured in DMEM (12-604F, Lonza Verviers SPRL, Belgium) supplemented with 5 % fetal bovine serum (S-001B-BR, Life Science Group L, UK); 100 IU/mL penicillin and 100 µg/mL streptomycin (17-602E, Lonza Verviers SPRL, Belgium), except for SKOV-3 cells which were maintained in RPMI-1640 medium. Cisplatin was used as positive control. The *Conus* extract (10 mg) was solubilized in 100 % DMSO.

Extraction

Fresh marine sample (*Conus geographus)* was extracted with a 1:1 (v:v) mixture of CH_2Cl_2 and CH_3OH (3 x 200 mL) for 24 h at 22 °C, to yield a green viscous extract (0.55g).

Determination of antiproliferative effect of extract

The cancer cells were seeded in 96-well plate at a density of 5000 cells/well and incubated for 24 h at 37°C and 5% CO_2. Thereafter, the cells were treated with serial dilution of the *Conus* extract (50, 25, 12.5, 6.25, 3.125, or 1.56 µg/mL). After 48 h, the viability of each cancer cell line was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 5 mg/mL) which measures the activity of mitochondrial succinate dehydrogenase in viable cells [9]. Following 4-h incubation, the resultant formazan crystals were solubilized using 10 % SDS/PBS/0.01 N HCl. After 14 h, the absorbance of the formazan solution was read at wavelengths of 570 nm and 630 nm in a BioTek plate reader (EL x 808, BioTek Instruments, Inc., Winooski, VT, USA). The experiment was performed three times and the standard deviation (SD) was calculated. The IC_{50} was calculated in terms of the concentration that causes 50 % inhibition of cell growth.

Analysis of cell cycle distribution

HepG2 cells were treated with 9.5 and 19 µg/mL *Conus* extract, or with 0.5% DMSO as negative control. After 48 h of incubation, the cells were washed twice with 1 x PBS (17-516F, Lonza Verviers SPRL, Belgium) and trypsinized [17-161E, Trypsin-Versene (EDTA), Lonza Verviers SPRL, Belgium]. The detached cells were centrifuged at 2500 rpm for 10 min, and the cells were fixed with 70% ice-cold ethanol for 2 h at -20 °C. The fixed cells were washed with 1 x PBS and centrifuged at 5000 rpm for 15 min. The effect of the treatment on the cell cycle was evaluated by staining the cells with propidium iodide (P1304MP, Invitrogen™) for 15 min in the dark. Fluorescence was measured using BD Accuri™ C6 Plus flow cytometer [9].

Statistical analysis

All statistical analyses were performed using GraphPad InStat software, version 3.05 (GraphPad Software, La Jolla, CA). Graphs were plotted using GraphPad Prism software, version 6.00 (GraphPad Software, La Jolla, CA).

RESULTS

The results indicated that the *C. geographus* extract showed good antiproliferative activity against SKOV-3, MCF-7, MDA-MB-231 and HepG2 cell lines. The antiproliferative effect of the extract was compared with that of cisplatin (positive control). Both showed significant antiproliferative effects against SKOV-3 cells, at IC_{50} values 22.7 ± 2.2 and 16.6 ± 3.0 µg/mL, respectively. They also exerted antiproliferative effects against MDA-MB-231, with IC_{50} values 68.7 ± 6.2 and 7.3 ± 1.0 µg/mL, respectively. The IC_{50} values for the antiproliferative effects of extract and cisplatin against MCF-7 cells were 47 ± 4.2 and 22.9 ± 1.87, respectively, while their

IC_{50} values for cytotoxicity against HepG2 were 19 ± 2.1 and 5.5 ± 0.35 µg/mL, respectively. Figure 1 shows the percentage of cell viability after treatment with different concentrations of *Conus* extract. The mechanism of cytotoxic effect of the extract against HepG2 cells was evaluated by assessing its effect on cell cycle using DNA flow cytometry technique, relative to untreated cells. The cell cycle results are shown in Figure 2 and Table 1, with values estimated as percent of cell viability after treatment of HepG2 cells with the obtained IC_{50} (19 µg/mL) and ½ IC_{50} (9.5 µg/mL).

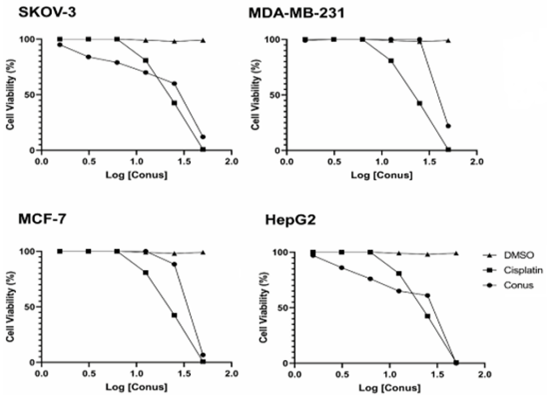

Figure 1: Cell viability after treatment with different concentrations of Conus extract. The cells were treated for 48 h and the viability was determined using MTT, with DMSO and cisplatin as negative control and positive control, respectively.

DISCUSSION

The marine environment enhances the production of a vast array of natural metabolites with diverse molecular structures. Currently, more than 28,600 organic compounds of marine origin have been reported. Marine bioprospection is beneficial and rewarding in the area of cancer therapy. The development of anticancer agents is a reflection of the tremendous impact of natural organisms on the chemotherapeutic drug arsenal, with 49% of anticancer agents approved prior to 2014 being classified either as natural products or products derived directly. Cone snails are marine mollusks belonging to the family of *Conidae* which has 152 genera. The genus *Conus,* known as specialized predators, currently has more than 800 recognized species [10-12]. They sting humans with different adverse effects. *Conus* species produce different neurotoxic peptides in their injected venoms. The severity of these hazardous peptides in humans has been reported [13].

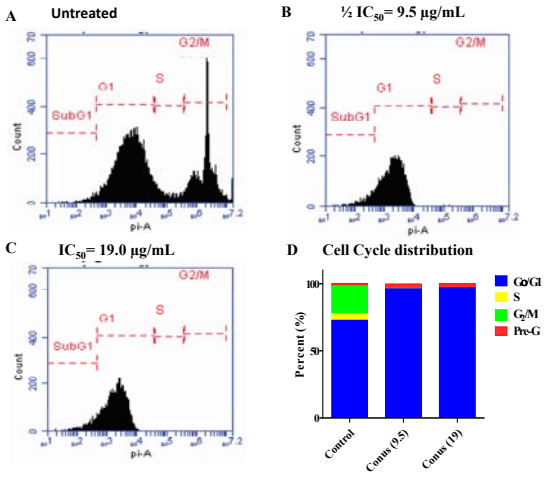

Figure 2 : Effect of Conus extract on the cell cycle distribution of HepG2 cells. HepG2 cancer cells were exposed to Conus extract for 48 h. Untreated cells (A), Conus extract tested at ½ IC_{50} (D) and IC_{50} (C), and bar chart of cell cycle distribution (D). Cell cycle distribution was determined as percentage, using DNA cytometric analysis.

Most of these proteinoid substances exhibit effects through the nervous system [10,12]. Conotoxins are important potential drug leads [14,15]. *Conus* venoms constitute exceptionally rich pharmacological resources [10,16-20]. *Conus geographus* has an uncommon method for catching fish. It engulfs multiple fish and simultaneously injects venom in them [19,21]. Several peptides from *Conus* venom are known for their neurotoxic effects. Moreover, there are reports on peptides with pharmacological effects [22-26].

A computer survey based on different scientific data bases (e.g., Scifinder) showed that there are limited publications on the cytotoxicity of *Conus geographus* against cancer cell lines.

Table 1: Flow cytometry scan (FACSCAN) for the effect of *Conus geographus* extract on HepG2

Parameter	Untreated cells	*Conus* (9.5µg/mL)	*Conus* (19 µg/mL)	Cisplatin
Pr-G1	1.8 %[a]	3.4 %	3.3 %	3.4 %
Go/G1	72.6 %	96.0 %	96.7 %	96.6 %
S	4.8 %	0.2 %	0.0 %	0.0 %
G2/M	20.8 %	0.0 %	0.0 %	0.0 %

Note: The percentages were calculated relative to untreated cells (control)

Thus, the present study was carried out to investigate the cytotoxic effect of *C. geographus* against some selected cancer cell lines. The results indicated that the extract of *C. geographus* increased the population of cells in the Go/G1phase from 72.6 % to 96.0, and 96.7 % at the IC_{50} value of 19 µg/mL, and ½ IC_{50} value of 9.5 µg/mL, respectively. At these concentrations, the extract increased the cell population at Go/G1 phase by 23.4 and 24.1 %, respectively. The increase in the population of non-proliferating cells was accompanied by subsequent decrease in cell population in S-phase from 4.8% to zero. Moreover, the population of cells in G2/M phase was decreased from 20.8 % to zero at IC_{50} and ½ IC_{50} (19 and 9.5 µg/mL).

It is known that the approach of a cell to the end of the G1 phase is controlled at an important checkpoint called G1/S, where the cell manages no replication of its DNA. At this point, the cell is tested for DNA damage to confirm that it has all the required cellular machinery to allow for effective cell division. Cells with intact DNA continue to S phase, while cells with damaged DNA that cannot be repaired are arrested through apoptosis, or programmed cell death.

CONCLUSION

The results obtained in this study indicate that *Conus geographus* exerts significant antiproliferative effect against HepG2 through cell cycle arrest at G0/G1 phase. Therefore, *C. geographus* is a potential source of a new anti-cancer agent.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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