CATALOGING OF TOXIN REPERTOIRE OF MOSTLY PREVALENT SNAKES IN NIGERIA

A Project Submitted in Partial Fulfillment of the Requirements for the Award of the

Degree of Bachelor of Science in Biochemistry

BY

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DECLARATION

I hereby declare that this work is the product of research efforts undertaken under the supervision of Dr. Susanta Pahari and has not been presented and will not be presented elsewhere for the award of a degree or certificate. All the sources have been duly acknowledged.

_____ _____

Student name Sign/Date

Student I.D

CERTIFICATION

This is to certify that this study was carried out by Husnah Turadu Usman 1060 in the Department of Biochemistry, School of science and information technology, Skyline University Nigeria, under my supervision.

Dr. Susanta Pahari _____

Supervisor date/sign

APPROVAL

The panel of examiners recommends the candidate……. for the award of the Degree of Bachelor of Science in Biochemistry subject to effecting all the corrections pointed out during the oral examination.

_____ _____

External Examiner date

_____ _____

Internal Examiner date

Dr. Susanta Pahari

Project Supervisor

Dr. Susanta Pahari

Head of Department

DEDICATION

This report is dedicated to my beloved parents for their unconditional love and support in my life.

ACKNOWLEDGEMENT

I wish to register my profound gratitude to God Almighty for His guidance and grace throughout my life.

My special gratitude goes to Skyline University Nigeria for their effort to see that this work saw the light of the day. I appreciate all my amazing lecturers in the department, and my wonderful supervisor, Dr. SusantaPahari, for his engaging, seasoned lectures, inspiration, and guidance. I also would like to thank Mr. Miracle Uwa luivinus, to them all, I say blessings all the way, Amen.

My regards to my amazing parents, who financially supported my educational pursuit, and my beloved siblings, love you all.

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ABSTRACT

Variety in toxin structure is a universal peculiarity in snakes and happens both interspecifically and interspecifically. However, there are about hundreds of toxins available in snake venom gland, but not all the toxins are available in equal proportion for a particular snake. In this work, what kind of family of toxins are available in one of the prevalent snake in Nigeria, *Echis ocellatus, Echis ocellatus* has been catalogued from NCBI database, those collected sequences have been aligned. Then the pattern of variation has been observed, analyzed. It has been observed that not all of the toxins mutate with equal rate, some are highly mutating and others are quite slow. However, mutations being the driver of changes of toxin sequences.

CHAPTER ONE

1.1 INTRODUCTION

Snake venom is harmful saliva that contains various toxins and is utilized to immobilize and consume victims [2]. These snake harmful toxins include α-neurotoxins, β-neurotoxins, Dendrotoxin, Cardiotoxin, Myotoxins, Sarafoxin, Hemorrhagins, Hemotoxins, (serine protease) etc. These toxins also serve as a deterrent to threats. Snake venom is injected into the body through a bite simply by special fangs. The venom consists of around 20 different chemicals, of which the majority are protein and polypeptides [3]. The poisonous and fatal associated with the complex combination of healthy proteins, enzymes, as well as other chemicals are renowned. [4]. The prey is rendered immobile by the venom [5]. The venom contains enzymes that promote the digestion of prey as well as many other compounds that can have biological effects that are not lethal [6].

1.2 AIM

To check on the variation in snake toxic substances between *Echis ocellatus* to other snakes. The toxic variety seen between snake species is the consequence of an intricate collaboration between a multitude of hereditary and postgenomic factors. This kind of variety eventually brings huge distinctions in toxin pathology and lethality and can sabotage the stability of antidote treatments used to deal with snakebite casualties.

1.3 OBJECTIVES

- To catalogue the repertoire of toxins of the carpet snake, *Echis ocellatus, which is one of the most prevalent species in Nigeria*.
- To follow the mutational pattern and intensity of available toxins in *Echis ocellatus* (carpet snake).

1.4 SIGNIFICANCE OF STUDY

To examine the components overseeing toxin variety we chose a connected gathering of restoratively significant viperid snakes, that is, *Echis ocellatus* and other verities of the snake. These varieties have been chosen in light of their clinical significance through Africa and our past portrayals of interspecific variety in contaminant creation, dietary inclination, and feed lethality.

CHAPTER TWO

REVIEW OF LITERATURE

Snakes are limbless reptiles from the serpentes suborder [7]. There are over 3400 types of Snakes in the world. More than 20 families are grouped into them. Some families contain only one or two species, some are incredibly small. Nearly all species are classified by the five main families below:

- **Colubridae**: The most important category of snake is obviously the Colubridae family. Within this family alone there is more than 1, 900 species. That is, over 35% of the 3400 species that reside on the entire world. Colubrids are example of this snake specie. Pretty much all of them are not venomous.

- **Boidae**: A family Boidae contains 40-45 species. The green serpent may be the world 's most significant snake and the heaviest snake. All bids are powerful constrictors.

- **Elapidae**: Elapidae (also known as elapids) are a category of snakes that include number of venomous species, most of these are cobras, taipans and mambas. This family involves some of the most venomous snakes worldwide. Elapids produce a neurotoxin that attacks their prey's central nervous system (Respiration).

- **Pythonidae**: The household Pythonidae includes some from the entire world 's longest snakes, which consists of the Southeast Asian reticulated python. The snakes from this family happen to be collectively referred to as "pythons", though there are many specific species occurring in many parts of the world.

- **Viperidae**: This family of snakes are referred to as Viperidae, which includes rattlesnakes, vipers, adders along with other species. All snakes are venomous in the family Viperidae. Members of this family produce hemotoxic venom that hits their prey's tissue and blood.

The most prevalent snakes in Nigeria

There are four categories of venomous snakes within Nigeria, which include: Viperidae, Elapidae, Colubridae and Actraspididae. However, the three types of cover snake include (*Echis ocellatus*), dark-necked cobra (*Naja nigricollis*) and puff viper (*Bitis arietans*) are the real key snakes related with envenoming in Nigeria [8]. Echis ocellatus has become consisted of like 497 per each 100, 00 populaces annually, with a normal mortality of 12 percent. Nibbles happen even more frequently while casualties were cultivating, crowding, or strolling [8]. Snake toxin contains prothrombin which activates pro-coagulant, hemorrhaging and cytolytic portions which may cause discharge, in-coagulable blood, shock, and neighborhood tendencies.

2.1 VARIETY OF SNAKE TOXINS

On snakes, diversity in toxin designing is definitely an omnipresent peculiarity and happens interspecifically. For snakebite casualties, the specific antibodies found in serums are insufficient against heterologous toxins in various toxins. The quick development of various poison coding quality families in various snake heredities is seen as the primary driver of toxin variety. This view ignores the understudied impact that cycles following up on quality records and meaning might have on the development of the toxin proteome. These toxins include;

2.1.1 NEUROTOXINS

Neurotoxins are toxins that damage low tissue and cause neurotoxicity [9]. Neurotoxins are a class of exogenous neurological insults that can influence the development of sensory tissues. Neurotoxins include lead, ethanol (drinking liquor), nitric oxide, botulinum contaminant (for example Botox) and tetrodotoxin. A few substances, for case, nitric oxide and glutamate will be fundamental for the legitimate ability in the body. These toxins include;

Fasciculins

Fasciculins are a category of harmful proteins used in specific snake toxins. these toxins are mostly found in green mamba, studies have uncovered beautiful structures in some green mamba toxins specifically FAS1 and FAS2 [10]. Fasciculins are believed to cause extraordinary fasciculation in the muscle fascicles of vulnerable pets like snakes 'prey. The following impact kills the prey by either killing it or deadening it so that the snake can swallow it. Most of these toxins destroy acetylcholinesterase (AChE) neurons by obliterating them. Hence, every cannot be separated. Eventually this causes compulsory muscle constriction which may lead to passing out.

Dendrotoxins

Dendrotoxins are a class of presynaptic neurotoxins secreted by mamba snakes (Dendroaspis) that block certain subtypes of the voltage-gated potassium shunt in neurons and enhance acetylcholine delivery to neuromuscular junctions. Given their high potency and selectivity for potassium channels, dendrotoxins are incredibly useful as pharmacological tools to target the assembly and capacity of these particulate channel proteins. Dendrotoxins impede neurotransmissions by preventing the exchange of positive and negative particles across the neuronal layer, resulting in the failure of nerve impulses, thereby desensitizing the nerves.

α-neurotoxins

α-Neurotoxins are a meeting of neurotoxic peptides discovered within side the toxin of snakes within side the households Elapidae and Hydrophiidae. They can reason lack of motion, respiration disappointment, and demise. Individuals from the three-finger poison protein family, are horrific of post-synaptic nicotinic acetylcholine receptors (nAChRs) within side the neuromuscular neurotransmitter that catches situation severely and irreversibly, forestalling synaptic acetylcholine (ACh) from establishing the particle channel. α-Neurotoxins unfairly tie firmly and non-covalently to nAChRs of skeletal muscles, finally impeding the hobby of ACh on the postsynaptic layer, restraining particle stream, and prompting lack of motion. nAChRs comprise limiting locations for snake toxin neurotoxins.

2.1.2 CYTOTOXINS

Cytotoxins are the artificial guns that Kills T-cells that use to obliterate infected cells. Infections take over stable cells and stunt them into making loads extra infections. At the factor when those infections get out, they could taint notably extra stable cells. By killing tainted cells earlier than those infections go out, cytotoxins shield your sound cells.

Various sorts of cytotoxins tasks in numerous ways. A few cytotoxins make openings within side the molecular layer, so the molecular isn't always protected from an outside perspective. Without a complete layer, the molecular kicks the bucket. Cell loss of life given this kind of destruction within side the cell layer referred to as lysis. Under cytotoxins we have,

Phospholipases

Phospholipase is a chemical that adjust the phospholipid atom right into a lysophospholipid (cleanser) → the brand new particle attracts in and ties fats which cracks molecular films. Phospholipase A2 is one specific type of phospholipase located in snake toxins.

Phospholipases A2 count on a tremendous element within side the neurotoxic and myotoxic effects of snakebites. These proteins have atomic hundreds of 13-15 kDa and are ordered into bunches I and II, that are located as tremendous components with inside the toxins of Elapidae and Viperidae, individually [11].

Cardiotoxins

Cardiotoxins are parts that are specifically toxic to the heart. They bind to specific targets on the outer layer of muscle cells and cause depolarization → the venom prevents muscle compression. These toxins can make the heartbeat unpredictable or stop beating and cause death.

An example is a Chinese cobra containing sixty amino corrosive polypeptide venom from the Taiwanese cobra *Naja atra*. It is an illustration of an assemblage of snake cardio/cytotoxins made up of more limited snake toxin three-finger venoms.

Hemotoxins

Hemotoxins are poisons that destroy red blood platelets, alter blood thickening, cause organ degeneration, and reduce tissue damage. The term hemotoxin is somewhat misleading because toxins that damage blood also damage other tissues. Injuring a blood specialist is often extremely distressing and can result in very long-lasting and, in severe cases, death. Hemotoxins are commonly used by venomous creatures, including snakes (snakes and garter snakes) and

bugs (earth-colored solitaires). The creature's toxins contain chemicals and various proteins that are hemotoxic or neurotoxic, or rarely both (as in the Mojave Diamondback, Japanese mamushi [12], and similar species. Side effects depend on the type, size, bite area, and amount of toxin infused. In humans, side effects include nausea, confusion, and migraines; these can be postponed for a long time. A typical group of hemotoxins includes snake toxin metalloproteinase, e.g., mucrolysin [13].

2.1.3 MYOTOXINS

Myotoxins are small, critical peptides found in snake toxins (e.g. rattlesnakes) and reptile toxins (e.g. Mexican beaded lizards) [14]. This includes a non-enzymatic component that causes severe muscle damage. These peptides act quickly, causing an instant loss of motion to prevent prey from escaping and eventually dying due to loss of diaphragm motion.

2.2 *ECHIS OCELLATUS*

Echis ocellatus (carpet snake) belongs to the Viperidae family which is considered as the therapeutically largest species of snake in West Africa. Its envenomation is poisonous and dangerous, causing more deaths than any other established African species.

Description and Behavior

The most extreme total length (body + tail) is 65 cm (26 in), possibly more, while the typical total length is 3,050 cm (1,220 in) [15]. They are described by their swollen eyes and short nose, an average species of the *Echis ocellatus* family is terrestrial, nocturnal, and crepuscular, usually going out in the early afternoon to hunt their prey, Prey as small vertebrates, similar to blood

animals. Reptiles and terrestrial and aquatic creatures were proactively considered to prey on small spineless creatures like centipedes and scorpions. It is an extremely energetic snake that forms its body in the shape of an "S" and rubs itself with its scales, emitting an alarm sound [15].

Range and Habitat

It is found in West Africa in Mali, Ivory Coast, Burkina Faso, Ghana, Togo, Benin, southern Niger and Nigeria. They are mainly found in the savannah and occasionally in forested areas [15].

Reproduction

Sexually mature females lay between 6 and 20 eggs, mostly towards the end of the dry season from February to March. Hatchlings measure 1,012 cm (3.94.7 in) at full length.

Venom

Their venom consists of pro-coagulant, anticoagulant, hemorrhagic, nephrotoxic, and neurotoxic substances and symptoms of their stings include local discomfort, swelling, bleeding necrosis, and deformity that can lead to amputation. Coagulopathy, hemorrhage, shock, renal failure, and blindness are examples of systemic symptoms. The poisoning rate is about 80%, the mortality rate is about 10%20% [16,17].

2.2.1 COMPOSITION OF ECHIS OCELLATUS TOXIN

The *E. ocellatus* toxin is highly toxic and consists primarily of peptides and proteins to a significant extent; Snake toxin metalloproteinase (SVMP) has hemorrhagic, nephrotoxic, cardiotoxic, and

anticoagulant effects. Its toxin's major proteins; include phospholipase A2, metalloprotease, a serine protease, C-type lectins, and disintegrins.

PHOSPHOLIPASE (PLA2s)

Phospholipases A2 play an important role in the neurotoxic and myotoxic effects of snakebite. These proteins have atomic masses of 1315 kDa and are arranged in groups I and II, which are individually found as significant parts in Viperidae toxins [18]. Several PLA2 have potent myotoxic effects, often leading to severe necrosis, and many of these toxins also promote inflammation by inducing edema, cytokine production and leukocyte recruitment, pain by inducing thermal allodynia, and mechanical hyperalgesia, paralysis by blocking neuromuscular transmission and increase the discharge. by inhibiting coagulation [19]. They are enzymes that hydrolyze the bond between the second fatty acid "tail" and the glycerol molecule and cleave the fatty acid at position two of the phospholipids [20]. PLA2 enzymes can be present in mammalian tissues as well as in arachnid, insect, and snake venom [20].

SNAKE VENOM METALLOPROTEASE (SVMPs)

Snake venom metalloproteinase (SVMPs) are zinc-dependent proteinases ranging in size from 20 to 110 kDa and are classified into three groups based on their structural domains: PI, PII and PIII [21]. These toxins are important components of viper venom and play a crucial role in their toxicity. Toxin SVMPs have evolved from ADAM proteins (a disintegrin and a metalloproteinase), specifically ADAM28, with PIII being the underlying variation that includes metalloproteinase,

disintegrin, and cysteine-rich spaces [22]. SVMPs contribute extensively to the hemorrhagic and coagulopathic toxin shedding that follows viper snake bites, and the diversity of SVMP isoforms found in their venom likely facilitates synergistic effects, such as acting simultaneously in multiple phases of blood coagulation [23].

A metalloproteinase, often referred to as a metalloprotease, is a protease enzyme with a metal catalytic mechanism. Most metalloproteases require zinc, although some also require cobalt. Three ligands are used to attach the metal ion to the protein.

SNAKE VENOM SERINE PROTEASE (SVSPs)

Snake venom serine proteinases (SVSPs) are serine proteinases belonging to the S1 family, with molecular weights ranging from 26 to 67 kDa and two distinct structural domains. They are enzymes that cleave peptide bonds in proteins known as peptidases. Serine is the nucleophilic amino acid in the active site of the (enzyme). SVSPs, like SVMPs, have been found in the venom of a variety of snake families, although they are more numerous in viper venoms and much less common in elapid and colubrid snake venoms [24].

C-TYPE LECTIN

C-type lectins (CLECs) are lectins that are domains of carbohydrate-binding proteins. Type C's nickname comes from its calcium-binding needs. Cell adhesion, immune response to pathogens, and apoptosis are functions of proteins containing C-type lectin domains [25,26,27].

Based on the order of the numerous protein domains of each protein, he grouped the C-type lectins into seven subgroups (I to VII). In 2002, this classification was revised, resulting in the creation

of seven new groupings (VIII to XIV) [28]. Three new subgroups (XV to XVII) have recently been added.

CHAPTER THREE AND FOUR

METHODOLOGY

3.1 Retrieval of *Echis Ocellatus* toxin sequence:

The toxin protein sequences were retrieved using:

National center for biotechnology information (NCBI)

The NCBI maintains a number of databases related to biotechnology and biomedicine and is a valuable resource for bioinformatics tools and services. GenBank, a database of DNA sequences, and PubMed, a bibliographic database of the biomedical literature, are two important databases. Other datasets include the NCBI Epigenomics database. NCBI database was used to retrieve the toxin protein sequence. The Protein Database stores the textual record for individual protein sequences obtained from a variety of sources including the NCBI Reference Sequence (RefSeq) project, GenBank. Protein registries are available in various formats, including FASTA and XML, and are linked to other NCBI resources.

Steps:

- Search NCBI in google
- Select protein in the database search tool. Search in the protein database the name of the protein and then click on desired protein.
- Click on FASTA in other to display the protein sequence

3.2 Basic local alignment search tool (BLAST) program:

The BLAST program was used to Search for toxin sequence similarity BLAST is an algorithm that measures sequence similarity between biological sequences, such as B. nucleotide sequences of DNA and amino acid sequences of proteins calculated. [7] BLAST is a powerful technique for locating sequences that resemble the query sequence within the same or different species. Searches NCBI databases and servers for the query string and returns the results to the user's browser in the specified format. Most BLAST input sequences are in FASTA or GenBank format [29].

Steps:

- Select the BLAST program
- Enter the sequence or upload a file containing the sequence.
- Select the parameters for the search
- Run the BLAST program

3.3 Alignment of sequences:

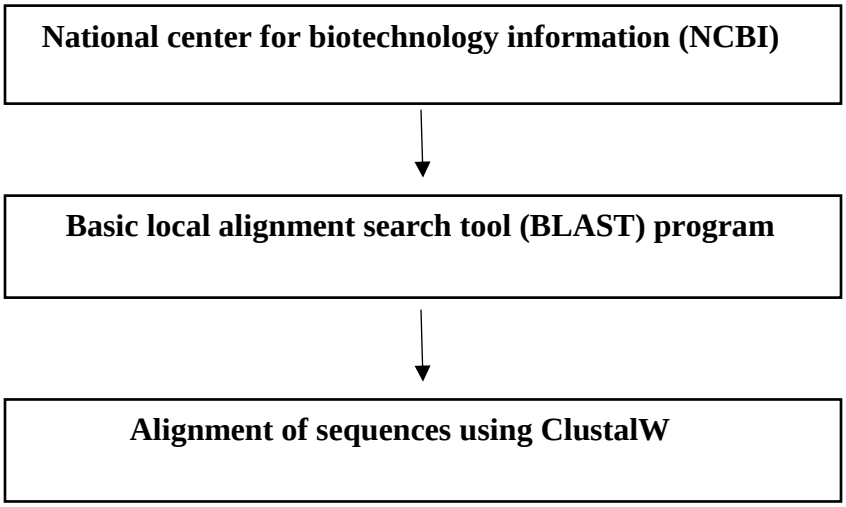
The alignment of the sequence was done using the ClustalW tool:

Clustal W is a collection of commonly used computational tools for multiple sequence alignment in bioinformatics. [2] During the development of the algorithm, there were several versions of Clustal, which are mentioned here. Each tool and its algorithm are also thoroughly studied in their respective fields [30]. Variants of the Clustal software align sequences using a heuristic that

develops a multiple sequence alignment from a series of pairwise alignments. This approach creates a distance matrix by evaluating the sequences as a whole and then using the UPGMA/neighbor join method.

Steps:

- Select the ClustalW program
- Select the sequence or alignment you wish to align
- Select the align/assemble button from the toolbar and choose multiple alignment



CHAPTER 4

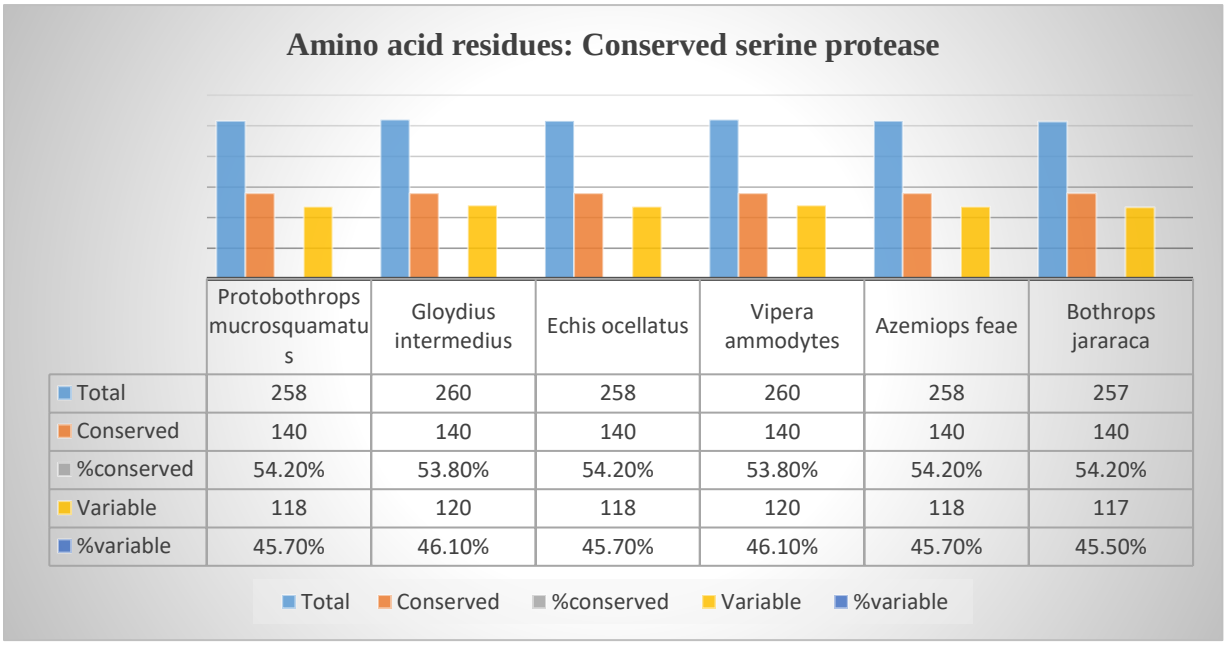
RESULTS

Total number of amino acids

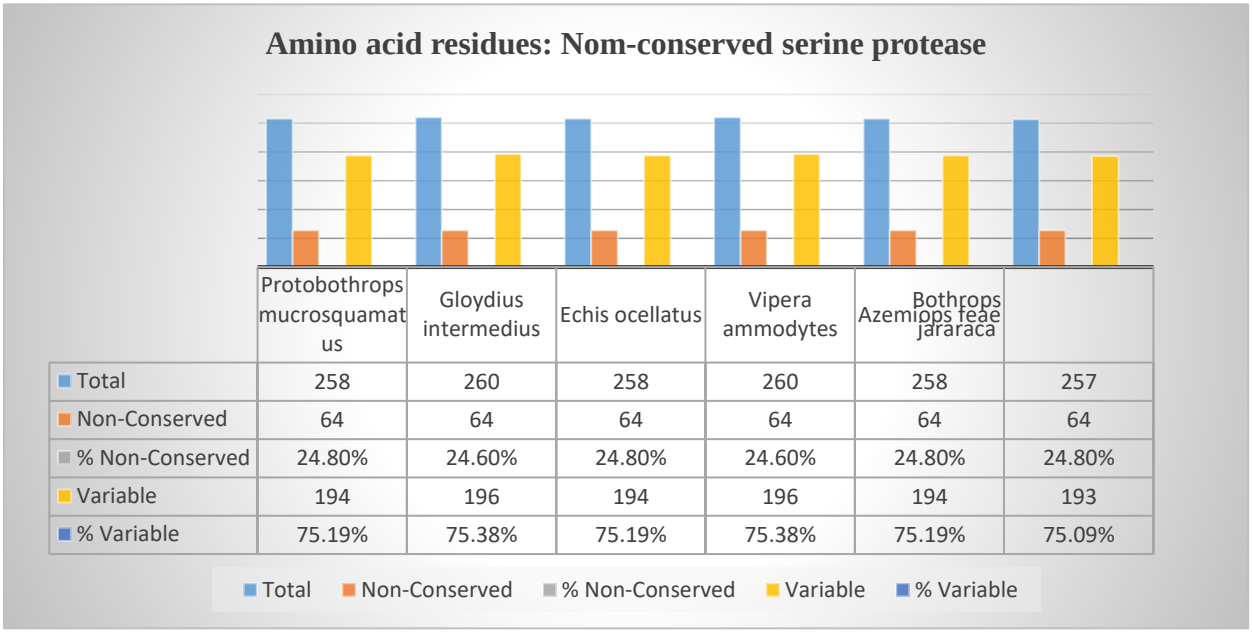
Phospholipase A2	**% of conserved amino acid:**	**% of non-conserved amino acid:**
*Ovophis monticola:**138***	44.2%	31.8%
*Protobothrops flavoviridis: **138***	44.2%	31.8%
*Crotalus mitchellii: **138***	44.2%	31.8%
*Vipera ammodytes: **138***	44.2%	31.8%
*Echis ocellatus: **138***	44.2%	31.8%
*Daboia russelii limitis: **137***	44.2%	31.8%
Serine protease		
Protobothrops mucrosquamatus: **258**	54.2%	24.8%
Gloydius intermedius: **260**	53.8%	24.6%
Echis ocellatus: **258**	54.2%	24.8%
*Vipera ammodytes:***260**	53.8%	24.6%
Azemiops feae: **258**	54.2%	24.8%
Bothrops jararaca: **257**	54.2%	24.8%
C-type lectins		
Echis ocellatus: **148**	35.1%	37.8%
Vipera transcaucasiana: **150**	34.6%	37.3%
Pseudocerastes urarachnoides: **146**	35.6%	38.3%
Crotalus tzabcan: **148**	35.1%	37.8%
*Oreganus helleri:***142**	36.6%	39.4%
Metalloprotease		
Echis ocellatus: **414**	89.9%	27.0%
Vipera ammodytes: **416**	89.9%	26.9%
Pseudocerastes urarachnoides: **420**	88.0%	26.6%
Bothrops jararaca: **409**	90.4%	27.3%

Agkistrodon contor: **411**	90.0%	27.2%

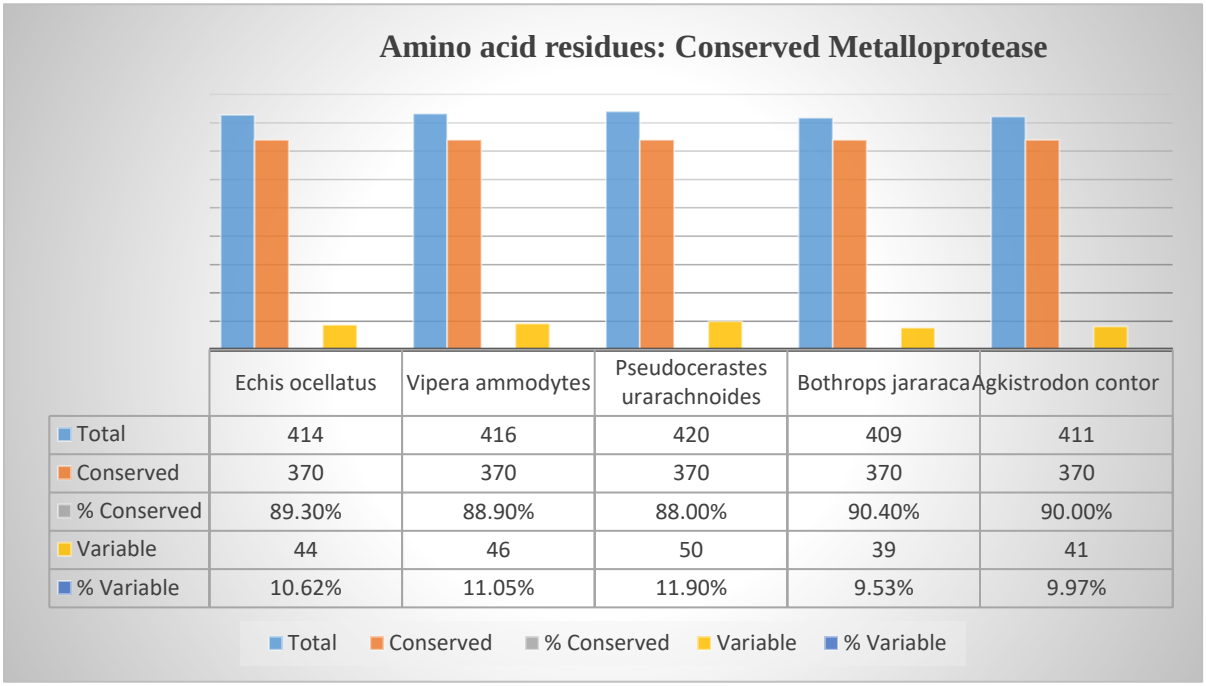
Charts



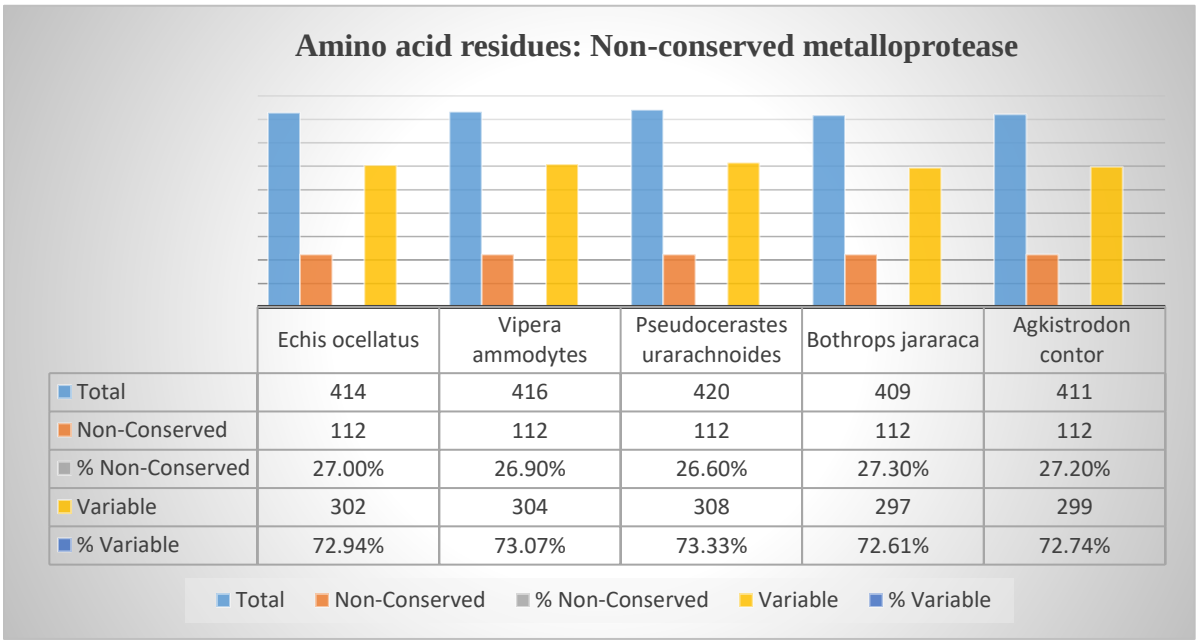
	Protobothrops mucrosquamatus	Gloydius intermedius	Echis ocellatus	Vipera ammodytes	Azemiops feae	Bothrops jararaca
Total	258	260	258	260	258	257
Conserved	140	140	140	140	140	140
%conserved	54.20%	53.80%	54.20%	53.80%	54.20%	54.20%
Variable	118	120	118	120	118	117
%variable	45.70%	46.10%	45.70%	46.10%	45.70%	45.50%



	Protobothrops mucrosquamatus	Gloydius intermedius	Echis ocellatus	Vipera ammodytes	Azemiops feae	Bothrops jararaca
Total	258	260	258	260	258	257
Non-Conserved	64	64	64	64	64	64
% Non-Conserved	24.80%	24.60%	24.80%	24.60%	24.80%	24.80%
Variable	194	196	194	196	194	193
% Variable	75.19%	75.38%	75.19%	75.38%	75.19%	75.09%

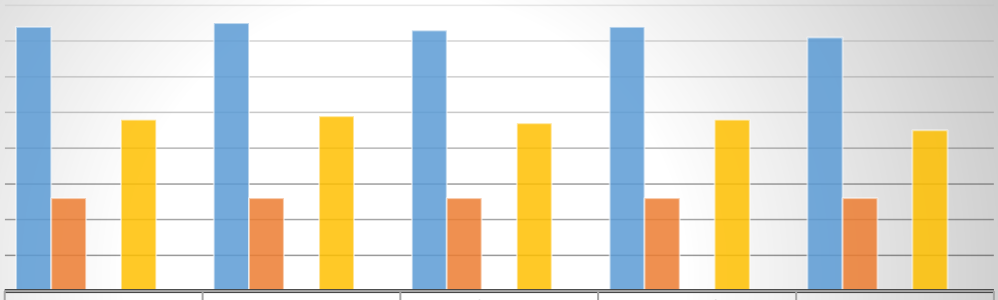


	Echis ocellatus	Vipera ammodytes	Pseudocerastes urarachnoides	Bothrops jararaca	Agkistrodon contor
Total	414	416	420	409	411
Conserved	370	370	370	370	370
% Conserved	89.30%	88.90%	88.00%	90.40%	90.00%
Variable	44	46	50	39	41
% Variable	10.62%	11.05%	11.90%	9.53%	9.97%



	Echis ocellatus	Vipera ammodytes	Pseudocerastes urarachnoides	Bothrops jararaca	Agkistrodon contor
Total	414	416	420	409	411
Non-Conserved	112	112	112	112	112
% Non-Conserved	27.00%	26.90%	26.60%	27.30%	27.20%
Variable	302	304	308	297	299
% Variable	72.94%	73.07%	73.33%	72.61%	72.74%

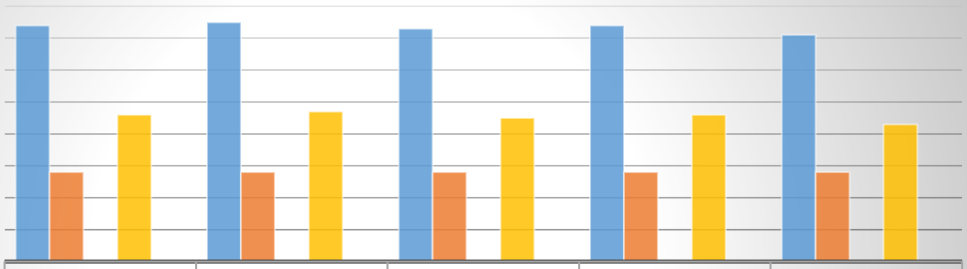
Amino acid residues: Conserved C-type lectin



	Echis ocellatus	Vipera transcaucasiana	Pseudocerastes urarachnoides	Crotalus tzabcan	Oreganus helleri
Total	148	150	146	148	142
Conserved	52	52	52	52	52
% Conserved	35.10%	34.60%	35.60%	35.10%	36.60%
Variable	96	98	94	96	90
% Variable	64.86%	65.33%	64.38%	64.86%	63.38%

Total Conserved % Conserved Variable % Variable

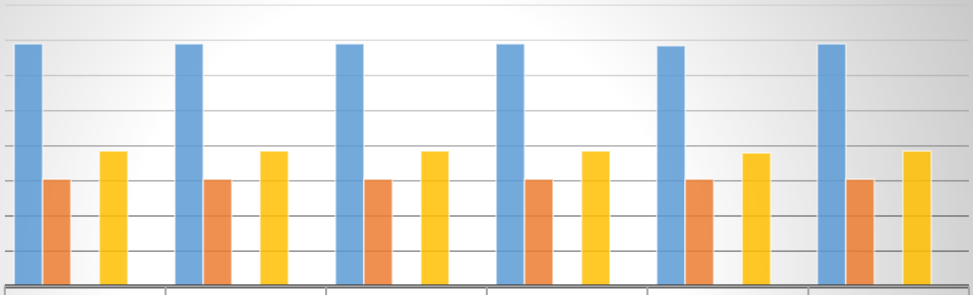
Amino acid residues: Non-conserved C-type lectin



	Echis ocellatus	Vipera transcaucasiana	Pseudocerastes urarachnoides	Crotalus tzabcan	Oreganus helleri
Total	148	150	146	148	142
Non-Conserved	56	56	56	56	56
% Non-Conserved	37.80%	37.30%	38.30%	37.80%	39.40%
Variable	92	94	90	92	86
% Variable	62.16%	62.66%	61.64%	62.16%	60.13%

Total Non-Conserved % Non-Conserved Variable % Variable

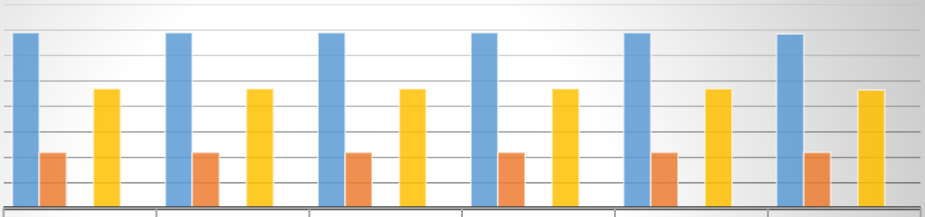
Amino acid residues: Conserved phospholipase A2



	Protobothrops flavoviridis	Crotalus mitchellii	Vipera ammodytes	Echis ocellatus	Daboia russelii	Ovophis monticola
Total	138	138	138	138	137	138
Conserved	61	61	61	61	61	61
% conserved	44.20%	44.20%	44.20%	44.20%	44.20%	44.20%
Variable	77	77	77	77	76	77
%variable	55%	55%	55%	55%	55%	55%

Total Conserved % conserved Variable %variable

Amino acid residues: Non-conserved Phospholipase A2

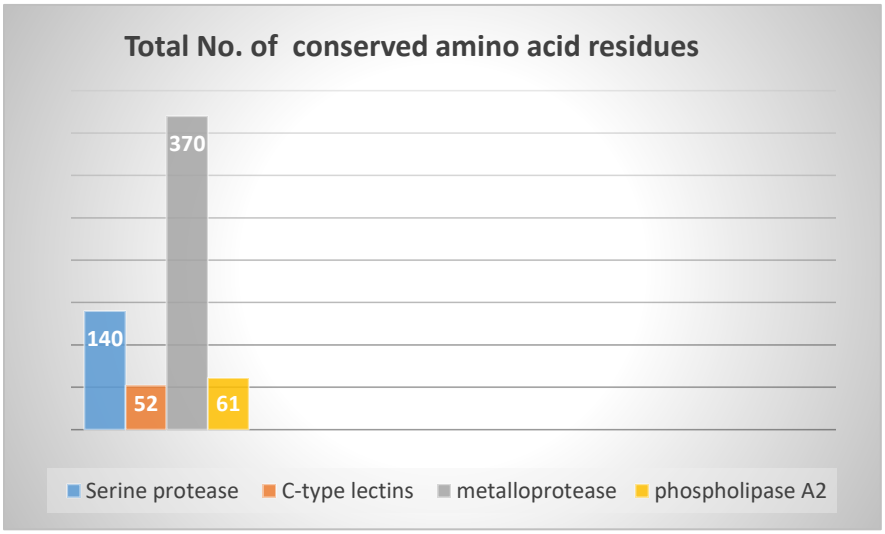


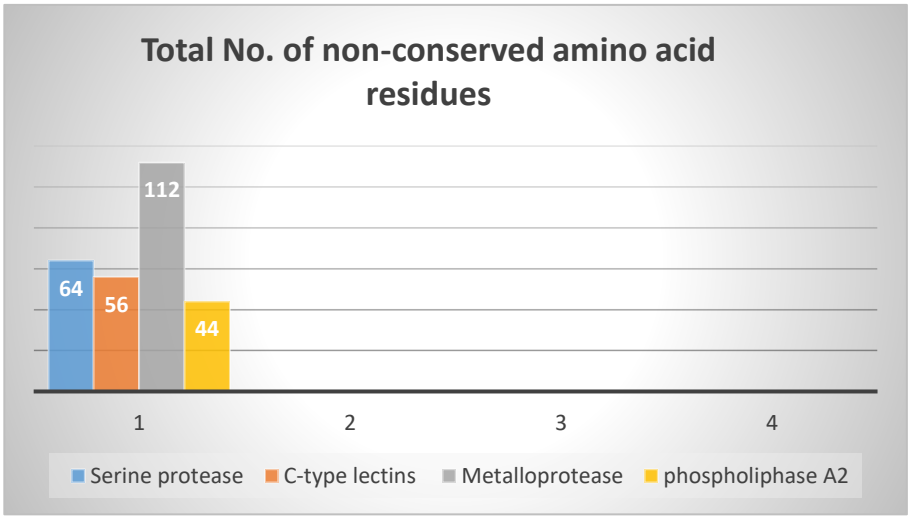
	Ovophis monticola	Protobothrops flavoviridis	Crotalus mitchellii	Vipera ammodytes	Echis ocellatus	Daboia russelii limitis
Total	138	138	138	138	138	137
Non-Conserved	44	44	44	44	44	44
% Non-Conserved	31.80%	31.80%	31.80%	31.80%	31.80%	31.80%
Variable	94	94	94	94	94	93
% Variable	68.11%	68.11%	68.11%	68.11%	68.11%	67.88%

Total Non-Conserved % Non-Conserved Variable % Variable

Total No. of conserved Amino acid residues	Total No. of non-conserved Amino acid residues
Phospholipase A2: *Ovophis monticola:* 61 *Protobothrops flavoviridis:* 61 *Crotalus mitchellii:* 61 *Vipera ammodytes:* 61 *Echis ocellatus:* 61 *Daboia russelii limitis:* 61	**Phospholipase A2:** *Ovophis monticola:* 44 *Crotalus mitchellii:* 44 *Vipera ammodytes:* 44 *Echis ocellatus:* 44 *Daboia russelii limitis:* 44
Serine protease: *Protobothrops mucrosquamatus:* 140 *Gloydius intermedius:* 140 *Echis ocellatus:* 140 *Vipera ammodytes:* 140 *Azemiops feae:* 140 *Bothrops jararaca*: 140	**Serine protease:** *Protobothrops mucrosquamatus:* 64 *Gloydius intermedius:* 64 *Echis ocellatus:* 64 *Vipera ammodytes:* 64 *Azemiops feae:* 64 *Bothrops jararaca*: 64
C-type lectins: *Echis ocellatus:* 52 *Vipera transcaucasiana:* 52 *Pseudocerastes urarachnoides:* 52 *Crotalus tzabcan:* 52 *Oreganus helleri:* 52	**C-type lectins:** *Echis ocellatus:* 56 *Vipera transcaucasiana: 56* *Pseudocerastes urarachnoides: 56* *Crotalus tzabcan: 56* *Oreganus helleri: 56*
Metalloprotease: *Echis ocellatus:* 370 *Vipera ammodytes:* 370 *Pseudocerastes urarachnoides:* 370 *Bothrops jararaca:* 370 *Agkistrodon contor*: 370	**Metalloprotease:** *Echis ocellatus:* 112 *Vipera ammodytes:* 112 *Pseudocerastes urarachnoides:* 112 *Bothrops jararaca:* 112 *Agkistrodon contor*: 112

Charts


Total No. of conserved amino acid residues
370
140
52
61
Serine protease
C-type lectins
metalloprotease
phospholipase A2


Total No. of non-conserved amino acid residues
112
64
56
44
1
2
3
4
Serine protease
C-type lectins
Metalloprotease
phospholiphase A2

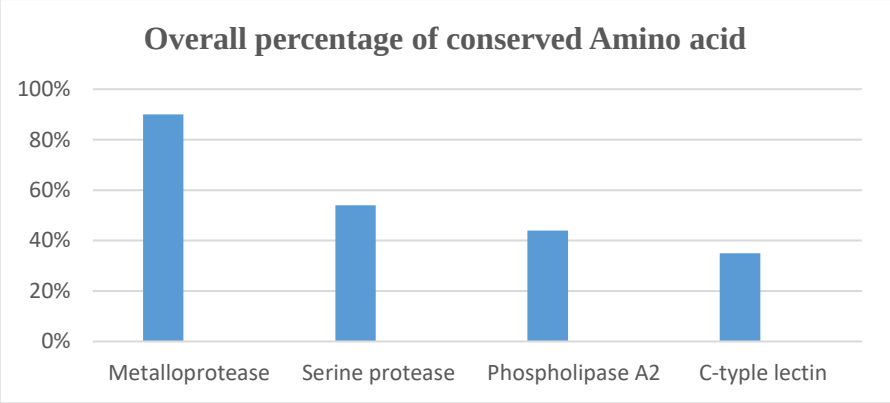
CHAPTER 5

DISCUSSION AND CONCLUSION

DISCUSSION

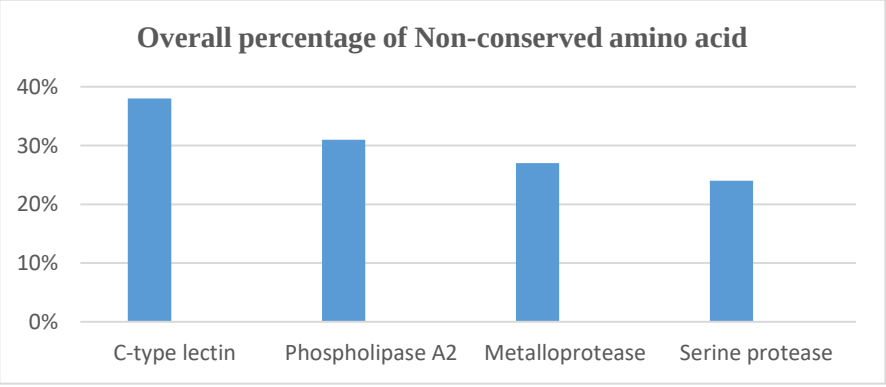
- **Conserved amino acids**

Metalloprotease	Serine protease	Phospholipase A2	C-type lectin
90%	54%	44%	35%



- **Non-conserved amino acid**

C-type lectin	Phospholipase A2	Metalloprotease	Serine protease
38%	31%	27%	24%



Conclusion

From the above results, metalloprotease has the approximate highest conserved units of amino acid residues in this snake (*Echis ocellatus*), followed by serine protease the phospholipase A2 and then C-type lectin has the lowest conserved units of amino acid residues, while C-type lectin has the highest non-conserved units of amino acid residues, followed by phospholipase A2, then metalloprotease and then serine protease, which has the lowest units of non-conserved amino acid residues.