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# Toxicological Assessment of Naja naja Venom: DNA Damage in Albino Rats

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**Abstract:** This study investigates the toxicological effects of Naja naja venom on DNA integrity in albino rats, with a focus on understanding its genotoxic potential. The venom of the Indian cobra (Naja naja) contains a complex mixture of proteins, enzymes, and toxins that may cause cellular damage, including DNA fragmentation. In this experiment, albino rats were administered varying doses of Naja naja venom, and the resultant DNA damage was assessed using the comet assay, a sensitive technique for detecting DNA strand breaks. Additionally, histopathological analysis of major organs was conducted to evaluate the venom's overall toxic effects. The results demonstrated significant DNA damage in the peripheral blood cells of the rats, with increased comet tail length and DNA fragmentation proportional to the venom dose. Histopathological findings showed cellular degeneration and necrosis in key organs, including the liver and kidneys. This study highlights the genotoxic effects of Naja naja venom, suggesting its potential as a hazard to DNA integrity. The findings underline the need for further research on the molecular mechanisms underlying venom-induced DNA damage and its implications for human health and safety.

**Keywords:** Naja naja venom, DNA damage, Genotoxicity, Albino rats, Toxicological assessment, Comet assay, Histopathology, DNA fragmentation, Venom toxicity.

**Introduction:** Venomous snakes, including Naja naja, commonly known as the Indian cobra, produce a diverse range of toxins that have significant biological effects. Naja naja venom is composed of a complex mixture of proteins, enzymes, and other biomolecules, which collectively cause harmful physiological effects in organisms. The venom contains neurotoxins, cytotoxins, phospholipases, and metalloproteinases, which target different cellular structures and processes, leading to a variety of toxic responses, including cell death,

inflammation, and neurotoxicity. While the acute effects of Naja naja venom on the cardiovascular and nervous systems have been extensively studied, the genotoxic potential of the venom—specifically its ability to cause DNA damage—has received less attention. Understanding the genotoxic effects of venom is crucial for assessing the overall toxicity and long-term health risks associated with snakebites.

DNA damage is a critical event in the toxicological evaluation of venoms, as it can lead to mutagenesis, cancer, and other genetic disorders. Genotoxic substances, including those found in venoms, are capable of inducing various types of DNA damage, such as strand breaks, base modifications, and chromosomal aberrations. These genetic alterations can compromise cellular function, potentially leading to long-term health consequences. In recent years, there has been growing interest in the molecular mechanisms underlying the toxic effects of snake venoms, especially in terms of their impact on genetic material.

In this study, we focus on the toxicological assessment of Naja naja venom, particularly its potential to cause DNA damage in albino rats, a commonly used model for evaluating the genotoxicity of environmental and biological toxins. Albino rats are ideal subjects for such studies due to their well-documented sensitivity to various toxicants and the availability of established protocols for assessing DNA damage. Using the comet assay, a sensitive technique for detecting DNA strand breaks, this research aims to quantify the extent of DNA damage caused by different doses of Naja naja venom. Additionally, histopathological examination of vital organs will provide insight into the broader toxic effects of the venom.

This study aims to fill the gap in knowledge regarding the genotoxic effects of Naja naja venom and provide valuable data for the development of better medical interventions for snakebite victims. By evaluating the DNA damage caused by this venom, we aim to enhance our understanding of its potential long-term health risks and contribute to the growing body of research on the toxicological impacts of snake venoms.

## METHODOLOGY

### 1. Study Design and Animal Selection

This study was designed to evaluate the genotoxicity of Naja naja venom in albino rats. A total of 30 healthy male albino rats (Rattus norvegicus), aged 8–10 weeks and weighing 180–220 g, were obtained from a certified animal breeding facility. The rats were acclimatized for one week under standard laboratory conditions, including a 12-hour light/dark cycle, controlled temperature (22 ± 2°C), and access to water and food ad libitum. Prior to the experiment, the animals were fasted for 12 hours to ensure their digestive systems were empty. The study was approved by the Institutional Animal Ethics Committee (IAEC), and all animal handling and procedures conformed to the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### 2. Venom Preparation

The Naja naja venom was obtained from a reliable supplier of snake venom and was stored at -20°C to preserve its stability. Prior to administration, the venom was thawed and diluted in physiological saline to the required concentrations. Three different doses of venom were selected to evaluate a dose-response relationship in terms of DNA damage: 0.25 mg/kg body weight (low dose), 0.5 mg/kg body weight (medium dose), and 1.0 mg/kg body weight (high dose). A control group was included, which was administered the same volume of saline without venom, to account for any potential effects caused by the injection procedure itself.

### 3. Experimental Groups

The rats were randomly divided into four groups (n = 7 per group):

Control Group (Group 1): Rats were administered saline only, serving as a baseline for comparison.

Low Dose Group (Group 2): Rats received 0.25 mg/kg body weight of Naja naja venom.

Medium Dose Group (Group 3): Rats received 0.5 mg/kg body weight of Naja naja venom.

High Dose Group (Group 4): Rats received 1.0 mg/kg body weight of Naja naja venom.

The venom was administered via intraperitoneal injection to ensure uniform distribution and absorption. After administration, the rats were observed for 24, 48, and 72 hours to monitor any signs of acute toxicity such as changes in behavior, mobility, and general health.

### 4. Comet Assay (Single Cell Gel Electrophoresis)

The comet assay was performed to assess the DNA damage in peripheral blood cells of the rats, which is a widely used method for detecting DNA strand breaks in individual cells. Blood samples were collected from the tail vein of each rat at 24, 48, and 72 hours post-venom administration. The collection was performed under light ether anesthesia to minimize stress.

The blood samples were processed immediately. A small volume (10 µL) of blood was mixed with 70 µL of low-melting agarose (0.75% in phosphate-buffered saline, PBS), and the mixture was spread on a microscope slide pre-coated with agarose to form a gel. The slides were

placed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10) for 1 hour at 4°C to lyse the cells and remove proteins. Following lysis, the slides were submerged in an electrophoresis buffer (1x TBE, pH 8.0) for 30 minutes to allow DNA unwinding.

After unwinding, electrophoresis was performed at 25 V and 300 mA for 20 minutes. The slides were stained with ethidium bromide (10 µg/mL) to visualize the DNA under a fluorescence microscope (excitation at 520 nm and emission at 590 nm). The DNA damage was quantified by measuring the tail length and the percentage of DNA in the tail using image analysis software. The tail length was correlated with the extent of DNA fragmentation.

5. Histopathological Examination

To further assess the systemic effects of Naja naja venom, histopathological analysis was performed on the liver, kidneys, and heart of the rats. These organs were chosen based on their relevance in detoxification and circulatory function. After euthanizing the rats via cervical dislocation, the organs were immediately excised, weighed, and fixed in 10% formalin for 24 hours. Once fixed, the tissues were processed using standard protocols for paraffin embedding.

Tissue sections (5 µm thick) were cut and stained with Hematoxylin and Eosin (H&E) for microscopic examination. Histopathological changes such as necrosis, inflammation, and cellular degeneration were assessed using a light microscope (Olympus CX41) at 100x and 400x magnification. The severity of tissue damage was scored using a semi-quantitative scale, where 0 = no damage, 1 = mild damage, 2 = moderate damage, and 3 = severe damage. These histopathological findings were correlated with the DNA damage observed in the comet assay.

6. Statistical Analysis

The data from the comet assay and histopathological analysis were statistically analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test for multiple comparisons. The results were expressed as the mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$. All analyses were performed using GraphPad Prism software (version 8.4.3).

7. Ethical Considerations

The study adhered to all ethical guidelines for the use of animals in research. All efforts were made to minimize animal suffering and distress, and euthanasia was performed humanely. The results of the study aim to contribute to the understanding of the toxicological impacts of Naja naja venom on genetic material and to support the development of better treatments for snakebite victims.

**RESULTS**

1. Comet Assay Results

The DNA damage observed in peripheral blood cells of albino rats exposed to Naja naja venom was quantified using the comet assay. A dose-dependent increase in DNA damage was noted in all venom-treated groups when compared to the control group.

Control Group: No significant DNA damage was observed in the blood cells of the control rats. The majority of the cells appeared intact, with minimal tail formation and no noticeable DNA fragmentation.

Low Dose Group (0.25 mg/kg): Rats exposed to the low dose of venom exhibited a moderate increase in DNA damage. Tail length measurements showed a significant increase compared to the control group, with approximately 25% of cells showing DNA fragmentation. The comet tail length was proportionally smaller compared to the higher doses, indicating less extensive damage.

Medium Dose Group (0.5 mg/kg): Rats in the medium dose group demonstrated a more pronounced increase in DNA damage. Around 45% of the cells showed noticeable DNA fragmentation, with significantly longer comet tails compared to the control. This group exhibited moderate DNA damage, suggesting a more substantial genotoxic effect at this dose.

High Dose Group (1.0 mg/kg): The rats receiving the highest dose of venom showed the most significant DNA damage. Over 65% of the cells exhibited fragmented DNA with long comet tails, indicating severe DNA strand breaks. The high dose group showed the most significant differences compared to the control, reinforcing the dose-dependent nature of DNA damage caused by Naja naja venom.

These results clearly demonstrate that Naja naja venom induces DNA damage in a dose-dependent manner, with higher doses leading to greater DNA fragmentation in peripheral blood cells of the rats.

2. Histopathological Findings

Histopathological examination of vital organs (liver, kidneys, and heart) revealed significant tissue damage in venom-treated rats compared to the control group.

Liver: In the control group, the liver showed normal architecture with intact hepatocytes and minimal inflammation. However, rats treated with Naja naja venom, particularly at higher doses, exhibited varying degrees of liver damage. The low dose group showed mild congestion of blood vessels, while the medium and high-dose groups exhibited moderate to severe necrosis, cellular degeneration, and inflammatory cell

infiltration in liver tissue.

Kidneys: In the control group, the kidneys appeared structurally intact with normal glomeruli and tubules. In venom-treated rats, especially those in the medium and high-dose groups, renal tissue showed signs of tubular necrosis, glomerular damage, and congestion. These changes were more pronounced with increasing venom doses.

Heart: The heart tissues of control rats were structurally normal, showing no signs of inflammation or necrosis. However, in venom-treated rats, especially at the higher doses, there was evidence of mild congestion and focal necrosis of myocardial cells, particularly in the high-dose group.

These histopathological findings complement the comet assay results, as they show significant organ damage in conjunction with DNA fragmentation, further confirming the toxicological effects of Naja naja venom.

## DISCUSSION

The findings from this study underscore the genotoxic potential of Naja naja venom, as evidenced by the dose-dependent increase in DNA damage in peripheral blood cells of albino rats. The comet assay revealed significant DNA fragmentation in the venom-treated groups, with the most pronounced effects occurring at higher venom doses. This result aligns with the known toxicology of snake venoms, which contain a variety of enzymatic components that can cause cellular and genetic damage.

The venom of Naja naja contains a mixture of toxins such as phospholipases, metalloproteinases, and neurotoxins that can induce cellular damage through various mechanisms. These toxins are known to disrupt cell membranes, degrade proteins, and interfere with cellular processes, which may lead to the generation of free radicals and oxidative stress. Oxidative stress has been implicated in DNA damage, as reactive oxygen species (ROS) can directly attack DNA, leading to strand breaks and mutations.

The dose-dependent nature of DNA damage observed in this study further supports the hypothesis that the severity of venom-induced genotoxicity is directly related to the amount of venom introduced into the system. The highest dose caused the most extensive DNA fragmentation, likely due to the overwhelming effects of venom components, which may induce more extensive oxidative damage or alter cellular repair mechanisms.

Histopathological analysis provided additional insights into the systemic effects of Naja naja venom. Liver and kidney tissues exhibited significant signs of damage, including necrosis, inflammation, and cellular degeneration, which are consistent with findings in previous studies on snake venom toxicity. The observed cardiac damage, although less severe, also highlights the venom's broader toxicological impact. These organ-specific damages further support the notion that venom-induced DNA damage may not be restricted to peripheral blood cells but could extend to various organ systems, potentially leading to long-term health complications.

The overall toxic effects of Naja naja venom suggest that its exposure may result in a wide range of genetic and organ-specific damages, which could have serious implications for human health, particularly in the case of snakebite victims. The ability of Naja naja venom to cause DNA fragmentation points to its potential as a genotoxic agent that could contribute to the development of mutations, cancers, or other genetic disorders if not addressed appropriately.

## CONCLUSION

This study provides valuable evidence of the genotoxic and cytotoxic effects of Naja naja venom in albino rats. The comet assay results demonstrated significant DNA damage in a dose-dependent manner, confirming that the venom can induce DNA strand breaks and other forms of genetic damage. Histopathological examination of vital organs further highlighted the systemic toxic effects of the venom, with noticeable damage to the liver, kidneys, and heart. These findings contribute to a deeper understanding of the molecular and cellular impacts of snake venom, particularly its potential to cause genetic alterations.

Given the widespread implications of venom-induced DNA damage, this study emphasizes the need for further research into the molecular mechanisms by which snake venoms cause genotoxicity. Additionally, the findings underscore the importance of developing effective treatments for snakebites that can mitigate both the immediate and long-term effects of venom exposure on human health. Future studies should focus on the potential for Naja naja venom to cause carcinogenic mutations and the role of DNA repair mechanisms in counteracting venom-induced genetic damage.

## REFERENCES

Asselta R and Peyvandi F (2009), Factor Vdeficiency SeminThrombHemost., Vol. 35,No. 4, pp. 382-9.

Bahman Maroufi, Kaboudanian SussanArdestani, Amina Kariminia, HussainNaderimanesh (2005) “The effect of vitaminE on splenocytes apoptosis of gammairradiated BALB/C mice”, Iranian Journal ofAllergy , Asthma and Immunology., Vol. 35,No. 1, pp.

77-82.

Bailey P Wilce (2001), “J Venom as a sourceof useful biologically active molecules”,Emergency Medicine, Vol. 13, pp. 28-36.

4. 4. Brachet J and Jeener R (1944),“Macromolecular Cytoplasmic ParticlesRich in Pentose Nucleic Acid. I. Generalproperties Relation to Hydrolase, Hormonesand Structural Proteins”, Enzymologia., Vol.1, pp. 196-212.

Gillo L (1966), “Biochemistry of snakevenom”, Toxicon. Ann. Soc Roy Sci MedNat Bruxelles, Vol. 19, pp. 121.

Gosselin L, Bacq C M, Gosselin F C, ReyC, Kozma S and Osterieth P M (1977),“Phospholipids of the Milk-fat-globuleMembrane and the Mouse Mammary Tumorvirus isolated from the milk of infected mice”,Biochem. Soc. Trans., Vol. 5, pp. 142-144.

Hantgan P (2005), “Snake venom revealsclues about heart drug”, Baptist Medical.,Vol. 1, pp. 1-3.

Holley R W (1968), “Biochemistry of snakevenom”, Toxicon. Progr.Nucleic Acids Res,Vol. 8, pp. 37.