بسم الله الرحمن الرحيم

International University of Africa

Deanship of Postgraduate Studies

Research Thesis entitled

Toxicological Studies on Some Snakes Venoms Using Experimental Rats

A thesis submitted to The Department of Pharmacology and Toxicology, Faculty of Pharmacy in fulfillment of the requirement for Ph.D. degree in Toxicology

Candidate:

Ruaa Yasir Altayeb Mohammed
(B. pharm, 2013, Alnilain University)
(M.Pharm. Clinical Toxicology, 2018, Omdurman Islamic University)

Supervisor:
Professor. Tarig Mohammed H. El-Hadiyah
(Department of Pharmacology, Faculty of Pharmacy, International University of Africa)

CO-Supervisor:
Professor. Samia Mohammed Ali El-Badwi (Department of Pharmacology, Faculty of Veterinary Medicine, University of Khartoum)

Feb, 2022

Dedication

This thesis is dedicated to my husband, who has been a constant source of support and encouragement during the challenges of this work and life, and led me through the valley of darkness with light of hope and support.

This work is also dedicated to my parents who have always loved me unconditionally and taught me to work hard for the things that I aspire to achieve.

To my beloved sisters and brothers, and to all my family and friends.

Acknowledgements

First and foremost, I am grateful to some people who worked hard with me from the beginning till the completion of the present research particularly my supervisor: Prof. **Tarig Mohammed H. El-Hadiah** who has been always generous during all phases of the research, thanks for his meticulous supervision, motivation, guidance for writing this thesis, revising and discussing all aspects of this study.

Also I highly appreciate the efforts expended by: **Prof. Samia Mohammed Ali El-Badwi**.

I would like to express my wonderful thanks to my family for their generous support; they provided me throughout my entire life and particularly through the process of pursuing the PhD degree.

Summary of the study

Background: snake venoms are complex mixtures of enzymatic and non-enzymatic components with specific pathophysiological effects that differ widely between and within species. Assessment of the toxicological effects of venomous snake species in Sudan is a critical step for an efficient determination of the venom activity and increase the understanding of geographical intraspecific Variation in venom composition.

Objective: This study aimed to investigate the toxicological effects of snake venoms of *Naja haje, Naja nubiae and Cerastes cerastes* on experimental rats

Methods: Lethality test was conducted in 3 groups of albino rats (5 each). Group 1: *N.haje* venom (minimal lethal dose 0.1mg/kg, i.p.), group 2: *N.nubiae* venom (minimal lethal dose 0.4mg/kg, i.p.), group 3: *C.cerastes* venom (minimal lethal dose 0.5mg/kg, i.p.). Death time of each group was recorded. As *N.haje* snake venom was found to be the most poisonous one among the three snake venoms, further toxicological tests were carried out on this venom. LD_{50} of *N.haje* snake venom was determined using karber method. Rats were divided into 3 groups (5 each). Group 1: control group injected with normal saline, group 2: injected with toxic dose (0.08mg/kg) of N.haje venom, group 3: injected with lethal dose (0.5mg/kg) of N.haje venom. These groups were observed for behavioral and neurological changes. Blood samples were collected from all rat groups for complete blood counts analysis and biochemical examinations then these rats were scarified and liver and kidneys were dissected for histopathological studies. To study fetotoxicity, pregnant rats were divided into 2 groups (5 each). Group 1: control group, group 2: *N.haje* venom toxic dose: 0.08mg/kg, i.p. Both groups

were observed for the continuity of pregnancy and fetuses were examined for presence of any external abnormalities.

Results: *N.haje* snake venom was the most poisonous one among the three snake venoms as it had the lowest minimal lethal dose (0.1mg/kg) and quick death time (46 ± 3 min). Minimal lethal doses and mean time death of *N.nubiae* and *C.cerastes* were 0.4 mg/kg, 0.5 mg/kg and 90min±15, 180min±20 min, respectively. LD_{50} of *N.haje* venom was 0.28mg/kg. Convulsions and paralysis were most significant neurotoxic effects of *N.haje* venom). Results of injected groups with *N.haje* venom two different doses (lethal, toxic) were as following: Convulsions and paralysis were most significant neurotoxic effects of *N.haje* venom. Biochemical parameters showed a significant decrease in glucose level, highly significant increase in liver enzymes (AST, ALT) and significant increase in urea and creatinine levels. The histological examination revealed alterations in liver and kidney tissues (hepatocytic swelling, infiltration of lymphocytes, tubular vacuolization, glomerular damage, inflammatory cellular infiltration and hydropic degeneration change). Rats that received lethal dose showed hematological changes appeared as a highly significant increase in White blood cells count and significant increase in platelet count. Toxic dose (0.08mg/kg) of *N.haje* venom didn't affect continuity of pregnancy or induced external malformations on fetuses.

Conclusion: it could be concluded that, both lethal and toxic doses of *Naja haje* snake venom are neurotoxic, hepatotoxic and nephrotoxic. Lethal dose of the venom was hematotoxic. Toxic dose didn't show teratogenic effect on fetuses.

Recommendation: Further toxicological studies concerning the two others snakes (*Naja nubiae, Cerastes cerastes*) presented in this study and mechanism of their toxic effects should be conducted.

ملخص الدراسة

الخلفية: سم الثعبان هو خليط مركب من بروتينات إنزيمية و لا إنزيمية ذات وظائف فسيولوجية مرضية محددة التي تختلف بإختلاف نوع الحية و بين افراد النوع الواحد.

الهدف: يهدف هذا البحث إلى دراسة التأثيرات السمية لسموم الكوبرا المصرية، الكوبرا النوبية و الأفعى القرناء على جرذان التجارب.

الطريقة: في البدء تم إجراء اختبار لتحديد الأفعى الآكثر سمية و الأسرع فتكا وذلك بتقسيم مجموعة من جرذان التجارب الى ثلاث مجموعات، المجموعة الأولى حقنت بالحد الأدنى من الجرعة المميتة لسم الكوبرا المصرية و الثانية حقنت بالحد الأدنى من الجرعة المميتة لسم الكوبرا النوبية و المجموعة الثالثة حقنت بالحد الأدنى من الجرعة المميتة لسم الأفعى القرناء.أجريت مزيد من التجارب على سم الكوبرا المصرية؛ تم حساب الجرعة نصف القاتلة باستخدام طريقة كاربر؛ من ثم تم تقسيم مجموعة أخرى من جرذان التجارب الى ثلاث مجموعات(٥ جرذان لكل مجموعة)، حقنت المجموعة الأولى بمحلول فسيولوجي (كلوريد الصوديوم) و التي استخدمت كمجموعة ضابطة، المجموعة الثانية حقنت بجرعة سامة مقدارها(0.08مج/كجم) من سم الأفعى المصرية، اما المجموعة الثالثة فقد حقنت بجرعة قاتلة (0.5 مج/كجم) من سم الكوبرا المصرية؛ على هذه المجموعات الثلاث تمت ملاحظة التغيرات العصبية و السلوكية ومن ثم سحبت عينات من الدم لتحليل صورة الدم و للفحص البيوكيميائي و أخيرا تم اعدام الجرذان وتشريحها و استخراج الكبد و الكلى من أجل تحليل الأنسجة. قسمت مجموعة من الجرذان في مرحلة الحمل المبكر الى مجموعتين؛ حقنت المجموعة الأولى بمحلول فسيولوجي (كلوريد الصوديوم) كمجموعة ضابطة، المجموعة الثانية حقنت بجرعة سامة مقدارها(0.08مج/كجم) من سم الأفعى المصرية، وتمت الملاحظة اثناء فترة الحمل ومن ثم فحص الأجنة.

النتائج: وجدت الدراسة ان سم الكوبرا المصرية هو الأكثر سمية و الأسرع فتكا من بين الأفاعي الأخرى (الكوبرا النوبية و الأفعى القرناء) اذ يساوي الحدالأدنى من الجرعة المميتة لسم هذه الكوبرا(0.1مج\كجم) مع متوسط زمن حدوث الوفاة مساو ل46±3 دقائق. بينما الحد الأدنى للجرعة المميتة و متوسط حدوث الوفاة للكوبرا النوبية و الأفعى القرناء هما(0.4 مج\كجم و 0.5 مج\كجم) و(90±15و 3ساعات±20دقيقة) على التوالى

الجرعة نصف القاتلة لسم الكوبرا المصرية هي(0.28 مج/كجم). ظهرت التأثيرات السمية العصبية لسم الكوبرا متمثلة في تشنجات و شلل ، بالإضافة الى انخفاض في مستوى الجلكوز في الدم في كلتا المجموعتين. فيما يخص التغييرات البيوكيميائية فقد أظهرت التحاليل ان سم الأفعى المصرية سبب

إرتفاعا ذا دلالة إحصائية في مستويات إنزيمات الكبد اسبارتات ترانس امينيز و الانين ترانس امينيز و مستويات معلمات الكلى اليوريا و الكيراتينين لكلا المجموعتين. بين فحص الأنسجة أن سم الكوبرا المصرية قد أحدث تغييرات في أنسجة الكبد و الكلى لكلا المجموعتين. و بينت تحاليل صورة الدم الكاملة أن سم الكوبرا المصرية سبب إرتفاعا ذا دلالة إحصائية في عدد كريات الدم البيضاء و الصفائح الدموية في المجموعة التي حقنت بالجرعة القاتلة، ايضآ وجدت الدراسة أن الجرعة السامة من سم الكوبرا المصرية لم تتسبب في حدوث تشوهات خلقية للأجنة و كذلك لم تؤثر على استمرارية الحمل بشكل طبيعي.

الخاتمة: الجرعة المميتة و الجرعة السامة من سم الكوبرا المصرية ذاتا تأثيرات سمية عصبية وتسببان ضعف في وظائف الكبد و الكلى و ذلك استدلالا بارتفاع مستويات انزيمات الكبد و معلمات الكلى اضافة الى التغيرات في أنسجتهما. أظهرت الجرعة المميتة من سم هذه الكوبرا تأثيرات سامة في الدم و ليس لدى الجرعة السامة أي تأثيرات على الأجنة.

التوصيات: يوصى بان تجرى مزيد من الدراسات على سموم الأفاعي الأخرى المذكورة في الدراسة (الكوبرا النوبية و الأفعى القرناء).

Table of Contents

List of Images

List of Tables

List of Abbreviations

ALT	Alanine transaminase
ALP	Alkaline phosphates
AST	Aspartate transaminase
CNS	Central nervous system
EDTA	Ethylene diamine tetra acetic acid
Hb	Hemoglobin
HCT	Hematocrit
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
MCH	Mean corpuscular hemoglobin
RBCs	Red blood cell
R.p.m	Round per minute
SPSS	Statistical package for social sciences software
WBCs	White blood cell
WHO	World health organization

Chapter One

Introduction

and

Literature Review

1. Introduction and literature Review

1.1Introduction

Toxicology is a field of science, overlapping with pharmacology, chemistry biology, and medicine that involves the study of the adverse effects of chemical substance on living organisms and the practice of diagnosing and treating exposures to toxins and toxicants. There are generally four types of toxic entities, chemical, physical, radiation and biological [(1)].

Biological toxins are poisonous substances produced by microorganisms, animals, insects and plants that produce harmful clinical effects when inhaled, injected or absorbed [(2)]. Snake envenomation is among the most hazardous biological toxin and snake bites are the highest among animal toxin poisoning. The total number of snake bites has been estimated to be 5.4 million per year, 1 million of which were recorded in Africa which is the most affected continent with snake envenomation as a result of poor medical facilities and shortage of technical knowledge which making management of envenomation cases difficult [(3)].

In Sudan, the geographical distribution of snake species isn't clearly established and most snake bites and deaths aren't nationally updated. Therefore, the study of the biogeographic distribution of snakes should be part of a global national research network involving ecologists, biochemists and clinicians [(3)].

1.1.1General classification of snakes and their venoms:

Venomous snakes primarily belong to four families: Viperidae (vipers), Elapidae, Atractaspididae and colubridae. Snakes from these families are found worldwide, but envenomation is a significant health problem primarily in tropical area where rural workers are the primary victims. In Europe, most venomous snakes are of the genus *Vipera*. In Asia, cobras and kraits are found but the most dangerous Asian snake is the Russell's viper (Daboia Spp). Australia is home to strikingly high

number of venomous snake including brown snake and taipan snakes. In North America, venomous snakes include a variety of rattlesnakes, copperhead and coral snakes. In Africa venomous natives include cobras (*Naja* Spp), mambas (*Dendroaspis* spp), and saw-scaled vipers (*Echis* Spp).[4]

In Sudan, most of venomous snakes are belong to Vibridae and Elapid families such as *Cerastes gasper*, *Echis* species, *Bitis arietans* "particularly found in east Sudan", *Naja haja arabicus* " knows as Egyptian cobra" and *Malpolon moilensis* "False cobra" [5].

1.1.2 Snake venoms:

Snake venoms are complex mixtures of 50 or more components that function to immobilize, kill, and pre-digest prey. In human victims these substances produce local digestive or cytotoxic effects on tissues as well as hemotoxic, neurotoxic, nephrotoxic and other systemic effects. The relative predominance of cytotoxic, hemotoxic and neurotoxic venom components depends on the species of the snake and on geographical and seasonal variables. [6]

Components of snake venom can be divided into:

- Enzymatic proteins: these enzymes include phospholipase $A_{2,}$ proteinase, phosphodiasterases, L amino acid oxidase and nucleotidase. Besides their catalytic properties that's have an important role in the digestive action of the venom, these enzymes as well induce various pharmacological effects including neurotoxic, myotoxic, cardiotoxic, hemorrhagic, hemolytic, procoagulant and anticoagulant effects[7,8].
- Non enzymatic proteins: these proteins include neurotoxins, myotoxins, cardiotoxins, ion channel inhibitors and anticoagulant proteins [7, 8].

  Snake venoms are almost 90% water and in addition to enzymatic and non enzymatic proteins the venom can contain additional constituents such as lipids, biogenic amines and carbohydrate. Thus, either the snake

venom proteins are enzymatic or non-enzymatic they target several organs, tissues, physiological systems and interfere with their normal functions resulting in multiple organs or system failure and often death [3].

1.1.3 Types of venom toxins and their effects:

A. Neurotoxins:

There are two different types of neurotoxins in snake venom, presynaptically or postsynaptically acting neurotoxins.

Presynaptic neurotoxins are member of phospholipase A_2 enzymes family. They targets the terminal axon of the neuromuscular junction causing destruction of the axonal structure which results in a disruption of the synaptic vesicles with a complete cessation of transmitter release. Signs and symptoms of a bitten victim are a flaccid paralysis starting with the cranial nerves, ptosis coming first then ophthalmoplegia, loss of airway protection, paralysis of respiratory muscles and diaphragm, finally limb paralysis with loss of deep tendon reflexes.

Postsynaptic neurotoxins are polypeptides with 60-70 amino acids. They acting by adherence to the two subunits of the receptor and block the formation of ion-channel. [9]

B. Myotoxins:

Phospholipase A_2 enzymes cause damage to musculature by destroying the skeletal muscles. When muscles are destroyed this followed by release of myoglobin. Myoglobin damages the tubulus cells of the kidney leading to secondary kidney failure which can be treated by hemodialysis [9].

C. Hematotoxins:

Snake venom enzymes damage blood hemostasis by several mechanisms, on a cellular level the functioning and amount of platelets are affected and on a

humoral level the blood coagulation is deranged. The main venom hematotoxins are procoagulants, anticoagulants, aggregation inhibitors or promoters and hemorrhagins that cause local injury to vessel walls [9]. Moreover, hematotoxic venoms can have cardiovascular effect presented by a dramatic fall in blood pressure and this attributed to the presence of snake venom metalloprotease enzymes (SVMPs) which indirectly causing hypotension by increasing vascular permeability via the deterioration of capillary basement membrane leading to reductions in blood pressure [10].

D. Local damage:

Most snake bite cause local damage around the bite (necrosis, blister) as a result of the venoms content of enzymes, leukotriene, histamine, kinins, phospholipase, collagenases and metallic ions. Few minutes following the bite, the region around the fang marks starts to swell and become painful, then within hour's ecchymosis, necrosis and blister develop and the swelling expand over the whole limb. The venom may stay local and be fixed to tissues but also can reach the circulation and thereby induce systemic toxicity [9].

E. Systemic symptoms:

As a result of snake bite, the venom is administered by subcutaneous route, spreads through lymphatic and superficial venous vessels and reaches the circulatory system slowly producing systemic symptoms. Less severe systemic signs are anxiety, malaise, weakness, nausea, and metallic taste in mouth, increased sweating and salivation. More severe signs manifest as abdominal pain, diarrhea, vomiting, dyspnea and confusion. The most serious sign is circulatory collapse with hypotension and tachycardia. In some cases the venom can result in overt anaphylaxis with a fatal or near fatal reaction. Generally signs and symptoms of snake venom may vary depending on

several factors such as the type of the snake, age of the snake and the location of the bite [9].

1.1.4 Management of snake bites:

The traditional first aid methods such as incision or sucking the wound are proved to be ineffective. Alternatively, it's effective to immobilize the bitten limb and the victim to diminish the distribution of the venom. It would be beneficial to wrap the limb with an elastic bandage to decrease or stop the transport of lymph and compress the superficial veins. In hospital, treatment requires description of signs and swelling to judge the progression, hematological and neurological investigations, anti-venom therapy, anticoagulant therapy and surgical intervention if necessary. Broad spectrum antibiotics could be given if there are signs of infection, also provide tetanus prophylaxis if needed [9,6].

1.2 Literature review

1.2.1 Review on the three snakes (*Naja haje, Naja nubiae* and *Cerastes cerastes*):

1.2.1.1 Naja haje snake:

Naja haje or the Egyptian cobra is a species of venomous snake in the family Elapidae. The generic name (Naja) means cobra and the word (haje) is derived from the Arabic word hayya which means snake [10].

The Egyptian cobra is a large species; the head of this cobra is large, depressed and slightly distinct from the neck which has long cervical ribs able to expand to form a hood such as all other cobras (Image 1.1). The body of *N.haje* is cylindrical with a long tail, generally the length of this species depends on the geographical location, population and subspecies, even though, its average length is 1.4 meters. The most identifiable characteristics of this species are it is head and hood. Color of *N.haje* is also varying but most of specimens are shade of brown with darker or lighter mottling and usually a tear drop mark below the eye [11].

Image1-1: *Naja haje* (Natural history museum, Sudan)

A. Geographical distribution:

Naja haje ranges across most of North Africa (Egypt, Sudan, Libya, and Morocco) across the savannas of West Africa to the south of Sahara south to the Congo basin and east to Tanzania and Kenya [(11)].

B. Habitat:

Naja haje appears in a wide variety of habitats such as steppes, dry to moist savannas, arid semi-desert regions with some water and vegetation. This species is often found near water and also found in agricultural fields; moreover, it exists in the presence of humans where it frequently enters houses. It is attracted to villages by rodent pests and domestic chickens [(11,12)].

C. Venom:

Venom of *N.haje* consists chiefly of neurotoxins and cytotoxins [(13)]. The range of venom yield of a single bite is approximately 175 to 300mg [(14)]. the venom affects the nervous system resulting in stopping of nerve signals transmission

to muscles then at later stage the transmission of signals to the lungs and heart is also stopped leading to death due to complete respiratory failure. Envenomation by N.haje snake causes severe swelling, local pain, necrosis, bruising and other symptoms like headache, nausea, abdominal pain, vomiting, collapse or convulsion accompanied with possible moderate to severe flaccid paralysis [15].

1.2.1.2 *Naja nubiae* snake:

Naja nubiae (the Nubian cobra) is a species of spitting cobra in the family Elapidae native to Africa. It is a relatively small spitting cobra that's spit the venom into the eye or skin of the victim. The maximum recorded length of this cobra is 148cm and it is color is brownish-gray overall [16] (Image1-2).

Image1-2: Naja Nubiae (Natural* history museum, Sudan*)

A. Geographical distribution:

The Nubian cobra distributes across Egypt (Nile valley), Sudan (Nile valley, Darfur), western Eritrea, Chad and Niger, where it seem to occupy primarily relatively mesic habitats [16].

B. Venom:

Venom of *Naja nubian* cobra characterized by cytotoxic type of envenomation that's involves swelling at the bite site with blistering and bruising that may lead to necrosis, additionally this venom has some neurotoxic properties [17].

1.2.1.3 *Cerastes cerastes* snake:

It is a venomous species in the family viberidae, generally known as the desert horned viper. *Cerastes cerastes* is an easily distinguishable snake due to the presence of a pair of supra-ocular horns (Image 1-3); it is often hiding by burying themselves in the sand. The average total length of this horned viper is 30-60cm with a yellowish, pale brown ground color that's almost matches the substrate color where the animal found [18].

Image 1-3: Cerastes cerastes snake (Natural* history museum, Sudan*)

A. Distribution and habitat:

They are found in north Africa (Sudan, Egypt, Libya), also can be found in Iraq, Saudi Arabia, Syria, Jordan. This species prefer dry, sandy areas and tend to avoid coarse sand [18].

B. Venom:

Envenomation usually causes hemorrhage, necrosis, swelling, nausea, vomiting and hematuria. The presence of high content of phospholipase A_2 may cause cardiotoxicity and myotoxicity. The average venom yield of a single bite is 19-27mg to 100mg [18,19].

1.2.2 Previous experimental studies:

In Egypt, Salman M.M.A (2014) studied the effects of different doses of *Cerastes cerastes* venom on the biochemical parameters in serum of guinea pigs. Results of this study appeared that, injection of single dose of this venom induced a significant decrease in the levels of total serum protein, uric acid, globulin and albumin, whereas, there was a significant elevation in the levels of serum glucose triglycerides, urea, creatinine, cholesterol, AST, ALT and ALP. According to these findings, this study concluded that, venom of *Cerastes cerastes* snake induced hepatic and renal dysfunctions in the envenomed guinea pigs [(20)].

Another study was conducted in Jordan by Abadala S (1992) to investigate the impacts of *C.cerastes* snake venom on hematological parameters of guinea pigs revealed that, venom of *C.cerastes* had significantly reduced the count of WBCs, plasma clotting time and erythrocyte deformability and had no effect on RBCs, Hob concentration or hematocrit value [(21)].

Results obtained by EL-Amir A M et al. (2020) on his study of the assessment of the bio-physiological effects of i.p injection of 1/10 LD_{50} dose of *Cerastes cerastes* venom in mice, presented significant elevation in serum AST, ALT, ALP, urea and creatinine direct bilirubin, as well as a significant decrease in RBCs and HB while platelets and WBCs count showed high significant increase [(22)].

Further study was designed by Alsadoon et al.(2014) in Saudi Arabia to investigate the hematological effects and oxidative stress induction of *C.cerastes* snake venom in lung, heart and spleen in albino mice and the findings appeared a significant difference in WBCs with an increase in neutrophils, monocytes, eosinophil, basophil and a significant decrease in concentration of hemoglobin. Moreover, this study revealed that, venom of *C.cerastes* was associated a significant increase in oxidative stress levels and induced congestion of the alveolar capillaries in lung, inflammatory cellular infiltration, myonecrosis in heart and splenomegaly [(23)].

The toxicological effects of *C.cerastes* venom on serum indices of male albino rats was carried out in Egypt by Salih S.M et al. (2015) who reported that injection of viper *C.cerastes* crude venom caused liver and kidney dysfunction which indicated by a significant decrease in albumin, glucose, cholesterol, total serum protein besides significant elevation in levels of AST, ALT, alkaline phosphate, urea and creatinine in the all envenomed rats [(24)].

In Saudi Arabia, Alsadoon et al (2013) conducted a study to explore the ability of *C.cerastes* crude venom to produce histopathological alterations on the tissues of liver and kidney and the findings proved that venom of *C.cerastes* snake is potent toxin-mediated hepatorenal toxicity additionally, it induces disturbance in carbohydrates and lipid metabolism [(25)].

In Sudan, Khalid H et al. (2015) conducted a study to measure the cytotoxic effect of *Naja nubiae* and *Echis ocellatus* venoms by using a cell based assay. Result of this study revealed that both venoms resulted in a remarkable inhibition of cell viability with *N.nubiae* venom being more cytotoxic than *E.ocellatus* [(17)].

A study of toxin and assessment of snake venomics of the African spitting cobra (*N.nubiae*) which carried out by Petras D et al. (2011) in America showed that, the high content of cytotoxins and PLA_2 may account for the extensive tissue necrosis characteristic of the envenoming by *N.nubiae* species. Moreover, the high abundance of type 1 a-neurotoxin in the Nubian cobra may be responsible for the high lethal toxicity of this venom in rodents [(26)].

In Egypt, Saad A et al. (2021) studied the effect of the spitting cobra (*N.nubiae*) on vascular permeability of liver and kidney tissues of albino rats based on the extravasation of the azo dye evans blue, results illustrated a high significant rate of evan blue extravasation to hepatic and renal tissues by the colorimetric determination of the dye concentration which means the venom of *N.nubiae* can

cause increased hepatic and renal vascular permeability that's may explain the inflammatory effect induced by this venom [27].

Another study conducted in Egypt by Al-Qurashy S et al. (2014) to assess the hepatotoxicity and oxidative stress induced by *Naja haje* venom. The Egyptian cobra venom was found to enhance the lipid peroxidation and nitric oxide production in both serum and liver of envenomed rats, in addition to reduction in glutathione, catalase, glutathione reductase and glutathione-S-transferase activities while, activities of superoxide dismutase and glutathione peroxidase were increased significantly. More over the caused hepatic injury which indicated by histopathological changes in liver tissues. According to the above findings, this study concluded that *N.haje* venom is a potent inducer of toxin-mediated hepatotoxicity [28].

Findings of the study presented by Shaban A et al. (2003) on the histological and biochemical effects of *N. haje* snake venom in mice and rats showed that, injected *N.haje* venom produced marked increase in the activities of AST, ALT, ALP and LDH in addition to significant elevation in urea, creatinine and serum glucose. The histological findings appeared vacuolated hepatocytes, scattered necrotic and hemorrhagic areas with congestion and dilatation of blood vessels besides sinusoids. The venom also caused severe degeneration of the cardiac muscles with loss of striations and considerable hemorrhage in between myocardial bundles [29].

Further study on the venom of *N.haje* snake was designed by Shaker et al. (2018) to investigate the toxicity of this venom. Results appeared that the venom caused high significant elevation in liver and cardiac enzymes, as well urea and creatinine. Complete blood pictures showed significant decrease in hemoglobin and platelet count whereas the counts of WBCs and RBCs were highly significant decreased. The venom also induced histological alterations in the tissues of heart, skin, liver and kidney [30].

In Libya, Saeid A et al (2021), studied the oxidative stress induction and nephrotoxicity of *N.haje* venom in rats injected with lethal dose and sub-lethal dose of the venom, findings of this study revealed a high significant increase in urea and creatinine levels in the two different doses and a high significant increase in lipid peroxidation and nitrite which indicate probabilities of nephrotoxicity due to cobra venom [31].

In Brazil, Martha M et al. (2011) conducted a study to investigate the effect of a moderate dose of *B. jararaka* snake venom on injected into pregnant mice on gestation day 5 ($GD_{5)}$, results presented by this study showed the injection of B. *jararaka* venom on GD_5 didn't change fetuses weight but produced high incidence of skeletal anomalies with absence of external malformations [32].

1.3 Rationale of the study:

Envenoming and deaths resulting from snake bites has always been a global health issue especially in rural areas and areas with inadequate medical services particularly in tropical and subtropical countries. In Sudan, agricultural workers and children are the most affected groups.

In 2018, the world health organization recognized the snake bites as a high priority neglected tropical disease (NTD) and stated a global goal to end the epidemics by 2030 [3].

There is a large degree of variability in venom compositions at all taxonomic levels. Additionally, within the same species the toxic components of the snake venom vary greatly among populations and across geographical areas [3].

Assessment of the toxicological effects of venomous snake species in Sudan is a critical step for an efficient determination of the venom activity and increase the understanding of geographical intraspecific Variation in venom composition and improving snake bite treatment by designing specific anti-venom.

1.4 Objectives of the study:

1.4.1 General objective:

This study was designed to investigate the toxicological effects of snake venoms, of *Naja nubiae, Naja haje,* and *Cerastes cerastes* on experimental rats.

1.4.2 Specific objectives:

1. To conduct preliminary lethality tests on the venoms of Naja nubiae, Naja haje, and Cerastes cerastes, which are the most commonly snakes present in Sudan.
2. The most poisonous snake venom will be subjected to further experimentation as follow:
    a. To determine the median lethal dose (LD_{50}).
    b. To investigate the behavioral toxicity and neurotoxicity of this venom.
    c. To measure the effect of the venom on some hematological parameters and blood glucose level.
    d. To measure the effect on some biochemical liver parameters and (ALT, AST, ALP) and kidney parameters (urea, creatinine).
    e. To assess the histopathological changes in liver and kidney.
    f. To test the effect of the venom on pregnant rats at early stage of pregnancy and their fetuses.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Materials

2.1.1 Chemicals

Chemicals	Source
Diethyl ether	AppliChem, Germany
Formalin 10%	Microxpress, India
Normal saline 0.9%	AIN Sudan

2.1.2 Instruments

Instrument	Company	Source
Automated hematology analyzer	Sysmex	Japan, Hyogo
Biochemistry analyzer	Biosystem	Biosystem, India, Kolkata
Blood glucose analyzer	Dirui	China, Changchun

2.1.3 Lab tools:

EDTA containers, plain containers, capillary pipettes, surgical forceps

2.2. Animals

2.2.1. Rats:

Males and females Wistar albino rats weighing 180 ± 30 gm were used. Rats were obtained from Experimental Animal Unit; Faculty of Veterinary Medicine, University of Khartoum, then housed at the experimental animal room in faculty of pharmacy, International University of Africa. Animal room was under controlled conditions of temperature (25 c^o) and relative humidity (50%) as well as 12 hours

light/ dark cycle with light between 7 am to 7 pm. Rats were allowed free access to food (standard rodents chow) and water *ad libitum*.

2.2.2 Snakes:

Following type of snakes were used:

Naja nubiae (family Elapidae), *Naja haje* (family Elapidae) and *Cerastes cerastes* (family Viperidae)

These snakes were obtained from Sudan Natural History Museum (Khartoum, Sudan). These snakes were collected from different regions of Sudan by a professional hunter. Each snake was housed individually under standard conditions and fed on frogs, chicks and lizards.

2.3 Methods

2.3.1 Venom collection and preparation:

Fangs of snake were put on container covered by plaster then venom was milked by massaging the gland below the eye in dim light (Image 2.1), collected venom was lyophilized using thermo freeze dryer, then stored in a light resistant container at 4° c and reconstituted in saline solution prior to use.

Image 2.1: Venom milking

2.3.2 Method of selection of most poisonous venom:

Preparation of venom working solution:

Ten mg of snake venom was weighed and dissolved in 1 ml normal saline solution (stock concentration) then a diluted dose was prepared by taking 0.1ml of the stock and dissolved in 0.9 ml saline solution.

2.3.3 Selection of minimal lethal and toxic (non-lethal) venom doses:

To determine minimal lethal dose of each one of the three venoms, different doses were tried in groups of animals (5 rats each), starting with 1mg/kg then decreased stepwise and least venom dose that caused death within 24 hours was considered minimal lethal venom dose. Half of this minimal lethal dose was considered maximum toxic (sub-lethal) dose. These trials were conducted for venoms of the three snakes (*Naja haje*, *Naja nubiae* and *Cerastes cerastes*).

2.3.4 Determination of LD_{50} of *N.haje* snake venom:

Median lethal dose (**LD_{50}**) was calculated using karber method [33]. Thirty rats were divided into 6 groups (5 rats each). A series of doses ranging between, LD_{100} (0.5mg/kg) and LD_0 (0.05mg/kg) were determined. These doses were: 0.25, 0.18, 0.12 and 0.0625mg/kg. Each group received a specific dose. Number of dead rats

in each group was recorded during 24 hours post injection and LD_{50} was calculated using this equation: $LD_{50} = LD_{100} - \sum (A*B)/N$

Where:

A = the difference between two successive doses of administered venom

B = the average number of dead rats in two successive doses

N = total number of rats in a group

2.3.5 Measurement of neurological and behavioral changes:

Both envenomed groups and control group were observed for neurological and behavioral toxicity. Changes in response induced by the treatment in each group were recorded in the observation sheet at 15 min, 30 min and 1 hour

2.3.6 Collection of blood samples and organs:

Using micropipettes, under mild ether anesthesia, blood was collected from supra orbital plexus from each rat and poured into EDTA containers for complete blood picture analysis.

Similarly, part of blood was poured into plain container, left for 20 min at room temperature to clotting and centrifuged for 30 min at 3000 r.p.m, and then serum was separated carefully for analysis.

Following blood collection, rats were sacrificed by cervical dislocation and dissected then; liver and kidneys were rapidly removed, fixed in 10% neutral buffered formalin and kept until the day of histopathological analysis.

2.3.7 Measurement of hematological changes:

Complete blood pictures (White blood cells, Red blood cells, Hemoglobin, Platelets, Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Hematocrit, lymphocyte, neutrophil, eosinophil, basophil) were measured by Sysmex® analyzer (full automation

instrument) which performs hematological analysis according to the RF/DC detection method, Hydrodynamic Focusing (DC Detection), flow cytometry method (using the semiconductor laser) and SLS-hemoglobin method.

2.3.8 Biochemical assay:

The separated serum was analyzed for determination of AST, ALT, ALP, urea and creatinine levels according to kinetic method by using full automation instrument Biosystem® which act by colorimetric analysis method, whereas the determination of glucose level was carried out by blood glucose analyzer device .

2.3.9 Histopathological examination:

Liver and kidneys were removed from the buffered formalin and washed out under running tap water for 1hour until complete removal of all traces of chemicals. Following washing, tissues were dehydrated by immersing in an ascending series of ethanol concentration and embedded in paraffin wax for making blocks. Sections of 5 microml were cut by microtome and stained with aqueous hematoxylin and alcoholic eosin for preparing permanent slides which were examined under light microscope. Histological alterations were recorded according to Ishak et al scoring system [(34)]:

0 = absent, + = mild, ++ = moderate, +++ = severe

2.3.10 Assessment of Teratogenicity:

Method described by ALharbi et al [(35)] and Eltahir et al [(36)] was used. Adult female Wistar albino rats were allowed to mate with proven male rats in a ratio of 1 male to 1 female. Presence of vaginal plug was taken as an indicator of copulation and that day considered as day one of pregnancy. Toxic dose (0.08 mg/kg) of *N.haje* venom was injected on day 7 of pregnancy (GD_7) and observed for continuity of pregnancy. Delivery of fetuses was allowed to be by spontaneous parturition. Number of live and dead fetuses was recorded, live fetuses were weighed individually and each fetus was examined for any external abnormalities.

2.4. Experimental design and treatment procedure:

2.4.1 Experimental design:

All treatments were by intraperitoneal (i.p) route of administration

Design to determine minimal lethal dose of each snake venom:

Initially, rats were divided into 3 groups (5 rats each):

Group 1: 1mg/kg, i.p., *Naja haje* venom

Group 2: 1mg/kg, i.p., *Naja nubiae* venom

Group 3: 1mg/kg, i.p , *Cerastes cerastes* venom

According to finding of these doses, consecutive doses for each venom were used and finally, minimal lethal doses were determined.

These groups were observed for lethality during 24 hours. Times of deaths were recorded. Snake venom with lower lethal dose and shorter time of death, was considered the most poisonous venom among the three.

LD_{50} study design:

To determine LD_{50} of *N.haje* snake venom, which was found to be the most poisonous one, rats were divided into 6 groups (5 rats each):

Group 1: received 0.5 mg/kg (LD_{100})

Group 2: injected with 0.25 mg/kg

Group 3: envenomed by 0.18 mg/kg

Group 4: received 0.12 mg/kg

Group 5: injected with 0.0625 mg/kg

Group 6: received 0.05 mg/kg (LD_0)

Design for neurological, behavioral, hematological, biochemical and histopathological studies:

15 rats were divided into 3 groups (5 rats each) as follows:

Group 1: injected with normal saline (0.9 % NaCl) served as control group

Group 2: received a single toxic non-lethal dose (0.08mg/kg) of *N.haje* venom dissolved in saline solution

Group 3: envenomed with a lethal dose LD_{100} (0.5mg/kg) of *N.haje* venom dissolved in saline solution

Teratogenicity study design:

Pregnant female rats were divided into 2 groups (5 rats each):

Group 1: control group recieved (0.9% Na Cl, i.p.)

Group 2: injected with toxic non-lethal dose (0.08mg/kg, i.p.) *N.haje* venom

2.4.2 Treatment procedure:

Behavioral, neurological changes:

Control and tested rats were injected and observed for any behavioral and neurological changes. Signs were recorded in observation sheet at time intervals: 15 min, 30 min and 60 min post injection.

For the control group and rats group that have received the toxic non-lethal dose (0.08mg/kg) blood samples were collected at 2 hours post treatment, whereas, group of rats that's envenomed with the lethal dose (0.5mg/kg) blood was collected just before death when the symptoms of toxicity became more serious.

Histopathological study:

Following the collection of blood samples, rats were sacrificed then liver and kidney were removed for histological examination.

Teratogenic aspects:

Toxic dose of *N.haje* venom (0.08 mg/kg) and saline solutions were injected into pregnant rats on day 7 of pregnancy (GD_7) then groups were observed for the continuity of pregnancy and after delivery fetuses were examined for external malformations.

2.5. Statistical analysis:

Data were statistically analyzed using SPSS software version 24 and presented as mean and standard error (SEM). Parameters of envenomed groups were compared to control group using one way analysis of variance (ANOVA). Results were considered significant at $P < 0.05$.

2.6. Ethical consideration:

Permission of this experimental study had been obtained from department of pharmacology, Faculty of Pharmacy, International University of Africa (appendix)

Chapter Three

Results

3. Results

3.1 Lethality of *Naja haje, Naja nubiae* and *Cerastes cerastes* snakes venom

Concerning the minimal lethal dose and time of mortality, table 3.1 shows that, the minimal lethal doses of *N.haje*, *N.nubiae* and *C.cerastes* are 0.1 mg/kg, 0.4mg/kg and 0.5 mg/kg respectively. Mean time of death for rats that's received *Naja haje* snake venom is 46 min ± 3 , 90 ± 15 min for the group that envenomated by *Naja nubiae* snake venom and 180 ± 20 min for group which injected by *Cerastes cerastes* snake venom.

3.2 Determination of the median lethal dose of (LD_{50}) of *Naja haje* snake venom

Table 3.2 reveals calculation of LD_{50} of Naja haje snake venom using karber method. LD_{50} was found to be equal to 0.28 mg\ kg

Table 3.1 Lethality of *Naja haje* , *Naja nubiae* and *Cerastes cerastes* snakes venom

Name of snake	**Dose (mg/kg)**	**Time of death** **(Mean± SEM)**
Naja haje	0.1mg/kg	46 ± 3 min
Naja nubiae	0.4mg/kg	90 ± 15 min
Cerastes cerastes	0.5mg/kg	180 ± 20 min

Table 3.2 (LD_{50}) of *Naja haje* snake venom

Group	**No of rats**	**Dose (mg/kg)**	**No of dead animals**	**Mortality %**	**Mean of death in 2 doses (B)**	**Dose difference (A)**	**(A*B)**
1	5	0.5	5	100	-	-	
2	5	0.25	2	40	3.5	0.25	0.875
3	5	0.18	1	20	1.5	0.07	0.105
4	5	0.125	1	20	1	0.055	0.055
5	5	0.0625	0	0	0.5	0.0625	0.031
6	5	0.05	0	0	0	0.0125	0
							$\sum$ 1.06

$LD_{50} = LD_{100} - \sum (A*B) / N$

$LD_{50} = 5 – 1.06/ 5,,$ $LD_{50} = 0.287$ mg/kg

3.3 Behavioral effects induced by Naja haje venom

Table 3.3 demonstrates the behavioral changes induced by *Naja haje* venom. Group 2 reported a decrease in motor activity at 30 min following the envenomation. Group 3 also recorded a decrease in motor activity which started within the first 15 min, while the control group showed absence of these behavioral changes.

3.4 Neurological effects induced by *Naja haje venom*

Table 3.4 shows that, both envenomed groups with the toxic and lethal dose of Naja haje venom had convulsion and paralysis when compared with the control group

Table 3.3 Behavioral effects of *N.haje* venom

Group number	Treatment groups	Dose (mg/kg)	Motor activity at different time intervals		
			15 min	30 min	60min
G1	Control (normal saline)	10ml/kg	0	0	0
G2	Toxic dose of *N.haje* venom	0.08mg/kg	-	-	0
G3	Lethal dose of *N.haje* venom	0.5mg/kg	-	-	Died

-: decrease in motor activity, 0: normal motor activity

Table 3.4 Neurological effects induced by *Naja haje venom*

Group number	Treatment groups	Dose (mg/kg)	Convulsions	Paralysis
G1	Control (normal saline)	10ml/kg	-	-
G2	Toxic dose of *N.haje* venom	0.08mg/kg	+	+
G3	Lethal dose of *N.haje* venom	0.5mg/kg	+	+

+ = Present, - = Absent

3.5 Hematological effects of *Naja haje* venom

3.5.1 Effect of *Naja haje* venom on RBCs

Table 3.5 presents that, the mean score of red blood cells count is 4.85 for group 1, 5.06 ± 0.1 for group 2 and 5.14 ± 0.14 for group 3. Statistical analysis shows no significant difference in red blood cells count between the 3 groups ($P > 0.05$).

3.5.2 Effect of *Naja haje* venom on hemoglobin

As shown on table 3.6, the mean value of hemoglobin level is 14.1 ± 0.76, 16 ± 0.6, 14.9 ± 1 for group 1, group 2 and group 3 respectively. Statistical comparison appears that, there is no significant change in the level of hemoglobin between the 3 groups ($P > 0.05$).

Table 3.5. Effect of *Naja haje* venom on RBCs

Groups No	Treatment groups	Dose mg/kg	RBCs Count Mean ± SEM (cell *10^6/mcl)	P value
G1	Control (normal saline)	10 ml/ kg	4.85 ± 0.1	-
G2	N.h venom (toxic dose)	0.08mg/kg	5.06 ± 0.1	> 0.05
G3	N.h venom (lethal dose)	0.5mg/kg	5.14 ± 0.1	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

Table 3.6. Effect of *Naja haje* venom on Hemoglobin

Groups No	Treatment groups	Dose (mg/kg)	Hemoglobin concentration Mean ± SEM (g/dl)	P value
G1	Control (normal saline)	10ml/kg	14.1 ± 0.76	-
G2	N.h venom (toxic dose)	0.08mg/kg	16 0.6	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	14.9 ± 1	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

3.5.3 Effect of *Naja haje* venom on WBCs

In regards to the count of white blood cells, table 3.7 appears that, the mean value of WBCS is 4.9 ± 0.34 for group 1, 7.2 ± 1.09 for group 2 and 12.5 ± 2.07 for group 3. Statistical analysis reports no significant difference in the mean of WBCs count between group 2 and group 1 ($P > 0.05$) where as a highly significant increase in WBCs count of group 3 is found when compared with group 1 ($P < 0.01$). There is a significant difference in the count of WBCs between group 3 and group 2 when compared statistically ($P < 0.05$).

3.5.4 Effect of *Naja haje* venom on neutrophils count

Table 3.8 reveals that, the mean of neutrophils count is 19.2 ± 1, 21.4 ± 1.5, 19.2 ± 0.1 for group 1, group 2 and group 3 consecutively. Statistical analysis shows no significant difference in the count of neutrophil between the 3 groups ($P > 0.05$).

Table 3.7 Effect of *Naja haje* venom on WBCs

Groups No	Treatment groups	Dose (mg/kg)	WBCs Count Mean ± SEM (cell*10^3/mcl)	P value
G1	Control (normal saline)	10ml/kg	4.9 ± 0.3	-
G2	N.h venom (toxic dose)	0.08mg/kg	7.2 ± 1.1	>0.05
G3	N.h venom(lethal dose)	0.5mg/kg	12.5 ± 2	<0.01** <0.05#

G2 and G3 were compared statistically to Group 1, G3 compared statistically to group 2

#P value <0.05 considered statistically significant

**P value <0.01considered statistically highly significant

Table 3.8. Effect of *Naja haje* venom on neutrophils count

Groups No	Treatment groups	Dose mg/kg	Neutrophils count Mean ± SEM (cells/mm^3)	P value
G1	Control (normal saline)	10 ml/kg	19.2 ± 1	-
G2	N.h venom(toxic dose)	0.08mg/kg	21.4 ± 1	>0.05
G3	N.h venom(lethal dose)	0.5mg/kg	19.2 ± 0.1	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

3.5.5 Effect of *Naja haje* venom on hematocrit

As seen in table 3.9, the mean score of hematocrit for group 1 is 46.2 ± 2, 40.4 ± 1 for group 2 and 48 ± 0.8 for group 3.the analysis didn't find significant difference in hematocrit level between the 3 groups ($P > 0.05$).

3.5.6 Effect of *Naja haje* venom on lymphocytes count

Table 3.10 illustrates the mean of lymphocytes count as following, 66.2 ± 1.7 for group 1, 62.8 ± 2.3 for group 2 and 66 ± 1.5 for group 3. Statistical comparison appears no significant change in the level of lymphocytes count between the 3 groups ($P > 0.05$).

Table 3.9. Effect of *Naja haje* venom on hematocrit

Groups No	Treatment groups	Dose (mg/kg)	Hematocrit %	P value
			Mean ± SEM (%)	
G1	Control(normal saline)	10 ml/kg	46.2 ± 0.34	-
G2	N.h venom (toxic dose)	0.08mg/kg	40.4 ± 1.2	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	48 ±0.8	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

Table 3.10. Effect of *Naja haje* venom on lymphocytes count

Groups No	Treatment groups	Dose (mg/kg)	Lymphocytes Count	P value
			Mean ± SEM (cells/mcl)	
G1	Control(normal saline)	10 ml/kg	66.2 ± 1.7	-
G2	N.h venom (toxic dose)	0.08mg/kg	62.8 ±2.3	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	66 ± 1.0.5	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

3.5.7 Effect of *Naja haje* venom on MCV

Data on table 3.11 present that, the mean score of MCV for group 1 is 83.6 ± 1.1, 82.2 ± 1.3 for group 2 and 87.8 ± 2 for group 3. When these means compared statistically, the analysis reports no significant change in the level of MCV between the 3 groups ($P > 0.05$).

3.5.8 Effect of *Naja haje* venom on MCH

As shown in table 3.12, the mean value of MCH for group 1 is 27 ± 0.7, 28 ± 0.4 for group 2 and 28.2 ± 0.8. There is no significant change in MCH level between the mean scores of the 3 groups ($P > 0.05$).

Table 3.11. Effect of *Naja haje* venom on MCV

Groups No	Treatment groups	Dose (mg/kg)	MCV Level	P value
			Mean ± SEM (femtoliters/cell)	
G1	Control (normal saline)	10 ml/kg	83.6 ± 1.1	-
G2	N.h venom (toxic dose)	0.08mg/kg	82.2 ± 1.3	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	87.8 ± 2	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

Table 3.12. Effect of *Naja haje* venom on MCH

Groups No	Treatment groups	Dose (mg/kg)	MCH Level	P value
			Mean ± SEM (pictograms/cells)	
G1	Control (normal saline)	10 ml/kg	27 ± 0.7	-
G2	N.h venom (toxic dose)	0.08mg/kg	28 ± 0.4	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	28.2 ± 0.86	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

3.5.9 Effect of *Naja haje* venom on MCHC

Table 3.13 demonstrates that, the mean score of MCHC level is 31.6 ± 0.6, 31.6 ± 0.7, and 30.18 ± 0.6 for group 1, group 2 and group 3 sequentially. Statistical analysis appears no significant difference in MCHC level between the 3 groups ($P > 0.05$).

3.5.10 Effect of *Naja haje* venom on monocyte

Data on table 3.14 exhibit that, the mean values of monocyte count is 16 ± 0.9 for group 1, 15.6 ± 1.1 for group 2 and 14.6 ± 0.74 for group 3. Statistical comparison reports no significant difference in the level of monocyte count between the 3 groups ($P > 0.05$).

Table 3.13. Effect of *Naja haje* venom on MCHC

Groups No	Treatment groups	Dose (mg/kg)	MCHC Level	P value
			Mean ± SEM (g/dl)	
G1	Control (normal saline)	10 ml/kg	31.6 ± 0.6	-
G2	N.h venom (toxic dose)	0.08mg/kg	31.6 ± 0.7	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	30.2 ± 0.6	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

Table 3.14. Effect of *Naja haje* venom on monocyte

Groups No	Treatment groups	Dose (mg/kg)	Monocytes (%)	P value
			Mean ± SEM	
G1	Control (normal saline)	10 ml/kg	16 ± 0.9	-
G2	N.h venom (toxic dose)	0.08mg/kg	15.6 ± 1.1	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	14.6 ± 0.74	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

3.5.11 Effect of *Naja haje* venom on eosinophil

Table 3.15 presents that, group 1, group 2 and group 3 have the same mean score of eosinophil count which is equal to 1 ± 0.

3.5.12 Effect of *Naja haje* venom on basophil

As seen on table 3.16 the mean of basophil count for the 3 groups is zero.

3.5.13 Effect of *Naja haje* venom on platelets count

Data on table 3.17 illustrate that, the mean value of platelet count for group 1 is 368 ± 19.4, 571 ± 81 for group 2 and 615.8 ± 40.5 for group 3. Statistical analysis shows, no significant difference between group 2 and group 1 ($P > 0.05$) and a significant increase in platelet count in group 3 when compared with group 1 ($P < 0.0$), while there is no significant difference in platelet count between group 3 and group 2 ($P > 0.05$).

Table 3.15. Effect of *Naja haje* venom on eosinophil

Groups No	Treatment groups	Dose (mg/kg)	Eosinophil Count	P value
			Mean ± SEM (cells/microliter)	
G1	Control (normal saline)	10 ml/kg	1 ± 0	-
G2	N.h venom (toxic dose)	0.08mg/kg	1 ± 0	-
G3	N.h venom (lethal dose)	0.5mg/kg	1 ± 0	-

Table 3.16. Effect of *Naja haje* venom on basophil

Groups No	Treatment groups	Dose (mg/kg)	Basophil Count	P value
			Mean ± SEM (cell/microliter)	
G1	Normal saline (control)	10 ml/kg	0	-
G2	N.h venom (toxic dose)	0.08mg/kg	0	-
G3	N.h venom (lethal dose)	0.5mg/kg	0	-

Table 3.17 Effect of *Naja haje* venom on platelets count

Groups No	Treatment groups	Dose (mg/kg)	Platelets Count Mean ± SEM (cells/microliter)	P value
G1	Normal saline (control)	10 ml/kg	368.8± 19.4	-
G2	N.h venom (toxic dose)	0.08mg/kg	571 ± 81	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	615.8 ± 40.5	<0.05*

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2*P value <0.05 considered statistically significant

3.6 Biochemical effects of *Naja haje* snake venom

3.6.1 Effect of *Naja haje* venom on AST enzyme

Table 3.18 reveals the mean values of AST enzyme level for the 3 groups in this manner, 33 ± 7.1 for group 1, 227 ± 5.5 for group 2 and 231 ± 2.3 for group 3. Statistical comparison between the means of AST enzyme level of the 3 groups reports a very highly significant increase in AST enzyme level of group 2 and group 3 when compared with group 1 ($P < 0.001$) and no significant in the level of AST in group 3 when compared with group2.

3.6.2 Effect of Naja haje venom on ALT enzyme

By looking at the data of table 3.19, the mean of ALT enzyme level is 23.8 ± 0.8, 60 ± 2.1 and 62 ± 0.4 for group 1, group 2 and group 3 consecutively. Statistical analysis demonstrates very highly significant increase in the level of ALT enzyme in group 2 when compared with group 1 ($P < 0.001$), moreover a very highly significant increase in ALT enzyme level of group 3 when compared with group 1 ($P < 0.001$), whereas there is no significant difference in the level of ALT enzyme between group 3 and group 2 ($P > 0.05$).

Table 3.18 Effect of *Naja haje* venom on AST enzyme

Groups No	Treatment groups	Dose (mg/kg)	AST activity Mean ± SEM (IU/L)	P value
G1	Normal saline (control)	10 ml/kg	33 ± 7.1	-
G2	N.h venom (toxic dose)	0.08mg/kg	227 ± 5.5	<0.001***
G3	N.h venom (lethal dose)	0.5mg/kg	231 ±2.3	<0.001***

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

***P value < 0.001 considered statistically very highly significance

Table 3.19 Effect of *Naja haje* venom on ALT enzyme

Groups No	Treatment groups	Dose (mg/kg)	ALT activity Mean ± SEM (IU/L)	P value
G1	Normal saline (control)	10 ml/kg	23.8 ± 0.8	-
G2	N.h venom (toxic dose)	0.08mg/kg	60 ± 2.1	<0.001***
G3	N.h venom (lethal dose)	0.5mg/kg	62.6 ± 0.4	<0.001***

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

***P value < 0.001 considered statistically very highly significance

3.6.3 Effect of *Naja haje* venom on ALP

As shown on table 3.20, the mean value of ALP is 226 ± 23 for group 1, 91 ± 51 for group 2 and 195 ± 97 for group 3. When these mean values compared statistically with each other, non-significant changes in ALP level between the 3 groups is found ($P > 0.05$).

3.6.4 Effect of *Naja haje* venom on serum urea

Data on table 3.21 show the mean values of serum urea level for the groups as following, 10.5 ± 0.66 for group 1, 33.4 ± 7 for group 2 and 43.4 ± 1.4 for group 3. Statistical analysis indicates a significant increase in the level of plasma urea of group 2 when compared with group 1 ($P < 0.05$) in addition to a highly significant increase in serum urea of group 3 when compared with group 1 ($P < 0.01$), while there is no significant difference in the level of urea between group 3 and group 2 ($P > 0.05$).

Table 3.20. Effect of *Naja haje* venom on ALP

Groups No	Treatment groups	Dose (mg/kg)	ALP activity Mean ± SEM (IU/L)	P value
G1	Normal saline (control)	10 ml/kg	226 ± 23	-
G2	N.h venom (toxic dose)	0.08mg/kg	91 ± 0.51	>0.05
G3	N.h venom (lethal dose)	0.5mg/kg	195 ± 97	>0.05

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

P value > 0.05 considered statistically not significant

Table 3.21 Effect of *Naja haje* venom on serum urea

Groups No	Treatment groups	Dose (mg/kg)	Urea Concentration Mean ± SEM (mg/dl)	P value
G1	Normal saline (control)	10 ml/kg	10.5 ± 0.66	-
G2	N.h venom (toxic dose)	0.08mg/kg	33.4 ± 7	<0.05*
G3	N.h venom (lethal dose)	0.5mg/kg	43.4 ±1.4	<0.01**

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

*P value < 0.05 considered statistically significant

**P value < 0.01 considered statistically highly significance

3.6.5 Effect of *Naja haje* venom on serum creatinine

Table 3.22 illustrates the mean scores of serum creatinine as follows, 0.38 ± 0.08, 0.92 ± 0.08 and 0.88 ± 1 for group 1, group 2 and group 3 respectively. Statistical analysis appears a highly significant increase in serum creatinine level of group 2 in comparison with group 1 ($P < 0.01$) as well as a highly significant increase in the serum creatinine level of group 3 when compared with group 1 ($P < 0.01$) in addition to no significant difference in the level of serum creatinine between group 3 and group 2 ($P > 0.05$).

3.7 Effect of *Naja haje* venom on blood glucose level

As seen on table 3.23, the mean value of the random blood glucose level is 120.5 ± 0.18 for group 1, 73.4 ± 12 for group 2 and 76.6 ± 10.4 for group 3. There is a significant decrease in in blood glucose level of group 2 when compared with group 1 ($P < 0.05$) furthermore a highly significant decrease in blood glucose level of group 3 when compared with group 1 ($P < 0.01$), while there is no significant change in blood glucose level between group 3 and group 2 ($P > 0.05$).

Table 3.22 Effect of *Naja haje* venom on serum creatinine

Groups No	Treatment groups	Dose (mg/kg)	Creatinine concentration Mean ± SEM (mg/dl)	P value
G1	Normal saline(control)	10 ml/kg	0.38 ±0.08	-
G2	N.h venom (toxic dose)	0.08mg/kg	0.92 ±0.08	<0.01**
G3	N.h venom (lethal dose)	0.5mg/kg	0.88 ± 0.1	<0.01**

G 2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2

**P value < 0.01 considered statistically highly significance

Table 3.23 Effect of *Naja haje* venom on blood glucose level

Groups No	Treatment groups	Dose (mg/kg)	Blood glucose concentration Mean ± SEM (mg/dl)	P value
G1	Normal saline (control)	10 ml/kg	120.5 ± 0.18	-
G2	N.h venom (toxic dose)	0.08mg/kg	73.4 ± 12	<0.05*
G3	N.h venom (lethal dose)	0.5mg/kg	76.6 ± 10.4	<0.01**

G2 and G3 were compared statistically to Group 1, G 3 compared statistically to group 2 ,

*P value < 0.05 considered statistically significant

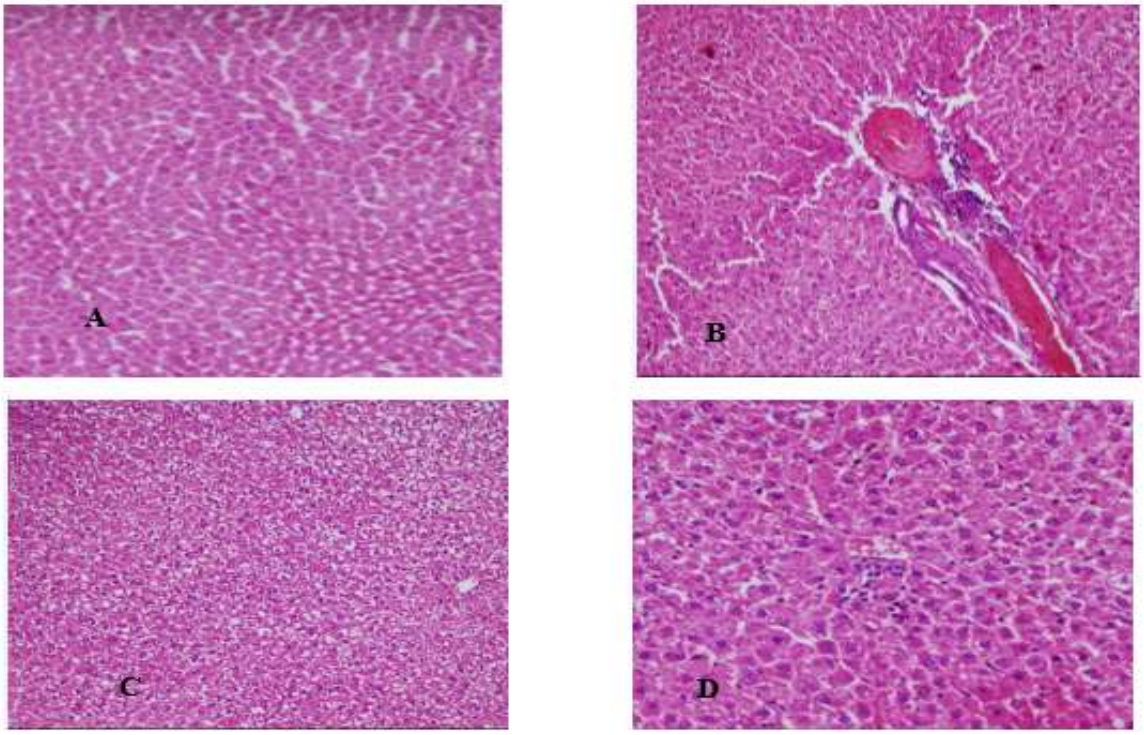
**P value < 0.01 considered statistically highly significance

3.8 Histopathological changes induced by Naja haje snake venom in Liver and Kidney rat tissues

3.8.1 Histolopathological changes induced by Naja haje venom in rat liver

As shown on image 3.1, image (A) liver of rat in the control group with normal morphological characteristics. Images (B-D), hepatic tissue of rat in the envenomated group, showed inflammatory response in liver indicated by cellular infilteration and hepatocytic swelling.

Data of microscopic observation in table 3.24 reveals absence of infiltertation of lymphocytes and hepatocytic swelling in the control group, whereas a mild infilteration of lymphocytes appears in group 2 and group 3, moreover there is a moderate swelling of liver cell in group 2 (++) and severe hepaotocytic swelling in group 3 (+++)



A: Normal liver picture

B: liver peri vascular inflammatory infiltration in the portal tract

C: liver with ballooning of hepatocytes

D: liver inflammatory infiltration in central area

Table 3.24 Histolopathological changes induced by *Naja haje* venom in rat liver

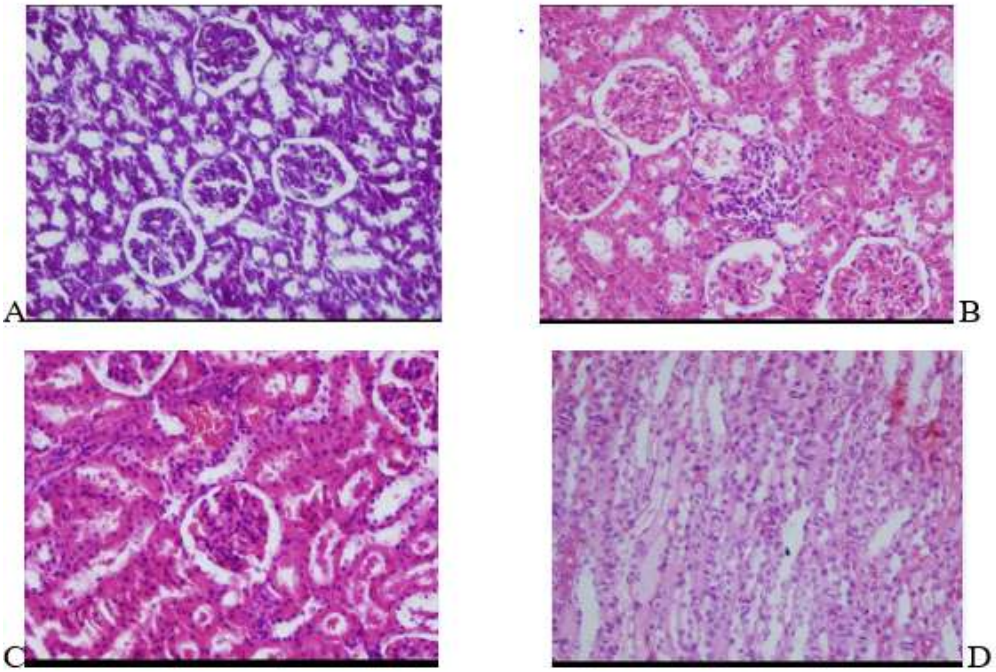
Microscopic Observation				
Group number	**Treatment groups**	**Dose (mg/kg)**	**Infilteration of Lymphocytes**	**Hepatocytic swelling**
G1	Control Normal saline	10ml/kg	0	0
G2	toxic dose N.h venom	0.08mg/kg	+	++
G3	lethal dose N.h venom	0.5mg/kg	+	+++

0: no change, +: mild, ++: moderate, +++: severe

3.8.2 Histopathological changes induced by Naja haje in rat kidney

By looking at image 3.2, image (A) shows untreated control kidney with normal architecture. Images (B-D) present histological changes in renal tissue of rat injected by Naja haje snake venom. Envenomated kidney appears with moderate inflammatory cellaular infilteration, damage of glomeruli, vacuolization of some kidney tubules and hydropic degeneration change.

Table 3.25 demonstrates the microscopic observation of the impact of Naja haje snake venom in rat kidney. There is a minor hydropic degeneration change, besides minor glomerular damage and moderate inflammatory cellular infilteration in group 2. Group 3 appears with mild tubular vacuolization, moderate hydropic degeneration change, furthermore presence of moderate glomerular damage and inflammatory cellular infilteration, whereas control group records absence of these histological changes.



A: Normal kidney picture

B: Inflammatory cellular infiltration

C: Glomerular damage

D: Hydropic degeneration change

Table 3.25 Histopathological changes induced by *Naja haje* in rat kidney

Microscopic Observation						
Group No	**Treatment groups**	**Dose (mg/kg)**	**Tubular Vacuoliza-tion**	**Hydropic Degeneratio n- Change**	**Glomerular Damage**	**Inflammato ry- Cellular Infilteration**
G1	Control Normal saline	10ml/kg	0	0	0	0
G2	toxic dose N.h venom	0.08mg/ kg	0	+	+	++
G3	lethal dose N.h venom	0.5mg/k g	+	++	++	++

0: no change, +: mild, ++: moderate

3.9 Effect of *Naja haje* venom on rat fetus

As shown on table 3.26, maternal weight at day 1 of pregnancy is 220 ± 10.5 for the control group and 220.5 ± 17 for the envenomated group. The number of fetuses born for each group is 0.5 ± 0.0, the mean of fetal weight is 4.2 ± 0.3 for group 1 and 4.3 ± 0.1 for group 2. Fetal death is zero in both groups in addition to absence of gross abnormalities. Statistical analysis shows no significant difference in the maternal weight at day 1 of pregnancy between group 1 and group 2 ($P > 0.05$) and no significant change in the fetal weight ($P > 0.05$).

Table 3.26 Effect of *Naja haje* venom on rat fetus

Group No	Treatment group/ Dose	Maternal Weight at day 1 of pregnancy	No of Fetuses Born	Fetal Weight(g)	Fetal death	Gross Abnormalities
		Mean±SEM		Mean±SEM		
G1	Control (normal saline 10ml/kg)	220 ± 10.5	5	4.2 ± 0.3	0	0
G2	N.h V 0.08 mg/kg	220.5 ± 17	5	4.3 ± 0.1	0	0
		P value >0.05		P value >0.05		

G2 statistically compared with G1

P > 0.05 considered statistically not significant

Chapter Four

Discussion,

Conclusion

And

Recommendations

4. Discussion, Conclusion and Recommendations

4.1 Discussion

Snake venom is a complex mixture consists of various substances such as enzymes, toxins, growth factor activators and inhibitors with a diversity of biological activities that result in multiple metabolic disorder, altering cellular and enzymatic activities in animals, besides releasing many pharmacological substances [25,37,38].

4.1.1 Lethality of (*Naja haje, Naja nubiae, Cerastes cerastes*) venom:

Generally, lethality of snake venom depends on a combination of its potency, volume of venom injected into the prey and the size of the victim. In the present study, time taken for death following envenomation with different snakes (*Naja haje, Naja nubiae* and *Cerastes cerastes*) considered as an indicator of the lethality of the venom.

Naja haje venom appeared to be the most poisonous one among the 3 snakes as the mean time of death for rats received this venom is 46 ± 3 min, *Naja nubiae* in the second place with mean time of death equal to 90 ± 15 min and *Cerastes cerastes* appeared to have the least lethality with mean time of death 180 ± 20 min.

These findings are supported by the facts that *Cerastes cerastes* (the horned viper snake) of family *viperidae* which is more hemotoxic than other families of snakes. Viper venom contains several components which promote or inhibit hemostatic mechanisms including coagulation, platelet function, fibrinolysis and vascular integrity [39]. Moreover, the bio distribution of viper venom from the site of injection to its numerous targets is supposably slow and partial, consistent with the fact that molecules in viper venom are medium and high molecular weight (20 – 60

KDa) compound that could have a longer residence time at the site of inoculation thereby reducing the venom bioavailability [40].

On the other hand, *Naja haje* and *Naja nubiae* (Cobras) are of *elapidae* family they are mainly neurotoxic. Neurotoxic venom tends to act more quickly [41] by affecting the nervous system, stopping transmission of nerve signals to muscles, heart and lungs causing death due to complete respiratory failure [42].

Cobra venom consist of small size molecules (MWT<15 KDa), such small molecules could have a shorter residence time at the site of injection causing an instant and complete bioavailability in the blood [40].

In the current study, the Egyptian cobra venom (*N.haje*) was found to have rapid lethality than the Nubian cobra venom. *N.haje* snake is a non-spitting cobra and its venom is mainly neurotoxic (act more quickly), whereas *Naja nubiae* is a spitting cobra that's have the ability to spit or spray venom from their fangs into the eyes or skin of the victim, spitting cobras are characterized by cytotoxic pattern of envenomation [43]. The abundance of cytotoxic PLA_2 and cytotoxins in the venom of *N.nubiae* is proposed to be the main factor for its clinical features of cytotoxicity.

Kazandjan et al.[44] stated that, most spitting cobra venoms are remarkably cytotoxic a part from the neurotoxic and cardiotoxic effects typical of other cobra species. So our findings are consistent with the general consideration that spitting cobras are primarily cytotoxic with little, if any neurotoxicity [45,46]. Furthermore, the rank of world atlas classified venom of *N.nubiae* to be less toxic than *N.haje* venom which supported results of this study.

4.1.2 Median lethal dose (LD_{50}) of Naja haje venom:

The estimation of snake venom lethal dose (LD_{50}) is an essential step for an accurate evaluation of the toxic activity of specific venom and is also frequently used to select the relevant anti-venom batch in addition to establish the neutralizing capacity of each vial, according to WHO, venom lethality is expressed as median lethal dose (LD_{50}) [47].

In this study, the LD_{50} of N.h venom was determined via intraperitoneal injection of the venom in rats by karber method, according to this method the approximate LD_{50} for *N.haje* snake venom was determined to be equal to 0.28 mg/kg. In the study of Robert et al. [48], the subcutaneous LD_{50} of N.h venom in mice was reported to be 1.75 mg/kg. Other finding obtained by Ghazala et al. [47] demonstrated that, the intravenous LD_{50} of the crude venom of N.h venom in rats was found to be 0.28 mg/kg, whereas LD_{50} equal to 0.25 mg/kg of *N.haje* venom by intramuscular injection into rats was found by Saeed et al. [31].

LD_{50} methods have many intrinsic variables and factors that affect the toxicity results; one of these factors is the route of administration. Lethality of the venom varies with change in the route of administration; generally the LD_{50} values obtained in animals injected intravenously were lower than those acquired by intraperitoneal and intramuscular injection [40,47].

Lethality of snake venom differs from species to species and even among individuals of the same species and this referred to multiple factors such as geographical location; most of the venom obtained from the same species with different geographical location has different LD_{50} values [47]. Furthermore, seasonality, age of snake, genetic variations, and health of the snakes could potentially affect the LD_{50} values of the venom.

The typical species of the targeted animals, its body weight and health also considered influencer variables which impact the lethality of the venom and the

values of the LD_{50}. In the study performed by R.M Douglas [49], to investigate the response of different animals to the same snake venom he found that, sheeps and horses are more susceptible to elapid venom than the other animals in the experiments (mice, guniea pigs and monkeys) and stated that different animals have different reactions to the same snake venom.

4.1.3 Behavioral aspects:

In the present study, both envenomed groups with two different doses of *N.haje* venom (toxic and lethal) showed decrease in motor activity at 15 and 30 minute time intervals (Table 3.3). This is referred to the action of snake neurotoxins which mainly intent the neuromuscular junction of skeletal muscle where the motor nerve terminal and the nicotinic acetylcholine receptors at the motor endplate are the dominant target sites.[50]

Behavioral manifestations after snake bites have been mentioned in several case histories each involving varying degrees of motor weakness.[51]

Comparing findings from different studies is difficult as there is a lack in the published experimental works studied the effect of *N.haje* venom on the motor activity of rats.

4.1.4 Neurological aspects:

Neurotoxicity is a common feature of envenomation by elapids such as *Naja* species. Acute neuromuscular paralysis is the principal type of neurotoxicity and it's an important cause of mortality and morbidity related snake bite.

In the current study, group of rat that received the lethal dose of the venom (0.5 mg/kg) showed severe convulsions then peripheral paralysis within 15 min of

envenomation, whereas the group that treated by the toxic dose (0.08 mg/kg) exhibited moderate convulsion and peripheral paralysis after 30 min of the envenomation.

The neurotoxic effects of the Egyptian cobra are referred to the presence of highly potent á neurotoxins which are post synaptic neurotoxins that bind and antagonize the nicotinic acetylcholine receptor at the neuromuscular junction resulting in systemic paralysis, respiratory failure and death [52,3]. In similar context Esmat et al.[29], illustrated that N.h venom is highly lethal due to its content of alpha neurotoxin that's kill by paralyzing respiratory muscles in few minutes.

Convulsions are one of the known preparalytic symptoms and signs after envenomation.

Campbell [53] revealed that, the onset of paralysis after envenomation with cobra venom has been shorter than its onset of bites from other snakes such as Australian and new quinea elapid.

4.1.5 Hematological aspects:

In the present study, some hematological parameters had been measured to assess the hematological alterations in the envenomed rats.

Regarding the count of white blood cells, there was a highly significant increase ($P < 0.01$) in the group rats received the lethal dose (0.5 mg/kg).

The main function of WBCs as phagocytes is to defend against xenobiotic or invading microorganisms. WBCs departure from reservoir of bone marrow to the blood under circumstances like trauma, stress, fever or a medical agent administration [54,55]. Lifshitz et al. [56] assumed that, a sympathetic effect as a result of the stress experienced by the victims could release temperorarly WBCs from the marginal pools. Another suggestion is the venom can release inflammatory cytokines from macrophages. Maes et al. [57] stated that, snake bites could be considered as a type of stress exposure that leads to disputation in the production of WBCs and has an effect on different blood constituents.

The finding of the elevation of WBCs following a lethal dose of *Naja* species is in agreed with the study of Riaz et al. [58] and Alsadoon et al. [59].

In this study, insignificant increase in the count of white blood cells was observed in the group of rats that's injected with the toxic or sub lethal dose (0.08 mg/kg) which is in agreement with Azza et al. [22] who reported that, injection of a sub lethal dose of *Naja.haje* venom exhibited non-significant elevation in the WBCs.

On the other hand, high significant decrease in WBCs in mice treated subcutaneously with ½ LD_{50} of *N.haje* cobra venom ,was observed by Shaker et al. [30] and Riaz et al. [58] whom reported that, the observed leukopenia was apparently an effect of peripheral destruction of cells in reticulo endothelial system or liver impairment.

Differential count of white blood cells (lymphocytes, neutrophils, eosinophil, basophil and monocytes) showed non significant difference ($P<0.05$) in both

treated groups, which agreed with Shaker et al. [30] who reported that, injection of sub lethal doses of N.h venom in albino mice showed insignificant change in the count of neutrophils, monocytes and eosinophil. Similar findings obtained by Riaz et al. [58].

In our study, hemoglobin, red blood cells count and erythrocytes indices (MCH, MCHC, MCV) exhibited in significant change in both envenomed groups. This result is compatible with the study of Sarang et al. [61] who stated that, hematological parameters such as HB, RBCs, and its indices weren't affected by venom injection.

Significant decrease in RBCs was found by Azza et al. [22] and Shaker et al. [30] they attributed this reduction due to the stress of envenomation which wasn't observed in the present study. Diversely, a significant increase in RBCs obtained by Abdou et al.[62] and Riaz et al. [58] whom assumed the elevation in erythrocytes could be due to liver failure or kidney impairment.

The present study revealed a significant increase in platelets count in the group treated with the lethal dose. This thrombocytosis may describe the initiation of clotting process responding to bleeding and intravascular hemorrhage. This finding is in consistence with Shaker et al. [30] and Riaz et al. [58].

Fluctuation of platelets count with time was observed by Alsaadon et al. [59] and hypothesized this fluctuation during the experiment might indicate the clotting process had arised to resist hemorrhage or bleeding then reduced parallel to RBCs.

4.1.6 Biochemical aspects:

Owing to its fundamental anatomy, ability to clear xenobiotics from blood and high metabolic potential, the liver represents an organ with a high susceptibility to toxic effects of toxicants. [63]

Liver enzymes are the main biomarkers in assessing hepatic injury. Levels of AST and ALT enzymes are important in monitoring and evaluating liver inflammation and necrosis that's result in release of these enzymes into the circulation due to increased cell membrane permeability and destruction of the cells [64].

In the current study, the Egyptian cobra was found to induced high significant increase ($P<0.01$) in the activity of AST and ALT in both injected groups and this attributed to breakdown of hepatic cellular organelles and intracellular release of these enzymes [65]. Elevation in AST and ALT levels could be attributed to the presence of a hepatotoxic agent in the snake venom [66,67].

This finding is similar to those of Shaker et al. [30] who demonstrated that, biochemical parameters of injected animals with two different doses of *N.haje* venom showed high significant increase in the levels AST and ALT. Further studies supported our findings were performed by Tohamy et al. [38] and Algurashi et al. [68], the latter attributed the elevation of AST and ALT in rats envenomed with *N.haje* cobra venom to damage of liver cells. Significant increase in the levels of AST and ALT also found by Elamir et al. [22] and Esmat et al. [29].

Regardless of the differences in dose, route of administration, time post envenomation and species all the studies of the effects of snake envenomation showed elevation in AST and ALT levels which indicated that liver is the primary target organ of snake venom.

Results of the present study appeared that, the Egyptian cobra venom was found to produced significant increase in the concentration of urea in both groups with more significant increase ($P<0.01$) in group injected with the lethal dose than group

treated with the toxic dose ($P<0.05$), in addition to a highly significant increase ($P<0.01$) in creatinine concentration in both injected groups.

Levels of urea and creatinine in blood have been found to be a fairly reliable indicator of kidney function. Abnormally high levels of these two parameters worn of possible failure or malfunction of the kidneys. [69]

Snake venom enzymes specially phospholipase A_2 and protease, initiate inflammatory processes that include generation of vasoactive mediators and proinflammatory cytokines resulting in renal and systemic hemodynamic alterations. [70]

Nephrotoxicity of snake venoms have been attributed to three mechanisms, hemodynamic effects of the venom, direct nephrotoxicity and immunologic reaction [71].

Significant high levels of urea and creatinine followed receiving of *N.haje* venom is in agreement with Tohamy et al. [38], who reported that injection of *N.haje* venom induced a significant disturbance in kidney function. In the similar context, Al-quraishy et al. [68] claimed that, venom of the Egyptian cobra caused an impairment of renal function as indicated by high levels of urea and creatinine in rats. Study of Saaeid et al. [31] by using two different doses ($_{1\2}$ $LD_{50,}$ LD_{50}) of *N.haje* venom referred the significant elevation in levels of urea and creatinine to a nephrotoxic effect of the cobra. Moreover our findings are incompatible with the studies result of Shaker et al. [30], Esmat et al. [29] and Elamir et al. [22].

Another possibility for the increase in serum urea level in envenomed groups might refer to an increase of nitrogen retention and/or due to corrupted renal function [72].

4.1.7 Change in blood glucose level:

In the current study, the Egyptian cobra venom was found to induce hypoglycemia in both envenomed groups. This reduction in blood glucose level indicates a disturbance in carbohydrate metabolism which could be as a result of some endogenous insulin-releasing effect of venom components [(59)].

Stress from envenomation could be another factor in reducing the level of blood glucose, such stress might occur as direct effect of the venom stimulating the release of insulin or indirectly by inhibiting the release of catecholamine due to exhaustion or blockade of the adrenal sympathetic supply [(73)]. Furthermore, hypoglycemia following N.h venom injection has been referred to be due to either an increased out-pouring of insulin or to an inhibition of the diabetogenic factors of the anterior pituitary and suprarenal cortex [(74)]. In similar context, the study of the effect of the LD_{50} of desert cobra on rats by Alsaadon et al. [(59)], revealed a significant decrease in blood glucose level of the envenomed rats. Additionally Riaz et al. [(58)], demonstrated a reduction in in blood glucose concentration at 1 hour after envenomation with *Naja* cobra and hypothesized that, the observed hypoglycemia could be due to the stimulatory of phospholipase A on the facilitated transport of glucose, attributing that the venom exhibits kinetic resembling insulin. [(75)]. Differently, several studies revealed the finding that, different types of snake venoms are responsible for hyperglycemia in different species (Esmat et al.[(29)], Ezzat et al.[(47)] ; Omran et al.[(77)] and Sarang et al.[(61)]) whom reported that, hyperglycemia is probably due to mobilization of glycogen in the liver and muscles by a direct inhibition of glucokinase or indirectly by stimulating the release of epinephrine which increase the breakdown of glycogen [(78)].

4.1.8 Histopathological aspects:

Liver damage is among the common and most serious signs of cobra snake envenomation [79]. In the present study injection of two different doses of the Egyptian cobra venom (toxic and lethal dose) produced histopathological alteration.

The histopathological effects of *Naja haje* cobra venom appeared that, livers of the group of rats that received toxic dose of venom (0.08 mg/kg) revealed mild infiltration of lymphocytes and moderate swelling of hepatocytes, whereas the other group that received a lethal dose of the venom (0.5 mg/kg) exhibited severe hepatocytes swelling and moderate infiltration of the cells. The hepatocytes swelling and cellular infiltration might indicate a status of inflammation of liver. Walter and Israel [80] proved that, the appearance of infiltrating cells is a feature of inflammation.

Cellular swelling might be referred to the action of venom phospholipase enzyme, which causes perturbation of the cell membrane permeability with consequent influx of Na^+ and water [81]. Chethankumar and Srinives [82] reported that, the exposure of cellular membranes to *Naja haje* venom phospholipase enzyme significantly decrease the Na^+/ K^+ ATPase activities, thereby altering the ionic gradient, disorganizing membrane lipid bilayer and finally leads to cell death.

As stated in Mukharjee and Maity [83], the progression of hepatic cellular swelling together with the effect of the venom phospholipase on the membranous phospholipids during envenomation might be one of the factors responsible for the rupture of hepatic cell membranes and the occurrence of cellular damage.

The microscopical observations of liver rats in this study agreed with that of Tohamy et al. [38] who reported that, administration of ½ LD_{50} of *Naja haje* venom on mice had been found to cause a severe inflammatory response of the liver which

indicated by inflammatory cellular infiltration, hepatocytic swelling besides cytoplasmic vacuolation.

Rahmy and Hemmaid [84] reported that, cobra venom induced a hepatotoxic action which reflected by histological and histochemical alterations of the hepatic tissues.

Several studies revealed that, *Naja haje* envenomation causes cellular swelling, cytoplasmic vacuolization and granulation, besides intrahepatic hemorrhage, liver necrosis and hyperplasia of kupffer cells. [84,86,87,38,85]

The alterations in liver structure of envenomated rats in this study are confirmed with the elevation of liver enzymes.

Results of the present work demonstrate that, a range of histopathological alterations in renal tissues had been caused by the venom of *N.haje* snake.

Mild hydropic degeneration change, mild glomerular damage and moderate inflammatory cellular infiltration were appeared in renal tissues of group rats that received toxic dose of the venom (0.08 mg/kg). While, renal tissues of rats envenomed with the lethal dose (0.5 mg/kg) showed a mild tubular vacuolization, moderate hydropic degeneration change, glomerular damage and inflammatory cellular infiltration.

Renal injuries following envenomation could be due to either direct or indirect effect of venom constituents [88]. The direct effects mainly referred to presence of venom components that's have an acute effects on the function a long with organization of the renal tissue, since the indirect action might be due to the deadly effect generated by reactive metabolites or mediators produced in the kidney during envenoming [89].

Chang [90], interpreted the appearance of inflammation and congestion in kidneys on the basis of the fact that, renal excretion is the main route of excretion of elapid toxins. Furthermore, considering the glomerular and renal tubule epithelial cells are strategically intervened between the extra and intramilien, they are probable

targets for numerous nephrotoxic agents additionally glomeruli are the first structure of the nephron to come in contact with the circulating venom. As long as toxic substances are circulated throughout the body with blood flow and whole blood samples of higher organism are filtered in kidney, then, renal injury considered one of the most common and serious symptoms of cobra envenomation.[91]

Results of the current observations were in agreement with the findings obtained by Tohamy et al. [38] who reported appearance of inflammatory cellular infiltration, vacuolization in tubules and shrinkage of glomeruli in most cases in renal structure of envenoming mice injected with ½ LD_{50} *N.haje* venom. Similar findings obtained by AL-Mamun et al. [91] who reported that, *N.naja* caused inflammatory cellular infiltration, tubular vacuolation, and shrinking of glomeruli in renal tissues of envenomed mice.

Several types of lesions (tubular, glomerular, interstitial or vascular) were also recorded by Skaker et al. [30] and Scheemann et al. [92].

4.1.9 Fetal aspects:

During pregnancy snake bites could be a risk to gestation maintenance which depend on the pregnancy period, maternal envenomation degree a long with the time of the beginning of envenomation [93-95]. Although snake bite envenomation during pregnancy not being entirely elucidated, the exposure to that venom during this period could lead to fetal growth retardant, teratogenic effect and mutation [95].

Present results showed that *N.haje* venom administered at toxic dose (0.08 mg/kg) on GD_7 didn't induce abortion nor external anomalies. The fetuses appeared with normal skull form, ears, eyes, mouth, tail, foot conforming and anal drilling when compared with the control group. Furthermore, there was non significant change in fetal weight between the two groups.

After extensive searching and reading, experimental records about the effect of *N.haje* snake venom on the continuity of pregnancy and induction of external malformations of fetuses weren't found in literature.

Study of Maria et al. [32] to investigate the effect of *Bthrops jaraka* snake venom on pregnant mice on GD_5 (Gestation Day) reported that, experimental groups revealed presence of external malformation, yet, it occurs in just one fetus what couldn't be related to venom exposure.

It remains uncertain whether snake venom crosses the placenta, although indirect evidence of placental transfer has been already represented in cases where adverse fetal effects occurred in the absence of adverse maternal effects.[96-98]

4.2 Conclusion

Results obtained in this study can be summarized as:

- ❖ Among the three snakes venom (Naja haje, Naja nubiae, Cerastes cerastes) Naja haje is the most poisonous snake with the lowest minimal lethal dose rapid onset of lethality
- ❖ Further toxicological studies conducted on N.haje venom :
  - The LD_{50} of N.haje snake venom is equal to 0.28mg/kg
  - Neurotoxicity of the venom presented as paralysis and convulsions in both lethal and toxic dose
  - Lethal and the toxic dose of the venom induced hypoglycemia
  - Both lethal and toxic doses of venom induced liver and renal dysfunction reflected by elevation in levels of liver enzymes (AST,ALT) and kidney parameters (urea, creatinine)
  - The two doses (lethal, toxic) caused pathohistological alterations in liver and kidney tissues.
  - Leukocytosis, thrombocytosis are the hematological effects of the lethal dose of venom
  - The toxic dose didn't induce gross abnormalities

Finally, it can be concluded that, lethal and toxic doses of Naja haje snake venom are neurotoxic, hepatotoxic and nephrotoxic. While the lethal dose of the venom had an additional hemotoxicity.

4.3 Recommendations:

1. Toxicological effects of *Naja haje* snake venom on other organs (heart, brain, lung and spleen) should be studied
2. Fractionation of the components of *Naja haje* snake venom
3. Further toxicological studies concerning the two other snakes (*Naja nubiae* and *Cerastes cerastes*) presented in this study should be conducted

References

References

1. Casarett and Doulls. Toxicology: The basic science of poisons. Fourth edition. New York, USA. Pergaren Press. 1991

2. Joseph P. Kozlovac, Robert J. Hawley. Biological Toxins: Principles and Practices, 5th edition. Washington,USA. ASM Press. 2016.

3. Eljaoudi R, Larreche S, Chakir S et al. snake bites in morocco: progress and challenges. Toxicology and toxic effects. 2019. 3(1):009-014

4. Casarett and Doulls. Essential of Toxicology. Third edition. New York, USA. McGraw Hill/ Medical. 2015

5. Corkill N.L. Notes on Sudan snakes. Sudan government museum (natural history) puplication. 1935

6. Kent R. Olson, Illen B. Anderson, Neal L. Benowitz et al. Poisoning & drug overdose. Sixth edition. California, USA. Mc Grow-Hill companies .2012

7. Oukkache N, Lalaoui M, Ghalim N. General characterizationof venom from the Moroccan snakes Daboia mauritanica and Cerastes cerastes. Journal of venom animal toxins including tropical disease. 2012, 18(4):411-420

8. Makran B, Fahmi L, Pla D, Sanz L et al. snake venomics of Daboia mauritanica from Morocco and assessment of the para-specific immunoreactivity of an experimental monospecific and a commercial antivenoms. Journal of Proteomics. 2012, 75:2431-2441

9. Greim H, Synder R. Toxicology and Risk Assessment: A Comprehensive Introduction, 2nd edition. Hoboken, USA.Wiley. 2018

10. Wuster W, Wolfgang, Wallach et al. In praise of subgenera: taxonomic status of cobras of the genus Naja Laurenti (Serpentes: Elapidea). Zootoxa. 2012, (1):26-36.

11. Mastenbroek R. Captive care of the Egyptian cobra Naja haje.Litteratura Serpentium. 2013, 34 (2):200-215

12.Juan J Calvete. Naja haje- General Details, Taxonomy and Biology, Venom, clinical effects, treatment, firstaid, antivenoms. Clinical toxicology resource. 2012, 25(3):44-62

13. Joubert, FJ, Taljaard N. Naja haje (Egyptian cobra) venom. Some properties and the complete primary structure of three toxins (CM-2, CM-11 andCM-12). European journal of biochemistery. 1978, 90(2):359-367

14. Fey Dr, Grieg B. Sub-cutaneous LD_{50s}. Australian venom research unit. University of Queensland. 2012, 9(3):27-36

15. Bogert, C.M. (1943). Dentitional phenomena in cobras and other elapids with notes on adaptive modifications of fangs. Bulletin of the American museum of natural history. 81(3):285-360

16. Wuster, W.& Broadly D.G . A new species of spitting cobra (naja) from north-eastern Africa (Serpentes: elapidae). Journal of zoology, London. 2003, 259 (4):345-359

17. Khalid H, Mukhtar M, Konstantakopoulos N. Cytotoxicity of naja nubiae (Serpentes:Elapidae) and Echis ocellatus (Serpentes:Viperidae) venoms from sudan. Journal of toxins. 2015, 10(4):1-7

18. Mallow D, Ludwig D, Nilson G. True Vipers: Natural History and Toxinology of Old World Vipers. Florida, USA. Malabar. 2003

19. Spawls S, Branch B. the dangerous snakes of Africa. Dubai: ralph Curtis books. California, USA.Oriental press. 2020

20. Salman M.A. Effects of Different doses of Cerastec cerastes crude venom on biochemical parameters in serum of Guinea pigs at different times. Biological Forum – An International Journal. 2014, 6(2):329-339

21. Abdalla S, Bilto Y, Disi A. Effects of Sand viper (Cerastes cerastes) venom on isolated smooth muscle and heart and on hematological and cardiovascular parameters in the guinea-pig. Toxicon. 1992, 30(10):1247-55

22. ELamir A.M, Mohamed S.G, Shaker L.S et al. Studies on venoms of the Egyptian cobra (NAJA HAJA), the hornes viber (CERASTES CERASTES), and the honey bee (APIS MELLIFERA): comparison safety study for 1/10 $LD_{50.}$ Journal of the Egyptian society of parasitology. 2020. 51 (1): 201-212

23. Al-Sadoon M.K, Diab M.S, Bauomy A.A et al. Cerastes cerastes gasperettii venom induced hematological and oxidative stress in male mice. Journal of Pure and Applied Microbiology. 2014, 8:693-702

24. Saleh S.M, Rashad N. Toxicological effects of viper Cerastes cerastes crude venom on serum indices of male albino rats. The Egyptian Journal of Forensic Science and Applied Toxicology. 2015, (2):173-182

25. AL-Sadoon M.K, Abdel Moneim A, Diab MS et al. Hepatic and renal tissue damages induced by Cerastes cerastes gasperettii crude venom. Life Science Journal. 2013, 10(4): 191-197

26. Petras D, Sanz L, Segura A et al. Snake venomics of African spitting cobras: toxin composition and assessment of congeneric cross-reactivity of the pan-African EchiTAB-Plus-ICP antivenom by antivenomics and neutralization approaches. Journal of Proteome Res. 2011, 10(3):1266-1280

27. Saad A, Abdel-Rahman M, Alaa EL-Deen M etal. The Effect of Venom of Egyptian Spitting Cobra Naja nubiae on Vascular Permeability of Hepatic and Renal Tissues. Avicenna journal of medical biochemistry. 2021, 9(1):22-25

28. AL-Guraishy S, Dkhail M, Esmat A. Hepatotoxicity and oxidative stress induced by Naja haje crude venom. Journal of venomous animals and toxins including tropical diseases. 2014, 8(4):20-42

29. Shaban E, Hafez M. Ability of gamma-irradiated polyvalent antivenin to neutralize the toxicity of the Egyptian cobra (Naja haje) venom. The Egyptian journal of hospital medicine. 2003, 13: 135-152

30. Shaker L, Osama A. Abbas, Sayed O et al. Physiological effects of Egyptian cobra venom on albino mice and its antibacterial effect. International journal of recent scientific research. 2018. 9 (5):26997-27007

31. Saeid A, Mukhtar Z. Effect of Naja haje (Egyptian cobra) crude venom-induced oxidative stress on the kidney of Wister albino rats. Global Libyan journal. 2021, 5(2):1-20

32. Martha M, Kirsten T, Juliani D et al. Maternal exposure to Bothrops jararaca snake venom: effects in mice offsprings. Veterinary medicine. 2011. 29(3):209-13

33. Ghosh M.N. Fundamentals of Experimental Pharmacology, second edition. Calcutta, India. Scientific Book Agency. 1984

34. Ishak K, Baptista A, Bianchi L et al. Histological grading and staging of chronic hepatitis. Journal of Hepatol. 1995, 22(4):696-699

35. AL-Harbi MM, AL-Shabanah OA, AL-Gharably MA, Islam MW. The effect of maternal administration of enalapril on fetal development in the rat. Research communications in chemical pathology and pharmacology. 1992. 77:347-358

36. EL Tahir KEH, AL Tahir AY and Ageel AM. Pharmacological studies on sesame and and Nigela sativa fixed oils: effects on the sensitivities of the adrenoceptors, baroreceptors, platelets and uterus of the rat. Saudi Pharm. J. 1999. 7:205-215

37. Cherifi F, Laraba-Djebari F. Isolated biomolecules of pharmacological interest in hemostasis from Cerastes cerastes venom. Journal of venomous animals and toxins Including tropical disease. 2013, 19(11):1-6

38. Tohamy A.A, ALY F.M, Abdel Moneim A E et al. biological effects of Naja haje crude venom on the hepatic and renal tissues of mice. Journal of king saud university-science. 2014, 26:205-212

39. John W. Harvey. Veterinary hematology: A Diagnosteic Guide and Color Atlas. First edition. Florida, USA. Saunders.2012

40. Oukkache N, EL Jaoudi R, Ghalim N et al. Evaluation of the lethal potency of scorpion and snake venoms and comparison between intraperitoneal and intravenous injection routes. Toxins. 2014. 6: 1873-1881

41. Hifumit T, Sakai A, Kondo Y et al,. Venomous snakebites: Clinical diagnosis and treatment. Journal of Intensive Care. 2015, 3(1):1-9

42. Bogert, C.M. (1943). Dentitional phenomena in cobras and other elapids with notes on adaptive modifications of fangs. Bulletin of the American museum of natural history. 81:285-3608

43. World Health Organization. Guidelines for the prevention and clinical management of snakebite in Africa. WHO regional office for Africa, Brazzaville, Congo, 2010, http://www.afro.who.net.

44. Kazandjian T.D, Petras D, Robinson S.D et al. convergent evolution of pain-inducing defensive venom components in spitting cobra. Science. 2021, 371(6527):386-390

45.Warrell D.A. Clinical toxicology of snakebite in Africa and the middle east/Arabian peninsula handbook of clinical toxicology of animal venoms and poisons. Florida,USA. CRC Press. 1995.

46. Chippaux J.P. Les serpents d"Afrique occidentale et central, IRD edition, paris, France. 2006

47. Parveen G, Ali H, UL Hassan H. Lethal dose (LD_{50}) of the big four snakes-a mini review. International journal of molecular biology. 2018. 3(2):76-77

48. Ian D, Robert L. The global snakebite crisis-a public health issue misunderstood not neglected. Wilderness and Environmental Medicine. 2009,20(1):43-56

49. Douglas R.M. Which are the most poisonous snakes in south Africa and the world. CULNA national museum magazine. 1994. 47:12-13

50. Silva A, Wayne C, Geoffrey K. Antivenom for neutomuscular paralysis resulting from snake envenoming. Toxins. 2017, 9(4):143-160

51. Sanmuganathan P S. Myasthenic syndrome of snake envenomation: a clinical and neurophysiological study. Journal of Postgrad Medicine. 1998, 74(876):596-599

52. Barber CM, Isbister GK, Hodgson WC. Alpha neurotoxins. Toxicon. 2013, 66 (7):47-58

53. Campbell CH. The effects of snake venoms and their neurotoxins on the nervous system of man and animals. Contemp Neurol Ser. 1975, 12:259-293

54. Otitoju, Nwamarah, J. U, Otitoju, G.T.O et al. Effect of Moringa olifera aqueous leaf extract on some hematological indices in Wistar rats. Chemical and Process Engineering Research. 2014, 4(4):45-56

55. Thrall. ML, Wieser. G, Allison. RW et al. Veterinary Hematology and clinical Chemistry, 2nd edition. Chichester,UK. Wiley-Blackwell 2012.

56. Lifshitz M, Maimon N, Livant S. W.aegyptia envenomation in a 22-year-old female: Acase report. Toxicon. 2003, 41(4):535-537

57. Maes M, Van der Planken M, Van Gastel A. Influence of academic stress on hematological measurements in subjectively healthy volunteers. Psychiatry Res. 1998, 80(3):201-212

58. Riaz Z, Zaman M.Q, Ullah Z, Rehman H et al. Bio-Physiological effcts of LD_{50} of crude venom of black cobra (Naja Naja karachiensis) in mice. The journal of animal &plant sciences. 2015, 25 (5):1344-1348

59. AL-Sadoon M.K, Fahim A, Salama S.F et al. The effects of LD_{50} of Walterinnesia aegyptia crude venom on blood parameters of male rats. African journal of microbiology research. 2012, 6(3):653-659

60. ELamir A.M, Mohamed S.G, Shaker L.S et al. Studies on venoms of the Egyptian cobra (NAJA HAJA), the hornes viber (CERASTES CERASTES), and the honey bee (APIS MELLIFERA): comparison safety study for 1/10 $LD_{50.}$ Journal of the Egyptian society of parasitology. 2020. 51 (1): 201-212

61.Lakhmale S, Acharya R.N, Sulakshan S et al. A protective effect of Symphorema polyandrum wight seeds against Naja naja venom- pharmacological evaluation. Indian journal of natural products and resources. 2016, 7(4):328-333

62. Abdou R.H, Ibrahim A.E. Effects of Egyptian cobra (Naja haje) venom on post mortem changes and some biochemical parameters in rats. International journal of scientific research. 2015, 4(1):186-190

63. Michael J, Eric R etal. The liver as atarget for chemical-chemical interactions. Advances in pharmacology.2008,4(43):37-63

64. Abdel Moneim. AE, Othman. MS, Mohmoud SM et al. Pomegranate peel attenuates aluminium induced hepatorenal toxicity. Toxicology mechanisms and methods. 2013, 23(8):624-633

65. Mohamed A.H, EL-Serougi M.S, Hnna M.M. Observations on the effects of Echis carinatus venom on blood clotting. Toxicon. 1969, 6(3):215-219

66. Sant S.M, Rembe V.S, Salgaonkar D.S. Lesions produced by Echis carinatus venom in experimental animals. Postgrad. Med. 1974,20(2):70-79

67. Temb V.S, Sant S.M and Purandare N.M. A clinic-pathologic study of snake bite cases. Journal of Postgrad. Med. 1975, 21(1):36-47

68. Al-Quraishy S, Dkhil M.A, Esmat A. Hepatotoxicity and oxidative stress induced by Naja haje crude venom. Journal of venomous animals and toxins including tropical diseases. 2014. 20(1):42-52

69. Michael C, Duane S, Sabina A et al,. Association of creatinine and creatinine clearance on presentation in acute myocardial infarction with subsequent mortality. Journal of the American College of Cardiology. 2003, 42(9):1535-1543

70. Sitprija V. Animal toxins and the kidney. Nature clinical practice and nephrology. 2008, 4(11): 616-627

71. Stiprija V and Boonpucknavig V. Snake venoms and nephrotoxicity. Berlin, Germany. Springer-Verlag. 1979

72. Abdel-Nabi IM. Effect of crude Cerastes cerastes venom and fraction B on the clinical biochemical parameters of white rat. Journal of Egyptian German Society of Zoology. 1993, 10(2):315-326

73. Abu Sinna G, AL-Zahaby AS, Abd EL-Aal A et al. The effect of the viper Cerastes cerastes venom and venom fractions on carbohydrate metabolism. Toxicon. 1993, 31(6):791-801

74. Mohamed AH, EL-Serougy MS, Kamel AM. Effect of W.aegyptia venom on blood glucose in diabetic animals. Toxicon. 1965, 3(2):163-165

75. Blecher M. Effects of insulin and phospholipase A on glucose transport across the plasma membrane of free adipose cells. BBA-Lipid Met. 1967, 137(3):557-571

76. Ezzat A.R and EL-aal A.A. Effect of Naja haje venom on adrenal activity in rabbits. Qatar University. Sci. 1989, 9(1):169-176

77. Omran M.A, Abdel-Nabi I.M, EL-Naggar M.H. Serum biochemical and hormonal parameters for the toxic effects of Egyptian Cobra (Naja haje) envenomation. Toxins. 1997, 6(1):69-83

78. Mohamed A.H, Saleh A.M, Ahmed S et al. Histopathological effects of Naja haje snake and a venom gland extract of the scorpion Buthus quinquestriatus on the liver, suprarenal gland and pancreas of mice. Toxicon. 1978,16(3):253-262

79. Adzu M.S, Abubakar K.S, Gamaniel. Effect of Annona senegalensis root bark extracts on Naja nigricollis venom in rats. Journal of Ethnopharmacol. 2005,96(3):507-513

80. Walter J.B, and Israel M.S. Cells response to injury in: general pathology, 6th edition. Churchill Livingstone, Edinburgh, London. 1978

81. Segelke BW, Nguyen D,Chee R et al. Structures of two novel crystal forms of Naja naja phospholipase A2 lacking Ca^2 reveal trimeric packing. Journal of molecular biology. 1998, 279(1):223-232

82. Chethankumar M, Srinivas L. Gangliosides as potential inhibitors of Naja naja venom PLA_2 induced human erythrocyte membrane damage. Afr J Biochem Res. 2008,2(1):8-14

83. Mukherjee AK, Maity CR. The composition of Naja naja venom samples from three districts of West Bengal, India. Comp Biochem Physiol A Mol Integr Physiol. 1998, 119(2):621-627

84. Rahmy T.R and Hemmaid K.Z. Histological and histochemical alteration in the liver following intramuscular injection with a sub-lethal dose of the Egyptian cobra venom. Toxins. 2000, 991):21-32

85. Abdou R, Ibrahim A. Effects of Egyptian cobra (Naja haje) venom on post mortem changes and some biochemical parameters in rats. International journal of scientific research. 2015. 4 (1): 186-189

86. Nanayakkara DP, Ratnayake RMP, Ranasinghe JG. Histopathological changes on brain, kidney and liver of mice following intramuscular administration of krait venom. Ceylon journal of science (Biological Sciences). 2009, 38(1):1-10

87.Ghani LMA, EL-Asmer MF, Abbas OA & Rahmy. Histological and immunohistochemical studies on the nephrotoxic effects of Najanigriocollis snake venom. Egyptian Journal of Natural Toxins. 2010, 7(1):29-52

88. Martins AM, Monteiro HS, Junior EO et al. Effects of Crotalus durissus cascavella venom in the isolated rat kidney. Toxicon. 1998, 36(10):1441-1450

89. Niesink RJM, de Vries J, Hollinger MA. Toxicology principles and applications. CRC Press, Inc. and Open University, Netherlands. 1996

90. Chang CC. The action of snake venom on nerve and muscle in: Snake venoms, springer-verlag Berlin, Heidelberg, New York. 1979

91. AL-Mamun M.A, Hakim M.A, Zaman M.K et al. Histopathological alterations induced by Naja naja crude venom on renal, pulmonary and intestinal tissues of mice model. British Biotechnology Journal. 2015, 6(3):119-125

92. Schneemann M, Cathomas R, Laidlaw ST et al. Life-threatening envenoming by the Saharan horned viper (Cerastes cerastes) causing micro-angiopathic haemolysis, coagulopathy and acute renal failure. Clinical cases and review. 2004, 97(11):717-727

93. Zugaib M, Barros ACSD, Bittar RE et al. Abrupuio placentae following snake bite. Am J Obstet GynecolInvest. 2004, 57(2):114-6

94. Pardal PPO, Mazzeo T, Pinheiro ACL. Snake bite in pregnancy: a preliminary study. Journal of venom animal toxins. 1997, 3(2):280-6

95. Pantanowits L, Guidozzi F. Management of snake and spider bite in pregnancy. Obstet Gynecol Survey. 1996, 51(10):615-20

96. Langely RL. A review of venomous animal bites and stings in pregnant patients. Wilderness Environ Med. 2004, 15(3):207-15

97. Brown SA, Seifert SA, Rayburn WF. Management of envenomations during pregnancy. Clin Toxicol (Phila).2013, 51(1):3-15

98. Habib AG, Abubakar SB, Abubakar IS et al. Envenoming after carpet viper (Echis ocellatus) bite during pregnancy: timely use of effective antivenom improves maternal and foetal outcomes. Trop Med Int Health. 2008, 13(9):1172-5

Appendices