DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY
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TOPIC:

THE EFFECT OF HYDRO-METHANOL LEAF EXTRACT OF *Icacina trichantha Oliv.* ON SEIZURES AND SKELETAL MUSCLE RELAXATION IN MICE

BY

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(PHYSIOLOGY/PHARMACOLOGY TECHNIQUE)

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

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CERTIFICATION

We clarify that this thesis was carried out by Miss Bevelyn AJUBE in the Department of Science Laboratory Technology, Faculty of Life Science, University of Benin, Benin City, Nigeria.

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DEDICATION

I dedicate this work to GOD Almighty for His support through this journey.

ACKNOWLEDGEMENTS

First, I would like to express my deepest my sincere thanks to GOD almighty, my pillar, my creator. He has been the source of my strength throughout this journey. I would also like to extend my heartfelt gratitude to all those who have contributed to the completion of this report.

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ABSTRACT

Studies and advancements in neuroscience have been sparked by the prevalence of CNS diseases. Consequently, variety of therapies, medications and treatments with the goal of improving the lives of those affected have been created with an emphasis on herbal medicine. Major research has been done on different parts of *Icacina trichantha* such as the tubers, stems and leaf. It is traditionally used to treat hypertension, asthma, fever and neurological disorders. Pharmacological studies on the medicinal potential of the plant tuber, leaf and stem have been conducted, however there has been little research done on the leaves of the plant regarding CNS activity.

Fresh leaves of *Icacina trichantha* were harvested, cleaned, dried and extracted over the course of 72 hours by cold maceration using (1:1) distilled water and methanol. Pentylenetetrazole induced seizure, Maximum electroshock induced Seizure, Rota-rod test and static- rod test was carried out in mice using graded doses 100, 200 and 400 mg/kg orally of the plant extract.

Results from the experiment revealed that mice administered with 200 mg/kg of the plant extract was protected from pentylyenetetrazole induced seizure. While no protection in maximal electro-shock induced seizure was observed, had no effect on the animal models used to evaluate skeletal muscle relaxation across all groups treated with the plant extract as compared to the control. This indicates that the plant possesses anti-convulsant activity but not effective on skeletal muscle relaxation.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The central nervous system (CNS) is made up of the brain and spinal cord and is regarded as the body's central processing station (Simonato *et al*, 2013). There are several factors that causes disorders of the central nervous system such as trauma, infections, degeneration, structural defects, tumors, autoimmune disorders and stroke (Seunguu, 2022; Newman, 2022).

These neurological disorders pose a significant social and economic burden that endangers the public's health, particularly in low-income and developing areas. This condition is a disease of the central or peripheral nervous system brought on by a physical injury to the Brain, Spinal cord or nerves (Simonato *et al*., 2003; Jenner *et al*., 1998). It has been recorded that, 12% of deaths worldwide are caused by neurological chronic disorders, with cerebrovascular diseases accounting for 85% of these deaths (6.8 million deaths annually) almost 80 of fatalities occur in low-income nations (report of united nations, 2007).

There are many examples of these neurological diseases such as Dementia, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disorders, cerebrovascular diseases such as stroke or migraine and other headache disorders, benign brain tumors and mental disorders also known as psychiatric diseases which are primarily expressed as inappropriate thoughts, feelings or behavior and cause distress. (Simonato *et al*., 2003; Jenner *et al*., 1998).

1.2 Global Relevance

Epilespy is a neurological illness that affects both men and women of all ages and across all socioeconomic and racial groups, while it is more common in young people under the age of 20 and in adults over the age of 60 (Duncan, 2006). According to estimates, there are at least

50 million epileptics globally (Sander, 1990), Over 80 of epileptics reside in developing nations, where the disease remains untreated (Garcia, 2003; Boussinesq, 2002).

In addition, epilepsy has a large psychological and emotional impact, it has been a socially and culturally stigmatized condition throughout history. People with epilepsy frequently experience stigma and psychosocial stress as a result of such devaluation (Cockerell,1994).

It is a brain disorder that occurs when at least two reflex seizures happen within 24 hours from one another (Fisher *et al*,2014). Seizures can occur as a result of epilepsy. Epilepsy is a group of non- communicable neurological disorders characterized by recurrent epileptic seizures (Ghosh, 2021) An unusually high level of neuronal activation in the brain triggers seizures. These seizures are linked to electrographic pattern, awareness, sensation and behavioral changes (Fisher *et al*, 2015; Reddy and Kuruba, 2013). A person with epilepsy is considered seizure-free if they have not had seizure medication since they were first diagnosed with the five years ago (Fisher *et al*,2014). despite the antiepileptic medications on the market around 33% of patients still experience seizures (Castel-Branco *et al*, 2009).

1.3 Animal Models of Epilepsy

The following are some models of generalized seizures:

1.3.1 Pentylenetetrazole Induced Seizure

It is also known as pentalenetrazole, leptazol, Metrazol, pentetrazol, pentamethylenetetrazol, corazol or PTZ is a drug formerly used as a circulatory and respiratory stimulant, High doses causes convulsions as discovered by the Hungarian American neurologist and psychiatrist Ladislas J. Meduna in 1934. It has been used experimentally to study seizure phenomena and to identify pharmaceuticals that may control seizure susceptibility, it is also a prototypical anxiogenic drug. Pentylenetetrazol produces reliable discriminative stimulus which is largely mediated by the GABA receptor (Minkel, 2007).

PTZ is a gamma aminobutyric acid (GABA)- A receptor antagonist (Squires, 1984). PTZ increases the activity of neurons by suppressing the function of inhibitory synapses. Animals that are subject to this rule develop generalized seizures (Tourov, 1996). There are certain drugs used in the treatment of epilepsy such as Phenobarbital and diazepam.

Phenobarbital is also known as phenobarbitone or phenobarb, is a medication of the barbiturate type. It is recommended by the world health organization (WHO) for the treatment of certain types of epilepsy in developing countries (Illangraratne *et al*., 2012). It can be administered intravenously, Intramuscularly or orally, phenobarbital is occasionally used to treat anxiety, difficulty sleeping and drug withdrawal, as well as facilitate surgery. when injected intravenously, it often starts acting after five minutes, while when given orally, it normally takes 30 minutes to work. Its effects last between four hours and two days (Marx, 2010).

Drugs that raise the seizure threshold are found using the pentylenetetrazole subcutaneous model (Ninds, 2014). Potential treatment for non-convulsive seizures includes drugs that are effective against this seizure paradigm (Mandhane *et al*., 2007; Ninds,2014). Pentylenetetrazole induced seizure's convulsive dose is 85mg/kg for rats and 70mg/kg for mice. These doses result in clonic seizures in 97% of animals, lasting at least 5 seconds or more. The animal is then examined for 30 minutes after that (Ninds, 2014).

1.3.2 Maximum electroshock seizure

Maximum electroshock (MES) is intended to stimulate acute seizures. It is used to predict treatment effectiveness against grand mal seizures by evaluating antiepileptic drugs at an early stage, particularly during preclinical research (Castro-Branco *et al*., 2009; Loscher, 2011).

This model's behavioral and electrographic seizure patterns are comparable to those in humans (Swinyard *et al*.,1989) additionally, it finds substances that prevent the spread of seizures (Ninds, 2014). Prior to maximum electroshock seizures assessment, a drop of solution is used to moisten the ear lobes. An electrode is clipped to both Animals ears for an electrical stimulation is delivered for 0.2 seconds at 50 mA to mice and 150 mA to rats at 60 Hz (Ninds, 2014). Tonic seizures are recognized by the extension of the fore and hind limbs as well as the filling of the body after seizure is induced. After the tonic phase of seizures, the clonic phase starts and lasts for 20 to 30 seconds before the animal returns back to normal (Castro-Branco *et al*., 2009). An Agents capacity to reduce or eliminate the tonic extensor characteristic of maximum electroshock seizure is a sign that it has an inhibitory effect on the seizure induced by maximum electroshock seizure (Swinyard *et al*., 1989; Ninds, 2014).

1.3.3 Picrotoxin Induced seizure

Picrotoxin as a GABA antagonist, can be used to assess a treatment's effectiveness for absence seizures (Ogbonnia *et al*., 2003). Weighed Adult mice of either sex are divided into groups with a minimum of three test drug dose levels; and a positive and negative control. Diazepam (3mg/kg) and the substance used to prepare the test agents (10ml/kg) are administered to the positive and negative control groups, respectively. Picrotoxin (2mg/kg I.P) is administered to all the animals Thirty minutes after the test drug and controls are provided, and the latency or onset of myoclonic spasms (Muscular jerks) and tonic phases of seizures (THLE) and death are recorded for Sixty minutes afterwards. Animals that did not experience seizures or subsequent fatalities throughout the Sixty minutes observation period are regarded as protected. However, for the sake of convenient statistical analysis, Animals without seizures have sixty minutes delay or time of onset.

1.3.4 Bicuculline Induced seizure

The technique for bicuculline is the same as that for pentylenetetrazole (PTZ) and picrotoxin, with the exception that monitoring after bicuculline administration lasts 120 minutes rather than 60 minutes, the monitoring parameters are not the same. The dose of bicuculline is 1mg/kg, after 30 minutes of administration to the test agents and controls. Diazepam (1-3 mg/kg, I.P) is used as a positive control.

1.3.5 Isoniazid (INH) Induced seizure

The procedure for isoniazid is the same as that for pentylenetetrazole (PTZ) and picrotoxin, with the dose at 300 mg/kg and the animals are observed for 120 minutes after administration, Diazepam (1-3mg/kg, I.P) or phenobarbital is used as positive control.

- Homocysteine Test (Deyn *et al*., 1992).
- Penicillin Model of absence Seizure (Deyn *et al*., 1992).
- WAG/Rij Model (Citraro *et al.,* 2014).
- Timed PTZ Infusion Test (Mandhane *et al*., 2007).
- Gamma Hydroxybutyrate Model (GHB) (Deyn *et al*., 1992).
- Models of Pharmaco resistant seizures which include Corneal Kindled mouse (Ninds, 2014), Hippocampal kindled rat model (Ninds, 2014).

1.4 Skeletal Muscle Relaxation

Skeletal muscle is a very well-organized organ with several bundles of muscle fibres. A muscle cell with the sarcomere as its fundamental cellular unit is represented by each myofiber, which contains many myofiber. Muscle tissue is made up of bundles of fascicles and bundles of myofibers, with the extracellular matrix covering each layer in turn (Lieber, 2009)

A muscle relaxant is a drug that affects skeletal muscle function and decreases the muscle tone, it may be used to alleviate symptoms such as a muscle spasms, pain and hyperreflexia. They are of two major Muscle therapeutic groups such as neuromuscular groups and spasmolytics

Neuromuscular blockers act by interfering with transmission at the neuromuscular end plate and have no central nervous system activity while spasmolytics also known as centrally acting muscle relaxant are used to alleviate musculoskeletal pain and spasms and to reduce spasticity in a variety of neurological conditions (Miller, 1998), common muscle relaxants diazepam, baclofen, tizanidine etc.

Diazepam is a medicine of the benzodiazepine family that acts as an anxiolytic, it is commonly used to treat a range of conditions, including anxiety, seizures, alcohol withdrawal syndrome, muscle spasms, insomnia and restless legs syndrome (Calcaterra and Barrow, 2014). It may also be used to cause memory loss during certain medical procedures. it can be taken orally, as a suppository inserted into the rectum, intramuscularly, intravenously or used as nasal spray. When injected intravenously (Valtoco, 2020), effects begin in one to five minutes and las up to an hour. Orally, effects begin 15 - 60 minutes (Dhaliwal and Saadabadi, 2022).

1.5 Animal models

1.5.1 Static Rod Test

The static rod is one such task that helps determine the motor capabilities of the subject that's affected by different pharmacological compounds, lesions, or diseases. It is made up of simple clamps and dowels. The rotarod is a static apparatus unlike the rotarod test (Deacon, 2013). It measures body coordination and balance (Fleming *et al*., 2006), In the static rod test

the animal is placed on vertical metallic rod on a clamp, the time the adult mice take to descend down to the base of the pole is recorded.

1.5.2 Rotarod Performance Test

The test measures the parameters such as riding time seconds or endurance, some of the functions of the test include evaluating balance, grip strength and motor coordination of the subjects; especially in testing the effect of experimental drugs or after traumatic brain surgery.(Dunham and Miya ,1957) have suggested that the skeletal muscle relaxation induced by test compound could be evaluated by testing the ability of mice to remain on a revolving rod. Before the beginning of all experiments, the riding ability of the animals in the rotarod is checked. Thus, the mice are initially put on a rotating rod, and mice that immediately dropped off within 30 seconds are removed from the experiment, male mice with a weight between 20 and 30g undergo pre-test on the apparatus. only those animals that have demonstrated their ability to remain on the rod for at least 3 minutes are used for the test.

1.6 Aim And Objectives

The study aim at evaluate the effect of hydro-methanol leaf extract of *Icacina trichantha* oliv. on seizures and skeletal muscle relaxation in mice.

The objectives of the study were;

1. To evaluate the plant extract on seizure.
2. To evaluate the plant extract skeletal muscle relaxation properties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Herbal Medicine

Herbal medicine has been practiced for centuries in both underdeveloped and developing nations, making it the oldest kind of healthcare. Nature has provided food, housing, clothing and medicines for treating illnesses for prehistoric people. These people could tell active plants from harmful ones and useful herbs with positive effects (Kunle *et al*., 2012).

There are 50,000 plant species with alleged therapeutic qualities (Barboza *et al*., 2009). Approximately 80% 0f the world's population still relies on herbal medicine as their primary form of health care, particularly in underdeveloped nations (Kunle *et al*., 2012; Okigbo *et al*., 2009 and Kamboj, 2000). The use of plants as medicine dates back 60,000 years ago according to ancient babylons (Qazi and Molvi, 2016).

The world health organization (WHO) describes herbal medicine as a field of study that uses herbs, herbal materials, herbal preparations and completed herbal products with components as active ingredients (WHO, 2000), these herbs are derived from various plant parts which includes leaves, stems, flowers, roots and seeds (Bent, 2008). Herbal medicines contain a variety of phytochemicals from different classes including saponins, flavonoids, glycosides, tannins, alkaloids and terpenoids (Saxena *et al*., 2013). Over time, phytochemicals potential to promote human health has been scientifically confirmed (Hasler and Blumberg, 1999), for instance, herbal sedatives and stomachic combinations mostly consist of aromatic plant species with therapeutic essential oils that have antibacterial gastrointestinal-soothing and antispasmodic characteristics (Ndhlala *et al*., 2011)

Some countries make use of herbal medicine for its accessibility and affordability. In some overpopulated nations like India, has almost no access to modern treatment; as a result, they

are forced to rely on herbal medicine for their essential medical requirements (Pandey *et al*., 2013), Traditional synthesized medications are thought to be less healthy than plants. Compared to reports of herbal toxicity, reports of the negative side effects of conventional medications have been found to be significantly more common (Pal and Shukla, 2003).

There are Various challenges currently faced by herbal medicine in the society today such as safety and toxicological concerns of herbal medicine, although the products of herbal medicine have a long history of being secure (Moreira *et al*., 2014), however the misuse of these medicines may result in negative effects from hazardous ingredients (George, 2011) Another major concern facing herbal drug market is the challenges of quality control, Lack of knowledge about herbal medicine within government regulations and need for scientific & clinical evaluation of herbal medicine.

A national policy on herbal medicine may address issues with intellectual property (WHO, 2005), the definition of herbal, medicine's place in the healthcare system and the creation of the required rules and laws. Regarding herbal medicine, national regulations differ from nation to nation. Herbal medications can be either prescription or over-the counter.

As originally suggested by the WHO guidelines for the assessment of herbal medicines, very few programmes have been formed to date to examine the efficacy and safety of herbal medicines. These recommendations have been useful in defining the place of herbal medicine in the medical community. However, the information needed to provide an accurate assessment of the efficacy, quality and safety of herbal medicine is insufficient, raising questions about the use of herbal medicines.

2.2 *Icacina trichantha oliv.* (*Icacinaceae)*

Icacina trichantha oliv. belongs to the family *Icacinaceae* (Asuzu and Abubakar, 1995). It is a medicinal plant used by the natives such as the indigenous tribes in Nigeria. It is commonly

known as ‘False yam’. It’s a flowering shrub native to forested vegetation areas of southern Nigeria (Asuzu *et al.,* 1990).

2.2.1 Taxonomy

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Icacinales

Family: Icacinaceae

Genus: Icacina A.Juss.

Species: *Icacina trichanthaa Oliv*.

Common name: False yam

(Source: Fay, 1987).

2.2.2 Local Names

Icacina trichantha is commonly known as false yam in English language. It is known by different names in different Nationalities and ethnic groups in Nigeria and Africa. It is sometimes locally abundant and troublesome as a weed in some parts of Nigeria. (see Table 1.1)

Table 1.1; Local names of *Icacina trichantha* Oliv (Burkhil, 1985)

COUNTRY	NAME	LANGUAGE	REFERENCES
Nigeria	Gbegbe	Yoruba	(Burkhil, 1985).
Nigeria	Ibugo	Igbo	(Burkhil, 1985)
Nigeria	Unumbia	Igbo	(Burkhil, 1985)
Nigeria	Unumbe	Igbo	(Burkhil, 1985)
Senegal	Kouraban	French	(Burkhil, 1985; Fay, 1987)
Ghana	Takwara	Ewe	(Burkhil, 1985; Fay, 1987)
Sudan	Pane	Arabic	(Burkhil, 1985; Fay, 1987)

Figure 1: Diagram of *Icacina trichantha* oliv. (Photo credit: Ajube bevelyn)

2.2.3 Origin and Distribution

The plant is a drought- resistant shrub which is up to 2 m with scandent growth above, and commonly found majorly in West and Central Africa (Asuzu and Abubakar, 1995)

2.2.4 Botanical Description

2.2.4.1 Leaves

The leaves are broadly elliptic, abruptly acute at the apex and rounded acute at the apex and rounded at the base, sometimes, they may be thinly pilose with simple, fasiciled hairs beneath. leaf length is about 8.0 – 10.0 cm while the width is up to 17.0cm (Hutchinson *et al*., 1958; Agyakwa *et al*., 1998)

2.2.4.2 Flowers

The flowers are densely crowded and subsessisle with calynx nearly as long as the petals which are usually villous outside. (Hutchinson *et al*., 1958; Agyakwa *et al*., 1998)

2.2.4.3 Fruits

The fruits are tomentose on the surface, ellipsoid to globose in shape and they are about 2.5 cm long (Hutchinson *et al*., 1958; Agyakwa *et al*., 1998)

2.2.4.4 TUBERS

It is characterized by the large, fleshy yam-like underground tubers, as big as several kilograms in weight, the tubers is rich in starch. Its large tuber is well known among the natives and traditional medicine practitioners for its nutritional and medicinal applications (Gbolade, 2012).

2.3 Ethnomedicinal Benefits

The plant is extensively used in rural areas the leaves and tubers have folkloric uses in the treatment of malaria, constipation and food poisoning in Nigeria (Asuzu and Abubakar ,1995a; Che *et al*., 2016). The leaves and seeds when crushed and macerated in local gin can be used for the treatment of hypertension and asthma. The tubers juice can be used for treating mumps (Ubom, 2010). In rural areas, tinctures of the tuber are often kept in households as a readily available remedy for treating common ailments and wounds such as fever and snake bites (Gbolade, 2012).

2.4 Phytochemical Constituents

The presence of phytochemicals such as flavonoids, terpenoids, tannins, glycosides, reducing sugars, steroids have been reported in different extracts and parts of *Icacina trichantha* (Onakpa *et al*., 2014; Otun *et al*., 2015). These phytochemicals may be responsible for the Antihyperglycemic, Anti-convulsion, sedative, analgesic and antimicrobial properties ascribed to the plant. (Dalziel,1937; Burkill,1985; Asuzu,2013; Onakpa and Asuzu,2013; Onakpa *et al*., 2014; Alawode *et al*., 2018). Alkaloids, tannins, phenols and saponins have all been found in phytochemical analysis of I,trichantha leaf extract (Otun, 2015). Alkaloids have a wide range of pharmacological activities including antimalaria, antiasthma, anticancer. Analgesic and antibacterial activities (Kittakoop, 2014 and Raymond, 2010), Saponins possess several biological properties such as anti-oxidant, anti- tumor, hyocholesterolemic, hypoglymeic and anti-inflammatory activities (Shen et al., 2017; Kitagawa et al., 2016) The presence of Flavonoids in the extract have some pharmacological activities such as anticancer (Zhao L *et al*., 2019) antimicrobial, antiviral, antiangiogenic (Zhao K *et al*., 2018; Camero *et al*., 2018) antimalaria, antioxidant, neuroprotective, antitumor and anti- proliferative

properties (Mazidi *et al*.,2019) Additionally, it guards against cardio metabolic diseases (Aguiar *et al*., 2019) and shows improved cognitive performance with ageing.

2.5 Rationale of the study

The burden of neuropsychiatric illnesses has not been fully addressed by health systems, necessitating the urgent need for alternative therapy (WHO,2013). Compared to synthetic or pure substances, many people believe plant- based medications to be much more effective and cause less side effects (Rout *et al*., 2012). There is a constant need to assess new drugs for the treatment of central nervous system diseases because of their high morbidity and socioeconomic cost. Despite the acclaimed ethnomedicinal uses of *I.Trichantha* aqueous leaf extract, Its toxicological profile and neuropharmacological properties have not been evaluated.

2.6 Pharmacological studies on *Icacina Trichantha*

2.6.1 Chemical Composition, antioxidant and antimicrobial potentials

The chemical makeup of *Icacina trichantha* plant leaf extracts were extracted using n-hexane, ethyl acetate and ethanol by using GC/GCMS sequentially was examined. The crude lraf extracts were subjected to a preliminary phytochemical screening, which identified the presence of tannins, flavonoids, phenols and glycosides, all of which are known to promote the bioactive properties of the plant in traditional medicine. In current research investigation, the antibacterial activity of *Icacina trichantha* against gram- negative bacteria including Escherichia Coli, Pseudomonas aeruginosa and klebsiella oxytoca was assessed. The bacteria strains were shown to respond better to the extracts of hexane and ethyl acetate than to ethanol. It was also examined, n- hexane extract had the strongest antioxidant activity (Kabir *et al.*, 2015).

2.6.2 The anti-inflammatory activity of *Icacina trichantha* tuber

The capacity of five fractions of the *Icacina trichantha* tuber to prevent croton oil-induced ear edoema in mice was examined. These fractions included hexane, chloroform, Ethylacetate, methanol and water. With an ID50(dose giving 50% edoema suppression) of 107g/cm2, the chloroform fraction was the most active one and significantly decreased ear edoema in a dose- dependent manner (Isaac *et al*., 1999).

2.6.3 The effects of *Icacina trichantha* tuber extract on the nervous system

The tuber of *Icacina trichantha* was extracted with 50% methanol and concentrated to dryness in vacuo to give a yield of 5.6% w/w. The extract induced sleep in rats treated with high doses (400 – 1000 mg/kg i.p). It potentiated pentobarbitone sleeping time in rat dose dependently and also induced significant local anaesthetic effects in guinea-pigs. The extract was able to give 80% protection to rats poisoned with pentylenetetrazole but failed to protect rats from strychinine poisoning. It induced significant dose- dependent analgesia in rats and showed significant muscle relaxant activity in mice (Asuzu and Abubakar, 1995)

2.6.4 Cytotoxic and genotoxic properties of leaf extract of *Icacina trichantha Oliv*.

The cytotoxic and genotoxic effects of the aqueous leaf extract of *Icacina trichantha oliv*. were evaluated using Allium cepa bioassay. Onion bulbs were exposed to various concentrations, 25 -100% v/v, of the extract for 48 - 96 h for macroscopic and microscopic analyses of the onion roots. The extracts significantly inhibited the root growth of allium cepa compared with the control in a concentration dependent manner also, photomicrographs revealed chromosomal abberrations ranging from chromosome laggard, vagrant, polar deviation, stickiness and bridges in the root tips cells of A.cepa (Timothy *et al,* 2014)

CHAPTER THREE

MATERIALS AND METHOD

3.1 Plant Collection

The leaves of *Icacina trichantha* used for the study was collected from capitol, Ovia North-East, Local Government Area, Benin city, Nigeria. The leaves were cleaned, shade dried, grounded to powder and stored in an air tight container till further use.

3.2 Animal Collection

Male mice with weight ranging from 18-30g were purchased from the department of pharmacology and toxicology Animal House, University of Benin, Benin City, Nigeria. The animals were housed in the animal facility of the department of pharmacology and kept in plastic cages, they were fed with standard feed with access to water and were acclimatized for a period of 14 days.

3.3 Materials And Chemicals

Animal cages, Gloves, Oral gastric tube, Distilled water, Feed materials, Cotton wool, Markers pen, Needle syringe, Weighing balance, Universal bottles, Marking tape, Tissue paper, Maceration bottle, Methylated spirit, Chloroform, Analytical weighing balance.

3.4 Equipments

Maximum Electroshock apparatus, Rota-rod apparatus

3.5 Drugs Used

The drugs used include; Pentylenetetrazole (Sigma Aldrich), Phenobarbitol (Phenobarbitol co.), Diazepam (Tablet; Swipha)

3.6 Materials and Chemicals for Extraction

Maceration bottle, Distilled water, stainless plates, conical, Flask beaker, measuring cylinder, Methanol, weighing balance, glass funnel, mucin cloth, sealant. Oven.

3.7 Extraction Process

During this extraction process cold maceration was carried out.500g of plant extract was weighed and poured into the maceration jar. 1:1 volume of methanol and water was measured and poured into the maceration bottle containing the weight plant and mixed thoroughly, the maceration bottle is then sealed and shaken vigorously. It soaked for 72 hours, on the last day of soaking, the extract was then filtered using a mucin cloth thoroughly soaked in methanol. The mucin cloth was spread on the glass funnel and the extract was then poured into portions, the filtrate was then poured into six stainless plates and placed in the oven to dry at 40 degree.

3.8 Methodology

3.8.1 Pentylenetetrazole- Induced Seizure (PTZ)

Mice were allotted into five groups (n=5), Group I served as the control and was given distilled water orally. Groups II, III and IV respectively received 100, 200 and 400 mg/kg doses of the extract orally and Group V received diazepam (2 mg/kg) orally. After 30 minutes of treatment, Pentylenetetrazole (70 mg/kg) was administered intraperitoneally to all the animals. Animals were observed for anticonvulsant activity. The latency to convulse, number of animals that convulsed and the degree of protection was observed (Swinyard et al., 1989).

3.8.2 Maximal Electro-Shock (MES)

Mice were divided into five groups (n=5 each). Group I served as control and received distilled water orally. Group II – IV (extract-treated groups), were administered 100, 200 and 400mg/kg doses orally. Group V was treated with standard drug phenobarbitone (30 mg/kg,

p.o). convulsion was Induced 1 hour after, by applying electrical stimulus of 100 mA, at a frequency of 50 Hz for 0.2 s through maximum electroshock (Ugo Basile ECT unit, Model 57800).

3.9 Test for skeletal muscle relaxation property

3.9.1 Rotarod Test

Mice were randomly distributed into five groups (n =5). Group I served as a control and was served distilled water orally. Group II, III and IV respectively received 100, 200 and 400 mg/kg doses of the extract orally, while group V received diazepam (4 mg/kg) orally. After 30 minutes of treatment, the mice were placed and observed on Ugo basil rotarod at 6rpm. The fall watch at time points 30 min, 60 min, 90 min and 120m. The experiment was conducted in a sound- free laboratory and after each observation, the rotarod were cleaned with 70% ethanol and allowed to dry completely to remove residual odour.

3.9.2 Static Rod Test

Mice were randomly distributed into five groups (n=5). Group 1 served as control and received distilled water orally. Group II, III and IV respectively received 100, 200 and 400 mg/kg doses of the extract orally, while Group V received diazepam (4 mg/kg) orally. One hour after treatment, each mouse was placed on an elevated rod and the fall off time was observed for 3 min for each mouse using a stop clock

3.10 Statistical analysis

To examine the contrast between the data being compared, a one-way analysis of variance (ANOVA) with Dunnet's or Tukey's post hoc tests was used at $p < 0.0001$, differences were considered significant.

CHAPTER FOUR

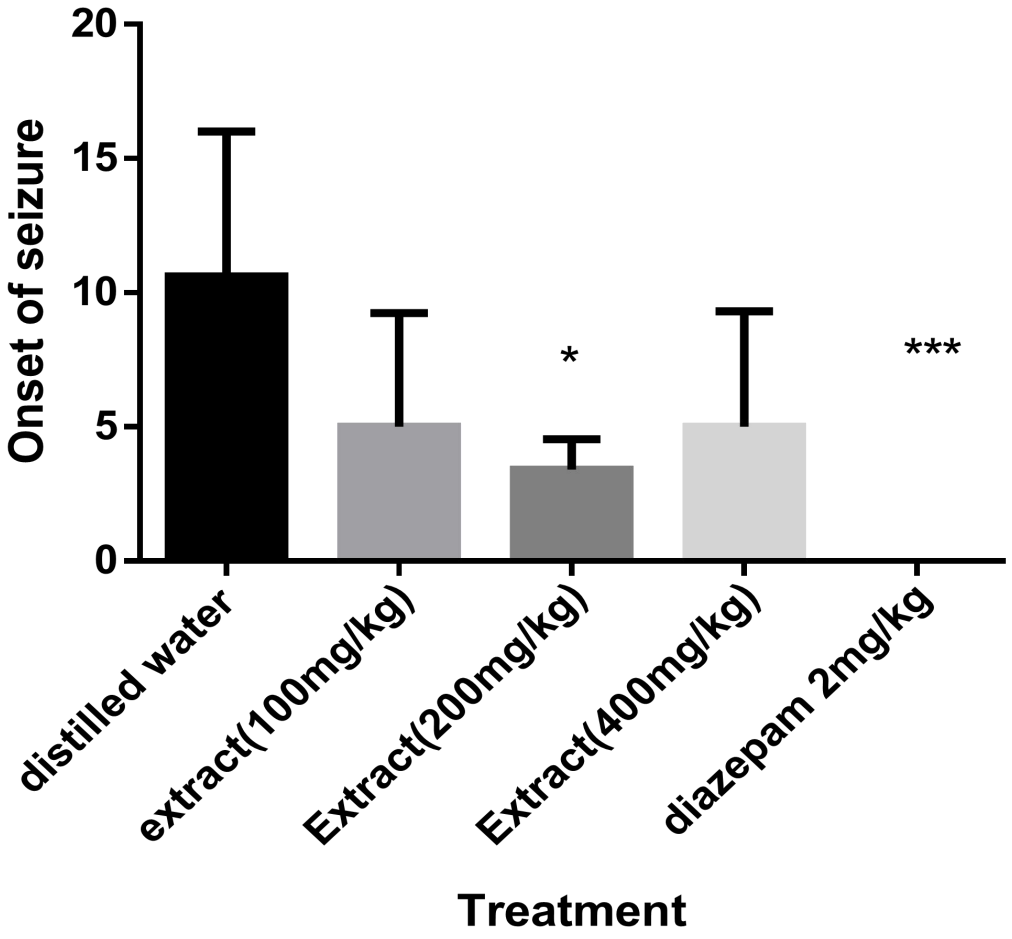

Figure 4.11: Effect of hydro-methanol of the leaf extract of *Icacina trichantha* On Pentylenetetrazole (PTZ) Induced seizure.

Figure 4.11 shows that PTZ at a single dose of 70 mg/kg produced seizures across all groups (100 and 400 mg/kg) except 200 mg/kg, suggesting that at 200 mg/kg it was statistically significant. Diazepam (2 Mg/kg) was significant statistically as it protected against hind limb tonic seizures.

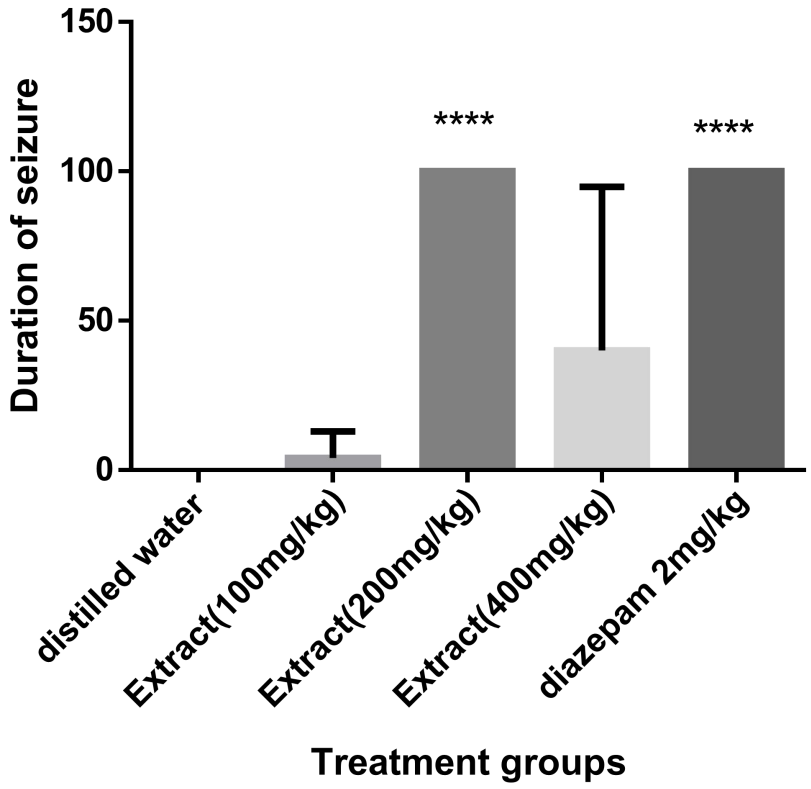

Fig 4.12: Effects of hydro-methanol of the leaf extract of *Icacina Trichantha* on duration of seizure in pentylenetetrazole (PTZ) Induced seizure in mice.

Fig 4.12 shows that PTZ at a single dose of 70 mg/kg did not produce seizures across all groups (100, and 400 mg/kg) The plant was statistically insignificant except 200 mg/kg, suggesting that at 200 mg/kg it was statistically significant. Diazepam (2 Mg/kg) was significant statistically as it protected against hind limb tonic seizures.

Table 4.13; Effects of hydro-methanol leaf extract of *Icacina Trichantha* on Electro induced convulsion

GROUPS	DOSE (mg/kg)	NUMBER OF MICE PROTECTED	PERCENTAGE PROTECTION
CONTROL	D/W	0/5	0%
EXTRACT	100	0/5	0%
EXTRACT	200	1/5	20%
EXTRACT	400	0/5	0%
PHENOBARBITONE	30	5/5	100%

Table 4.13 shows that there were no protection against shock across groups (100 and 400 mg/kg) 200mg/kg of the extract and Diazepam (2 Mg/kg) was significant statistically as it protected against hind limb tonic seizures.

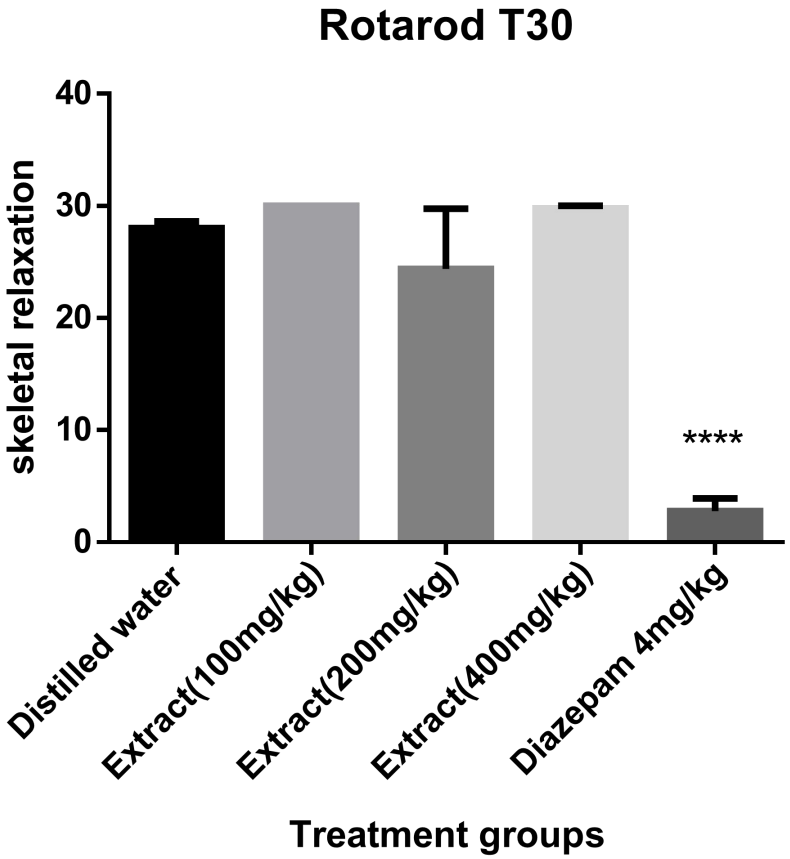

Fig 4.14; Effects of hydro-methanol of the leaf extract of *Icacina Trichantha* on Rotarod.

Fig 4.14 shows that there were no significance across all groups (100, 200 and 400 mg/kg) and Diazepam (2 Mg/kg) was significant at T30.

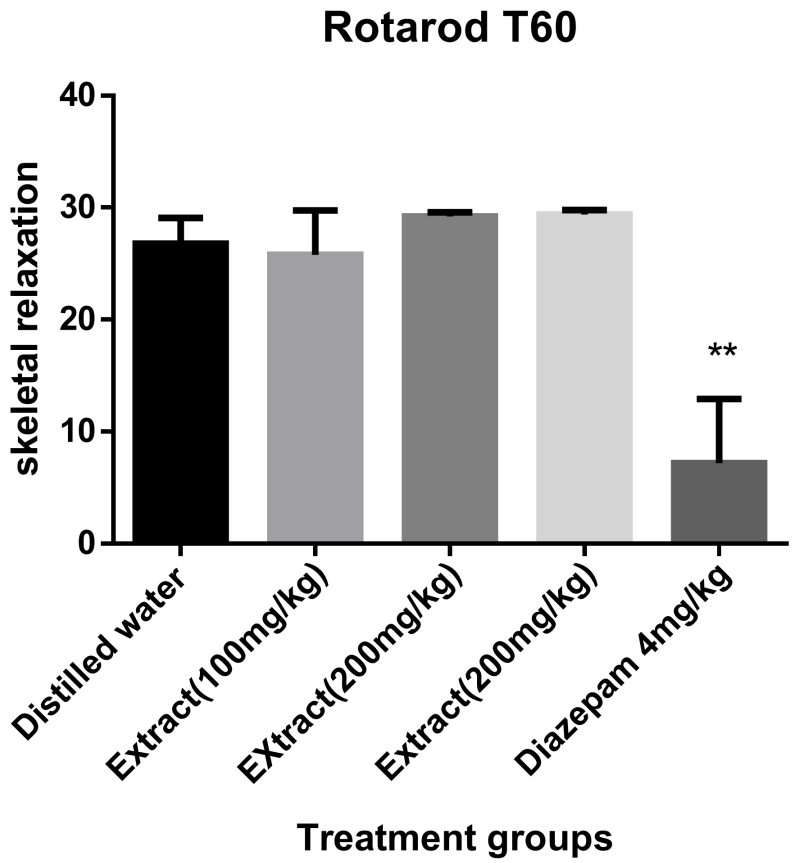

Fig 4.15: Effects of hydro-methanol of the leaf extract of *Icacina Trichantha* on Rotarod on T60.

Fig 4.15 shows that there were no significance across all groups (100, 200 and 400 mg/kg) and Diazepam (2 Mg/kg) was significant at T60.

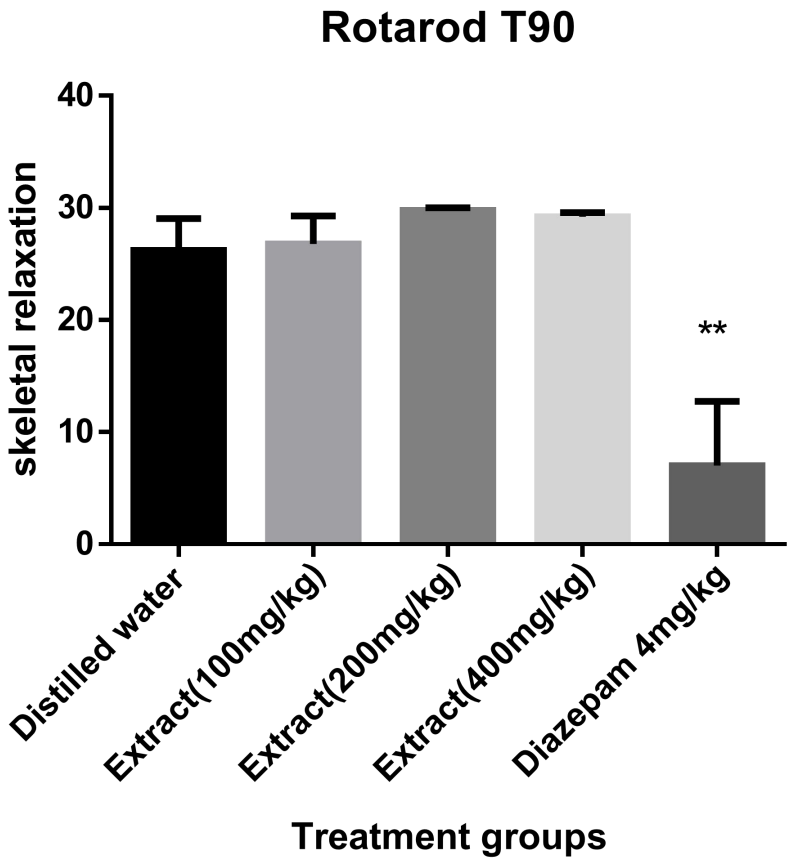

Fig 4.16; Effects of hydro-methanol of the leaf extract of *Icacina Trichantha* on Rotarod T90.

Fig 4.16 shows that there were no significance across all groups (100, 200 and 400 mg/kg) and Diazepam (2 Mg/kg) was significant at T90.

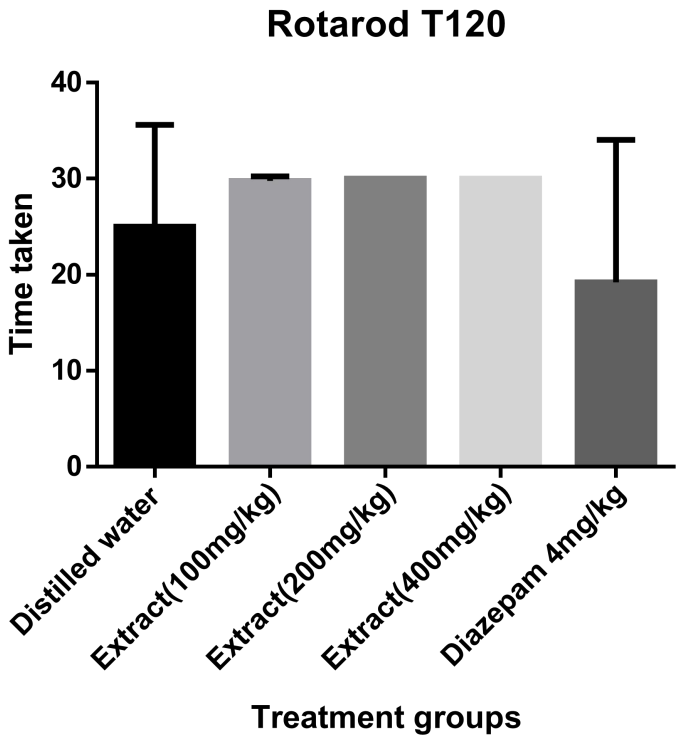

Fig 4.17; Effects of hydro-methanol of the leaf extract of *Icacina Trichantha* on Rotarod T120.

Fig 4.17 shows that there were no significance across all groups (100, 200 and 400 mg/kg) and Diazepam (2 Mg/kg) was insignificant at T120

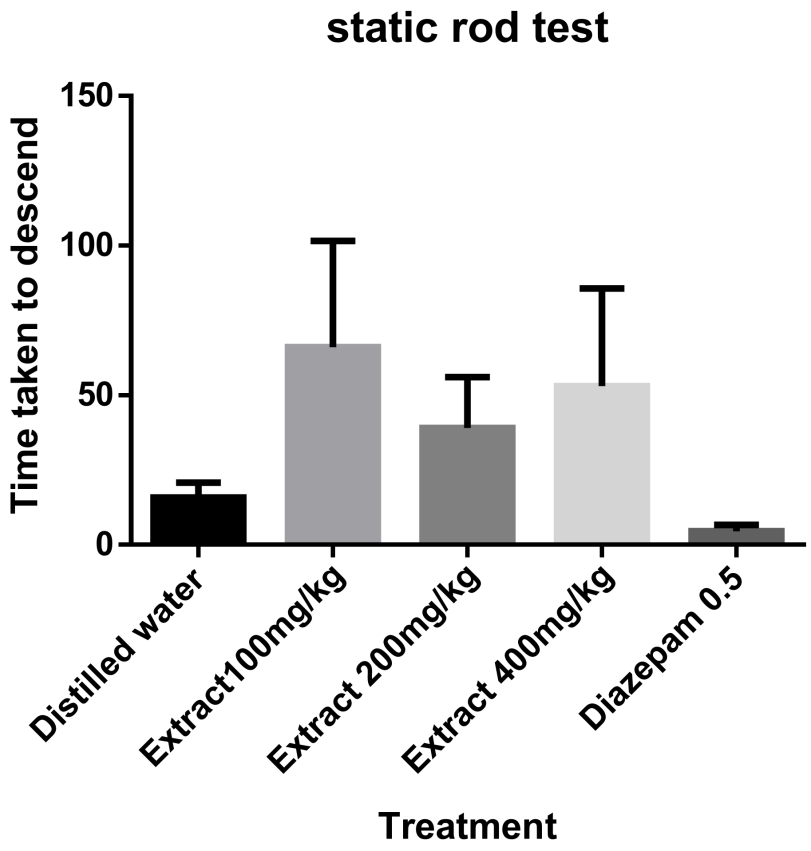

Fig 4.18; Effects of hydro-methanol of the leaf extract of *Icacina Trichantha* on static rod test.

Fig 4.18 shows that there were no significance across all groups (100, 200 and 400 mg/kg) and Diazepam (2 Mg/kg) was insignificant.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Electroshock and chemically induced models are conventionally used primarily to screen agents with antiepileptic property. These models induce seizures via different mechanisms. Pentylene induced convulsion identifies agents that increase the threshold and drugs which block seizures induced by this model are potential therapies for absence seizures (Ninds, 2014). Behavoural responses to these seizures include myoclonic jerks, clonic jerks and hind limb clonic extensor amongst others. An investigational drug increases seizure threshold if there is an absence of clonic phase (Ninds, 2014). Convulsive effect 0f pentylenetetrazole is exerted via inhibiting the activity of gamma amino butyric acid (GABA) at GABA Receptors (De sarro et al., 1999). Similarly the maximum electro- shock induced seizures and shows a behavourial pattern similar to humans (Swinyard et al., 1989). It is utilized in identifying compounds that prevent seizure spread (Ninds, 2014). This model is a predictive model that is effective against generalized tonic- clonic seizures (Krall et al., 1978; Toman *et al.,* 1946) and the endpoint in this test is tonic hind limb extension (Krall et al., 1978), the present study showed that the extract did not protect the mice against MES- induced convulsion. This suggests that the extract may not possess anticonvulsant activity.In skeletal muscle relaxation used to screen agents with muscle relaxation properties, A muscle relaxant is a drug that affects skeletal muscle function and decreases the muscle tone, In rotarod test, the skeletal muscle relaxation induced by test compound could be evaluated by testing the ability of mice to remain on a revolving rod. In static rod test , the test was conducted to measure the body coordination and balance .

5.2 Conclusion

1. The leaf extract of *I. trichan*tha possess neuropharmacological property as demonstrated in anticonvulsant test.

2. The leaf extract of I. Trichantha does not posses skeletal muscle relaxation properties .

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