INVITRO CRUDE COBRA SNAKE VENOM SIGNIFICANTLY DECREASES THE PRODUCTION OF RNA & DNA IN BREAST CANCEROUS TISSUE

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Crude cobra snake venom at the rate of 25 µg / ml reduces nucleic acids production in human breast cancerous tissue invitro. It suggests an ideal model for examining the anticancer activity and could be a better substitute in comparison to presently available anti tumour drugs, for therapeutic use in breast cancer in future.

Key words: Snake venom, breast cancer, RNA & DNA

INTRODUCTION
Breast cancer has become common in developing and developed countries. According to WHO reports the number of breast cancer is multiplying every year and it may reach to frightening number in next 10 to 20 years if not checked timely. Alarming increase in this disease a leading cause of death in women is a concern of all. Breast cancer is more fatal in white men than in white women and this is associated with high fat diet, particularly animal fat. Despite significant research in this area the disease remains with poor prognosis. Recently interest is developed to treat cancers in general and breast cancer in particular with animal toxins and especially snake venom.1,2 Omran et al have observed that snake venom has potential efficacy for antitumour therapy.3

Until an effective therapy is established new treatment modalities are being investigated Stephin et al recently reported that use of liposomal delivery system for snake venom disintegrin (homodimer) has a significantly prolonged circulatory half life compare with native CN, LCN is passively accumulated in the human breast tumour and limits the progression.4

Francis et al has also observed that snake venom disintegrin inhibits human ovarian cancer in the orthotopic nude mouse models.5

Presently available antitumours drugs eg cycloehosphomide and mitomy cin etc, are effective on one or other receptor but also develop countless side – effects. However both workers have reported that the venom interacts with different sub-types of integrin receptors hence shows prominent effects.

The present investigation is in continues to our previous study into6 to see the effect of cobra crude snake venom on inhibition of human breast cancer invitro.

MATERIAL AND METHOD
CHEMICALS
Electrolytes (Na+, K+ and Ca++) for standard calibration were purchased from Merck (USA). Bovine serum albumin (BSA) for protein calibration was obtained from Sigma. Nucleic acids (RNA & DNA) and Di-methyl benz-anthracene (DMBA) were supplied by Fluka for standardization, for inorganic phosphate calibration, Di-sodium hydrogen phosphate (12-hydrate) was purchased from Merck-Schuchardt (USA). Radiac wash (with EDTA) = RW was supplied by Atomic Rodents Corporation (New York), for cleansing the glass wares while chronic acid was prepared in this lab. All other reagents were of “Analar grade” and were supplied by BDH chemicals, LTD Poole (England) and Merck Schuchardt (USA) + Riedel – de Haein AG Seelze – Hannover (Germany).

Human breast tissues (cancerous and normal) were collected from different hospitals and atomic energy center of Sindh and also from Ihsan laboratories, Karachi. Disease was confirmed by oncologist through biopsy. Patients later were also treated by radiation and kept on chemotherapy.

Surgically excisioned tissues were kept separately (cancerous and normal). Tissues further were cut in small slices i.e. 1 mm thick and put in ice – cold normal saline there after kept in deep freezer before process later tissues were homogenized and incubated with and without snake venom.

COLLECTION OF SNAKE VENOM:
Cobra snakes were supplied by Laghari Snakes Association and from Jogi Colony of Thatta, Thur and Jamshoro. Fresh Snake Venom was collected by compressing the glands of the healthy Snakes in the laboratory. The charmars were also requested for Venom from Cobra snakes. The venom thus obtained, was then lyophilized. Cobra venom was also purchased from Sigma loeate. The venom, thus obtained was used for all biochemical quantitative (invitro) studies and was found to be equally successful in maintaining the biological activity of the poison up to the level of stored one and fresh one.

Fresh venom was placed directly in a fine sterilized glass container fixed in coloured and air-tight box. The whole procedure was carried out in the dark room at normal temperature. After two weeks venom got dried and changed into the solid
transparent crystals of light yellow color and was ready for use.

Determinations
1. DNA – content determinations were carried out in triplicate by modified Indole method of Certoti\(^7\) at 490 µm.
2. RNA – content determinations were carried out by Schneider\(^8\) method at 660 nm. All spectrophotometer estimations were made by bauch and lumb spectrophotometer spectron 21.
3. . All chemicals were supplied by Fluka (USA), ARC (New York), BDH, Ltd, Poole (England), E. Merck (USA) and Riedel-de Haein AG.
Seclze – Hannover (Germany). Venom was collected from living cobra snake and different dosage forms 10µg, 25µg and 50µg per ml were prepared.

Statistical Analysis
4. The level of significance was calculated by the method of students\(^t\) test. The RNA, DNA – content is expressed as µg/50mg tissues homogenate per 30 minutes at 37°C.

RESULTS
This study was designed to evaluate the effect of crude snake venom on a level of RNA and DNA of normal and breast cancer tissues invitro. The tissues were collected from hospitals and atomic energy centers mentioned in material method.

It is observed that in comparison to normal tissue the amount of RNA in cancerous tissue was higher about 84 % (Fig 1a). And when a cancerous tissue was treated with snake venom (25 µg/ml) the content was reduced by 25 % (Fig 1b). This is consistent to our previous studies.

Similarly when same procedure was repeated for DNA contents the results show similar pattern and amount of DNA increased in cancerous tissues by 57 % (Fig 2a). The DNA amount is reduced with snake venom (25 µg/ml) by 95 %. (Fig.2 b).

DISCUSSION
Despite the significant improvements in the management of breast cancer the survival rate is not more than 20% - 25%. Most common death in breast cancer patients is due to metastatic spread of cancer cells which invade into angiogenic blood vessels growing into the tumour. Thus it is imperative to find new treatment. The new trends in literature suggests to look for animal toxins especially snake venom as it has given better results. We are investigating is a therapeutic potential of disintegrin present in cobra snake venom contains a number of components with different pharmacological and Biological activities.

We observed that human breast cancer contains higher amounts of nucleic acids, (Fig 1a & Fig 2a) when compared with normal one. This may be due to polymerase enzyme activity responsible for increase in RNA content in rapidly growing cancerous tissues. Earlier it is reported that cytotoxin component of snake cobra venom has more cytotoxic effect on tumour cells than normal cells invitro.\(^2\) This is consistent with the observation of Oman et al\(^8\) who have Biochemically and Pharmacologically analyzed the cell that is induced by cobra venom in cancerous tissues with minimum dose of 20µg/ml.

He has further pointed out that cell death by two mechanism, necrosis and induction of apoptosis or may be in combination of apoptosis with same cell. This is also recognized by Shimizu et al.\(^9\)

Recently different components CN, a disintegrins are small disulphide rich Arg-Gly-Asp (RGD) containing peptides etc are being separated and their effects on different receptors and their sub – types in both cancerous and non cancerous tissue is being studied. It binds to cell adhesion receptors on normal and malignant cells. Francis et al have reported that CN not only significantly inhibited ovarian cancer dissemination in the nude mouse model, but it also dramatically prevented the recruitment of blood vessels to tumors at secondary sites.\(^5\)

Antitumour activity of CN is based on the high affinity interaction with several integrin displayed on both cancer cells and newly growing vascular endothelial cells.\(^4\) Both i.t and i.v administration of CN and LCN respectively affectively inhibit angiogenesis when compared with control. The diverse mechanisms of action provide CN with a distinct advantage over many other antitumour agents that only block a single pathway.\(^10,11\) Despite receiving several setbacks in recent clinical trials with routine antitumour agents the snake venom therapy remains a very promising in near future.

Muhammad Alla & Oman\(^12\) have observed that venoms and toxins from snake have influence on the growth of breast cancers cell lines T 470 & MRDMB – 468 cells. They have emphasized on early significant cell destruction with lower doses that is 20µg / ml and 50µ g / ml.

The results of our present invitro study (Fig 2b & Fig 1b) showing reduction in DNA / RNA contents in breast cancerous tissue.

Inactivation of enzyme responsible support the potential for snake venom ingredient a possible treatment of this disease. We also demonstrate appropriate feasibility; safe and effective dose i.e. 25µg/ml of snake venom to impair the normal cell growth of tumour cells.\(^2\)
Fig-1a. Effect of crude snake venom (25 µg / ml) on RNA of normal and breast cancerous tissue.

RNA (N)  RNA (Ca)
0.325    0.3525
84%

Fig 1b  Amount of RNA in normal and Ca-breast

RNA (Ca)  S.V
0.3525    0.2617
25%

Fig-2a. Effect of crude snake venom (25 µg / ml) on DNA of normal and breast cancerous tissue.

DNA (N)  DNA (Ca)
0.278    0.437
57%

Fig 2a  Amount of DNA in normal and Ca-breast

DNA (Ca)  S.V
0.437    0.021
95%

Fig 2b  Amount of DNA in normal and Ca-breast
This support the idea of the role of the natural products and toxins as antitumour agents. Further investigations are needed to explore and achieve the ultimate aim of better understanding of the mechanism of venom therapeutic strategies, which can play physiologically efficient and potent anticancer treatment useful in the clinical management of cancer.

CONCLUSION

Snake venom invtro at the rate of 25 µg / ml maximally reduces the RNA & DNA productions. Hence in future it could be a better choice to treat the cancers of different types.

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