EFFECT OF SNAKE VENOM ON NUCLEIC ACIDS AND TOTAL PROTEINS IN VARIOUS NORMAL AND CANCEROUS ANIMAL TISSUES

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Background: Venoms have occasional medical uses. This study was carried out to determine effect of cobra snake venom on nucleic acid and total proteins in various normal and cancerous animal tissues. **Methods:** In this vitro study the venom with varying concentrations was incubated with homogenates of liver, heart and kidney of normal tissues. Reduction in RNA / DNA levels was observed. **Results:** Our results indicate that nucleic acids are more sensitive. However the effect of snake venom on normal skin tissue was insignificant when compared with cancerous tissue. The dose response curve shows that the lowest concentration of venom at 25 µg / ml (a survival dose) produced maximum inhibition of both nucleic acids. The opposite and linear response was observed in protein contents. **Conclusion:** It can be assumed from the present study that the venom might have therapeutic effect at a dose of 25 µg /ml in cancerous tissues.

Keywords: Snake venom, Nucleic Acid, Tissue, Total Proteins

INTRODUCTION

Cancer is as old as life and it was recognized as a disease by the Egyptians as early as 1500 B.C. in all ages, people always have dreamed of a single key to unlock the mysteries of life. Similarly, initial step towards the cure of cancer by snake venom as was claimed by some physicians in early days and this hope persisted as a dream for quite long time.[1,2] On one hand despite there has been proper scientific approach in this area.[3] Whereas on the other side, 'Cancer', 'Malignancy' and 'Tumour' are the words that cause people to freeze in fear.

Cobra venom has been used for many years in medical research because it has an enzyme lecithenase that dissolves cell walls and virus membranes via interaction with specific ion channels .[4] The severity of venom's effects depend on several factors because of its chemical nature as it is a complex mixture of toxic components that include proteins and different peptides toxins, enzymes and other active agents.

Venoms have occasional medical uses for example some are used as pain killers in cases of arthritics (presently is used in Thar) or cancer and some serve as coagulants for people with hemophilia. Snake venom also revels clue about heart drug S.V proteins blocks receptors as the drugs do.[5]

The present study is designed to investigate therapeutic strategies. If succeeded in getting the desired results in the positive direction; it will open a door for the designing and development of a new antineoplastic drug from the venom in the future.

MATERIAL AND METHODS

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 chromic 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 Seclze – Hannover (Germany).

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 charmers 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.

The quantitative determination of total proteins was assayed by spectro-photometric method of Lowry's, et al.[6] The method of Lowry's et al[6] and modified by Peterson[7] was used for the protein determinations throughout the study.

The experiments were performed on animal (rabbits) tissues, i.e., liver, heart and kidney (normals) and skin (normal & cancerous). At the same time, DMBA- induced skin (rabbit) cancer was developed with alternate applications of DMBA, as initiator and croton oil, as promoter on selected area. Tumor developed in area which was already affected by initial applications of DMBA, a carcinogenic agent.[8]
After incubation, the venom treated and control homogenates were followed by the method of extraction of nucleic acids and total proteins[9] and then estimated quantitatively, described as follows:
Deoxyribonucleic acid was quantitatively assayed by a spectrophotometric method. The method involves the acidification of samples followed by extraction in chloroform. This indole method first published by Dische[10] in 1929 & further modified by Ceriotti in 1952 / 53.[9]
The quantitative determination of ribonucleic acid was assayed by spectrophotometric method of Schneider.[11]
The measurements of DNA and RNA in samples of tissue has been accomplished most generally by the spectrophotometric determinations of the coloured products of the reaction b/w sugar moiety in DNA and colouring reagent indole. The RNA was estimated by the reaction of Orcinol (color reagent) and base in the RNA extract. For DNA present in approx: 400 diploid mammalian cells.

RESULTS

In this part of study, the invitro effect of the cobra venom on different cellular macromolecules (nucleic acids and proteins) was quantitatively evaluated, in various normal and cancerous tissues of animals. Thus, an attempt has been made (a) to see the inhibitory effect of the venom, (if any) on the macromolecules of normal liver, heart and renal tissues, secondly (b) to assess the similar effect of the venom after skin cancer was experimentally induced in rabbits in our labs to study and compare.
In order to observe the effect of cobra venom on cellular nucleic acids and total proteins in varions animal tissues viz liver (Fig.1), heart (Fig.2), and kidney (Fig.3), the venom with varying concentrations was incubated with homogenates of the said tissues.
The data shows that during the invitro treatment of 30 minutes incubation at 37^{o} C, the venom produce significant change against their controls in the level's of nucleic acids and total proteins, in these normal tissues of the rabbit. The nucleic acid contents of all tissues, after venom treatment, show a mixed response while, the proteins fraction was found to have good linear correlation with the increasing dose of the venom, when a dose response-curve was established.
The changes in the nucleic acids as well as the protein in the normal and cancerous skin brought about by the venom are mentioned and the effect, of varying dose preparations, is shown in figure 4 & 5. From the data, it is evident that small but significant change in the amounts of RNA, DNA and the proteins could be observed in the normal tissues where as, in cancerous tissue samples which have large concentrations of nucleic acids as well as the proteins at basal level, showed a significant fall in the RNA and DNA contents, only.

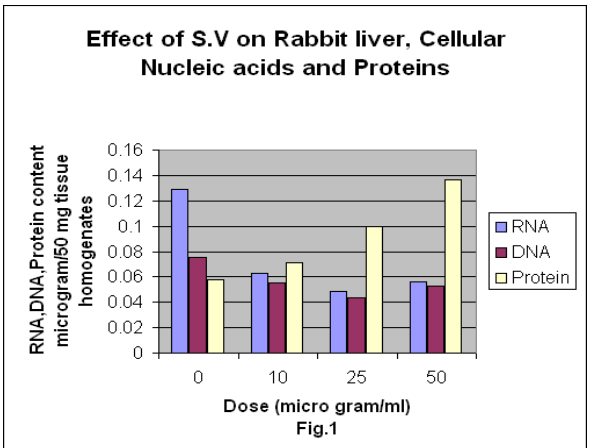

Fig.1

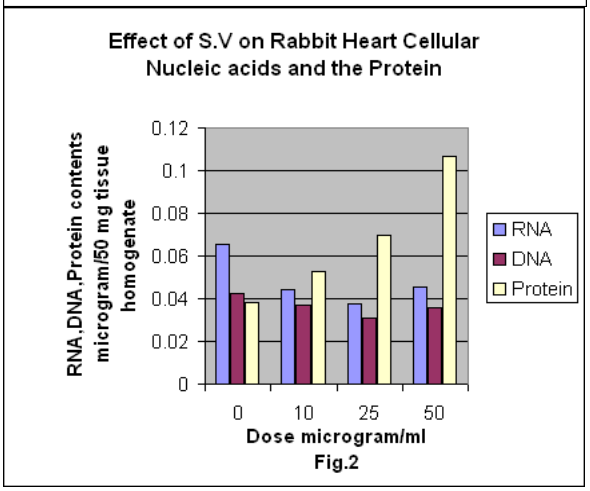

Fig.2

The corresponding values regarding the reduction of RNA / DNA – levels indicate that cancerous nucleic acids might be more sensitive towards the venom. The dose-response curve shows that the effective concentration of the venom i.e. 25µg ml produced maximum inhibition of both nucleic acids and conversely, the increasing doses did not maintain the same. Various concentration of S. venom caused gradual reduction in the RNA / DNA content of cancerous tissues, but as far as total proteins contents are concerned, there have an opposite response.

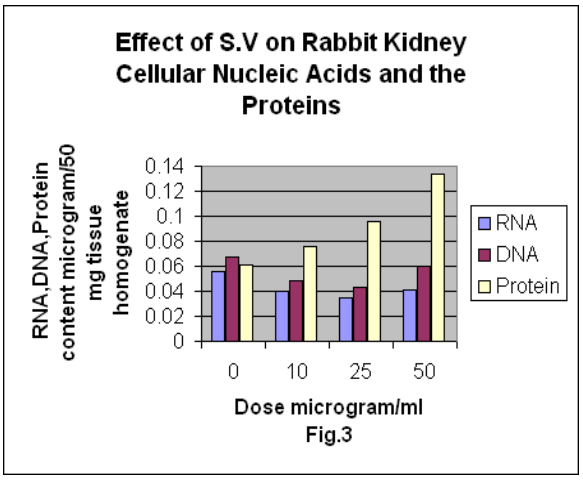

Fig.3

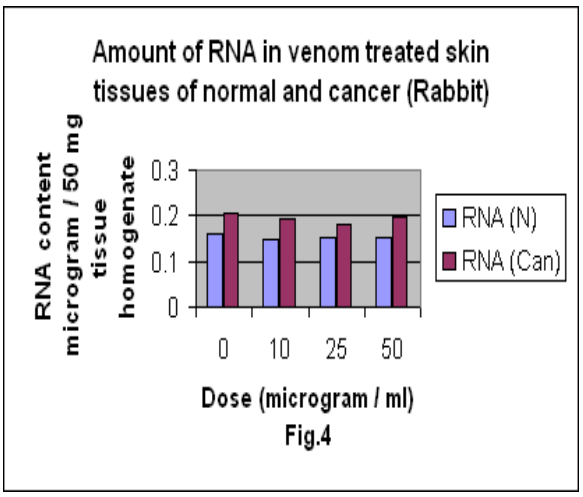

Fig.4

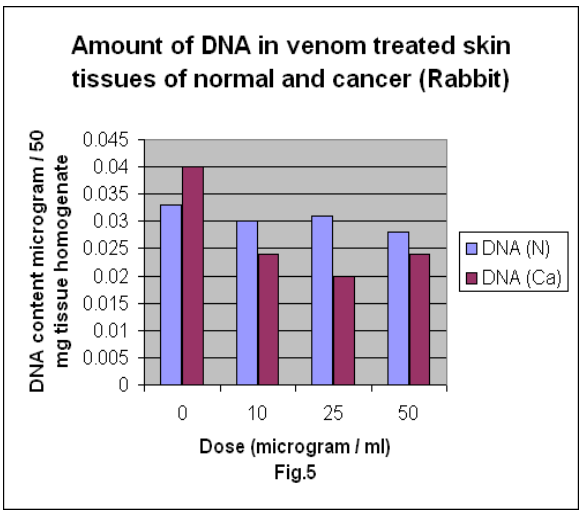

Fig.5

DISCUSSION

We have previously worked to establish the scientific basis of use of cobra snake crude venom in treatment of cancers.[12] We had also shown that crude snake venom could be used safely and effectively **invitro** and **invivo** to treat the cancers. Recently cobra venom is being studied by Paul Bailey and Jacqueline Wilce[4] as a source of useful biological active compound . Hantgan and colleagues[5] hope that it might reveal a clue to treat the various cancers. Different factors are also being separated to assess their direct and indirect effect of each individual fraction on RBC and other tissues from rabbit as well as on lipid metabolism. All fractions decrease significantly serum total lipids and increase inorganic phosphorous..[13] The present study was designated to investigate whether venom does have any effect to alter cellular proteins as well as the nucleic acids and if any; then to compare its influence in the normal as well cancerous animal tissues within the range of the dose regime being studied throughout. The data obtained so far showed that cancer tissues (rabbit skin) possessed considerably larger amounts of nucleic acids than did the normal tissues. This agrees with the observation of Brachet and Jeener[14] for both RNA/DNA that rapidly growing tissues always contain larger amounts of non-sedimentable DNA than differentiated tissues.

The inhibitory role of venom in various physiological processes including transport mechanism of cell membrane and mitofic division has been well-documented.[15-18]

Hantgan et al.[19] working on to develop heart drug have reported that snake venom proteins blocks the receptors just as the drugs do. Toxins bind specifically site on myocardium voltage – gated sodium channels [20]. Paul et al[4] have also observed that venom mixture of proteins and peptide toxins are potent inhibiters of sodium channel in activation. Increased RNA content (cancer tissue) has been correlated with endocrine activity of thyroid and the adrenal cortex[21] as well as anterior pituitary.[22] Whereas, increased DNA content indicates the occurrence of cell proliferation, although, DNA is essentially constant in amount per nucleus in non-proliferating cells.[23] Thus increase in the amount of mitotic activity would be reflected by an increase in total DNA of an organ and should indicate an increment in the cell number.[24] Increased RNA content in cancer tissues controls likely to be due to increased DNA dependent RNA polymerase activity. Our results clearly show suppression in DNA content when the cancerous tissues were treated with the venom. Various studies reported in the literature have demonstrated that cobra venom components like phospholipase cardiotoxin and many other enzymes especially endonucleolytice (DNAases and RNAases) do inhibit cancer growth.[25-27] In our results significant but low level change could be found in nucleic acids

level when normal tissues were treated with varying doses of the venom However the cancerous tissues responded well. The skin samples got their DNA and RNA contents reduced to a maximum with 25µg / ml venom. This strengthens our previous findings of venom's effects on cellular electrolytes fluxes and sodium-pump activity and it could be proposed that the said dose could be more practicable and needs further exploration to confirm these findings.

Some authors reported to have observed, in animal experiments, the growth inhibitions and even regressions of tumours following the direct administration of cobra venom into neoplasm.[28-29] In 1939 special attention was called by Dustin and his collaborators.[30] For invivo and invitro studies to see the influence of toxic substances on cell division and growth. Shaikh et al[12] also reported safe and effective dose in vivo and invitro. These results led to the suggestion that S.Venom might be helpful in the treatment of cancer or may help to develop key techniques for designing therapertic agents/medicines.

Cobra venom cytotoxin was found to have a more cytotoxic effect on tumour cells than normal cells upon incubation invitro.[31] A change in membrane permeability and nucleus has been proposed to be the primary causes of cell proliferation resulting in the metabolic disturbances[32] and genetic changes in nucleoproteins.[33]

In contrast to nucleic acids, the total cellular proteins contents showed proportionally increased levels in the cancer tissues when treated with varying concentrations of cobra venom. It might be that snake venoms are predominantly mixtures of proteins, some of which are enzymes and polypeptides. Or discrepant effect might be related to the relative action of different factors. However it needs further investigation. Interestingly the presence of these enzymes (proteases) is accompanied by their inhibitors in the same venom. Consequently, all these inhibitors are small basic polypetides with a physiological role possibly to dominate unwanted proteolysis within the venom.[4,34]

Not only that, it was also demonstrated that RNAases portion of ribonucleoprotein enzymes is the source of nuleolytic activity[35,36] which could catalyse their own replication at early life, dependent upon RNA chemistry rather than protein chemistry. It could be this reason that cancer is produced because of the active role of the ribosomal RNA rather than increased protein content.[37]

Furthermore, there are many reasons for believing that ribosomal proteins contribute little to basic ribosmal functions.[38] The ribosome itself has been reported to be a ribonucleoprotein enzyme and easily works with rRNA than the ribosomal proteins .[35]

Results conclusively show that venom in small doses can cause inactivation of stimulating enzymes and / or activating the inhibitory enzymes at that site. This might be one aspect through which venom interferes the nuclear functions by inducing alterations in the nucleus to restrict the active DNA – synthesis, responsible for enhancement or initiation of rapid cell division. This adds weight to the hypothesis concerning venom's mode of action at nuclear as well as cellular level.

It may also be demonstrated that appropriate concentrations to impair abnormal cell growth could be 25µg / ml. Recently Stephan et al[39] have reported that 20 µg can also have maximum change in tumour cells.

Further study is required to establish a convenient method to analyse the structure / activity relation ship. Which may give clue to formulate a potential anti tumour drug from natural toxins.

CONCLUSION

- 25µg / ml safe and effective dose invivo and invitro
- Tumour cells are more secretive than normal cells
- 7 – 12 DMBA as intialar and croton oil, as promoter are successful and inducing tumour in Rabbit skin
- Crude snake venom has more effect on nucleic acids

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