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Research Paper

INFLUENCE OF SNAKE *NAJA NAJA* VENOM ON DNA DAMAGE IN *ALBINO RAT*

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Snake venom has therapeutic effect on treatment of certain diseases. This study has carried out to determine the snake *Naja naja* venom on DNA fragmentation levels. An increase in DNA levels was observed and the electrophoretic studies revealed that there is a significant change in the DNA banding pattern and the banding pattern is species specific which plays an important role in species identification and taxonomy. The electrophoretic DNA patterns are consistent in 24 h, 48 h, 72 h of envenomated rats. The present study may be for the first time concentrated on DNA banding pattern in the envenomated rats and this banding pattern will have the significance in building up the different snake species in getting together under one umbrella of taxonomy.

Keywords: Snake *Naja naja* venom, DNA damage by Agarose gel electrophoresis

INTRODUCTION

Snake *Naja naja* 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. The severity of the venom's effect 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 like pain killers, arthritis or cancer and some serve as coagulants for people with hemophila. Snake venom also reveals clue about heart drug. Snake venom proteins blocks receptors as the drugs do. This study will open a door for the designing and development of a new antineoplastic drug from the venom and also play an important role in identifying the species taxonomy in the future.

Electrophoresis is a technique in which the migration of charged particles moves under the influence of electric field. Many biological molecules, such as amino acids, proteins, peptides, nucleotides and nucleic acids possess ionisable groups and therefore at any given pH solution as electrically charged species either cations or anions are under the influence of electric field.

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The DNA bands are visualized by adding ethidium bromide (EtBr), a fluorescent molecule which intercalate with the DNA bases, extending the length of linear and nicked circular DNA molecules and making them more rigid. When EtBr is added, UV radiations at 254 nm is absorbed by the DNA and transmitted to the bound eye. EtBr is a powerful mutagen and hence the gel should be handled carefully with gloves. The DNA bands can be visualized under UV and the data can be recorded by gel documentation appliances.

MATERIALS AND METHODS

Chemicals: Tris HCl, EDTA, NaCl, SDS, Proteinase K, Phenol, Chloroform, Absolute cold ethanol, Sodium acetate, RNAse

Agarose gel electrophoresis was estimated by modified protocol of phenol-chloroform extraction as described by Nelson (2008).

Sample Preparation

Tissues of rat samples were placed in microtubes, treated with 550 μL of lysis buffer solution (50 mM of Tris-HC1 pH 8.0, 50 mM of EDTA, 100 mM of NaCl) plus 1% of SDS and 7 μL of 200 μg mL^{-1} of proteinase K, and then were incubated in a thermo regulated bath at 50°C for 12 h. Then, the DNA was purified with two separate extractions of phenol (250 μL) and three separated extractions with chloroform (250 μl). The DNA obtained was precipitated with 750 μl of absolute cold ethanol and with 300 μl of sodium acetate, and then it was incubated at -20°C for 2 h. The DNA samples were centrifuged with 700 μl of 70% ethanol, and resuspended in TE buffer (10 mM of Tris HCl pH 8.0 and 1mM of EDTA before being treated with 30 μg mL^{-1} of RNAse. Later, the DNA obtained was incubated in a water bath at 37°C for 40 min, and then kept at -20°C.

DNA FRAGMENTATION ASSAY

DNA fragmentation assay was estimated by diphenylamine method as described by Bahman Maroufi (2005).

The extracted DNA was transferred to a micro centrifuge tube. The DNA were lysed with 0.5ml ice cold lysis buffer (10 mM Tris Hcl, pH 7.5, containing 1 mM EDTA and 0.2% Triton X-100). Fragmented DNA was separated from intact chromatin by centrifugation for 10 min at 13000×g, 4^0C (preparation B). The supernatant was carefully transferred to a test tube (preparation A). 0.5 mL of the lysis buffer was added to pellet containing preparation B. 0.5 mL of 25% trichloroacetic acid (TCA) was added to A and B preparations and vortex vigorously. The tubes were placed at 4°C and left the precipitate over night. The precipitates were centrifuged for 10 min at 13000×g. The supernatants were aspirated and discarded. 80 μL of 5% TCA was added to each pellet and the DNA was hydrolyzed by heating for 20 min at 83°C in a water bath. 160 μL of diphenylamine solution was added to the test tubes and to a blank containing 80 μL 5% TCA. All the test tubes were vortexed and then left overnight at room temperature. The collected supernatants were transferred to 96 well plate and optical densities were read at 620 nm by ELIZA reader. The percentage of fragmented DNA was calculated according to the following formula:

$$\%\, fragment DNA = \frac{OD620 tube A}{OD620 tube A + OD620 tube B}$$

STATISTICAL ANALYSIS

Data was expressed as the mean of the ± SD of 3-4 observations. Statistical significance was determined by students 't' test and $p < 0.05$ was

taken as statistically significant compared to control values.

RESULTS

On envenomation of snake *Naja naja* venom to albino rats, DNA fragmentation was estimated in liver, kidney, brain and heart tissues and found to be gradually increased from 24 h to 72 h (Table 1). In the present investigation the DNA samples of control and snake *Naja naja* venom treated albino rat in liver, kidney, brain and heart were used to run the agarose gel electrophoresis to know the DNA damage pattern along with the molecular marker (Plate 1) Snake *Naja naja* venom affect the major vital organs like liver, kidney, brain, heart and the DNA banding pattern was found to be altered in envenomated rats. The DNA banding pattern revealed that the snake *Naja naja* venom exposed liver, kidney, brain, heart interact with the DNA intact bands and there by DNA lysis takes place causing DNA damage indicating that there is a significant DNA fragmentation on time dependent manner, i.e., 24 h, 48 h and 72 h seen during the experimental condition. The DNA fragmentation gradually increased from 24 h, 48 h, 72 hrs (Plate 1; Figure 1; Table 1).

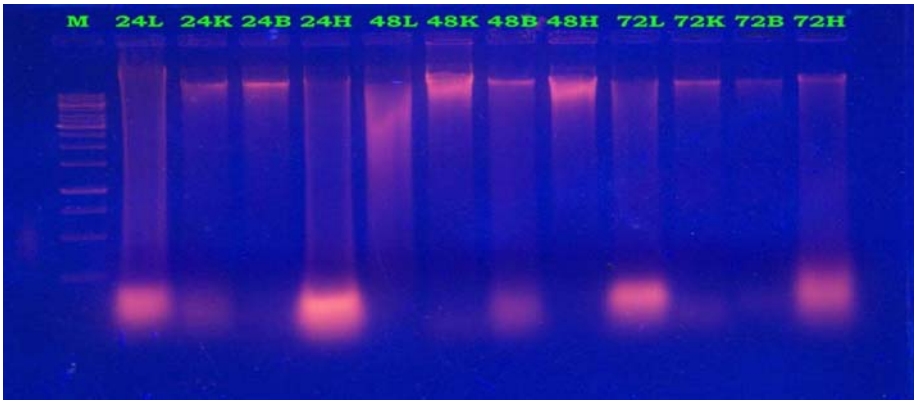
DISCUSSION

The present study was designated to investigate whether venom does have any effect to alter cellular proteins as well as nucleic acids and if any then to compare its influence in the normal as well as snake *Naja naja* venom treated rats. This agrees with the observation of (Brachet and Jeener, 1944) for DNA that rapidly growing tissues always contain larger amounts of non sedimental DNA than the differentiated tissues. (Hantgan, 2001) reported 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(Shaikh *et al*.,1986). (Bailey *et al*., 2001) have also observed that the venom mixture of proteins and peptide toxins are potent inhibitors of sodium channel in activation. The increased DNA content indicates the occurrence of cell proliferation, although DNA is essentially constant in amount per nucleus in non-proliferating cells. Our results clearly show the increase in DNA fragmentation in venom treated rats. Various studies reported in the literature have demonstrated that cobra venom components like phospholipase cardiotoxin and many other enzymes especially endonucleolytic (DNA ases) do inhibit cancer growth (Gillo, 1966). In our results significant but low level change can be found in nucleic acids when treated with various doses of the venom.

Cobra venom cytotoxin was found to have a more cytotoxic effect on tumour cells than normal cells upon incubation *In vitro* (Iwaguchi *et al.,*1973), A change in the cell permeability and nucleus has been proposed to be the primary causes of cell proliferation resulting in the metabolic disturbances and genetic changes in the nucleoproteins (Gosselin *et al*., 1977). Results conclusively show that the venom in small doses can cause inactivation of stimulating enzymes and activating the inhibitory enzymes at that site. This might be one aspect through which venom interferes the nuclear functions by inducing the alterations in the nucleus to restrict the active DNA synthesis, responsible for enhancement or initiation of rapid cell division.

DNA damage can be studied in a variety of organisms such as bacteria, cyanobacteria,

Figure 1: Electrophoretic Profiles of Snake *Naja naja* venom Treated Albino Rats in Liver, Kidney, Brain and Heart Tissues



Note: M: Marker, C: Control, L: Liver, K: Kidney, B: Brain, H: Heart.

Table 1: Changes In Dna Fragmentation Analysis Of Liver, Kidney, Brain, Heart Of Albino Rats In 24 Hrs, 48hrs, 72 Hrs Exposed to Snake *Naja Naja* Venom. Values In Parentheses Indicate Percent Change Over Control

Name of the tissue	Control	24 Hours	48 Hours	72 Hours
Liver				
Mean	8.917	14.833	17.833	24.833
SD	±2.010	±2.090	±2.041	±1.992
PC		-66.355	-100	-178.505
Kidney				
Mean	7.833	13.833	16.583	20.75
SD	± 2.090	±2.090	±2.333	±2.115
PC		-76.596	-111.702	-164.894
Brain				
Mean	5.75	9.833	13.5	18.75
SD	±1.969	±2.090	±2.470	±2.115
PC		-71.014	-134.783	-226.087
Heart				
Mean	4.75	7.833	11.5	16.75
SD	±2.043	±2.090	±2.470	±2.115
PC		-64.912	-142.105	-252.632

Note: All the values are mean ± SD of six individual observations; SD – Standard Deviation; PC – Percent change over control.

ONE WAY ANOVA					
Source of Variation	DF	Liver MS	Kidney MS	Brain MS	Heart MS
Between Groups	3	262.927**	176.083**	183.125**	159.792**
Within Groups	20	4.135	4.663	4.704	4.779
Total	23				
Note: NS: Not Significant, *-Significant (P<0.05), **- Highly Significant (P<0.01)					

phytoplankton, macro algae, plants, animals and humans. It may be spontaneous or environmental that affects all living cells in a number of ways (Horio, 2007). There are several kinds of DNA damages and for developing some artificial repair strategies against these damages in humans and other floura and fauna, detection of DNA damage is important. There are several methods available for detecting different kinds of DNA damage but with some or other limitations. PCR based assays are although very sensitive and easy to measure gene-specific DNA damage but cannot quantify and recognize the kind of damage. It is completely based on the template activity of damaged DNA during amplification an analysis depends on the intensity of the amplified band (Sunita Kumari *et al*., 2008).

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