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Can a Veterinary Drug be Repurposed for Human Cancers?: Cytotoxic Effect of *Tarantula cubensis* Venom on Human Cancer Cells

Suleyman ILHAN[1*]

ABSTRACT: *Tarantula cubensis* is known as Cuban tarantula having a venom that contains a diverse mixture of potent compounds with various biological activities. These peptides have been shown to have antitumor activities, therefore features of spider-venom peptides prompted scientists to test them as a potential anticancer drug. The purpose of the study was to investigate the potential cytotoxic effect of *Tarantula cubensis* venom (Logoplex®) on human cancer cells including prostate (PC-3), lung (H69), breast (MDA-MB-231), and ovarian (OVCAR-3). Moreover, non-tumorigenic MCF-10A cells were used to evaluate the possible cancer cell-specific effect of the extract. The increasing concentrations of Logoplex® were applied for 24, 48 and 72 h. MTT assay was used to assess cell viability. Concentration-response curves and the IC_{50} values were determined via Graphpad Prism software. Logoplex® caused a time- and concentration-dependent cytotoxic effect in MDA-MB-231, PC-3, OVCAR-3 and MCF-10A cells and the highest cytotoxicity was achieved at 72h. However, in H69 cells, there was a concentration-dependent cytotoxic effect and the highest cytotoxicity was achieved at 24h. IC_{50} values of Logoplex® in MDA-MB-231, OVCAR-3, PC-3, H69 and MCF-10A cells were determined as 159.3±2.1, 48.9±1.8, 40.2±1.2, 498.3±1.2 and 217.8±2.0 µg/mL, respectively. Logoplex® showed a lower cytotoxic effect against normal cells than the cancer cells suggesting a cancer cell-specific effect. According to the preliminary results of this study, although Logoplex® is a veterinary drug, its cytotoxic effect on human cancer cells suggests that it should be re-evaluated as a potential cytotoxic agent. Analyses to identify functional compounds of *Tarantula cubensis* venom, and future studies addressing its mechanism of action on cancer cells are recommended.

Keywords: *Tarantula cubensis*, venom, cytotoxicity, cancer cell.

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INTRODUCTION

Cancer is known to be the major cause of death worldwide. Despite the great advances achieved in the field of the modern age, it has not been possible to reach the desired point in cancer treatment. There are extensive studies for the development of less toxic anticancer drugs that continues intensely to find potential targets and promising anticancer approaches.

Venom is a diverse mixture of potent compounds such as various enzymes, polypeptides, ions, polyamines, and a variety of chemicals. Venom research is an important area for drug design and development, as many of these compounds have been shown to have potent pharmacological activities (Calderon et al., 2014; Oldrati et al., 2016).

Venoms having various pharmacological activities have been isolated and characterized by spiders, sea snails, snakes, scorpions and some other venomous animals (Olivera et al., 1984; Fry et al., 2005; Oldrati et al., 2013; Ilhan et al., 2020). Among them, spider venoms are known to have a potent biological activity due to their novel and rich sources of peptide content that reveal various biological effects (Estrada et al., 2007; Saez et al., 2010; Vassilevski et al., 2009). These peptides have been shown to have antitumor activities, therefore features of spider-venom peptides prompted scientists to test them as a potential anticancer drug (King, 2011).

Tarantula cubensis is known as Cuban tarantula and belongs to the Theraphosidae family. Its venom contains a mixture of many different toxins and digestive enzymes. Alcoholic extract of *Tarantula cubensis* venom (TCV) is commercially available in two forms Tarantula-Logoplex® (TL) and Theranekron®. Both extracts have been used as homeopathic drugs in veterinary medicine. It has been shown that TCV has anti-bacterial, anti-inflammatory, anti-tumor, antiphlogistic and wound healing properties (Gultiken and Vural, 2007; Ghasemi-Dizgah and Amirmozafari, 2015; Gultiken et al., 2015; Er et al., 2017) Although the antitumor effects of TCV have been studied in clinical veterinary medicine, most of these studies have preliminary results and are not detailed. Also, the possible cytotoxic effects of TCV on cancer cell lines of different origins have not been studied.

The present study aimed to investigate the cytotoxic effect of TL on tumor-derived cell lines including prostate (PC-3), breast (MDA-MB-231), lung (H69) and ovarian (OVCAR-3). Moreover, the effects on non-tumorigenic MCF-10A cells were investigated in order to evaluate the possible cancer cell-specific effect of TL.

MATERIALS AND METHODS

Tarantula cubensis venom extract

Tarantula-Logoplex® that contains alcoholic *Tarantula cubensis* venom extract was bought from Richter Pharma AG (Wels, Austria), obtained from the local veterinary clinic with the permission of the veterinarian (Manisa, Turkey).

Cell lines and culture conditions

Four tumor-derived cell lines including prostate (PC-3), breast (MDA-MB-231), lung (H69), ovarian (OVCAR-3) and a non-tumorigenic cell line MCF-10A were used to evaluate the cytotoxic effect of TL. The cancer cell lines were purchased from ATCC (USA). RPMI 1640 medium which includes 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich), 2 mM L-glutamine (LG), and 1% penicillin-streptomycin (P/S) were used to maintain cancer cells in 75 cm^2 polystyrene flasks. MCF-10A cells were obtained from the Health FBS, 1 % LG, 1 % P/S. All cell lines were maintained at 37 ºC incubator with 5% CO_2.

Assessment of cell viability via MTT assay

After treatment with TL, the cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay is a colorimetric method for determining cell viability. It is based on the ability of NADPH-dependent cellular oxidoreductase enzymes to convert the tetrazolium dye MTT to its insoluble formazan, which is purple. All cells were counted and seeded approximately 10^4 cells/well in a total volume of 200 µL in microtiter flat-bottomed plates. Plates were then incubated at 37 °C for 24 h for cell attachment. After 24 h period, cells were exposed to the increasing concentrations (10-500 µg/mL) of TL for various time points (24, 48 and 72 h). Fluorouracil (5-FU) was the reference standard. After each incubation period, 20 µL MTT mixture was added to each well and plates were incubated for 4 h at 37 °C incubator. Then the medium was discarded and DMSO (200 µL) was added to each well to dissolve formazan crystals. DMSO concentration was not toxic to cells since the concentration did not exceed 0.1%. The optic densities were measured at 570 nm and 690 nm using a spectrophotometer. (Tecan, Switzerland).

Derivation of dose-response curves and IC_{50} values

GraphPad Prism 5.0 was used to normalize the data. Log-transformed drug concentrations were then plotted against the concentration-response and the half maximal inhibitory concentration (IC_{50}) values were determined using nonlinear regression log vs. normalized response-variable slope (GraphPad Software, USA).

Statistical Analysis

Results were expressed as mean ± SD (standard deviation) and the data were analyzed by GraphPad Prism 5.0 software via Student's *t* test which determines significant differences between groups, and that between three or more groups were analyzed using one way analysis of variance test (ANOVA) followed by Dunnett's *t* test The obtained data were statistically significant at * $p<0.05$.

RESULTS AND DISCUSSION

The cytotoxic effect of TL was investigated on tumor-derived cell lines including prostate (PC-3), breast (MDA-MB-231), lung (H69) and ovarian (OVCAR-3) using MTT assay. The TL treatment was performed in 6 different doses at 24-, 48- and 72-hour intervals at concentrations of 10, 25, 50, 100, 250 and 500 µg/mL with a special emphasis on the assessment of time- and concentration-response relationships. After MTT application, the absorbance data obtained spectrophotometrically were evaluated statistically. The cell viability of five cell lines after TL treatment was illustrated in Figure 1.

According to the MTT assay results, at 24 and 48 hours, TL inhibited cell viability in MDA-MB-231 breast cancer cells at concentrations of 50 µg/mL and above. However, at 72 h, TL significantly inhibited cell viability at all tested concentrations. After treatment with 10, 100 and 500 µg/mL TL for 72 h, cell viability was inhibited by 18.5%, 53.5% and 61% in MDA-MB-231 cells, respectively (Figure 1)($p<0.05$). In OVCAR-3 ovarian cancer cells, TL significantly inhibited cell viability at all tested concentrations and at all time points. At 24 and 48 hours, a significant decrease in cell viability was seen at concentrations of 100 µM and above. However, a significant decrease in OVCAR-3 cell viability was observed after the application of 10 µg/mL concentration at 72 hours. There were 41%, 56.5%, 76.5% reductions in cell viability after 10, 100 and 500 µg/mL TL, respectively for 72 h in OVCAR-3 cells (Figure 1)($p<0.05$). In PC-3 prostate cancer cells, TL significantly inhibited cell viability at all tested concentrations and at all time points. TL treatment resulted in a 35%, 67.5% and 81% reduction in cell viability at concentrations of 10, 100 and 500 µg/mL, respectively for 72 h in PC-3 cells (Figure 1)($p<0.05$). Unlike other cancer cell lines, in H69 lung cancer cells, TL administration showed the

highest effect at 24 h. The cell viability of H69 cells was inhibited by 16.5%, 51% and 60% by 10, 100 and 500 µg/mL TL, respectively at 24 h. However, at 72 h, 10, 100 and 500 µg/mL TL treatment resulted in a 27.5%, 46.5% and 51.5% reduction in cell viability, respectively in H69 cells (Figure 1) ($p<0.05$).

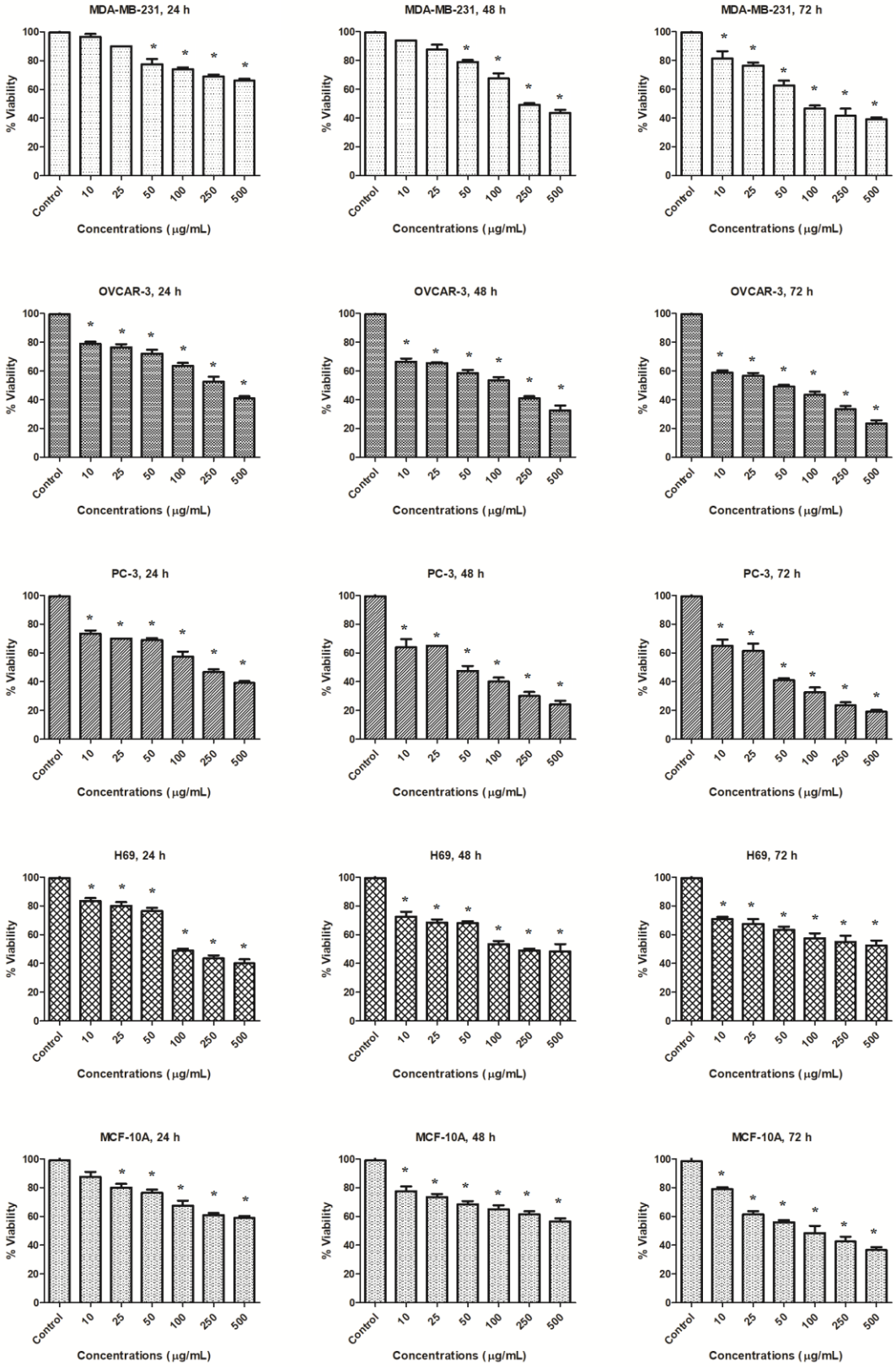

Figure 1: Effects of TL treatment on the viability of different cell lines

IC_{50} values determined for TL in five cell lines tested at 24, 48 and 72 hours were shown in Table 1. IC_{50} values were 159.3 ± 2.1, 48.9 ± 1.8, 40.2 ± 1.2, 498.3 ± 1.2 and 217.8 ± 2.0 µg/mL for MDA-MB-231, OVCAR-3, PC-3, H69 and MCF-10A cells, respectively at 72 h. Among human cancer cell lines, PC-3 prostate cancers were the most sensitive cell line to TL treatment while human breast cancer cell line H69 was the most resistant, depending on their IC_{50} values at 72 h. The IC_{50} values of 5-FU were 1.9±0.5, 1.4±0.2, 2.5±0.8, 3.1 ±0.4 and 0.9±0.2 µg/mL for MDA-MB-231, OVCAR-3, PC-3, H69 and MCF-10A cells, respectively at 72 h.

To screen the selective role of TL treatment towards cancer cells over normal cells, all experiments were repeated by using MCF-10A non-tumorigenic breast cells. Unlike 5-FU, TL showed a lower cytotoxic effect against normal breast cells than the cancer cell lines indicating cancer cell-specific effect (Figure 1) ($p<0.05$).

Table 1: IC_{50} values (µg/mL) of each cell line after treatment with TL for 24, 48 and 72 h

	IC_{50} Value		
Cell Line	**24 h**	**48 h**	**72 h**
MDA-MB-231	734.8 ± 0.4	360.1 ± 1.6	159.3 ± 2.1
OVCAR-3	341.5 ± 0.8	198.1 ± 0.2	48.9 ± 1.8
PC-3	288.2 ± 1.5	92.7 ± 0.4	40.2 ± 1.2
H69	95.2 ± 0.2	370.1 ± 0.6	498.3 ± 1.2
MCF-10A	592.5 ± 1.2	425.3 ± 1.8	217.8 ± 2.0

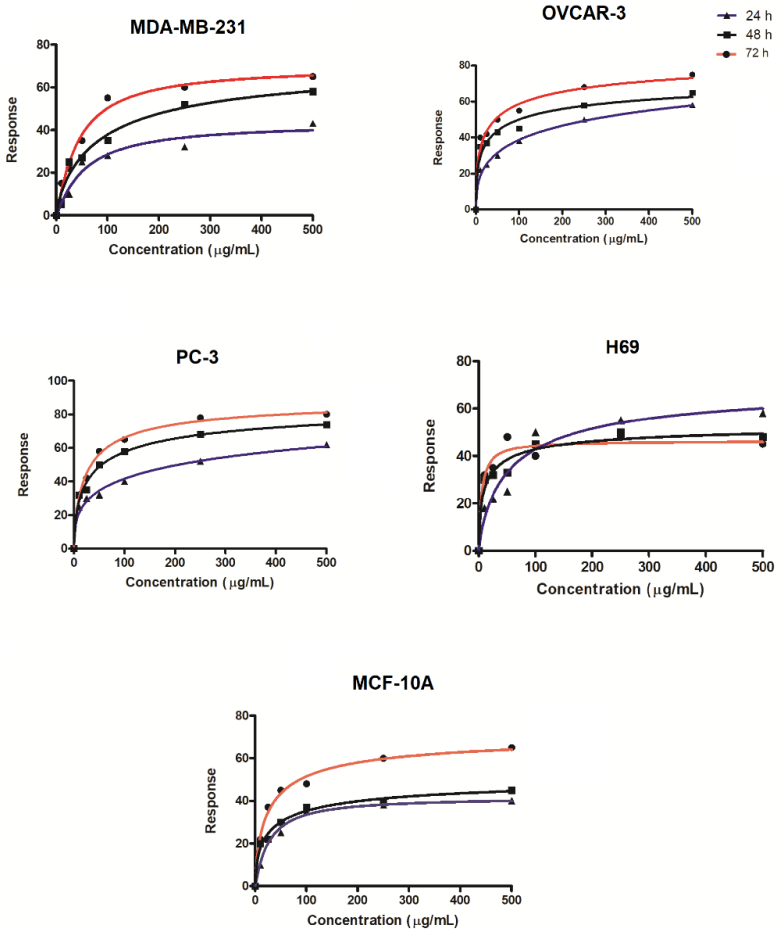

Figure 2: Concentration-response curves for the cytotoxic effect of TL in human cell lines.

It can be seen from the concentration-response curves that TL caused a time- and concentration-dependent manner cytotoxic effect in MDA-MB-231, PC-3, OVCAR-3 and MCAF-10A cell lines and the highest cytotoxicity was achieved at 72 h (Figure 2). However, in H69 lung cancer cells, by TL exposure, cell viability was inhibited in a concentration-dependent manner and the highest cytotoxicity was achieved at 24 h (Figure 2).

In the literature, there are limited studies investigating the *in vitro* cytotoxic effects of TL. Since it is known that Theranekron® has strong antitumor activity on canine breast tumors (Gultiken et al., 2015), cell culture studies have always been conducted using the MCF-7 cell line, which represents luminal A breast cancer. Er et al. investigated the cytotoxic and anti-apoptotic effects of Theranekron® in MCF-7 cell line and found the induction of cytotoxicity and apoptosis in a concentration- and time-dependent manner. Increased levels of tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) was detected as well as cell apoptosis (Er et al., 2017). Ghasemi-Dizgah et al., also evaluated the cytotoxic and apoptotic effect of *Tarantula cubensis* venom (Theranekron®) on MCF-7 and HN5 head and neck cancer cells. They incubated MCF-7 cells with concentrations of 20, 50, 100, 250 and 500 µg/mL for 12, 24 and 48 hours and found similar results indicating concentration-dependent induction of cytotoxicity and apoptosis in both cell lines (Ghasemi-Dizgah et al., 2017). In this study, unlike the literature, the cytotoxic effects of TL were investigated in aggressive MDA-MB-231 triple (-) breast cancer cells. TL was also quite effective in a more aggressive breast cancer cell line. Yenigun et al. also investigated the effects of TL on human gastric cell lines and showed that the IC_{50} value of TL was 100 µg/mL. Moreover, it was demonstrated that TL induced autophagic cell death in gastric cells.

In the study of Ghasemi-Dizgah et al., the authors also investigated the effects of Theranekron® on non-tumorigenic HEK293 human embryonic kidney cell line. Similar to the results of the current study, the authors revealed that Theranekron® showed significantly more cytotoxic effects against MCF-7 and HN5 cancer cell lines than the non-tumorigenic HEK-293 cells (Ghasemi-Dizgah et al., 2017).

CONCLUSION

In the current *in vitro* study, the concentration- and time-dependent response of TL was investigated on different human cells including prostate, ovarian, lung and breast cancer and non-tumorigenic cells. PC-3 prostate and OVCAR-3 were the most sensitive cell lines to TL. It showed a lower cytotoxic effect against normal breast cells than the cancer cell lines indicating cancer-specific effects. According to the preliminary results of this study, although TL is a veterinary drug, its cytotoxic effect on human cancer cells suggests that it should be re-evaluated as a potential cytotoxic agent. Analyses to identify functional compounds of *Tarantula cubensis* venom, and future studies addressing its mechanism of action on cancer cells are recommended.

Conflict of Interest

The article author declares that there is no conflict of interest.

Author's Contributions

I hereby declare that the planning, execution and writing of the article was done by me as the sole author of the article.

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