O103-Inhibition of Na^+/K^+-ATPase in SHSY5Y and U87MG Cancer Cells by the venom of Desert Black Cobra (*Walterinnesia morgani)* from Şanlıurfa, Turkey

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Introduction/Aim: Cancer and neurodegeneration are often thought of as disease mechanisms at opposite ends of a spectrum; one due to enhanced resistance to cell death and the other due to premature cell death. The ion pump Na^+/K^+-ATPase has an important role in cellular mechanism, where it modulates sodium reabsorption and homeostasis of the extracellular compartment. Also, many of the genes associated with either cancer and/or neurodegeneration play a central role in cell cycle control, DNA repair, and kinase signaling. In the present study the effect of the venom of Desert Black Cobra (*Walterinnesia morgani)* from Şanlıurfa was investigated functional changes in Na^+/K^+-ATPase activity on SHSY5Y and U87MG neuronal cancer cell lines.

Materials and Methods: For this purpose, cytotoxic effects of *W. morgani* venom were determined on SHSY5Y and U87MG cell lines by MTT assay. Na^+/K^+-ATPase activity was determined following treatment with different concentrations of *W. morgani* crude venom (IC_{50} and $2xIC_{50}$) by flow cytometry analysis. Also, the venom effect on Na^+/K^+-ATPase activity was performed via western blot analysis after cells exposure with different venom concentrations. Additionally, change of TEER (Transephitelial Electrical Resistance) values was determined in cultured colonal human intestinal cancer cells (CaCo-2).

Results: According to the cytotoxicity results, IC_{50} values were determined as 1.02 and 5.53 µg/ml by MTT assay against SHSY5Y and U87MG cell, respectively. Flow cytometry analyses were shown that *W. morgani* venom triggered inhibition on Na^+/K^+-ATPase activity in SHSY5Y and U87MG cells. Western blot analysis exhibited inhibition of Na^+/K^+-ATPase in a dose dependent manner. Also, *W. morgani* crude venom reduced TEER values of CaCo-2 cell lines with increased membrane permeability.

Conclusion: *Walterinnesia morgani* venom contain a large number of pharmacologically highly active substances through a specific mode of action each with the potential of becoming a potent drug. The formulations or combinations of this venom for targeted drug delivery could be used to cancer and neurodegenerative diseases treatments. Further detailed investigations need to focus on isolation of active peptide from *W. morgani* venom.

Key words: *Walterinnesia morgani,* Na^+/K^+-ATPase, Nerve Cancer Cells, Flow Cytometry, TEER

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All responsibility for the articles in the abstract book belong to their authors.

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ABSTRACT ID	TITLE / AUTHOR(S)	TOPIC(S)
O101	"*The Research of the Anti-Tumoral Effects on Mice Formed of Curcumin's Ehrlich Solid Tumor*" Seher YILMAZ*, Harun ÜLGER, Tolga ERTEKİN, Mehtap NİSARİ, Şerife ÇINAR, Arzu Hanım YAY, Niyazi ACER	Angiogenesis, Anticancer Drug Development
O102	"*Argyrophilic Nucleolar Organizing Region–Associated Protein Synthesis Amounts in Selection of the Most Reliable Dose of the Drugs Such as Rhamnetin in Cancer Treatments*" Tolga ERTEKİN*, Recep EROZ, Mehtap NİSARİ, Duygu BİRCAN, Mustafa NİSARİ, Erdoğan UNUR	Primary tumorigenesis and Cell cycle, Anticancer Drug Development
O103	"*Inhibition of Na^+/K^+-ATPase in SHSY5Y and U87MG Cancer Cells by the venom of Desert Black Cobra (Walterinnesia morgani) from Şanlıurfa, Turkey*" Çiğdem ÇELEN, Bayram GÖÇMEN, Yalçın ERZURUMLU, Mehmet ZÜLFÜ YILDIZ, Ayse NALBANTSOY*	Cell Death Mechanisms, Ion channels and Ion Transporters, Anticancer Drug Development
O105	"*Expression of miR-143, miR-145, miR-192, Tumor Suppressor miRNAs using qPCR in Colon Cancer Tissues*" Cigdem GUNGORMEZ*, Hatice GUMUSHAN AKTAS, Ersin BORAZAN	Tumor suppressor genes
O106	"*Knockdown of TIGAR Inhibits Proliferation and Induces Apoptosis and Autophagy via Oxidative Stress Markers in Lung Cancer Cell by Targeting TIGAR*" Osama Hamid SHAREEF and Can Ali AGCA*	Cell Death Mechanisms
O107	"*Cytotoxicity of Cetuximab in Parental and Epirubicin-HCl Resistant Liver Cancer Cells*" Ayse ERDOGAN*, Aysun OZKAN	Cell Death Mechanisms, Anticancer Drug Development
O108	"*Anticancer Activities of Heterocyclic Compounds against Breast and Colon Cancer Cell Lines*" Senem AKKOÇ*, İlhan Özer İLHAN and Veysel KAYSER	Anticancer Drug Development
O109	"*Raftlin, Presepsin Levels and Thiol-Disulfide Homeostasis in Acute Appendicitis*" Omer Faruk OZER*, Eray Metin GULER, Abdurrahim KOCYIGIT, Sahabettin SELEK, Bilge Sumbul GULTEPE, Mehmet YIGIT, Yeliz Emine ERSOY	Oxidative stress Diagnostic / Prognostic Markers
O110	Views of Student Nurses on the Ethical Issues Experienced by Patients Diagnosed with Cancer Feray KABALCIOĞLU BUCAK*, Mert KARTAL	Cancer ethics

Inhibition of Na^+/K^+-ATPase and CXCR-4 in Estrogen Receptor Negative MDA-MB-231 and Estrogen Receptor Positive MCF-7 Cancer Cells by the venom of Desert Black Cobra (*Walterinnesia morgani)* from Şanlıurfa, Turkey

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INTRODUCTION

Breast cancer is the second most common cancer type in women after skin cancer. In 2015, approximately 40.290 women expected die from breast cancer. The ion pump Na^+/K^+-ATPase is a key regulator of maintain ionic and osmatic balance in cells and also plays a vital role in the regulation of cellular homeostasis, cell differentiation and proliferation. Different expression and functioning of Na^+/K^+-ATPase has been observed in Alzheimer's disease, diabetes and different cancer types. In the present study, the effect of the venom of Desert Black Cobra from Sanliurfa was assess the effect of Na^+/K^+-ATPase activity on MDA-MB-231 and MCF-7 breast cancer cell lines.

MATERIALS & METHODS

Figure 1. *Walterinnesia morgani*

Cell culture and *in vitro* cytotoxicity assay

MDA-MB-231 (human breast adenocarcinoma, ER negative, epithelial cell line) and MCF-7 (human breast adenocarcinoma, ER positive, epithelial cell line) cancer cells were used for determining the cytotoxicity. Cell lines were purchased from ATCC and cultivated in DMEM/F12 (Gibco), supplemented with 10% FBS (Gibco), 2 mM L- glutamine, 100 U/ml of penicillin and 100 µg/ml of streptomycin (Lonza). Cytotoxicity of crude venom were determined by following the general procedure based on cell viability using a modified colorimetric MTT assay. The cells were incubated at 37 ^oC in a humidified atmosphere of 5% CO_2 for 24 h in 96-well microplates with an initial concentration of $1x10^5$ cells/ml. Subsequently, the cultured cells were treated with different doses of crude venom (50, 5 and 0.5 µg/ml) and incubated for 48 h at 37 ^oC. Percentages of surviving cells and half maximal inhibitory concentration (IC50) in each culture were calculated after incubation with MTT for 4 h following venom treatment. The IC50 values were calculated using GraphPadPrism 5 software (CA, USA).

Preparing the sample for FACS analyzes for CXCR4 and Na+ /K + -ATPase

MDA-MB-231 and MCF-7 cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h in 6-well microplates with an initial concentration of $5x10^5$ cells/ml. Then, the cultured cells were treated with different doses of crude venom (IC50 or 2xIC50) and incubated for 3 h at 37 °C. After trypsinization procedure cells were washed 2 times with PBS by centrifugation for 5 minutes at 1000 rpm. The cells were fixed with 4% paraformaldehyde at +4 C for 10 min., washed 2 times with PBS and then blocked with 3% BSA + 0.3% Saponin in PBS for 30 min. After washing, the cells were incubated with primary antibody (anti Na + /K + -ATPase antibody (Novus) or CXCR4 antibody (SantCruz)) diluted in 1% BSA + 0.3% Saponin in PBS according to manufacturer's recommendations for 1 h at room temperature. After antibody incubation and washing, the cells were incubated with secondary antibody (FITC conjugated anti-IgG, (BD)) for 30 min., washed twice with PBS and analyzed by FACS (BD, Accuri C5).

Protein preparation and Immunoblotting (IB)

MDA-MB-231 and MCF-7 cells lysates were prepared by homogenizing cultured cells in RIPA buffer (1xPBS, 1% nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS, pH 8.0). After removal of insoluble materials by centrifugation at 14.000 rpm for 10 min at 4 °C, protein concentrations were determined using BCA protein assay kit (Thermo Scientific). Typically, 40 µg of total cellular protein were used for immunoblotting. Samples were denatured in 6x Laemmli bu er at 95 °C for 5 min and were separated on handcast polyacrylamide. Gels were transferred onto PVDF mem- branes (Millipore). Following classical immunoblotting steps (blocking, incubating with primary and second- ary antibodies), proteins were visualized using enhanced chemiluminescence (BioRAD) by Fusion FX7 (Vilber Lourmat). Densitometric analyses of protein bands were performed using ImageJ so ware (http://imagej.nih. gov/ij/).

RESULTS & CONCLUSION

According to the cytotoxicity results, IC_{50} values were determined as 2.17± and 3.23± µg/ml by MTT assay against MDA-MB-231 and MCF-7 cells, respectively. Western blot analysis exhibited inhibition of Na^+/K^+-ATPase levels in a dose dependent manner. Especially, MDA-MB-231 cells more sensitized to effect of venom than MCF-7 cells. Flow cytometry analysis were shown that *W. morgani* venom triggered inhibition on Na^+/K^+-ATPase activity in both cell lines.

W. morgani venom possess number of highly active compounds that act a different mode of action on cells. Usage of potent effect of venom combinations or formulations may use to be with new drug targeting techniques for treatment of cancer and neurodegenerative disease. For best characterization of *W. morgani* venom action, forthcoming investigations will be focus on isolation of active protein and/or peptide from crude venom.

	IC_{50} (µg/ml)
MDA-MB-231	2.168
MCF-7	3.233

Table 1: IC_{50} values of cell lines following *W. morgani* treatment for 48 h.

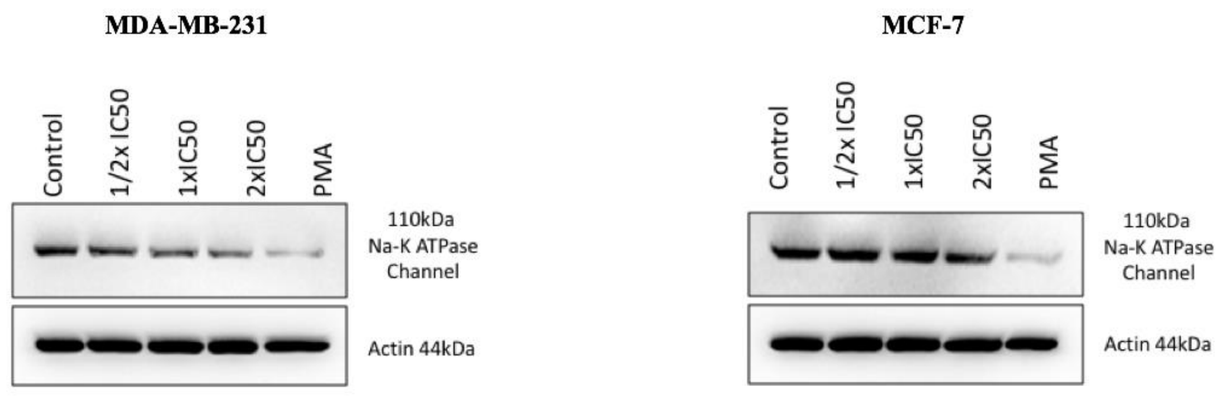

Figure 2. Effects of ½x, 1x, 2x IC_{50} venom extract (uM) on Na-K ATPase channel protein expression in MDA-MB-231 and MCF-7 cell lines.

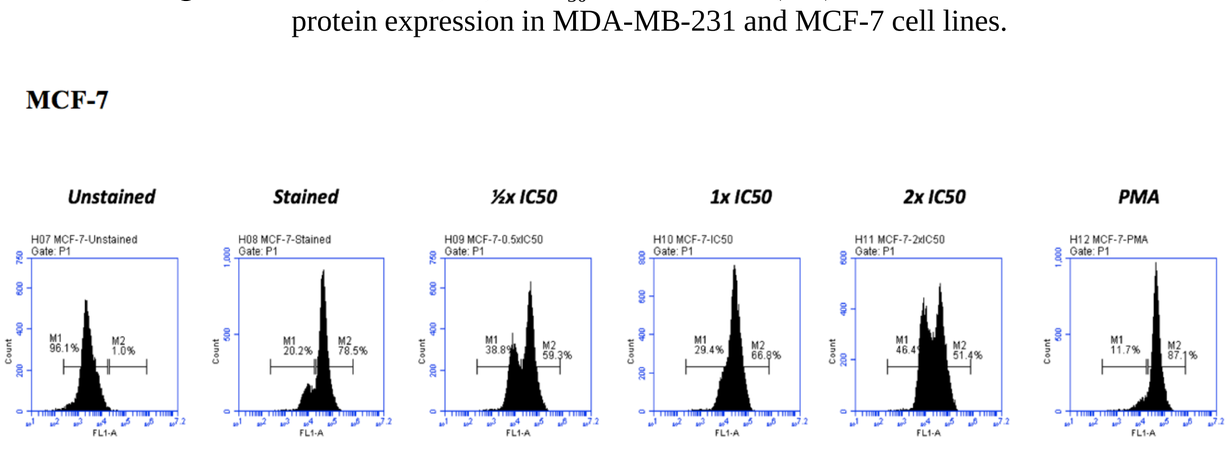

Figure 3. Effects of ½x, 1x, 2x IC_{50} venom extract (µg/ml) on CXCR4 protein level in MDA-MB-231 and MCF-7 cell lines. CXCR-4 levels were evaluated by using flow cytometry technique.

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