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Potential Anticancer Activity of Indian Cobra (*Naja naja*) Venom Components on Human Cancer Cell Lines

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Neema K N[a,b], Narasimha Swamy[c] , Priya B S[d], S Nanjunda Swamy[a*]

[a.] *Department of Biotechnology, Sri Jayachamarajendra College of Engineering, JSS Technical Institutions Campus, Mysuru. Karnataka, India.*

[b] *JSS Research Foundation Mysuru. JSS Technical Institutes Campus, Mysuru. Karnataka, India.*

[c] *Department of Physiology, Adichunchanagiri Institute of Medical sciences, Bellur.*

[d.] *Department of studies in Chemistry, University of Mysore, Manasagangothri, Mysuru-570006, Karnataka, India.*

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ABSTRACT

Indian cobra (*N. naja)* venom from the venomous Elapidae family found in the western part of Indian subcontinent was studied to examine its cytotoxic properties on human cancer cell lines- breast cancer (MDA MB-231) and colorectal adenocarcinoma (HCT-116) cell lines. Cytotoxicity of *N. naja* was expressed under the Sulforhodamine B (SRB) assay conditions. *N. naja* inhibits cytotoxicity in dose dependent manner in both MDA MB-231 and HCT-116 with 55% and 49% of inhibition respectively. This is due to the presence of reactive oxygen species primarily in the form of H_2O_2 that is generated in high amounts, which kills the tumor cells. The cell toxicity assay has been adapted as an alternative to assess toxicity in animals. It is a simple, economical, time-saving and sensitive method which produces results. In the present study, it can be concluded that Indian cobra venom and its components exhibit promising anticancer agent against human cell lines.

INTRODUCTION

Snake venoms and their toxins is an effective anticancer agent which have shown possible cytotoxic effects on human cancer cell lines, providing new perspectives in drug development. The search for cancer cure from all natural resources is in practice from long time because surgery, radiotherapy and chemotherapy are not providing adequate protection towards cancer cells. The currently available anticancer agents involve various side effects that are toxic to the normal cells and decrease the therapeutic indexes of these drugs [1]. They are also simultaneously affecting the normal cells along with cancerous cells; causing more serious side effects. This has lead to the research of cancer curing drugs from naturally available products. Calmette in 1993[2] first reported the treatment of cancer using lab animals with the use of snake venom. Venoms from Elapidae, Viperidae and Crotalidae were screened for cytotoxicity assays towards B16F10 melanoma cell lines, which showed cytotoxic activity majorly in Elapidae, compared to Viperidae and Crotalid causing cell aggregations [3]. Venom of each snake varies, but a major difference exists between various inter-species, juveniles and adults, even among the snakes of intra-species and also with different geographical regions. Snake venom contains species-specific proteins, exhibiting an array of toxicological activities. The chemical composition of the venom also depends on the interspecies, and very specifically for varied reasons. Daltry et al. [4] observed that the variation in chemical composition of venom depends on the habitat and closely related to the diet. Different species have different type of venom. The major enzymes present in the snake venom are given in Table 1.

Table 1.Various enzymes found in the Venom (BON**, C [5])**

Class	Type of enzyme	Species
Oxidoreductases	L-amino acid oxidase	All species
	Dehydrogenase	Elapidae
Hydrolases	Phospholipase A_2	All species
	Hyaluronidase	All species
	Protease	Elapidae
	Acetylcholinesterases	Elapidae
	Alkaline phosphatase	All species
	Amylase	All species
	5'-nucleotidase	All species
	Deoxyribonuclease	All species
	Ribonuclease	All species
	NAD-Nucleotidase	All species
	Prothrombin activator	All species

The enzymes mainly involved in various pharmacological activities are L-amino acid oxidase (LAAO), PLA2, hyaluronidase and proteases. The characterization of snake venom elements and mode of action of the venom components plays an important part for the medical treatment. Snake venom LAAO has its effects on various biological activities such as platelet aggregation, cell apoptosis and cytotoxicity. It has its potency towards anti-tumor anti-leishmaniasis, anti-microbial and anti-HIV activity [6]. Hence, all other snake venom components which include peptides, polynucleotide toxins, proteins, nucleotides, metals, lipids etc, are being explored for its potential in cancer therapy. Keeping this in mind, the aim of the present study is to further investigate the cytotoxic mode of action of cell death of the Indian cobra venom (*N. naja*) from western part of Indian subcontinent, against two human cancer cell lines i.e. Human colorectal carcinoma (HCT 116) and breast cancer cell lines (MDA MB-231).

MATERIALS AND METHODS

Lyophilized *Naja naja* venom was obtained from the *Haffkine Institute,* Mumbai, India. Sephadex G-75 gel was purchased from Sigma–Aldrich. Tris (hydroxymethyl)aminomethane analytical grade HCl, bovine serum albumin (BSA), sodium hydroxide, trichloroacetic acid(TCA), calcium chloride and sodium chloride were purchased from Merck. Cell lines - HCT 116 and MDA-MB 231 were maintained and studied at the Department of Biochemistry, JSS University Mysore. All chemicals were of analytical grade.

Bradford Protein Determination

Protein concentration was determined according to Bradford's method [7] using BSA as standard. Venom samples (0, 2, 4, 6, 8 and 10 µg) were mixed individually with 1 ml of Bradford reagent in microcentrifuge tubes. Absorbance of Bradford-protein complexes was measured after 20 minutes at 595 nm in microcuvettes using a Shimadzu UV-1800 spectrophotometer (Japan). A standard curve of BSA protein at different concentrations was plotted to enable the determination of the protein concentrations in the venom samples.

Measurement of cell viability using SRB assay

Sulforhodamine B (SRB) assay was performed according to the method of Skehan et al [8]. Experimentally, cells were fixed in $1/4^{th}$ volume of cold 50% (w/v) TCA for $4^{o}C$. After 1hr the media was removed and the wells were washed with water (200µl X 4 times) to free from

TCA and serum proteins. The plates were dried, incubated with 100µL 0.4% SRB for 30 minutes to stain the cellular proteins and washed quickly with 1% acetic acid (200µl X 4 times) to remove unbound SRB. MDA MB-231 and HCT 116 cell treated with different doses (0.4µg-50µg) of *N. naja* for 48h incubation time, respectively. The bound SRB was solubilized in 10mM Tris base solution (100µl/well) and the absorbance was measured at 490 nm in a Bio-Rad plate reader. The percentage cell growth inhibition is calculated by comparing the OD values with control DMSO vehicle treated cells and in comparison with oxaliplatin as positive control.

RESULTS

Cytotoxicity testing is assessed using one or more human cell lines which are under actively growing conditions and undergoing mitotic cell division and the reason behind this activity is the production of H_2O_2, a known reactive oxygen species (ROS), through the enzymatic reaction [9]. The cytotoxicity of various compounds from snake venom is explained by the alterations in the cellular metabolism that leads to several effects on cancerous cells [10]. To analyze the cytotoxic effect of *N. naja* on human colorectal carcinoma (HCT-116) and breast cancer cell lines (MDA MB-231), crude lyophilized *N. naja* powder was analyzed for its protein concentration using Bradford's reagent and later was incubated for 48h in a dose dependent manner using Sulforhodamine B (SRB) protocol. SRB cell cytotoxicity assay is said to be one of the most widely used methods applied to detect cell viability that can induce cytotoxicity in various cancer cell lines. SRB cell proliferation assay indicates that the treatment of different cancer cell line with various concentrations of snake venom protease (SVP) inhibits cell proliferation. This assay relies on the ability of SRB to bind cellular protein components and measure the total biomass.

The result of SRB assay determined that *N. naja* killed the cell lines HCT 116 and MDA MB-231 in a dose dependent manner. This suggests that H_2O_2 an ROS present in the venom component mediates *N. naja* inducing cytotoxicity. The cytotoxic effects of venom have the capacity to degrade or destroy tumor cells mainly because of the presence and activities rendered by LAAO, proteases, PLA_2. Fig 1 and 2 shows the response of MDA MB-231and HCT 116 cell treated with different doses (0.4µg-50µg) of *N. naja* for 48h incubation time respectively, and the percentage of inhibition was calculated in comparison with oxaliplatin as positive control. Concentration of 14µg initiated the inhibition process of *N. naja*, which further inhibited 55% of MDA MB-231 cell line and 49% of inhibition was observed for

HCT-116 cell at a maximum concentration of 50µg. This establishes the concentration dependent cytotoxic/anti-cancer activity of Indian cobra venom on two major cancer cell lines.

Figures and description

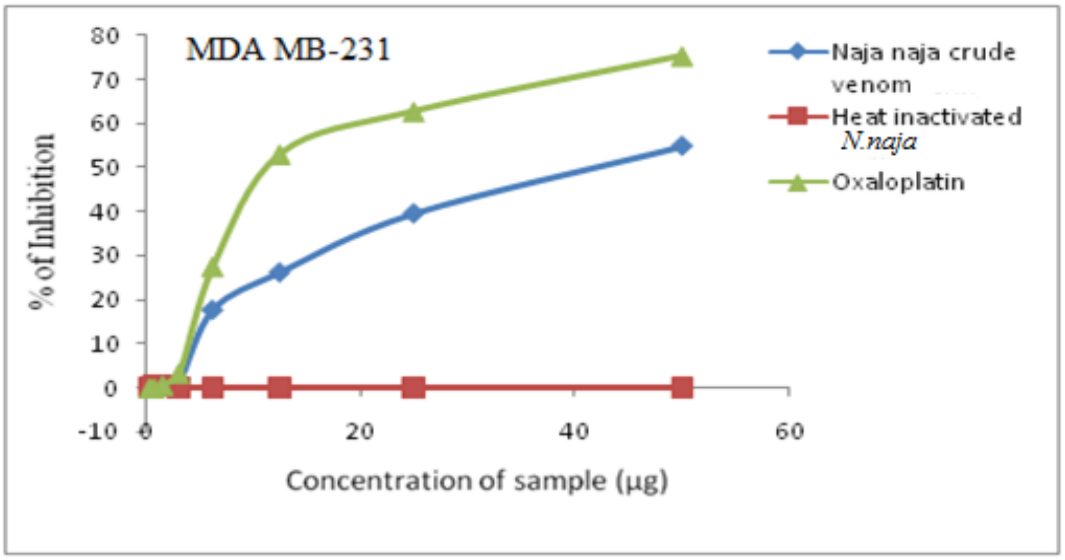

Fig 1: Cytotoxicity activity: - MDA-MB 231 cells were treated with different concentrations of *N. naja* (0.4 µg-50µg) for 48h with oxaloplatin as positive control

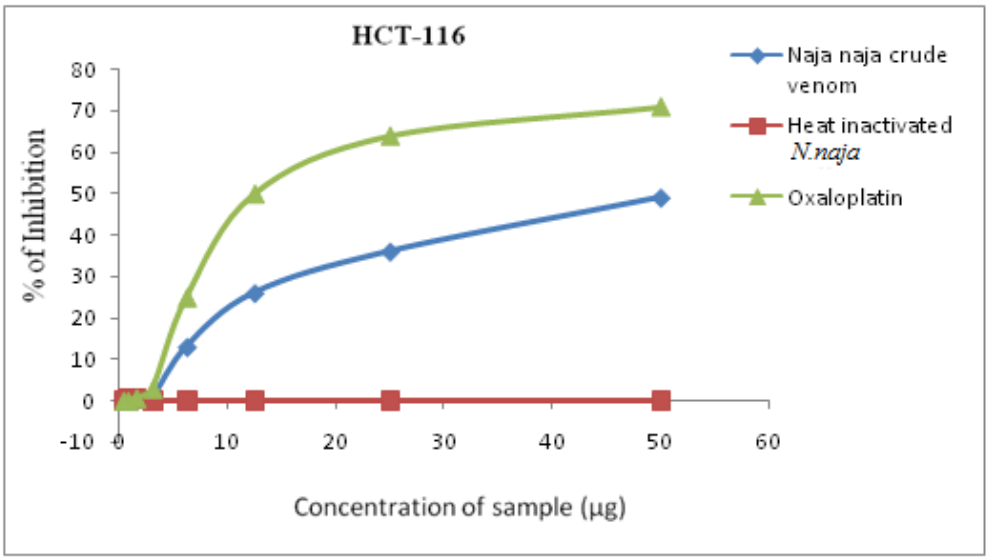

Fig 2: - HCT 116 cells were incubated for 48h with various doses of *N. naja* (0.4µg-50µg) and Oxaliplatin as positive control.

CONCLUSION

Breast cancer is the one of the major cancer disorder seen among women worldwide, especially in developing countries, as well as colorectal adenocarcinoma a major problem for public health, which are mainly attributed to the undefined signs and symptoms that is an impediment to early diagnosis and treatment. Snake venoms toxins are the natural sources which could become potential candidates for the treatment of various types of cancer. It is thus important to further study the pharmacological aspects, concerning therapeutic drugs from natural resource and to investigate their mechanism of action on cancer cells. The present work provide substantial role of *N. naja* in the development of therapeutic approach acting on human cell lines showing its cytotoxic activity and the way of cell proliferation/death against Human Colorectal carcinoma (HCT 116) and Breast cancer cell lines (MDA MB-231), which aids in elucidating the role as an anti-cancer drug.

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