ANTITRYPANOSOMAL EFFECT OF CRUDE SCORPION VENOM EXTRACT IN *TRYPANOSOMA EVANSI* INFECTED SWISS ALBINO RATS

BY

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ABSTRACT

Despite the control measures against trypanosomiasis over the past few decades, the disease still contributes to the current economic losses and socio-political crises in the sub-Saharan Africa. The recent interest of chemotherapists in aggressive search for novel antitrypanosomal drug agents informed this current investigation to elucidate the acute toxicity and antitrypanosomal efficacy of crude scorpion venom in *Trypanosoma evansi* infected rats. Eighty (80) medium sized scorpions, brownish in colour with robust metasoma ending in telson were collected from their habitat in Minna metropolis. Venom was collected by electric stimulation of each scorpion telson. Acute toxicity of the venom was established through oral injection of the scorpion venom using graded concentrations (10, 20 and 30 mg/kg body weight) with saline extract of crude venom. The antitrypanosomal bioassay of the venom was performed against established infection in *Trypanosoma evansi* infected rats. Group 1 was set up as negative control of 0.2ml normal saline /kg/body weight, group 2 as 5mg chloroquine /kg/body weight and group 3 to 5 as 10, 20 and 30 mg/kg/body weight of the scorpion venom. Acute toxicity showed an LD_{50} extrapolated to be 44.72 mg/kg body weight. Antitrypanosomal bioassay results showed scorpion venom significantly ($P<0.05$) reduced the level of parasitemia of the infected rats, with peak activity recorded on the last day of observation, a pattern distinctly different from the positive control and total parasite clearance was recorded on the 5^{th} day in the group treated with 20 and 30 mg/kg body weight venom concentration having 0.00 and 0.00 ± 0. Treatment of infected rats with scorpion venom extract significantly ($P<0.05$) ameliorated loss in some body enzymes tested. The crude venom extract also promoted fall in Packed Cell Volume of *T. evansi* infected rats, promoted body weight loss for the infected untreated group having 71.23 ± 1 against the initial weight of 123.45 ± 1 and elongated the survival time of *T. evansi* infected rats. Treatment of the infected rats with scorpion venom at 20 and 30 mg/kg body weight significantly restored the liver and spleen to body weight ratio to a level comparable with the normal rats. Findings from this study suggest that scorpion venom posses a potent and promising source of antitrypanosomal agent.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Human African Trypanosomiasis (HAT) is a neglected tropical disease that occurs in sub-Saharan Africa, within the distributional limits of tse-tse fly vector. Two forms of the disease exist (Feyera *et al.,* 2011; Mulaw *et al.,* 2011). The slow-progressing form, caused by *Trypanosoma brucei gambiense*, is found in western and central Africa. The fast progressing form, caused by *Trypanosoma brucei rhodesiense,* is found in Eastern and Southern Africa (Simarro *et al.,* 2010). A person can be infected for months or even years without major signs or symptoms of the disease, when more evident symptoms emerges the patient is often already in an advanced disease stage where the central nervous system would have been affected (WHO, 2018). It is classified as neglected due to the limited amount of research and control funding allocated to it compared with malaria, HIV and tuberculosis.

African Animal Trypanosomiasis also called "nagana" a Zulu word for powerless/useless parasitic disease caused by *T. b. brucei, T. b. rhodesiense, T. congolense* and *T. simiae* in pigs (Loker & Hofkin, 2015). *Trypanosoma evansi* causes 'surra' a disease in camel, rats, horses and buffalo (Reid, 2002). Trypanosome that causes serious economic losses in livestock from anaemia, loss of condition and effects on reproduction, losses in cattle are especially prominent. Animals other than livestock including dogs can be affected. Untreated cases can be fatal, and the mortality rate is high in some outbreak (Auty *et al.,* 2015).

Sleeping sickness and nagana have a great economic impact in Africa because it affects the settlement patterns of people including land use and farming (National Tsetse and Trypanosomiasis Investigation and Control Centre, 2016).

World Health Organization (WHO) in 2010 attributed trypanosomiasis as the deadliest disease in the world. The treatment of this menace has since been a burden because of the adverse drug reactions that most drugs present when given to the patients (Ugoji *et al.,* 2014).

The immune response of animals could be unable to eliminate trypanosomes completely, and the animals could become inapparent carriers. These inapparent infections can be reactivated if the animal is stressed, transplacental transmission can also occur (Center for Food Security and Public Health, 2017).

Human African Trypanosomiasis (HAT) is still reported from more than twenty (20) countries in Africa where it causes serious morbidity among the affected rural populations, and it continues to pose the threat of severe epidemics (Franco *et al.,* 2014).

The highest effect of trypanosomiasis however has been its impact over land use systems that have been adopted in the Luangwa valley in Zambia. In-depth knowledge is required to identify specific circumstances where win-win trade off can be achieved between the conservation of biodiversity and the reduction of the disease in the human population (Anderson *et al.,* 2015).

In Nigeria, as in some other western African countries, *Trypanosoma brucei gambiense* is the etiology of sleeping sickness or Human African Trypanosomiasis (Akish *et al.,* 2016).

The second stage called meningoencephalitic of the infection is usually severe, affecting the cerebrospinal unlike the first stage hemolymphatic stage which is usually less severe fluids of humans clinical presentations include dullness, intermittent somnolence and apparent confusion.

Also, intention tremor in all limbs and myoclinic jerks are often seen (Onyebiguwe *et al.,* 2010; Akish *et al.,* 2017).

Trypanosomes causing Human African Trypanosomiasis are classically transmitted by the bite of blood sucking tsetse flies (Diptera, genus *glossina*). *T. b. gambiense* can also be transmitted congenitally (Rochas *et al.,* 2004; Lindner & Priotto 2010; Lestrade-Carluer *et al.,* 2016).

Ever since the creation of mankind, humans have been battling with the challenge of arthropods such as flies, spider, scorpions etc. Scorpions are predatory arachnids and members of the order Scorpiones, they have long been known for their painful, venomous, fatal sting and for their specific morphology (Dehghani, 2015). Identifying various species of scorpion and their biological, nutritional and ecological features has become a common practice in zoology and is used in scientific research in different field such as genetics, physiology, ecology and medicines (Dehghani, 2008; Dehghani *et al.*, 2016).

Scorpions are the most primitive venomous, arachnids of the order Scorpiones (Ortiz *et al.,* 2015; Zhang *et al.*, 2015). They belong to the class Arachnida of phylum Arthropoda (Zhang *et al.,* 2015), have eight legs and are easily recognized by the pair of grasping pedipalps and the narrow, segmented tail, often carried in a characteristic forward curve over the back, ending with a venomous stinger (Ortiz *et al.*, 2015). Scorpions range in size from 9mm/0.3 in (*Typhlochactas mitelli*), 23cm/9 in. (*Heterometrus swammerdami*).

Venom produced by scorpion has attracted researchers because of its toxic properties which has help to modify our understanding about various biological phenomenon, it is a combination of various bioactive compounds which are been used in investigatory study of Pharmaceutical

research (Oukkache *et al.,* 2008). It possesses immunosuppressive, cytotoxic, apoptogenic and antiproliferative effects (Zargan *et al.,* 2011).

Scorpion venom is a rich source of biomolecules, which can cause uneasy physiological activity on envenomation and may also have therapeutic potential (Ding *et al.,* 2014). Despite of its numerous negative effects, it contains many biologically active components which are being used for the development of drugs in Pharmaceutical industries (Andreotti *et al.,* 2010; AbdulRahman *et al.,* 2016; Zhang & Zhang 2016).

Being the oldest venomous species, scorpion had been extensively studied for venom production (AbdulRahman *et al.,* 2016). Venom differs in composition from species to species and their effectiveness varies due to the changes in their toxins associated with environmental and genetic variations such as climate and diet (Rodriguez-Ravelo *et al.,* 2013; Pucca *et al.,* 2014).

The current chemotherapy of HAT relies on only six drugs (Suramin, Pentamidine, Melarsoprol, Eflorinithine, Arsobal and Mel B), five of which were developed more than 30 years ago (Shiferaw *et al*., 2015). Others such as Homidium, Isometamidium and Diminazene aceturate are used in animal infections. Each of these drugs has one or more of these challenges: expensive, not available to the populace that are greatly affected, highly toxic and need trained personnel for its administration. The continued use of the same trypanocides for years has resulted in drug resistance that has been largely responsible for the current chemotherapeutic failures (Geerts *et al*., 2010; Shiferaw *et al*., 2015). The poor prospect for a vaccine due to antigenic variation of the parasite is further compounded by unwillingness of the Pharmaceutical industry to develop new compounds because of uncertain and unprofitable, market or perhaps the localized nature of the disease.

1.2 Statement of the Research Problem

In spite of the efforts in the past few decades for the control and management of trypanosomiasis, the infection still pose great threat causing 50,000 to 500,000 mortality yearly. Being a neglected tropical disease, trypanosomiasis has added to heavy economic losses and socio-geopolitical crises, especially in the sub-Saharan Africa. Besides, efforts to develop novel vaccine against the disease still remained in the embryo. Hence, the disease still exerts devastating effect on life stock and human health. The exploitation of plant based remedy is facing challenges of ineffectiveness and parasite resistance problem.

1.3 Justification for the Study

The problem facing the effectiveness of the available antitrypanosomal drugs including their cost implication, unavailability to the populace, high toxicity and also lack of expertise for the administration call for need for a better alternative. Additionally, the current antitrypanosomal drugs have developed resistance which calls for the need to search for better alternatives. This problem has been earlier attributed to the main source of the drugs, chiefly plant. Therefore there is need to explore other natural product for their possible potentials against such disease.

1.4 Aim and Objectives of the Study

This study aimed at evaluating the antitrypanosomal efficacy of scorpion venom extract on *Trypanosoma evansi* infected Albino Rat.

Objectives of the study were to:

i Determine the acute oral (LD_{50}) toxicity of the venom on *Trypanosoma evansi* infected Albino Rat.

ii Determine the effect of scorpion venom extract in *Trypanosoma evansi* infected rats.

iii Assess the effect of scorpion venom extract on the body weight and haematological parameters of infected rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

Human African Trypanosomiasis (HAT) is a neglected tropical disease that occurs in sub-Saharan Africa within the distributional limit of the tsetse fly vector (Simarro *et al.,* 2010). Trypanosome causing HAT is basically transmitted by the bite of blood sucking tsetse flies in the order Diptera and genus *Glossina*. *Trypanosoma brucei gambiense* can also be congenitally transmitted (Rochas *et al.,* 2004; Lindner & Priotto 2010; Lestrade-Carluer *et al.,* 2016).

African Animal Trypanosomiasis (Nagana) is a parasitic disease that as well causes serious economic losses in livestock from anaemia, loss of condition and effects on reproduction. Loss in cattle are especially prominent, animal other than livestock such as dogs, pigs can also be affected. Trypanosomes are protozoan parasites in the family Trypanosomatidae. *Trypansoma brucei gambiense, trypanosoma brucei rhodiense* are the group of parasites causing sleeping sickness in man in west and central Africa. *Trypanosoma brucei brucei*. The difference between this disease and African animal trypanosomiasis is that these two organisms can evade the innate resistance humans possess against other tsetse transmitted African trypanosomes (Auty *et al.,* 2015).

2.1 Parasite of Trypanosomiasis

Trypanosoma brucei belongs to the trypanosomatide, a family consisting of exclusively parasitic organisms found world-wide in vertebrates and insects, these unicellular parasites have co-evolved with their hosts to such an extent that most of them are commensal rather than pathogenic (Steverding, 2008). The species *Trypanosoma brucei* includes three morphologically indistinguishable subspecies *Trypanosoma brucei brucei*, which causes nagana in livestock, is

not infective to humans. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodiense* both which affect humans as they developed the ability to resist apoliprotein A, a serum protein that triggers death on other trypanosomes (Vanhollebeke & Pays 2010; Pays & Vanhollebeke, 2016). *T. brucei* cells contain one central nucleus, one single mitochondrion with its own DNA comprising the kinetoplast situated at the posterior end of the cell and a flagellum attached to the cell by an undulating membrane. During its life cycle, alternating between a mammal and an insect (tsetse fly) host, *T. brucei* remains extracellular and undergoes important metabolic adaptations reflected by morphological changes. In the blood and tissue of mammals, trypanosomes can be observed as spindle shaped cells, 20-30um long, 2-5um wide and characterised by their wriggling movement. The interplay between immune response of the phost and antigenic variation of the parasite results in irregular fluctuations in parasitemia, reflected by irregular fevers accompanying destruction of trypanosomes (Lejon *et al.*, 1998; Bisser *et al.*, 2000; Lejon *et al.*, 2010).

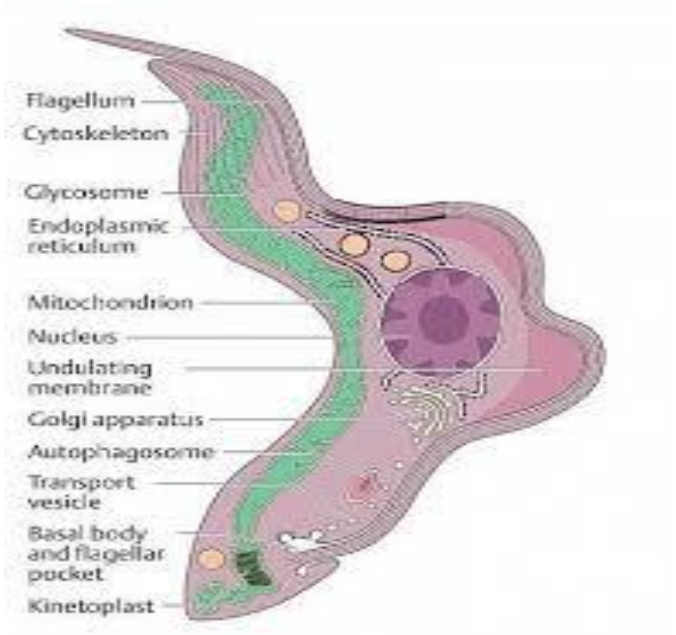

Figure 1: ***Trypanosoma evansi***

Source: (Rjeibi *et al*, 2015)

2.2 Intermediate Host of Trypanosome

The intermediate host and vector of African trypanosomes are species of the tsetse fly (*Glossina* spp.). Tsetse flies become infected when taking a blood meal containing trypomastigotes from an infected human. The parasites undergo asexual reproduction in the fly gut, transforming from procyclic trypomastigotes to epimastigotes. These then move to the salivary glands where they transform into the infective stage to humans, the metacyclic trypomastigote.

Humans are infected when an infected tsetse fly injects metacyclic trypomastigotes with the saliva into the blood while feeding. These then become trypomastigotes and multiply in the blood and other fluids, including the spinal fluid, which leads to disease. The primary habitat for tsetse flies is in vegetation around water sources. Humans are the primary host for *T.b. gambiense* whereas wild game mammals may serve as reservoir hosts for *T.b. rhodesiense*. Another subspecies, *T.b. brucei* infects wild game mammals but does not infect humans.

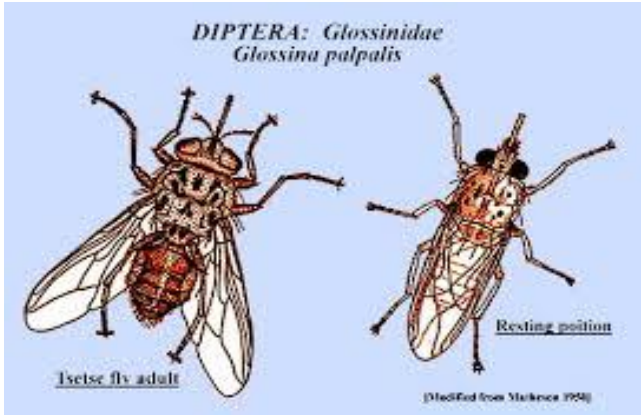

Figure II: *Glossina palpalis* the intermediate host of the parasite
Source: (Rjeibi *et al*, 2015)

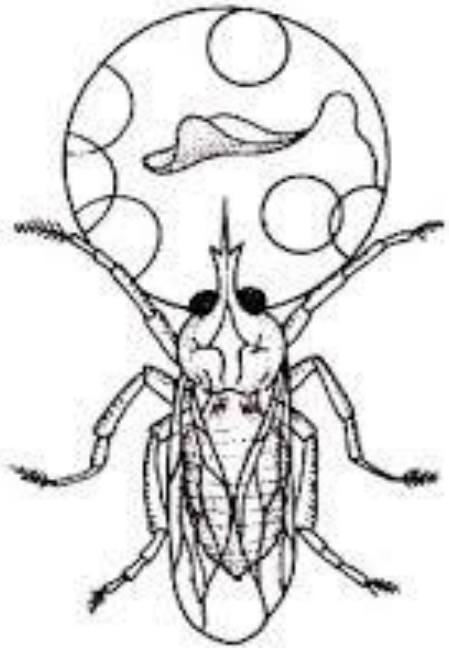

Figure III: *Glossina palpalis* releasing the parasite together with its saliva
Source: (William, 2019)

2.2.1 Life cycle of trypanosomes

During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes, are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission. The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. The cycle in the fly takes approximately 3 weeks.

Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*.

2.3 Distribution of Trypanosomiasis in Africa

In Africa, trypanosomiasis occurs in 37 countries including Nigeria, extending over 10million square kilometres which are about a third of the continent. It is recorded in this region that an estimated 50million cattle, about 30% of Africa's total cattle population, are vulnerable to the infection. Africa produces about Seventy (70) times less animal protein per hectare than Europe, and this is on the account of widespread incidence of trypanosomiasis (FAO, 2016).

2.4 Clinical Features of Human African Trypanosomiasis

The clinical manifestations of HAT depend on the parasite subspecies, host response and disease stage. Variations of virulence and pathogenicity have been attributed to different parasite strains. Both form of the disease generally lead to death if untreated, although healthy carriers and self-cure have been described for *gambiense* HAT (Jamonneau *et al.,* 2012). *Rhodesiense* HAT is typically acute, progressing to second stage within a few weeks and to death within six months (Checchi *et al.,* 2008). *T.b gambiense* HAT follows a chronic progressive course, with a mean duration estimated at three (3) years. The disease goes through two stages, first hemolymphatic stage followed by a second meningoencephalitic stage when trypanosomes cross the blood brain barrier and invade the central nervous system (CNS). Neurological disturbances, including sleep disorder, are typical of the second stage; however, most signs and symptoms are common to the two stages.

First stage *gambiense* present predominantly with long-lasting intermittent fever (day1 to week 1, separated by intervals of days or months), headache, pruritus and lymphadenopathy (mainly

posterior cervical, but also possible in the axillar, inguinal and epitrochlear regions).less frequent features are hepatosplenomegaly, edema and endocrine dysfunction (amenorrhea, infertility, miscarriage in women, reduced libido, impotence in men.

In the second stage, neuropsychiatric disorders add to the first-stage features, while fever becomes less frequent. The characteristic sleep disorder, which elicited the name sleeping sickness, consists of daytime somnolence plus sudden overwhelming sleep urges, and nocturnal insomnia. Polysomnographic records show a disruption of the sleep-wake cycle with frequent, short, sleep-onset episodes that occur equally during day and night (Buguet *et al.,* 2001; Mpandzou *et al.,* 2011 Njamnshi *et al.,* 2012). Other neurological signs comprise hyper-or hypo-tonicity, tremor of hands and fingers and choreiform, movements of limbs or trunk, fasciculation, motor weakness, ataxia and speech disorder (Blum *et al.,* 2006).

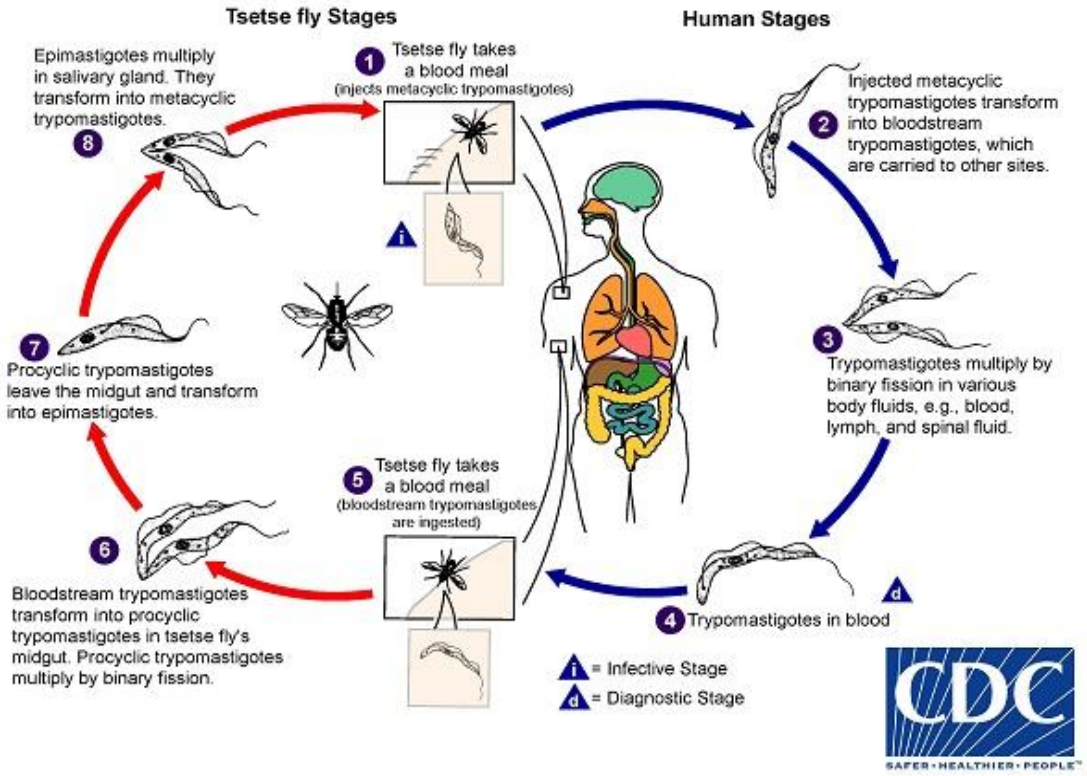

Figure IV: Diagramatic representation of trypanosomiasis
Source: (Centre for Disease Control, 2017)

2.4.1 Treatment of trypanosomiasis

The common chemotherapy drugs available for the treatment of trypanosomiasis or sleeping sickness are suramin, pentamidine, melarsoprol, eflornithine, diminazine aceturate and isomethamidium chloride. The high cost of these drugs and development of resistance has necessitated the search for alternative options with antitrypanosomal activity which could be effective in the treatment.

Trypanosomes possess variant surface Glycoprotein (VSG) which constitutes a characteristic molecular interface between the protozoan and the human host immune system, thereby invading

breakdown by complement alternative pathway (Musa *et al.*, 2015). The first two are used to treat the first stage, and the remaining three are used to treat the second stage of the disease. HAT drugs are not affected by the challenge of counterfeit because they are donated by WHO. The earlier HAT is treated the better the prospects of treatment tolerability and cure. Drugs for the first stage will generally not cure a second stage, and second stage drugs are not justified in first stage because of their toxicity and cumbersome logistics. Treatment of second stage requires drugs that cross the blood-brain barrier and such drugs tend to be toxic and complicated.

2.4.2 Control and elimination of human African trypanosomiasis

In the absence of vaccine or chemoprophylaxis, HAT is controlled through case detection and treatment and also to an extent vector control. For *gambiense* HAT the most effective control strategy is case finding and treatment, which reduces the human reservoir and thus decreases transmission. Cases are detected through active screening campaigns by mobile teams, consisting of up to eight (8) persons travelling in vehicles or boats and through passive screening in fixed health structures, (Simarro *et al.,* 2014). Diagnosis and treatment are resource intensive and require specific training, which is difficult to ensure in all countries and all endemic areas. In current elimination context, it is also important to reinforce passive surveillance, integrating it in the general healthcare system and focusing on self-presenting patients (Mitashi 2014; Franco *et al.,* 2014). Despite recent advances, the elimination process faces many challenges: reaching populations living in or fleeing from areas of civil unrest; sustaining the commitment of national authorities; partners and donors; clarifying and necessary addressing the asymptomatic human carrier; overcoming the limitations of the current diagnostic and treatment tools; developing tools and criteria to monitor, verify and validate HAT elimination at different geographical scales.

2.5 Classification of Trypanosoma

Kingdom: Protista

Phylum: Protozoa

Subphylum: Sarcomastigophora

Class : Kinetoplastea

Order: Kinetoplastida

Families: Trypanosomatidae,

Genus: *Trypanosoma*

Sub genus: *Trypanozoon brucei*

Species: *T.evansi* (salivaria species)

Source: (Hamilton *et al.*, 2004)

2.6 Habitat of Scorpion

Species of *Buthus* live in semi-arid to arid climate in various terrains, from mountain valleys to coastal plains mostly with sparse vegetation, even in deserts. As most scorpions they are predominantly nocturnal and hide in shallow burrows, most commonly below stones. (Hamilton *et al.*, 2004)

2.7 Geographical Distribution of Scorpion

The evolutionary history of scorpions goes back to the Silurian period 430million years ago. They have adapted to a wide range of environmental conditions and can now be found on all continents except Antarctica (Ruming *et al.*, 2010). They did not occur naturally in Great Britain, Ireland, Japan, South Korea, New Zealand and some of the Islands in the Oceania, but now have been accidentally introduced in some of these places by Human trade and commerce.

In addition to desert habitats, they have adopted to temperate, subtropical and tropical environments such as forest, savannas and grassland.

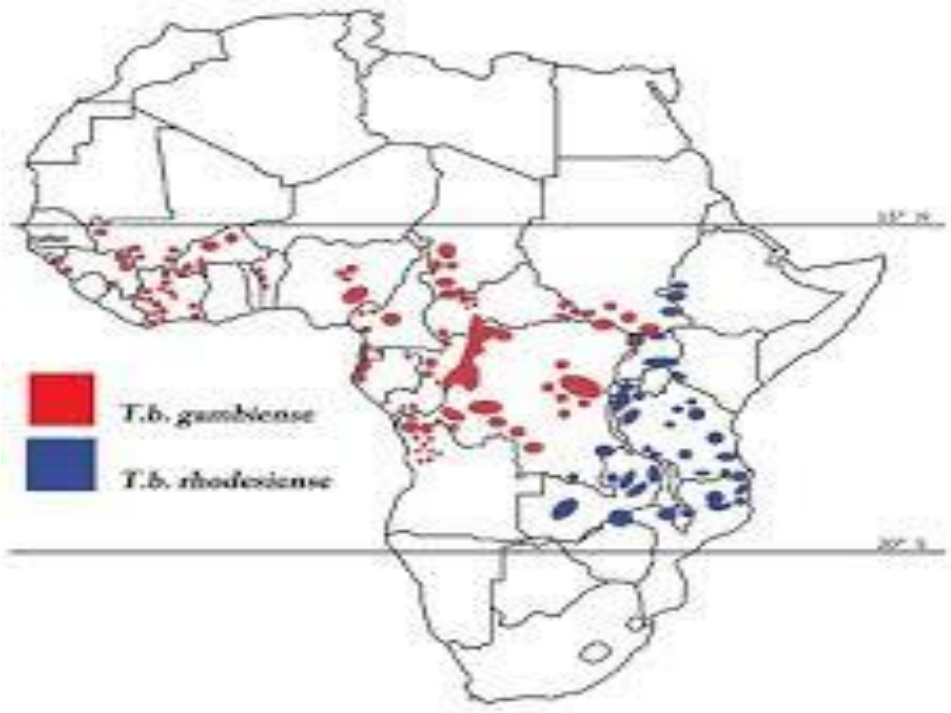

Figure V: Locations where *Trypanosoma brucei gambiense* & *T.b. rhodesiense* are found in Africa
Source: (Gibson, 2007)

2.8 Scientific Classification of Scorpion

One thousand Seven hundred and fifty (1750) species of scorpion have been described (František, 2009) with Eighteen (18) living families recognized to date, out of them fifty (50) species are considered as more poisonous (Bawaskar & Bawaskar, 2012).

Among all the families Buthidae is considered as the most fatal, poisonous and medically important family for its therapeutic properties (Michael & Victor 2003).

This classification is based on that of Soleglad & Fet 2003, which replaced the older, unpublished classification of Stockwell (1989). Additional taxonomic changes are from papers by Soleglad *et al.,* (2005). Being the oldest venomous species scorpion has been studied extensively for its venom. The toxic and pharmacological effect of the scorpion venom is largely due to its enhanced source of numerous small molecules and peptides. Several new drug targets have been identified from the scorpion venom through various researches, venoms of scorpions belonging to Buthidae family are considered most toxic and medically important for its curative potentials (Soleglad *et al.,* 2003).

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Chelicerata

Class : Arachnida

Subclass : Dromopoda

Order: Scorpiones

Families: Buthidea

Genus: *Hottentota*

Source: Solegad *et al.*, 2003)

2.9 Medicinal Importance of Scorpion Venom

The medical effect of venoms is important because of its complex bioactive components that are characterized by its high level of specificity. A reasonable number of studies have investigated and established the effects of snake, scorpion and bee venoms as effective tools in cancer therapy development (Vyas *et al.,* 2013; Ortiz *et al.,* 2015; & Zhang *et al.,* 2015). The scorpion venom peptides and toxins have been reportedly used to treat various diseases like cardiovascular diseases, acute and chronic convulsions, tetanus, subcutaneous nodules, HIV, epilepsy, brain tumor, human leukemia cell lines, male impotency, kidney tumor, prostrate tumor, breast cancer, skin cancers, pancreatitis and rheumatism (Wang *et al.*, 2005; Gupta *et al.,* 2007; Zargon *et al.,* 2010; Chen *et al.,* 2012; Sarzaeem *et al.,* 2012; Song *et al.,* 2012). Venom from scorpion have been reportedly used for antibodies production in animals like horse and sheep, which are used for the neutralization of the venom hazardous effects on man (Petricevich *et al.,* 2013) The therapeutic effect of the scorpion venom has shown to be linked with several diseases including cancer, particularly breast cancer (Feng *et al.*,2008; Al-Asmari *et al.,* 2015), Leukemia (Hayden *et al.,* 2006) and Glioma (Kesaven *et al.,* 2010).

The different constituents of crude scorpion venom are water, mucosa, oligopeptides, nucleotides, amino acids, ions, neurotransmitters, salts, low molecular weight peptides, metals, mucoproteins, mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, biogenic amines and many unidentified substances (Possanin *et al.*, 2009; Gwee *et al.*, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Study Area.

The study was conducted in laboratory of Animal Biology Department, Bosso campus of Federal University of Technology (FUT) Minna.

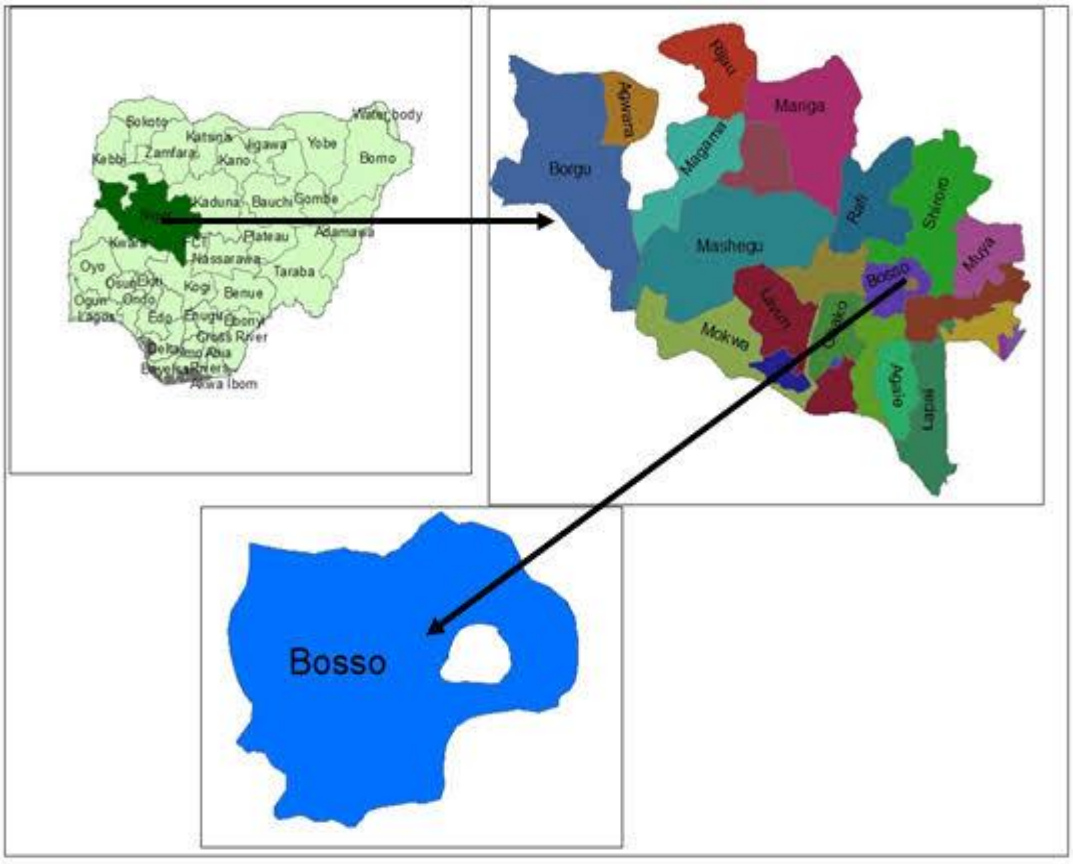

Figure VI: Map of Nigeria showing the where Niger state is located and Bosso local government where FUT minna is situated.

Source: (Olusegun *et al,* 2020)

3.2 Ethical Approval

Approval for the use of experimental animal was obtained from the Ministry of livestock, Minna.

3.3 Scorpion Collection

A group of research assistants were involved with the collection of scorpion in Shiroro town. Eighty (80) medium sized scorpions were collected within January and March in batches; they were kept in a well aerated large plastic and provided with live cockroaches and water (Al-Asmari *et al.,* 2007; Al-Asmari *et al.,* 2009). Dried cow dung was introduced into every

container before the scorpion were placed, the dung serve to provide an environment like in the wild were they were collected from for the study purpose.

Plate 1: Field picture taken during scorpion collection in Shiroro.

3.3.1 Location where scorpion samples were collected

Scorpion collected for this study was sourced for in Shiroro local government area, it has an area of 5,015 square kilometres and a population of about 235,404 as at 2006 census. Its coordinates are 9.57'25^0 N, 6.49'55^0 E. A populated place, town with agglomeration of buildings where people live and work.

Figure VII: Location of Shiroro in Niger state In Nigeria
Source: (Olusegun *et al,* 2020)

3.3.2 Species identification

Identification of species of scorpion collected was done in the laboratory using a published manual of African species of Scorpion. Kovarik, F., Ojanguren A. (2013). Family Buthidae. *Illustrated catalogue of scorpions*. Part II: Bothriuridae; Chaerilidae; Buthidae I: genera Compsobuthus, Hottentotta, Isometrus, Lychas & Sassanidotus *Prague* pp. 141-212.

3.4 Scorpion Venom Extraction

Numerous methods of venom collections have been described such as electric stimulation at varying voltage, manual extraction and maceration. In this study venom was extracted by electric

stimulation of the telson as described by Yaqoob *et al.,* 2016. Scorpions were placed on a white surface one after the other and a sticky tape was used to tape it down leaving out the tail, 2-3 drops of 10% normal saline to enhance conductivity. At the tail a pointed tip forceps was used to hold the telson and pointing at the slide to be used for the venom collection. With the help of a pointed electrode, electric current of 0.8Amps at 5volts was used to produce shock on the tail and this enhances quick release of venom without killing it.

The jelly-like, milky discharge collected on a slide and mixed with distilled water in 1:5 ratios (venom: distil-water) and immediately transferred into ependurph tubes. Centrifuge at 10000rpm for 10min, supernatant was extracted and refrigerated at -20^0 C until further use.

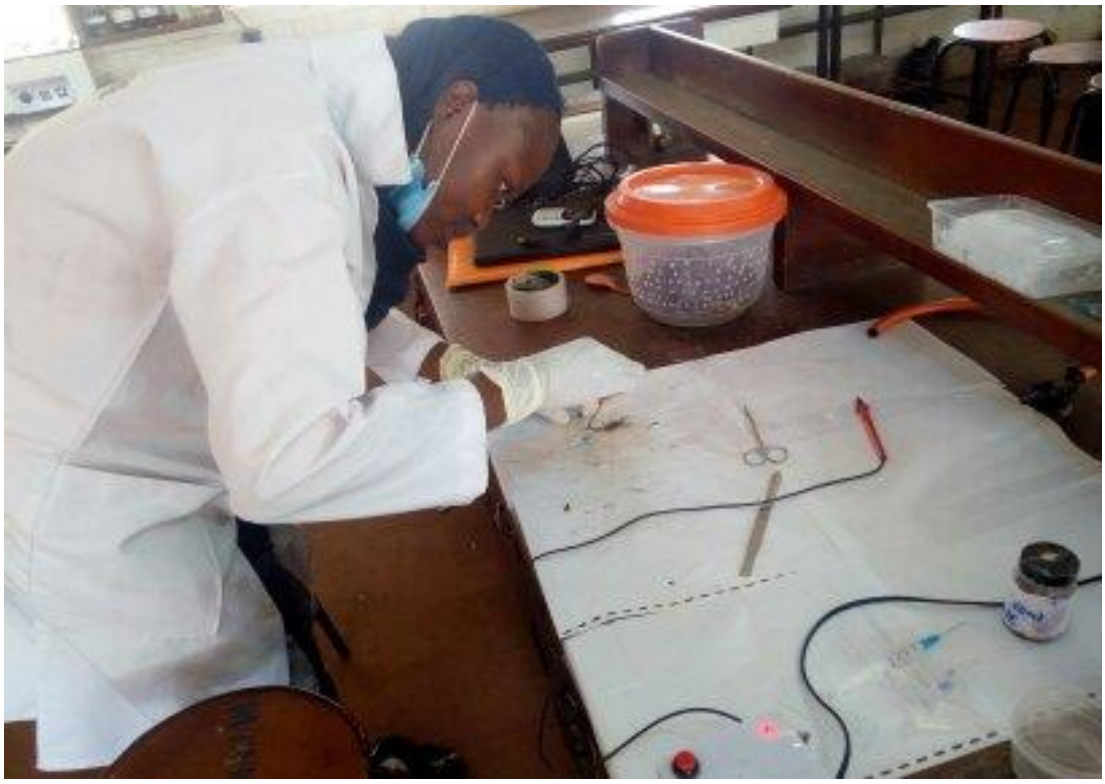

Plate II: ***Picture of student during Scorpion venom extraction using the electric stimulation method.***

3.5 Experimental Animals

The Swiss albino rats required for this experiment were obtained from the Nigeria Institute for Trypanosomiasis Research (NITR) Kaduna, Kaduna State (90-120grms), housed in plastic cages (Fifteen animals per cage) under laboratory condition of 27 ± 2 ^{0} C, 12-hours light/darkness cycle with standard pellet diet and water. They were acclimatized to the new environment for three weeks. The experiment was conducted in compliance with the International Guiding Principle for Biochemical research involving animals (Council for International Organizations of Medical Sciences, 1985; Observational research, audit and related, Revised edition 2012). All efforts are to ensure minimal use of animal and their suffering.

Plate III: ***Test animal procured from NITR in kaduna (90-120grams Swiss albino Rats)***

3.6 Test Parasites

The parasite species *Trypanosoma evansi* used for this study were obtained from the Nigeria Institute for Trypanosomiasis Research (NITR) Kaduna, Kaduna State. The parasites were

maintained in the laboratory by continuous transfer in Trypanosome free rats intraperitoneally throughout the period of the study.

3.7 Innoculation of Experimental Animals

This as an act of introducing parasites into the animal and this was repeatedly carried out to ensure the continuous availability of the parasite in the animal.

3.8 Determination of Median Lethal Dose (LD_{50})

The assessment of the lethal dose LD_{50} (the dose that kills 50% f test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survival animals are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD_{50}, therapeutic index and degree of safety of a pharmacological agent. This is the dose of injected scorpion venom extract that kill 50% of the rats within 24hours of study (Akhila *et al.*, 2007; Chinedu *et al.*, 2013). Using equation 1:

$$The\ LD50 = \sqrt{(D0\ X\ D100)} \quad (1)$$

LD_{50}= Lethal Dose 50

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

3.9 Determination of In *vivo* Antitrypanosomal Activities (Atawodi *et al.*, 2003)

18 Swiss albino rats were used for the curative study six groups of 3 rats each.

Group 1: infected and treated with 0.1mg/kg with crude venom extract and with distil-water

Group 2: infected and treated with 0.2mg/kg with crude venom extract and with distil-water.

Group 3: infected and treated with 0.3mg/kg with crude venom extract and distil-water

Group 4: infected and untreated (negative control)

Group 5: infected and treated with 3.5mg/kg of Diminazene aceturate (berenil) (positive control).

3.9.1 Determination of parasitemia

The determination of parasitemia change was done according to the procedures of WHO (2015) National malaria slide bank standard operating procedures. Geneva (In preparation). Using equation 2:

$$\%\ change\ in\ parasitemia = \frac{mean\ parasitemia\ on\ day\ 14 - mean\ parasitemia\ on\ day\ 0}{mean\ parasitemia\ on\ day\ 0} \quad (2)$$

3.9.2 Determination of body weight calculated using equation 3

The determination of parasitemia change was done according to the procedures of WHO (2015) National malaria slide bank standard operating procedures. Geneva (In preparation). Using equation 3:

$$\%\ change\ in\ body\ weight = \frac{Initial\ weight\ of\ animal - weight\ lost}{initial\ weight\ of\ animal}\ X\ 100 \quad (3)$$

3.9.3 Determination of mean survival time (MST) calculated using equation 4:

Mortality will be monitored daily and the number of days from the time of inoculation of the parasite up to death will be recorded for each mouse. The mean survival time (MST) will be calculated according to the method of (Zheng and Liang, 2011). Using equation (4)

$$Mean\ survival\ time = \frac{sum\ survival\ time\ for\ all\ mice\ in\ a\ group\ (days)}{Total\ number\ of\ mice\ in\ the\ group} \quad (4)$$

3.9.4 Hematological profile of *Trypanosoma evansi* infected albino rat (Saad *et al.*, 2018)

The packed cell volume (PCV), Haemoglobin concentration (Hb).

$$\%\ change\ in\ \ PCV = \frac{Maen\ on\ day\ 7 - mean\ PCV\ on\ day\ 0}{Mean\ PCV\ on\ day\ 0} x100$$

Total and differential white blood cells (WBC) was carried out once for a week from day zero (pre-infection), through the study period as well as post treatment profile.

3.9.5 Data analysis

The data obtained from the study were summarized and expressed as mean $\pm$ standard error of mean (SEM), Data analysis was performed using Statistical Package for Social Science (SPSS), version 19.0. One-way ANOVA followed by Tukey's multiple comparison tests carried out to compare the results obtained from different groups and determine statistical significance. Probability values less than 0.05 were considered signifant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Acute Oral Toxicity Profile of Crude Venom Extract of Scorpion.

The acute toxicity profile of crude venom extract of scorpion is shown in Table 4.1 There was no sign of toxicity observed up to 30mg/kg body weight. However, mortalities were recorded at higher doses above 30 mg/kg body weight. The LD_{50} extrapolated to be 44.72mg/kg body weight.

Table 4.1: Acute Toxicity of Crude Scorpion Venom Extract

Group	Dosage (mg/kg/bw)	No of rats	Mortality
Phase One			
1	**10**	**3**	**0/3**
2	**20**	**3**	**0/3**
3	**30**	**3**	**0/3**
Phase Two			
4	**40**	**3**	**1/3**
5	**50**	**3**	**3/3**
6	**100**	**3**	**3/3**

4.1.2 Antitrypanosomal Effect of Crude Scorpion Venom Extract in *Trypanosoma evansi* Infected Swiss albino Rats.

Effect of crude scorpion extract on parasitaemia counts of *Trypanosoma evansi* infected Swiss albino rats is shown in Table 4.2 The antitrypanosomal potency of the crude venom extract revealed dose dependent activities against the *Trypanosoma evansi* parasite. The antitrypanosomal activity was significantly highest in the group treated with 30mg/kg/body weight. On day five (5) of venom treatment, total parasite clearance was observed in the group treated with 30mg/kg body weight and standard drug when compared with the infected untreated group (negative control).

Table 4.2: Effect of Crude Scorpion Venom extract on Parasitaemia counts of *Trypanosoma evansi* infected Swiss albino Rats.

Dose mg/kg/b.d	weight	Day 1	Day 2	Day 3	Day 4	Day 5
10		20.14±0.12[a]	18.14±0.11[a]	15.31± 0.11[c]	10.45±0.11[d]	5.21±0.31[b]
20		23.13± 0.11[a]	21.20±0.09[b]	12.44±0.08[b]	3.45±0.09[c]	0.00[a]
30		21.25±0.07a	18.45±0.05a	8.12±0.09a	0.67±0.09[a]	0.00±0[a]
+ve control		25.45±0.12[a]	21.13±0.21[b]	11.23±0.14[b]	1.50±0.19[b]	0.00±0[a]
-ve control		21.15±0.17[a]	27.63±0.12[c]	31.23±0.32[d]	41.15±0.90[e]	43.21±0.12[c]

Data are Mean ± SEM of triplicate determination. Data followed by different superscript alphabet along the column are significantly different ($p<0.05$).

4.1.3 Effect of Crude Scorpion Venom extract on Packed Cell Volume (PCV)

Effect of crude scorpion extract on packed cell volume (PCV) of *Trypanosoma evansi* infected Swiss albino rats is shown in Table 4.3. After 3 days post infection with the *T. evansi* there was drastic drop in the mean PCV of all groups except the uninfected (normal) group. The observed loss in the mean PCV was ameliorated; 7 days post infection after treatment with the scorpion extract and standard drug. However the infected untreated group recorded gradual loss in PCV till the end of the experiment.

Table 4.3: Effect of Crude Scorpion Venom extract on Packed Cell Volume (PCV) of *Trypanosoma evansi* infected Swiss albino Rats.

Venom extract (mg/kg/bw)	before infection (%)	3days post Infection (%)	7days post infection (%)
10	39.34 ±0.08[a]	36.21±2.14[a]	42.14±2.09[b]
20	41.25±0.91[b]	39.14±1.31[b]	42.14±1.24[b]
30	41.72±1.23[b]	39.14±2.24[b]	43.21±0.39[b]
+ve control	42.07±1.45[b]	41.32±1.23[b]	43.51±0.14[b]
-ve control	40.12±0.89[b]	37.21±0.21[a]	29.15±1.23[a]
Normal control	40.13±0.87[b]	41.13±0.76[b]	40.14±4.02[b]

Data are Mean±SEM of triplicate determination. Data followed by different superscript alphabet along the column are significantly different ($p<0.05$).

4.1.4 Effect of Crude Scorpion Venom extract on Body Weight Change

Effect of crude scorpion extract on body weight changes of *Trypanosoma evansi* infected Swiss albino rats is shown in Table 4.4. Weight change of *T.evansi* infected rats treated with venom of scorpion extract. The mean weight change of the infected treated with scorpion venom dropped after three days post inoculation with *T.evansi* parasite. The animals in the group treated with crude venom and standard drug recorded a significant increase in weight on day 7 after treatment. However the infected untreated group recorded drastic decrease in weight throughout the experimental period.

Table 4.4: Effect of Crude Scorpion Venom Extract on body weight changes of *Trypanosoma evansi* infected Swiss albino Rats

Scorpion Dose (mg/kg/bw)	Day0	Day4	Day7
10	111.53 ±1.21[c]	100.23±2.34[c]	143.23±3.21[d]
20	131.23±2.14[e]	111.43±3.23[d]	138.12±2.45[c]
30	80.41±1.28[a]	70.45±2.81[a]	103.45±3.14[b]
+ve control	98.45±0.98[b]	97.15±1.65[b]	101.23±4.15[b]
-ve control	123.45±0.92[d]	112.41±1.63[d]	71.23±1.29[a]

Data are Mean ± SEM of triplicate determination. Data followed by different superscript alphabet along the column are significantly different ($P<0.05$).

4.1.5 Effect of Crude Scorpion Venom Extract on Relative Organ Body Weight

Effect of crude scorpion extract on relative organ body weight of *Trypanosoma evansi* infected Swiss albino rats is shown in Table 4.5. The relative heart, kidney and lung to body weight ratio in *T. evansi* infected Swiss albino rats were not significantly different from the control animals as well as the crude scorpion extract treated groups. However, the relative liver and spleen to body weight ratio in *T. evansi* infected Swiss albino rats were significantly higher when compared with the control groups. Treatment of the infected rats with crude scorpion venom at 20 and 30

mg/kg body weight significantly ($p<0.05$) restored the relative liver and spleen to body weight ratio to a level comparable with the normal control rats.

Table 4.5: Effect of Crude Scorpion Extract on relative organ body weight of *Trypanosoma evansi* infected Swiss albino rats

	Heart	Kidney	Liver	Spleen	Lungs
10.	0.54 ± 0.08^{a}	0.81 ± 0.01^{a}	4.98 ± 0.07^{c}	1.34 ± 0.12^{b}	1.35 ± 0.03^{a}
20.	0.52 ± 0.04^{a}	0.87 ± 0.01^{a}	3.40 ± 0.01^{b}	1.00 ± 0.01^{a}	1.07 ± 0.12^{a}
30	0.63 ± 0.10^{a}	0.87 ± 0.11^{a}	3.20 ± 0.04^{a}	0.90 ± 0.09^{a}	1.00 ± 0.13^{a}

+ve control	0.54±0.03[a]	0.88±0.08[a]	3.38±0.08[a]	0.98±0.07[a]	1.09±0.13[a]
-ve control	0.51±0.07[a]	0.80±0.02[a]	5.90±0.03[b]	1.71±0.04[c]	1.25±0.18[a]
Nrmal control	0.53±0.11[a]	0.89±0.10[a]	3.61±0.09[a]	0.94±0.03[a]	1.05±014[a]

Data are Mean±SEM of triplicate determination. Data followed by different superscript alphabet along the column are significantly different ($p<0.05$).

4.1.6 Effect of Crude Scorpion Extract on Biochemical Parameters

Effects of scorpion venom on serum biochemical parameters of *T. evansi* infected rats are shown in Table 4.6: the activities of aspartate transaminase (AST) in *T. evansi* infected rats were significantly higher, while the concentration of total proteins and albumin were significantly

lower when compared with standard and normal control. Treatment of the infected rats with scorpion venom significantly restored the normal levels of these parameters in a dose dependent fashion. Serum ALP and ALT activities in untreated rats were not significantly ($P<0.05$) different from that of the normal control group, standard treated groups and also scorpion treated groups.

Table 4.6: **Effects of Scorpion Venom on serum Biochemical Parameters of *Trypanosoma evansi* infected Swiss albino Rats**

	AST (U/L)	ALP(U/L)	Total proteins (mg/dL)	ALT (U/L)	Albumin (mg/dL)
10 mg/kg b.w SV	19.24±0.35^{b}	56.00±4.56^{a}	19.12±0.56^{b}	56.24±0.35^{a}	16.00±2.56^{c}
20 mg/kg b.w SV	19.41±0.68^{b}	57.32±5.43^{a}	17.41±2.35^{b}	60.41±2.68^{a}	11.84±1.34^{b}
30 mg/kg b.w SV	15.43±0.54^{a}	60.85±5.43^{a}	25.36±3.45^{c}	55.43±0.54^{a}	15.82±1.56^{c}
5 mg/kg DMA	19.41±0.68^{b}	58.48±4.77^{a}	19.47±2.34^{b}	59.41±0.68^{a}	10.89±2.45^{b}
Untreated control	35.41±0.68^{c}	58.32±5.10^{a}	13.41±2.35^{a}	58.41±0.68^{a}	7.84±1.34^{a}
Control	17.41±0.35^{ab}	59.25±3.45^{a}	25.36±3.45^{c}	55.43±0.54^{a}	15.82±1.56^{c}

Data are Mean ± SEM of triplicate determination. Values along the same column with different superscripts alphabet are significantly different ($P<0.05$)

4.2 Discussion

Over the last decades toxins and secretions from poisonous and venomous animals have been used as drugs and drug leads for treatment of numerous untreatable human ailments (Sudhanshu, 2013). Leech salivary secretion exert antioxidant activities (Omalu *et al.,* 2016), antimicrobial agents and has been reported to be used in treatments of back pain, Snake Venom serve as anticancer, anti-diabetics and anti-hypertensive agents, secretion from cone snail *Conus magus* used in treatment of chronic pain (Sudhanshu, 2013), while hemolymph from African land snail has been reported for their hepatocurative effect against CCl_4 intoxicated rats (Lawal *et al.,* 2015). Bee venom has been reported for antimalarial and antitrypanosomal activities (Shittu *et al.,* 2015; Shittu & Eyihur, 2015). The present study, has pioneered the establishment of antitrypanosomal activities of scorpion venom against *Trypanosoma evansi* infected rats.

Result of acute oral toxicity of the extract suggests that the extract could be relatively safe on acute exposure at dose of 30 mg/kg body weight because there was no sign of toxicity observed up to 30mg/kg body weight. Furthermore, clinical observations for acute studies show that the scorpion venom at doses up to 30 mg/kg body weight did not produce any grossly negative behavioural changes such as excitement, restlessness, respiratory distress, convulsions or coma. However, mortalities were recorded at higher doses above 30 mg/kg body weight. This indicates that therapeutic application of this venom should be done at the dose of 30mg/kg body weight or below. The LD_{50} extrapolated to be 44.72mg/kg body weight.

Due to the challenge of trypanosome resistance, toxicity of synthetic drugs and high cost of purchase, the present study evaluated the antitrypanosomal effect of the venom against *T. evansi* infected rats. Interestingly, it was discovered that the administration of scorpion venom extract to

T. evansi infected rats reduce the parasitemia level of infected rats significantly when compared with infected not treated group where the parasitic load increased infinitely. It is noteworthy that the venom exhibited complete parasite clearance after 7 days post infection treatment. This finding agrees with the report of Shittu and Eyihuri (2015), who documented that bee venom, produce 56.6% chemosupression of *Plasmodium berghei* parasitisized mice.

The antitrypanosomal activities observed in the venom extract could be due to the antibiotic potentials previously attributed to scorpion venom. This implies that scorpion venom contain inherent bioactive metabolites whose increase in concentration promoted antitrypanosomal activities as observed in the current study, (Umar *et al.,* 2012). Although, the mechanism by which these venom extract elicits it trypanocidal action was not determined, it has however been documented that many natural products including arthropods (where scorpion belongs) exhibit their antiparasitic activity through interference with the redox balance of the cellular defence against oxidative stress. This is because they possess structures capable of generating radicals that may cause per oxidative damage to alterations in redox balance (Sepulveda and Carssel, 2010). Furthermore, Park *et al.* (2010) reported that the basic therapeutic mechanism of insect is a synergy involving several compounds.

Haematological indices such as packed cell volume (PCV) were studied to assess the toxic effect of the parasite on blood component. Anaemia is one of the established major pathological features of African Trypanosomiasis (Noedl *et al.,* 2003). Therefore, the presence and severity of anaemia are good indicators of disease status. The significant decrease level of PCV of the infected untreated rats observed in this study is an indication of anaemic condition caused by the parasite. Similar findings have been reported by several investigators (Ogbadoyi *et al.,* 1999; Shitu *et al.,* 2015). In addition to this, the parasite also stimulates certain cells to produce R.O.S

thereby resulting in haemoglobin degradation (Loria *et al.*, 1999). The increases in PCV observed for infected rats treated with scorpion venom in comparison with infected not treated group suggests that the venom reduce the severity of *T. evansi* infection in rats. The findings from the present study agrees with the previous study, which reported that insect venom improved the PCV, HB, RBC of *P. berghei* parasitisized mice when compared to untreated control (Shittu and Eyihuri, 2015). Furthermore, Adult houseflies (*Musa domestica)* treatment has been reported to suppressed *P. berghei* replication, improved mice life span (34 days) and ameliorated parasite induced anaemia when evaluated for it antimalarial activities at 600 mg/kg against *P. berghei* parasitized mice.

The need for the analysis of biochemical parameters following treatment of parasitic infection has been suggested by previous studies (Shittu *et al.*, 2017; Umar *et al*., 2019). The biochemical indices monitored in the serum of rats are useful 'markers' for assessment of tissue damage due to parasitic infection and also valuable in assessing the treatment outcome of the drug/extract under investigation. The measurement of activities of various enzymes in the body fluids plays a significant role in disease investigation and diagnosis (Lawal *et al*., 2016). Biomarker enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Umar *et al*., 2017). The transaminase (ALT and AST) are 'markers' of liver damage during trypanosomiasis infection (Shittu *et al.*, 2017) with ALT being a more sensitive biomarker of hepatotoxicity than AST.

The increase in ALT activities in the infected un-treated may be related to parasites induced liver inflammation and is an indication of abnormal function of the liver. The elevation of these enzyme levels recorded here agrees with earlier reports from natural and experimental infected animals (Umar *et al*., 2007; Shittu *et al.* 2017). The results suggest probable infiltration of vital

body organs and inflammation particularly of liver, muscles, and kidneys by *T. evansi.* Elevated enzyme levels may also result from effect of trypanosome lyses resulting from the host's defense mechanisms (Kennedy *et al*. 2004). However, treatment with the scorpion venom (10, 20 and 30 mg/kg body weight) significantly restored the serum activities of AST, towards the reference value (table 4.6). This is an indication of the effectiveness of the venom in ameliorating the effect of *T. evansi* infection.

Alkaline phosphatase is a "marker" enzyme for the plasma membrane and endoplasmic reticulum. It is often employed to assess the integrity of plasma membrane and endoplasmic reticulum. In the present study, *T. evansi* infection as well as the treatment with scorpion venom did not cause any significant ($p>0.05$) alterations to the levels of serum alkaline phosphatase activities when compared with the control animals. This is an indication that the *T. evansi* infection did not cause loss of membrane component (including alkaline phosphatase) into the extracellular fluids or inactivation of the enzyme molecule in situ or depletion of important molecules required by the enzyme for maximum activity (Yakubu *et al*., 2003). Also, it indicated that there was no over activation of the enzyme or an increase in the rate of the synthesis of the enzyme in *T. evansi* infection.

The concentrations of total protein and albumin, are useful 'markers' of secretory, synthetic and excretory functioning of the liver (Yakubu & Musa, 2012). In the present study, *T. evansi* infection cause a significant ($p<0.05$) decreases in serum total proteins and albumim when compared with the normal control. The observed increase in the total protein and albumin content suggests a compromise of the synthetic ability of the liver arising from the *T. evansi* infection. Such decrease in total protein could, however, lead to over hydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the

liver and consequently the health of the infected animals (Yusuf *et al.,* 2018). However, rat treated with scorpion venom shows ameliorative effect as it cause significant increase in the concentrations of total proteins and albumins comparable to the normal control rats.

The loss in body weight of the *T. evansi* infected rats could be attribute to the pathological condition of the infected rats which affect their appetite and thus results in overall feed intake and weight loss (Lawal *et al.,* 2016). Loss of body protein in support of defence mechanism of the animals could also be responsible for the decrease weight of the infected rats. Organ body weight ratios are normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal. The absence of an effect on the computed heart, lung and kidney/body weight ratios suggest that the *Trypanosoma evansi* infection did not cause any form of swelling, atrophy and hypertrophy on the heart, lung and kidney (Berinyuy *et al.,* 2015). However, the relative liver and spleen to body weight ratio in *Trypanosoma evansi* infected Swiss albino rats were significantly higher when compared with the control groups. This is an indication *Trypanosoma evansi* infection has induced hepatosplenomegaly. Several studies have also reported the parasite induced hepatosplenomegaly. Treatment of the infected rats with crude scorpion venom at 20mg/kg and 30 mg/kg body weight significantly ($p<0.05$) improved the weight gain and restored the relative liver and spleen to body weight ratio to a level comparable with the normal control rats. This is an indication that treatment of *Trypanosoma evansi* infected rats with scorpion venom has ameliorated the overall pathological effect of the parasite in rats.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The data presented in the present investigation provide a strong base that scorpion venom posed antitrypanosomal activities as the acute oral toxicity (LD_{50}) extrapolated to be 44.72mg/kg body weight, antitrypanosomal bioassay results showed scorpion venom significantly reduced the level of parasitemia of the infected rats, treatment of infected rats with scorpion venom extract significantly ameliorated loss in some body enzymes and has potential to ameliorate loss in body weight and Packed Cell Volume caused by parasite invasion. The venom also significantly ameliorated *Trypanosoma evansi* induce alteration in biochemical parameters of liver and spleen integrity when compared to normal rats, thus could be consider a candidate for development of novel drug against trypanosomiasis.

5.2 Recommendations

The scorpion venom has shown promising antitrypanosomal activity, the next step would be to isolate, identify and characterize the active metabolites in the venom. The mechanism by which this scorpion venom exerts its effect can also be determined, which could be of help even in other areas of research and as well if there be need to formulate synthetic analogues of the compound in the future.

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