SUITABILITY OF CHICKS AS EXPERIMENTAL ANIMALS IN SOME PHARMACOLOGICAL STUDIES

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Declaration

I declare that the work in this thesis entitled 'SUITABILITY OF CHICKS AS EXPERIMENTAL ANIMALS IN SOME PHARMACOLOGICAL STUDIES' has been carried out by me in theDepartment of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project was previously presented for another degree or diploma at this or any other university

Onyum Catherine EDEH	_____	_____
Name of Student	Signature	Date

Certification

This thesis entitled 'SUITABILITY OF CHICKS AS EXPERIMENTAL ANIMALS IN SOME PHARMACOLOGICAL STUDIES' by Onyum Catherine EDEH meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to the first fruit of my womb EUCHARIA OINE ADA IGELE with whose co-operation I began this journey. My sister (OINEM) you are indeed.

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ABSTRACT

Pharmacology is the unified study of the properties of chemicals (ranging from medicines to horrendous poisons), living organisms and all aspects of their interactions. Animal experimentation is the term used to describe the experimental use of animals in education and research. *In vivo* and *in vitro* use of animals for experimentation is a vital component of pharmacology education (teaching and training), as it is in basic, applied and safety pharmacology research that leads to drug discovery, development and exposition of drug effects.Since the rate of research is increasing and challenges of acceptability, availability and affordability plague the use of traditional experimental animals such as mice, rats, guinea pigs, rabbits and cats, there is the need to find alternative animal models such as chicks.Furthermore, developmentof the principles of the '3Rs'namely: replacement, reduction and refinement has increased the search for suitable alternatives such as tissue culture, drosophila and non-rodent animals. This project addresses some of the many challenges associated with the use of traditional experimental animals in biomedical research. Specifically, it assesses the suitability of the more readily acceptable, available and affordable chick(en)s as alternative experimental animals in pharmacology education and research.The study outcomes show that chicks and chickens produced comparable results to those obtained with mice, rats, guinea pigs and rabbits in the following eight (out of ten tested) classical pharmacology experiments: dose-response relationship (*in vivo* and *in vitro*),antagonism and synergism, microsomal enzyme induction and inhibition, analgesic assay (using hot plate method), anticonvulsant screening (using pentylenetetrazole-induced seizure method), determination of median lethal dose (using conventional medicine, plant extract and pesticide) as well as parasympathomimetic and sympathomimetic effects on ileal smooth muscle. However, chicken isolated heart atria was not comparable to that of guinea pig as there was no activityrecorded after drug administration neither was it possible to elicit acetic acid-induced writhes in chicks under the usual

experimental conditions employed in mice. Thus, the more readily available, affordable and acceptable chick(en)s were suitable in 80% of the tested pharmacology experiments used in teaching and research in the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria.

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List of Abbreviations

AAVS- American Antivivisection Society

ABUCAUC- Ahmadu Bello University Committee on Animal use and Care

ACh- Acetylcholine

AEDs- Antiepileptic Drugs

ANOVA- One way Analysis of Variance

ANS- Autonomic Nervous System

ASPCA- American Society for the Prevention of Cruelty to Animals

AV- Atrioventricular

AVN-Atrioventricular Node

BZ- Benzodiazepine

CIM- Cimetidine

CNS- Central Nervous System

COX- Cyclooxygenase

CPL- Chloramphenicol,

CPZ- Chlorpromazine

DAM- Delayed Amelanotic

DDT- Dichlorodiphenyltrichloroethane

DNA- Deoxyribonucleic acid

EC_{50}- Maximum Concentration

ENS- Enteric Nervous System

FSH- Follicle Stimulating Hormone

GABA- Gamma-Aminobutyric Acid

GIT- Gastrointestine Tract

HLE- Hind Limb Extension

IAEC- Institution's Animal Ethics Committee

ILAE- International League against Epilepsy

IP- Intraperitoneal

IV- Intra Venous

LD_{50}- Median Lethal Dose

MES- Maximal Electroshock Seizure

MGluRs- Metabotropic Glutamate Receptors

NS- Normal Saline

NSAIDs- Non-Steroidal Anti-inflammatory Drugs

OTC- Over-the-counter

PGs- Prostaglandins

PHB- Phenobarbitone

PKA- Phosphokinase C

PKC- Phosphokinase C

PTX- Picrotoxin

PTZ- Pentylenetetrazole

REM- Rapid Eye Movement

RGE- Retinopathy Globe Enlarged

SA- Sinoatrial

SEM- Standard Error of Mean

SPCA- Societies for Protection and Care of Animals

TM_2- Transmembrane

UK- United Kingdom

UN- United Nation

USA- United States of America

CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Pharmacology is the unified study of the properties of chemicals (ranging from medicines to horrendous poisons), living organisms and all aspects of their interactions. Thus, the spectrum of pharmacology includes physiology, biochemistry, pathology, therapeutics and toxicology; and hence it is a core subject for pharmacy, medical, nursing and veterinary students at both undergraduate and postgraduate levels. *In vivo* and *in vitro* use of whole animal or isolated tissues for experimentation is a vital component of such pharmacology education (teaching and training), as it is in basic, applied and safety pharmacology research that leads to drug discovery and exposition of drug effects.

This project addresses some of the many challenges associated with the use of domestic animals such as mice, rats, guinea pigs and rabbits in biomedical research. Specifically, it assesses the suitability of the more readily acceptable, available and affordable chick(en)s as alternative experimental animals in pharmacology education and research.

1.2 Statement of the Research Problem

The use of animals in education and research dates back to the period when humans started to look for ways to prevent and cure ailments (Dinesh and Chetna, 2014). The mission of medicine is to eliminate suffering, maintain good health and prolong life. Animal experimentation is the term used to describe the use of animals in experiments in educationandresearch. For long, it has been an integral part of pharmacology education at medical colleges (Badyal*et al.*, 2010) and in research.

The debate surrounding animal use in experiments and teaching started way back in the 17th century (Richmond, 2002). The animal protection movement was started in 18th century by a

group of people known as abolitionists in England. Another worldwide initiative started in 1975 by Societies for Protection and Care of Animals (SPCA) who opposed all forms of animal research (Richmond, 2002).The dilemma to continue animal experiments in education and research continues with varied and confusing guidelines (Dinesh and Chetna, 2014). Although, rodents are small, easily housed/maintained and adapt well to new surroundings, because of the increasing rate of research, they are becoming scarce, relatively expensive, difficult to handle as they can easily biteand difficult to restrain.Animal experimentation is what the biomedical sciences in third world countries cannot do without as far as education and research are concerned.However, issues of ethics surround animal experimentation in terms of the care, use and eventual disposal of the animals (Ferdowsian and Beck, 2011).

Thus, the research problem is one of poor acceptability, availability and affordability of animals traditionally used in research which are mice, rats, guinea pigs, rabbits and cats.

1.3 Justification for the Study

The use of animals in research dates back to ancient Greece, with Aristotle (384-322 BCE) and Erasistratus(304-258 BCE) among the first to perform experiments on living animals (Cohen and Loew, 1984). Drugs, an important tool in healthcare, are introduced in therapeutics after experimental evaluation. Thus, most of present day drug discoveries were possible because of the use of animals in research. Research using animal models has been central to most of the achievements of modern medicine (Lieschke and Currie, 2007).They have contributed to most of the basic knowledge in fields such as human physiology and biochemistry and have played significant roles in fields such as neuroscience and infectious diseases (Jann and Steven, 2011). For example, the results have included the near-eradication of polio and the development of organ transplantation and have benefited both humans and animals (*Royal Society of Medicine, 2015).*

Chicken (*Gallus gallusdomesticus*) and their eggs have been used extensively as research models throughout the history of biology. In times past, they served as important models for normal human biology as well as pathological disease processes (Murphy, 1914).The science of embryology, which can be traced as far back as Aristotle's time, is largely based on the use of avian embryos and birds' egg (McArdle, 1999).A rich background of information, coupled with new technologies and relative ease of maintenance suggest an expanding utility for the chick embryo in research.

Chicks are very cheap, easy to handle and maintain, adapt to new environments, relatively have good surviving rates and are also very simple to work with, as they are friendly.Since the rate of research is increasing and problems of acceptability, availability and affordability affectsthe use of traditional experimental animals such as mice, rats, guinea pigs, rabbits and cats, there is need to find replacement animal models such as chicks. A number of such replacement animals have been tried e.g. *drosophila melanogaster* (Morgan, 1910), zebra fish (Streisinger, 1972) and *Aspergillusnidulans* (Micheli, 1729) for diverse reasons such as cost, breeding difficulties and non-suitability for pharmacological experiments. This work examines and reports a possible solution to the problem by way of use of chicks as replacement for the traditional experimental animals.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this research project is to assess the suitability of chicks and older chickens (cockerels, except otherwise stated), as replacements for other routinely used laboratory animals in some*in vivo* and *in vitro*pharmacologicalstudies.

1.4.2 Specific objectives

The objectives are to determine

1. Suitability of chicks in dose-response relationships with diazepam-induced sleep - *in vivo.*
2. Suitability of chicks in *in vivo* drug antagonism and synergism.
3. Suitability of chicks in microsomal enzymes induction and inhibition - *in vivo.*
4. Effects of parasympathomimetic and sympathomimetic drugs on smooth muscle using adult chicken (cock or hen) intestine - *in vitro.*
5. Effects of parasympathomimetic and sympathomimetic drugs on cardiac muscle using adult chicken (cock or hen) heart -*in vitro*
6. Suitability of chicks in analgesic action of drugs in different models of Pain - *in vivo.*
7. Suitability of chicks in anticonvulsant action of drugs in pentylenetetrazole-induced seizure model - *in vivo.*
8. Suitability of chicks in determination of median lethal Dose (LD_{50}) - *in vivo.*

1.5 Research Hypothesis

Chicks are not suitable as replacement for experimental animals in pharmacology.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Animal Models

A model organism or animal model is a non-human species that is studied to understand a particular biological processes with the view that the result obtain will give an insight into the workings of other organisms (Fields and Johnston, 2005). Animal models are widely used in research to study human disease, when human experimentation would be unfeasible or unethical (Griffiths, 2010).Studying model organisms can be informative, but care must be taken when extrapolating the results from one organism to another (Slack, 2013).

Animal modelsgive a better understanding of disease process without added risk of harming an actual human (Barre-Sinoussi and Montagutelli, 2015).Animal models have been used to address different types of scientific questionsranging from basic science to the development and assessment of novel vaccines and therapies. The use of animals is not only based on the similarities in the biology of most mammals, but also on the fact that human diseases often affect other animal species (Barre-Sinoussi and Montagutelli, 2015).It is particularly the case for most infectious diseases but also for very common conditions such as Type I diabetes, hypertension, allergies, cancer, epilepsy, myopathies etc. These diseases are similar and shared the same mechanisms that 90% of the veterinary drugs used to treat animals are identical or very similar to those used to treat humans. Many breakthroughs in basic science and medical research today have been made possible because of observations and testing on animal models. Most vaccines, which save millions of human and animal lives every year, have been successfully developed using animal models. The treatment of Type I diabetes by insulin was first established in dog's by Banting and McLeod in 1921 who received the Nobel Prize in 1923.

2.1.1 Types of animal models

From the earliest days of modern biology and medicine, animal experimentation has been used to provide insights into both human and general biology.

There are four (4) main categories of animal models:

1. **Induced or experimental models:**These are models that attempt to reproduce conditions found in the original species.
2. **Spontaneous or natural models:** Those are recognised as being similar to some condition in the original species.
3. **Negative or nonreactive models:** These are the normal counterparts of a disease model.
4. **Orphan models:** These are models of animal diseases for which no human or animal counterpart is known (Frenkel, 1969).

2.1.2 Criteria for selection of animal models in research

In experiment or research, model selection is the right of the individual researcher who therefore will be responsible for convincing the scientific world for his or her choice.

The selection of any animal models for research should be based on the following considerations:

1. Appropriateness as an analog
2. Transferability of information
3. Genetic uniformity of organisms
4. Background knowledge of biological properties
5. Cost and availability
6. Generalization of the results
7. Ease of adaptation to experimental manipulation
8. Facilities required to house the chosen model appropriately

9. Husbandry expertise - some models require not only special housing, but also special caree.g chicks
10. Ecological consequences
11. Ethical implications (Davidson *et al.*, 1987).

2.1.3 Most commonly used animal models in pharmacological experiments

Many different animal species are used around the world, but the most common ones include mice, fish, rats, rabbits, guinea pigs, hamsters, domestic animals (sheep, horse, goat, cow), birds, cats, dogs, pigs, and non-human primates (monkeys, and in some countries, chimpanzees)(Humane Society International, 1991).

2. 1.4 Regulation of animal use in experiments

The first animal protection law to regulate animal testing was in 1822 in the British parliament and was followed by the Cruelty to Animals Act in 1876. The legislation was supported by Charles Darwin, who wrote way back in 1871, “You ask about my opinion on vivisection. I quite agree that it is justifiable for real investigations on physiology; but not for mere damnable and detestable curiosity. It is a subject which makes me sick with horror, so I will not say another word about it; else I shall not sleep tonight”. This led to formation of American Society for the Prevention of Cruelty to Animals (ASPCA) in 1860s, followed by the American Antivivisection Society (AAVS) in 1883. Under this law, “any procedure can be performed on an animal if it is successfully proven that it is scientifically justified, as specified under the provisions of the Animal Welfare Act and the guide for the care and use of laboratory animals, published by the National Academy of Sciences”. The institution's animal ethics committee (IAEC), for care and use of animals advise the researchers for the use of animals in their respective research projects. These committees have the responsibility to ensure that alternatives, including non-animal alternatives, have been discussed, the

experiments are not unnecessarily duplicative and appropriate analgesia is given unless it would interfere with the research.

The use of animals in research is a privilege granted by society or various committees to the researcher with the expectation that it will benefit both humans and animals and also lead to improvement in their well-being (McCarthy, 1999; Perry 2007).

The principles of 3Rs have been used to regulate and to develop alternatives to animal experimentation:

1. **Replacement:** Refers to methods that avoid the useof animals. It can be absolute replacements (i.e. replacing animals with inanimate systems such as computer programs) or relative replacements (i.e. replacing animals such as vertebrates with invertebrate animals such as *Drosophila,* nematode worms, bacteria, fungi etc).
2. **Reduction:** Itinvolves minimizing animal use and enablesresearchers to obtain comparable levels of information from the use of fewer animals or maximizing the information obtained from the few number of animals used.
3. **Refinement:** Refers to modifications of experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. Examples include, non-invasive techniques, using appropriate analgesic regime for pain relief except where it will alter the outcome.

2.2 Chicks as an Animal Model

The chicken (*Gallus gallusdomesticus*) is a type of domesticated fowl, a subspecies of the red jungle fowl. It is one of the most common and widespreaddomestic animals, with a total population of more than 19 billion as of 2011. Chickens are more in the world than any other bird or domesticated fowl (UN's Food and Agriculture Organisation, 2011).Chickens are kept

primarily by humans as a source of food (consuming both their meat and eggs) and secondarily as pets. They were originally raised for cockfighting and for special ceremonies.Chickens were not kept for food until the Hellenistic period (fourth–second centuries BCE) (Xiang, 2014).

Genetic studies have shown multiple maternal origins in Southeast Asia, East Asia, and South Asia, but with the clade found in the Americas, Europe, the Middle East and Africa originating in the Indian subcontinent (Xiang, 2014).The domesticated chicken was imported from India to Lydia in western Asia Minor and to Greece by the fifth century BC (Toussaint-Samat, 2009).

SCIENTIFIC CLASSIFICATION OF CHICKS

Kingdom	Animalia
Phylum	Chordata
Class	Aves
Order	Galliformes
Family	Phasianidae
Genus	Gallus
Species	*G.gallus*
Subspecies	*G. g. domesticus*

(Linnaeus, 1758).

Figure 2.1: Plate of arooster or cock (left) and hen (right)

2.2.1 Terminology

Chickenare knownas young domestic fowl or fowl (Firefly Encyclopedia of Birds, 2003).In the UK and Ireland, adult male chickens over the age of one year are primarily known as*cocks*, whereas in the United States, Canada, Australia and New Zealand, they are more commonly called*roosters.* Males less than a year old are*cockerels*. Castrated roosters are called*capons*. Females over a year old are known as *hens*, and younger females as*pullets*. Although in the egg-laying industry, a pullet becomes a hen when she begins to lay eggs, at 16 to 20 weeks of age. The young are often called *chicks*(Firefly Encyclopedia of Birds, 2003).

Chickens are omnivores (Berhardt, 1986). In the wild, they often scratch at the soil to search for seeds, insects and even animals as large as lizards, small snakes or young mice (Info on Chicken Care, 2003).

Advances in basic and clinical sciences depend heavily on thesuccessful use of appropriate animal models. Today there is widespreaduse of the mouse with its various genetic modifications,but there are also limitations to this model, particularly when anessential gene is deleted in all tissues. This systemic genetic modification of the entire animal's physiology may make it difficult if not impossible to draw valid conclusions regarding thefunction of a specific gene in a specific tissue. However, thechoice of appropriate animal models is dependent on familiarityof scientists with different animal models, the availability ofanimal models, and the cost of the animal and housing requirements (Bahr, 2008).

The chicken, also referred to as the domestic hen, has servedscience well. The chick embryo has been the basis for understandingthe stages of early development and its control and is widelyused in embryology classes. The young chick was the popularanimal of choice for the discovery of steroid receptors, namelyprogesterone and estradiol receptors. The chick's oviduct, a richsource of these receptors following treatment with steroids,yielded large amounts of tissue for isolation, characterization, andcloning of the steroid receptors. A great deal of vitamin D researchwas done using the shell gland of the chicken. Awareness ofthe toxicity of some chemicals used in the environment, such asdichlorodiphenyltrichloroethane (DDT), came from observingthat birds exposed to this chemical laid soft-shelled eggs (Bahr, 2008).

2.2.2 Use of chickens in research

The chicken has had a long association with man more than 8,000 years ago when humans ceasedto be hunter-gatherers. The Red Jungle Fowl is thoughtto be the source of all poultry

(Fumihito*et al.*, 1994).Analysis of mitochondrial DNA suggests that domesticationtook place more than 8,000 years ago in what is calledThailand and Vietnam, the region in which the Red JungleFowl is still found today (Komiyama *et al.*, 2004). Thegreat philosopher Aristotle contained a description of achick embryo as a model for embryology in his famous work *HistoriaAnimalum*. Later examples include its use in the discoveryof blood circulation (Harvey, 1628), the transmission ofinfection (Pasteur, 1880), and the most famous, the descriptionof chicken breeds by Darwin in *The Variation ofPlant and Animals under Domestication* (Darwin, 1868). Theroots of avian genomics go back more than 100 years to theemerging field of genetics. Familiar terms such as alleles(Bateson and Saunders, 1902), genetic linkage (Sutton,1903), and epistasis (Bateson and Punnett, 1911) werebased on work on chicken morphological traits, such asfeather colour. The first genetic maps exploited sex linkagein chickens (Spillman, 1909) and were soon expandedto create the first genetic linkage maps of the chicken(Serebrovsky and Petrov, 1930; Hutt, 1936).

The chicken has also been an important model organism in developmentand immunology (Stern, 2005). Easily access to thechicken embryo using incubated eggs and the ease ofembryo manipulation make the chick an ideal system forthe study of vertebrate development (Stern, 2005). Thechicken has also been important in other fields such asimmunology, with the discovery of B cells, and in medicine,with the isolation of the first oncogenes (Brown *et al.*, 2003). The chick limb bud has been used as a modelof molecular patterning in vertebrates, with the discoveryof the apical ectodermal ridge that determines proximaldistalpatterning in the limb (Saunders, 1948) and thepolarizing region, a small group of cells at the posteriormargin of the limb bud that act as a signalling region tospecify the pattern of structures in the limb (Tickle *et al.*, 1975).

2.2.3 Use of chickens in pharmacological experimentation

The chicken has been used as a model in developmentalbiology for over 100 years (Stern, 2005). Example is the use of the chick limb bud to understandcell patterning during development. Classic grafting experiments(Tickle *et al.*, 1975) of posterior regions of the limb bud to the anterior region generated mirror duplicationsof the digit pattern.The chicken as a model for human eye defects is usedas an example of its use in medical research. Five chickenmutants have been used as models of retinal degeneration (Semple-Rowland *et al.*, 1998). Other blinddefects include blindness enlarged globe (Pollock *et al.*,1982), sex-linked retinal dysplasia and degeneration (Randall*et al.*, 1983), delayed amelanotic (DAM) strain (Komendaand Fite, 1983), and retinopathy globe enlarged(RGE)(Montiani-Ferreira *et al.*, 2005).

2.3 Dose-Response Relationship

The dose-response relationship is the change in effect on an organism caused by varying levels of doses to a stressor (usually a chemical) after a certain exposure time, or to a food (Crump *et al.*, 1976).

Studying dose-response and developing dose-response models, is central to determining safe, hazardous and beneficial levels and dosages for drugs, pollutants, foods, and other substances to which humans or other organisms are exposed. As the concentration of a drug increases, the magnitude of its pharmacologic effect also increases (Lockheed, 2009).

2.3.1 Concept of dose and concentration

The dose-response relationship is the cornerstone of Pharmacology/Toxicology. It quantitatively defines the role of the dose of a chemical in evoking a biological response. In the absence of chemical no response is seen. As chemical is introduced into the system the

response is initiated at the threshold dose and increases in intensity as the dose is raised. Ultimately a dose is reached beyond which no further increase in response is observed. The dose-response relationship can be demonstrated for interactions of chemicals with biological receptors leading to physiological responses, therapeutic effects of drugs, or for toxic, lethal, teratogenic, mutagenic or carcinogenic effects of chemicals. The data from these studies can be expressed as dose-response curves which can take the form of linear plots or a variety of reciprocal or logarithmic transformations (Altshuler, 1981).

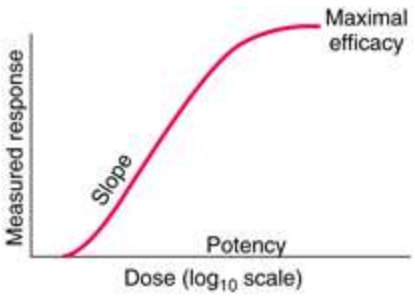
2.3.2 The arithmetic and log dose-response or log concentration curve.

The dose-response curve is used to determine;

1. Potency: It is a measure of the amount of drug necessary to produce an effect of a given magnitude. For a number of reasons, the concentration producing an effect that is fifty percent of the maximum is used to determine potency; it is commonly designated as the EC_{50}.

2. Efficacy (intrinsic activity): It is the ability of a drug to elicit a physiologic response when it interacts with a receptor. Efficacy is dependent on the number of drug-receptor complexes formed and the efficiency of the coupling of receptor activation to cellular responses.

Hypothetical dose-response curve



2.4 *In vivo* Drug Antagonism and Synergism

In vivo drug combinations may exhibit synergistic or antagonistic effects.Rational design of synergistic drug combinations remains a challenge despite active experimental and computational efforts. Because drugs manifest their action via their targets, the effects of drug combinations should depend on the interaction of their targets in a network manner (Fitzgerald *et al.,* 2006).Drug combinations have been envisaged by many to be a promising approach to treat complex diseases such as cancer, inflammation and type 2 diabetes(Keith, 2005). However, when used in combination, drugs interact in many unexpected ways and show a plethora of different outcomes (Yeh*et al.,* 2009). Among these interactions, drug synergy and antagonism have attracted special attentions.

2.4.1 Drug interactions

A drug interaction is a reaction between two (or more) drugs or between a drug and a food or any supplement. Drug interaction can decrease or increase the action of the drug(s) or cause adverse effects (Lynch 2019).A background medical condition can also cause drug interaction.For example, severe haemorrhage may occur when warfarin and salicylates (aspirin) are combined.

2.4.2 Antagonism

Drug antagonism occurs when the combined effect of two drugs is less than the sum of each drug given alone. Drug antagonism, in contrast, is often undesirable, but could be useful in selecting against drug resistant mutations (Chait*et al.,* 2007).

2.4.3 Synergism, potentiation and addition reactions

A synergistic reaction occurs when the combined effect of two drugs or chemicals is greater than the sum of the effects of each given alone. It can be in two forms: summation (additive) and potentiation.

Potentiation is an interaction between two or more drugs or agents resulting in a pharmacologic response greater than the sum of individual responses to each drug or agent, e.g. combination of sedative drugs with alcohol.

Additive effect is when the combined effectof two or more drugs or chemicals isequal to the sum of the effect of each drug or chemical given alone(Kamo and Yokomizo 2015).

2.5 Microsomal Enzymes Induction and Inhibition

Microsomal enzymes are a group of enzymes associated with a certain particular fraction of liver homogenate that plays a role in the metabolism of many drugs (Mosby's Medical Dictionary, 2009). Microsomal enzyme system is a collection of enzymes in the smooth endoplasmic reticulum of the liver cells that modify molecules to make them more polar and less lipid-soluble (Collins Dictionary of Medicine, 2005).

Large numbers of drugs, pesticides, herbicides, food additives, and environmental carcinogenic hydrocarbons are known to stimulate their own metabolism or the metabolism of other compounds. The evidence suggests that foreign chemicals exert this action by increasing the amount of drug-metabolizing enzymes in liver microsomes (Conney, 1967).Many drugs alter drug metabolism by inhibiting or inducing cytochrome enzymes.

2.5.1 Microsomal enzymes induction and therapeutic failure

Enzyme induction is the process by which exposure to certain substrates results in accelerated biotransformation with a corresponding reduction in parent drug.This leads to a decrease in the concentrations of drugs metabolized by the same enzyme (Ogu and Maxa, 2000).The drugs most frequently encountered as enzyme-inducing agents in man are barbiturates, rifampicin and phenytoin. Enzyme induction is usually associated with a reduction in the drug efficacy but may also alter the toxicity of certain substances (Park and Breckenridge, 1981).

Consequences of enzyme induction

- Increased rate of metabolism
- Decrease in drug plasma concentration
- Enhanced oral first pass metabolism
- Reduced bioavailability
- If metabolite is active or reactive, increased drug effects or toxicity

2.5.2 Microsomal enzymes inhibition and toxicity

Enzyme inhibitors are drugs which decrease the metabolism of other drugs by inhibiting microsomal enzymes.An enzyme inhibitor is a molecule that binds to an enzyme and decreases its activity leading to increase plasma concentration and in some cases toxicity. Examples are cimetidine, chloramphenicol, valproate, erythromycin etc.

Consequences of enzyme inhibition

- Increase in the plasma concentration of parent drug
- Exaggerated and prolonged pharmacological effects
- Increased likelihood of drug-induced toxicity

2.6 Effects of Parasympathomimetics and Sympathomimetics on Smooth Muscles

Smooth muscle is an involuntary non-striated tissue. It cells are found in the walls of hollow organs, including the stomach, intestines, urinary bladder and uterus and also in the walls of arteries and veins of the circulatory system and the tracts of the respiratory, urinary and reproductive systems (Biga*et al.,* 2008).

Parasympathomimetics or cholinomimetics stimulate parasympathetic nervous system in same manner as acetylcholine does. The parasympathomimetic drugs contract the intestinal smooth muscles while the sympathomimetic drugs relax it. Acetylcholine is the major excitatory neurotransmitter of the enteric nervous system (ENS), and its excitatory effect on

intestinal smooth muscle is mediated through the muscarinic type of cholinoceptor (Hansen, 2003). Acetylcholine (Ach) also mediates the excitatory effects of parasympathetic nerves that act on intestinal smooth muscle indirectly through an effect on the ENS (Hansen, 2003). It has been shown to increase the amplitude of spontaneous contractions in the rabbit small intestine, and the frequency of these contractions is in the circular but not longitudinal muscle layers (Grasa*et al.*, 2004). The muscarinic sub-type of receptor that directly mediates smooth muscle contraction in the GI tract is the M_3 sub-type (Uchiyama and Chess-Williams, 2004). This sub-type is coupled to Gq and the activity of phosphokinase C (Caulfield and Birdsall, 1998).

Sympathetic nerves of the ANS also modulate GI tract motility indirectly through the ENS, by producing an inhibitory effect on motility (Hansen, 2003). This inhibition occurs through two different mechanisms. Release of norepinephrine acts presynaptically to decrease activity in the cholinergic nerves of the ENS; this is through activation of the $\alpha_2$adrenoceptor sub-type (Wood, 2003). This sub-type of adrenoceptor is coupled to Gi and inhibition of adenylyl cyclase (Stephens and Mochida, 2005). Norepinephrine also acts directly on intestinal smooth muscle cells to cause relaxation through activation of β_3-adrenoceptors, which are coupled to Gs and protein kinase A (Tanaka *et al.*, 2005).

2.7 Effects of Parasympathomimetics and Sympathomimetics on Cardiac Muscles

Cardiac muscleis a type of involuntary striated muscle found in the walls and histologic foundation of the heart,specifically the myocardium. Cardiac muscle is one of three major types of muscle, the others being skeletal andsmooth muscles. The cells that comprise cardiac muscle are called myocardiocyteal muscle cells.They are multinuclearwhereas smooth muscle cells are mononuclear (Chummy, 2006).

Coordinated contraction of cardiac muscle cells in the heart propels blood out of the atria and ventricles to the bloodvessels of the left/body/systemic and right/lungs/pulmonary circulatory

systems. This phenomenon is understood assystole of the heart. Cardiac muscle cells, like all tissues in the body, rely on an ample blood supply to deliveroxygen and nutrients and to remove waste products such as carbon dioxide. The coronary artery doesthisfunction.

2.7.1 Metabolism

Cardiac muscle is adapted to be highly resistant to fatigue.It has alarge number of mitochondria, enabling continuous aerobic respirationvia oxidative phosphorylation, numerous myoglobins (oxygen-storingpigment) and a good blood supply, which provides nutrients andoxygen. The heart is so tuned to aerobic metabolism that it is unable topump sufficiently in ischaemic conditions. At basal metabolic rates,about 1% of energy is derived from anaerobic metabolism. This canincrease to 10% under moderately hypoxic conditions, but, under moresevere hypoxic conditions, not enough energy can be liberated bylactate production to sustain ventricular contractions (Ganong, 2015).

Parasympathomimeticdrugs stimulate muscarinicreceptors (cholinergic receptors) in the heart and reduceheart rate and force of contraction.On I.V administration, methacholine activatesmuscarinic receptors of blood vessels and heart.This stimulation also reduces atrial conductivity andconduction velocity of atrioventricular node (AVN).Parasympathomimetic drugs cause brief and rapid fall indiastolic and systolic blood pressures due to reducedperipheral blood flow resistance.

Methacholine has a more cardiovascular activityreducing conduction of impulses from the pacemakerand is good for treatment or controlling tachycardia ofatria origin.

Sympathomimetic drugs stimulate α1, α2, β1 and β2 -adrenoceptors located in the heart and arteriole smoothmuscles.Stimulation of cardiac β1 adrenoceptors mediates theeffects of stimulation of sympathetic nerves.The stimulation of β-adrenoceptors in the heart causeincreased rate, automaticity and increased velocity inconducting tissue. Myocardium

contractility and oxygenconsumption is also increased.Also, α1 adrenoceptors stimulation causes constriction ofarterioles due to contractions of their vascular smoothmuscles.

2.8 Analgesic Agents

Analgesic agents are any drug that relieves pain selectively without blocking the conduction of nerve impulses, markedly altering sensory perception, or affecting consciousness. This selectivity is an important distinction between an analgesic and an anaesthetic.Analgesicsare commonly known as painkillers. They work in various ways to relieve different types of pain experienced in the body (Rang *et al.,* 2003). Over-the-counter (OTC) analgesics that are generally used by the public are paracetamol, weak opioids such as codeine, and non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and aspirin (Rang *et al.*,2003).

2.8.1 Types of analgesic agents and their mechanism of action

Analgesics may be classified into two (2) types: anti-inflammatory drugs, which alleviate pain by reducing local inflammatory responses; and the opioids, which act on the brain. The opioid analgesics were once called narcotic drugs because they can inducesleep. The opioid analgesics can be used for either short-term or long-term relief of severe pain while the anti-inflammatory agents are used for short-term relief of pain and for modest pain, such as headache, muscle strain, bruising or arthritis.

2.8.1.1 *Anti-inflammatory analgesics*

Most anti-inflammatory analgesics are derived from three (3) compounds discovered in the 19th century; salicylic acid, pyrazolone, and phenacetin (or acetophenetidin). Although chemically unrelated, the drugs in these families have the ability to relieve mild to moderate pain through actions that reduce inflammation at its source. Acetylsalicylic acid, or aspirin, which is derived from salicylic acid, is the most widely used mild analgesic. It is considered

the prototype for anti-inflammatory analgesics, the two other major types of which include acetaminophen (a derivative of phenacetin) and the aspirin-like drugs, or nonsteroidal anti-inflammatory drugs (NSAIDs), which include compounds such as ibuprofen, naproxen, and fenoprofen. Pyrazolone derivatives, with some exceptions, are no longer widely used in many countries, because of their tendency to cause an acute infection known as agranulocytosis.

NSAIDs appear to share a similar molecular mechanism of action namely, inhibition of the synthesis of prostaglandins (natural products of inflamed white blood cells) that induce the responses in local tissue that include pain and inflammation (Vane, 1971). In fact, aspirin and all aspirin-like analgesics, including indomethacin and sulindac, which are derived from a heterocyclic organic compound known as indole, inhibit prostaglandin synthesis and release(Vane, 1971). Cyclooxygenase (COX), is an enzyme responsible for the synthesis of prostaglandins and related compounds. It has two forms, COX-1, which is found in most normal tissues, and COX-2, which is induced in the presence of inflammation. Because COX-2 is not normally expressed in the stomach, the use of COX-2 inhibitors (e.g., rofecoxib, celecoxib) seems to result in less gastric ulceration than occurs with other anti-inflammatory analgesics, particularly aspirin. However, COX-2 inhibitors do not reduce the ability of platelets to form clots, a benefit associated with aspirin and other COX-1 inhibitors.

2.8.1.2 *Opioids*

Opioids are drugs that produce morphine-like effects to reduce moderate or severe pain.Codeine is an example of an opioid; it has structural similarities to morphine, which in turn causes similar effects to reduce pain. There are three (3) main types of receptors, which all produce different types of responses; μ, δ and κ. The μ and the κ receptors produce the analgesic effects plus the unwanted side effects. The δ receptor does not cause many side effects. Codeine has a high affinity for the μ receptor and a low affinity for δ and κ receptors,

and therefore is defined as a weak opioid as its effects are less than those of morphine (Rang *et al.,* 2003). Codeine should be used for mild-to-moderate pain (Nicholson, 2004). It is not recommended for children, it is generally not used for asthmatics and can cause dependence.The side effect commonly associated with codeine is constipation, due to the increase tone in the gastrointestinal tract and decrease in gastric motility (Nicholson, 2004).

2.9 Antiepileptic Drugs

Epilepsy is one of the most common neurological disorders (Poole *et al.,* 2000, Ropper and Brown, 2005). Epilepsy is recognized as a syndrome of disturbed electrical activity in the brainthat can be caused by a variety of stimuli. This disturbed electrical activity leads tothe development of seizures. Seizures occur because of the abnormal discharge of neurons within the central nervous system (CNS) (Leppik, 1993). Worldwide, theprevalence is estimated to be 0.5 – 1%, and there is a life time incidence of 1 – 3% (White, 2003). It hasimportant medical, social and psychological consequences. Despite the introduction of several new therapeutic options in the 1990s, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizures (White, 2003).

The incidence of epilepsy in the general population is highest in newborn andyoung children with a second peak occurring in patients older than 65 years. It has beensuggested that there may be some genetic predisposition to the development of seizuresand epilepsy. Although the incidence of epilepsy is higher among patients with mentalretardation and cerebral palsy, neither condition is synonymous with epilepsy (Hauser, 1992).

Antiepileptic drugs (AEDs) act within the central nervous system in one of two ways: by reducing pathologicelectrical discharges or by inhibiting the propagation of aberrant electrical activity.This may occur through effects on specific ion channels, inhibitory neurotransmitters,or excitatory neurotransmitters. Though multiple neurophysiological effects of AEDshave been theorized and hypothesized, it is important to recognize that the true

mechanismsof action of these agents are poorly understood and may be multifactorial (Dichter, 1994).For most AEDs there ispoor correlation between maintenance doses and their resulting serum concentrations(Garnett, 1995). In addition there is important inter-individual variability in both therapeutic andtoxic response to medications (Garnett, 1995,; Schmidt and Haenel,; 1984, Schmidt *et al.*, 1986). Therefore, knowledge of the pharmacokineticsof AEDs is essential for understanding and interpreting serum concentrations of AEDs.This includes issues related to all aspects of drug disposition: absorption, distribution,metabolism, and excretion.It is important to recognize that many AEDs are frequently employed for offlabeluse. The majority of off-label use involves the treatment of psychiatric disorders,particularly bipolar affective disorder or manic depressive disorder. Other offlabeluses include such things as migraine prophylaxis, attention-deficit disorder, and neuropathic pain (Bowden, 1996).

2.9.1 Drug Therapy

Many drugs are available to treat epilepsy, several of which haveonly recently been released. Older, classic medications used to treat epilepsy include:Phenytoin,Phenobarbitone, Carbamazepine,Primidone,Ethosuximide,Valproic acid Diazepam and its derivatives

Newer drugs to treat epilepsy include:Felbamate,Gabapentin,Lamotrigine,Oxcarbazepine,Topiramate,Tiagabine,Levetiracetamand Zonisamide (Benbadis and Heriaud, retrieved 2018).

2.9.2 Types of seizure

The International League against Epilepsy (ILAE) is the world's main scientific body devoted to the study of epilepsy, and it has recently revised its classification of seizures.

2.9.2.1 *History of seizure classification:*

For decades, the most common words to describe seizures were grand mal and petit mal. Although the medical meaning of these terms was fairly precise, lay people often used them loosely when referring to any big or little seizure.

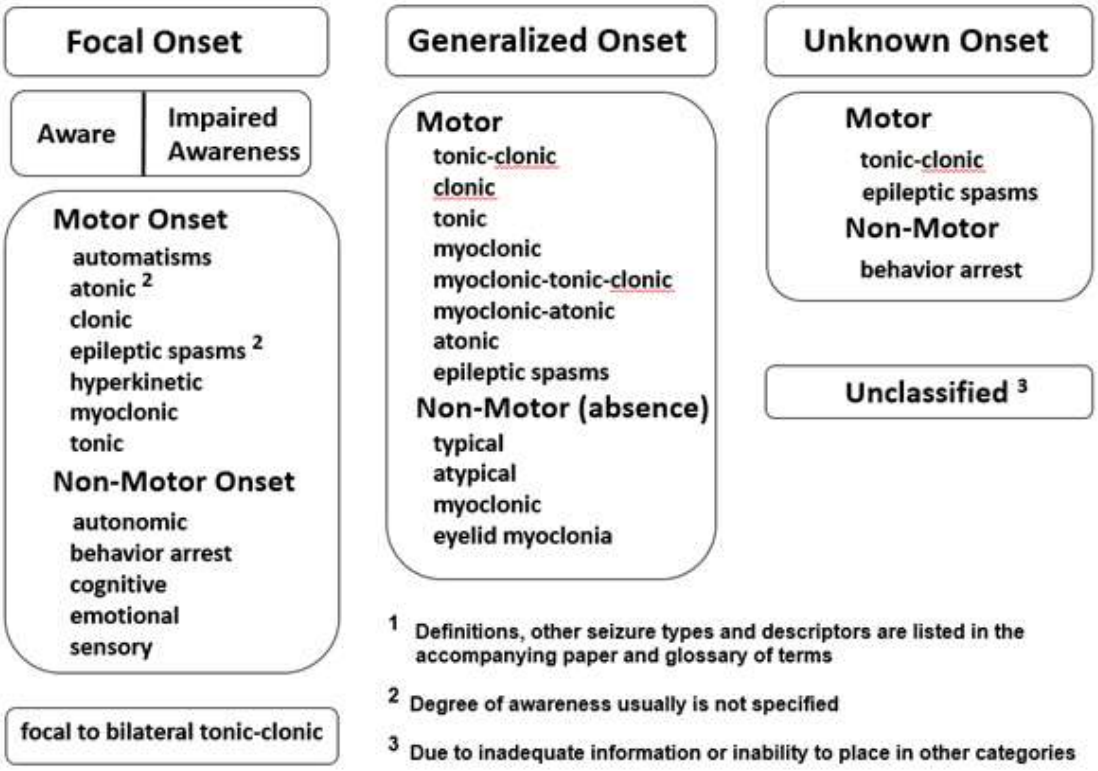
In 1981, a classification was developed that has been used for 35 years. This system divided seizures into partial (focal) onset and generalized onset seizures. The partial seizures were further divided into simple partial seizures (no change in consciousness) and complex partial seizures (impaired consciousness). Generalized seizures were divided into various subcategories.

This classification served well but had several drawbacks. Such drawbacks are that:

- several important seizure types were not specifically listed, for example, focal clonic seizures or infantile (epileptic) spasms.
- it was impossible to classify a seizure if the onset (the part or network of brain involved in generating or starting the seizure) was not known.
- many of the terms, such as psychic seizures or complex partial seizure were confusing. Some people with epilepsy felt that there was nothing “simple” about simple partial seizures.

These concerns led to the International League against Epilepsy current revision.

INTERNATIONAL LEAGUE AGAINST EPILEPSY 2017 CLASSIICATION OF SEIZURE TYPES EXPANDED VERSION



The ILAE 2017 seizure classification replaces the 1981 classification that was used for 35 years.

2.9.3 Maximal electroshock seizure (MES)

MES generates tonic–clonic convulsions mediated by the brainstem (Browning *et al.,* 1981). Over the years, (MES) model has remained one of the gold standards in early stagesof testing (Rogawski, 2006). MES stimulation can be applied through transcorneal or transauricular (ear-clip) electrodes from an electroshock apparatus at anintensity sufficient to elicit tonic hind limb extension (HLE) in 100%of the control animals. A seizure is generally considered to be maximal if increments in current intensity do not alter the pattern or theduration of its various components (Tedeschi*et al.,* 1956). The conventional MES testhas standardized parameters such as a 50-mA (mice) or 150-mA(rats) fixed current, a 50-60–Hz pulse frequency, a 0.6-ms pulsewidth and a 0.2-s stimulus duration (Woodbury and Davenport, 1952;Löscher and Schmidt 1988;Löscher*et al.,* 1991). Cornealelectrodes are mainly used. During stimulus application, the animalshould be restrained only by hand and released at the

moment ofstimulation to permit observation of the seizure throughout its entirecourse (Löscher and Lehmann, 1996;Mareš and Kubová, 2006).

2.9.4 Chemically-induced seizure

Pentylenetetrazole(PTZ) generates clonic seizuresmediated by the prosencephalon that can be followed bygeneralized tonic–clonic seizures (Browning and Nelson, 1986). The clonic movement offorelimbs is related to activation of structures not only in the limbic systembut also in the thalamus, neocortex, and nucleus basalis (Ackermann *et al.,* 1986).Pentylenetetrazole is an antagonist at the gamma-aminobutyric acid(GABA)A receptor complex (Ramanjaneyulu and Ticku, 1984). Pentylenetetrazole alters the ionic conductanceof sodium and potassium channels(Pellmar and Wilson, 1977)by inducing changes inintracellular Ca2+-related processes (Onozuka and Tsujitani, 1991) Several receptor systems areaffected by the administration of PTZ, including changes in ionotropicand metabotropic glutamate receptors (mGluRs) (Ekonomou*et al.,* 2001)Administration of PTZ is a commonlypreferred behavioral approach used for studying brain excitability (Klioueva*et al.,* 2001) and for developing AEDs (Löscher, 2002). Chemical kindlingseizures induced with PTZ are human absence epilepsy andmyclonic, generalized tonic-clonic (primary generalized)seizure models and it is a model for drug resistant epilepsy (Ali *et al.,* 2005). It is claimed to exert its activity via inhibiting gammaaminobutyricacid (GABA) activated channels (Macdonald and Barker, 1978). It is suggestedthat its activity is especially due to blockade of GABAAgated chloride receptors (Luthman and Humpel, 1997). GABAA receptors have someallosterical binding sites. Different drugs can influenceGABA mediated chlorine influx via those binding sites. Pentylenetetrazoleis a central nervous system convulsant. It shows its activityby binding to sites where picrotoxin (PTX) binds to GABAAreceptor and probably exerts its activity through interactionat the picrotoxin site within GABAA receptor subunitsecond transmembrane(TM2) domain (Huang, 2001).The

exact mechanism of the epileptogenic action of PTZ at the cellular neuronal level is still unclear but it has been generally reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) neurotransmission (De Sarro*et al.,* 2003). Enhancement of GABAergic neurotransmission has been shown to inhibit or attenuate seizures, while inhibition of GABAergic neurotransmission or activity is known to promote and facilitate seizure. Anticonvulsant agents such as diazepam, valproic acid and phenobarbitone inhibit PTZ-induced seizure by enhancing the action of GABA-receptors, thus facilitating the GABA-mediated opening of chloride channels (Gale, 1992; Olsen, 1981). Postsynaptic $GABA_A$-receptors are multi-unit complexes with binding sites for the endogenous ligand GABA, benzodiazepines, barbiturates and other ligands with a central chloride ion channel (Olsen and Leeb-lundberg, 1981).

2.10 Toxicity

Toxicology is the study of the interactionbetween chemical agents and biological systems. While the subject of toxicology is quitecomplex, it is necessary to understand the basic concepts in order to make logical decisionsconcerning the protection of personnel from toxic injuries (UNL Environmental Health and Safety, 2002).

Toxicity can be defined as the relative ability of a substance to cause adverse effects in living organisms. This "relative ability is dependent upon several conditions. As Paracelsus suggests,the quantity or the dose of the substance determines whether the effects of the chemical are toxic,nontoxic or beneficial. In addition to dose, other factors may also influence the toxicity of thecompound such as the route of entry, duration and frequency of exposure, variations betweendifferent species (interspecies) and variations among members of the same species (intraspecies) (UNL Environmental Health and Safety, 2002).

The effect can be on a whole organism, such as an animal, bacterium or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity).

2.10.1 Acute toxicity

Acute toxicity is defined as the unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours. The unwanted (or adverse) effect is any effect that produces functional impairments in organs and/or biochemical lesions, which could alter the functioning of the organism in general or individual organs (Walum, 1998).A study of acute toxicity however tends to establish the dose-dependent unwanted (or adverse) effect(s), which may take place and these includes all information that is important in the assessment of acute toxicity including mortality. The assessment of the lethal dose (LD_{50}) (the dose that kills 50% of 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 survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent (Akhila*et al.,* 2007). The toxicity assessment of pharmacological agents is a very important procedure that is usually carried-out before they are allowed to enter the market for sale. Conversely, different methods have been developed and adopted for acute toxicity testing. However, most of these methods have their short-comings.

2.10.2 Sub-chronic toxicity

Sub-chronic toxicity is defined as adverse effects occurring after repeated or continuous administration of a test sample for up to 90 days or not exceeding 10% of the animal's lifespan (De Jong and Geertsma, 2012). It is a consequence of the persistent or progressively deterioratingdysfunction of cells, organs or multiple organ systems, resulting from long-termexposure to a chemical. The highest dose administered is designed to causesome toxicity, but not lethality. Upon completion of the test, a whole host ofclinical and histological evaluations are recorded, including experimentalobservations and whole body and individual organ analyses (Pfaller*etal.*, 2001).

2.10.3 Chronic toxicity

The chronic toxicity study provides information on the possible health hazards likely to arise from repeated exposure over a considerable part of the lifespan of the species used. The study will provide information on the toxic effects of the substance, indicate target organs and the possibility of accumulation. It can also provide an estimate of the no-observed-adverse effect level which can be used for establishing safety criteria for human exposure. The need for careful clinical observations of the animals, so as to obtain as much information as possible, is also stressed (OECD, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chicks and other experimental animals

Day-old chicks (27-43g) were obtained from Chi Farms Limited, Km 20 Ibadan-Lagos Expressway, Ibadan, Oyo State, Nigeria; and were kept in the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were provided with Starter Mash Vital Feed$^{(R)}$ and water *ad libitum*as well as electric bulb-powered warmth; then acclimatize in the Animal House for 3 days or more. In some experiments, older chicks (e.g. 1 week) and adult chickens were used.

Mice (19-22 g), rats (160-200 g), guinea pigs (590-670 g) and rabbits (640-720 g) were used in the study. They were obtained from the Departmental Animal House and were provided with Growers Mash Vital Feed$^{(R)}$ and water *ad libitum*.

3.1.2 Ethics statement

The care and use of animals in this study was carried out according to International Ethical Standards (Institute of Laboratory Animal Research, 1996). Ethical guidelines on handling of experimental animals of the Ahmadu Bello University, Zaria, Nigeria, were also adhered to during the course of the study following approval by the Ahmadu Bello University Committee on Animal use and Care (ABUCAUC).

3.1.3 Equipment and apparatus

Electrical hot plate (Model No 7045)

Sleep wooden board

Microdynamometer (Model No 7050)

Syringes and needles

3.1.4 Chemicals, reagents and drugs

- 2,2-dichlorovinyl dimethyl phosphate (Sigma, USA)
- Acetic acid (May and Baker, England)
- Acetylcholine (Sigma, USA)
- Adrenaline (Sigma, USA)
- Apomorphine (Sigma, USA)
- Artemether (Vital Laboratories, India)
- Atropine (Sigma, USA)
- Calcium (Sigma, USA)
- Carbachol (Sigma, USA)
- Chloramphenicol (Shandong XierKanglai, China)
- Chlorpromazine (Shandong Shenglu, China)
- Cimetidine (Grand Health, China)
- Diazepam (Roche, Switzerland)
- Eserine (Sigma, USA)
- *Ficuscapensis*(Gift from SaniAbubakar)
- Isoprenaline (Sigma, USA)
- Methacholine (Sigma, USA)
- Morphine (Verve Human care, India)
- Nicotine (Sigma, USA)
- Noradrenaline (Sigma, USA)
- Normal saline (Juhel, Nigeria)
- Pentazocine (SokarHeathcare, India)
- Pentylenetetrazol (Sigma, USA)
- Phenobarbitone (May and Baker, England)
- Phenytoin (Sun pharmaceutical, India)

- Piroxicam (GMP Western Medicine, China)
- Propranolol (FoncerPharma, India)
- Salbutamol (TenatraChemie, India)
- Tyramine (Sigma, USA)

3.2 Methods

3.2.1 Dose-response relationship using diazepam-induced sleep

Twenty five (25) 4-day old chicks were divided into five (5) groups of five (5) chicks each and kept in separate cages. Groups 1 to 5 received 5, 10, 20, 40 and 80 mg/kg of diazepam intraperitoneally respectively. All the groups were monitored and observed for onset and duration of sleep.

3.2.2 Concentration-response relationship using acetylcholine-induced contractionsand parasympathomimetic and sympathomimethic effects on ileal tissue

Rabbit ileum which is the traditional tissue for the *in-vitro* demonstration of concentration-response relationship, and chicken ileum were used for this experiment. A chicken was humanely euthanised and the gastrointestinal tract wasremoved and kept in Tyrode solution, and aeration with air was provided. The ileum was cut off and transferred to a petri dish containing Tyrode solution, 2 cm portions of the ileum was cut, intestinal contents were removed, and the tissue was freed from mesenteric attachments. A thread was tied at each end making sure that the tissue is left open and the thread did not close the lumen. The tissue was mounted in an organ bath containing Tyrode solution, with temperature maintained at 34±1°C and was allowed to equilibrate for 30 minutes. Tension load was adjusted to 0.5 g, the magnification 5:1folds and bath volume of 25 ml was maintained.

Contractions were recorded using isotonic lever on microdynanometerwith a contact time of 60 s and speed of 0.25 mm/s. Responses to different concentrations of acetylcholine were recorded as changes in height (mm) from baseline. After recording the response with a particular concentration of the Ach, the tissue was washed thrice with Tyrode solution at an interval of 1 min; then allowed to rest for 5 min. The responses to increasing concentrations of acetylcholine were taken till the ceiling height was achieved. The same procedure was applied for the rabbit ileum.

Similarly, various parasympathomimetic(Acetylcholine, Carbacho, Methacholine and Nicotine) and sympathomimethic(Adrenaline, Noradrenaline, Isoprenaline and Tyramine) drugswere added to demonstrate the effect of parasympathomimetic and sympathomimethicdrugs on chicken ileum and their responses were recorded. The organ bath was washed out thrice and allowed to equilibrate for 10 minutes before addition of the next drug.The same procedure was applied for the rabbit ileum.

3.2.3 *In vivo* drug antagonism and synergism

Twenty (20) 4-day old cockerels (42-54 g) were used for this experiment. The experiment was divided into two groups; antagonistic and synergistic groups. Each group are kept in a separate cage. The antagonistic group were divided into groups I which is the control (apomorphine 0.5 mg/kg + normal saline 10 ml/kg) and II which is the test (apomorphine 0.5 mg/kg + chlorpromazine 2 mg/kg). Both groups were administered apomorphine 0.5 mg/kg subcutaneously. After 20 minutes, group I were administered normal saline 10 ml/kg intraperitoneally while group II were administered chlorpromazine 2 mg/kg intraperitoneally. The synergistic group were also divided into groups I which is the control (diazepam 15 mg/kg + normal saline 10 ml/kg) and group II which is the test (diazepam 15 mg/kg + chlorpromazine 0.5 mg/kg). Both groups were administered diazepam 15 mg/kg intraperitoneally and after 20 minutes, group I was administered normal saline 10 ml/kg intraperitoneally while group II was administered chlorpromazine 0.5 mg/kg intraperitoneally. All the chicks in the groups were observed noting the onset of the effect of the drugs.

3.2.4 Experimental demonstration of microsomal enzyme induction and inhibition

Experimental demonstration of microsomal enzyme induction and inhibition, traditionally carried out in mice and rats was attempted in 4-day old chicks. Sixty chicks (29-44 g) were divided into six (6) groups of ten (10) chicks each. They were pretreated with normal saline-

10 ml/kg (Group 1),phenobarbitones-10mg/kg (Group 2), phenobarbitones-20mg/kg (Group 3), chloramphenicol-15 mg/kg(Group 4), cimetidine-200mg/kg (Group 5), and cimetidine-400mg/kg (Group 6) for three days.

On the fourth day, each of the chicks were given diazepam 15mg/kg and the sleeping time was recorded. The experiment was conducted three times and mean recordings calculated.

3.2.5 Analgesic studies

3.2.5.1 Hot plate method

The modified method ofEddy and Leimbach(1953) was employed. The hot plate consists of an electrically heated surface withthe temperature controlled for 40° to 45°C. The animals are placed on the hot plate and the time until either licking or jumpingoccurs is recorded by a stop-watch as latency time. This latency is recorded at 0, 30, 60, 90 and 120 mins followingsubcutaneous administration of morphine and intraperitoneal administration of pentazocine.

In the actual experiment, 3 day old chicks(34-45 g) of five (5) groups of five (5) chickswere used. Group I received normal saline 10 ml/kg, groups II and III received pentazocine 10 and 20 mg/kg respectively, while groups IV and V received morphine 10 and 20 mg/kg respectively. The latency times were recorded as described above.

3.2.5.2 Acetic acid induced writhing test

The method of Koster*et al.*(1959) was employed and two groups of five chickswere used. Group I received normal saline 10 ml/kg while group II received piroxicamintraperitoneally.Thirty minutes later,0.1 ml of a0.6 % solution of acetic acid was injected intraperitoneally to both groups. Numbers of abdominal writhes were to be counted in each 10 min period starting at 10 minutes after acetic acid injection.

3.2.6Pentylenetetrazole-induced convulsion in chicks

The method of Swinyard*et al.* (1989) was employed. Thirty (30) 4-day old chicks (47-55 g) were divided into three (3) groups of ten (10) chicks each. Groups I, II and III were pretreated with normal saline 10 mL/kg (control), phenobarbitone 20 and 30 mg/kg intraperitoneally respectively.After30 minutes all the groups were administered pentylenetetrazole 80 mg/kg intraperitoneally and were monitored and observed for onsets of seizures and mortality.

3.2.7Demonstration of parasympathomimetic and sympathomimetic effects on isolated heart atria *in vitro*

The guinea pig heart atria which are the traditional tissue used for the isolated heart atria and chicken heart atria were used. Chicken was humanely euthanised and the heart was dissected. Ringer Locke's solution was used to keep the heart tissue in optimal condition. Fat around the heart tissue was trimmed and the atrioventricular (AV) junction was exposed. The heart was cut into two pieces; the atria and ventricles. At this point extreme care was taken while cutting the heart to make sure the sinoatrial (SA) node did not get damage in the process because the SA node keeps the atria of the heart beating as it generates impulses.The atria of the heart was isolated and set up in the organ bath. The isolated atria were allowed to equilibrate in the organ bath for 15 minutes.

Various parasympathomimetic and sympathomimethicdrugs were added and their responses were recorded. The organ bath was washed out thrice and allowed to equilibrate for 10 minutesbefore addition of the next drug.The same procedure was carried out for the guinea pigheart atria.

3.2.8 Determination of median lethal dose (LD_{50}) in conventional medicine,herbal medicine and pesticide

Acute toxicity study was carried out using the method of Lorke, (1983). In the first phase, nine (9) chicks were randomlydivided into three (3) groups of three (3) chicks each and weregiven 10, 100 and 1000 mg/kg body weight of the drug(Artemetheri.p), extract (*Ficuscapensis*methanol stem bark p.o) and chemical (Dichlorvosi.p).They were observed for 4 hours post administration for signs of toxicity and death within 24 hours.

In the second phase of thestudy, one (1) chick per three groups was used andthe doses were based on the outcome of the first phase. The chicks were also observed for signs of toxicity and mortality for 24 h. Geometric mean of the smallest dose that killed a chick and the highest dose that did not were taken as the mean Lethal dose (LD_{50}) of the substance.**3.3 Statistical Analyses**

Data were analysed using SPSS version 20. One way analysis of variance (ANOVA), repeated measures ANOVA and Bonferoni post hoc test were used. Results were expressed as mean $\pm$ standard error of mean (SEM). Data were represented in tables, graphs and charts. P-value ≤ 0.05 were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS/DATA PRESENTATION AND ANALYSIS

4.1Dose-response Relationship Using Diazepam-induced sleep*(in vivo)*

The result has shown that diazepam administered to 4-day old chicks caused a dose-dependent increase in sleeping time.The lowest dose (5 mg/kg) resulted in a sleeping time of 75.40±7.63 minutes while the two highest doses (40 and 80 mg/kg) resulted in sleeping time >1440 minutes. The log dose-response plot is shown in Figure 4.1below.

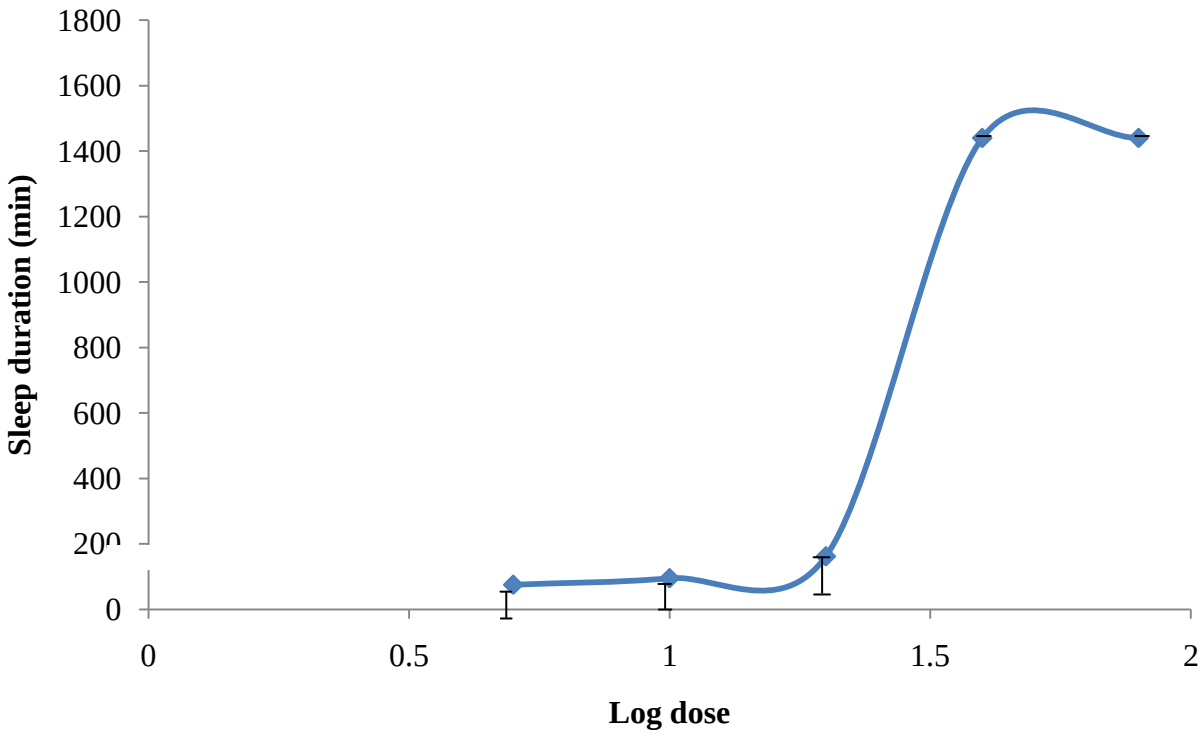


Figure 4.1Log dose-response curve using diazepam-induced sleep in Chicks

Data are expressed as mean± SEM (n=5 per group)

4.2 Concentration-response Relationship Using Acetylcholine-Induced Contraction of Ileum (*in vitro)*

The results below indicated that there was an acetylcholine induced concentration-dependent contractions of ileal smooth muscles over a range of 0-320 ng/ml. The resultant Logarithmic sigmoidal curves are shown below in Figure 4.2

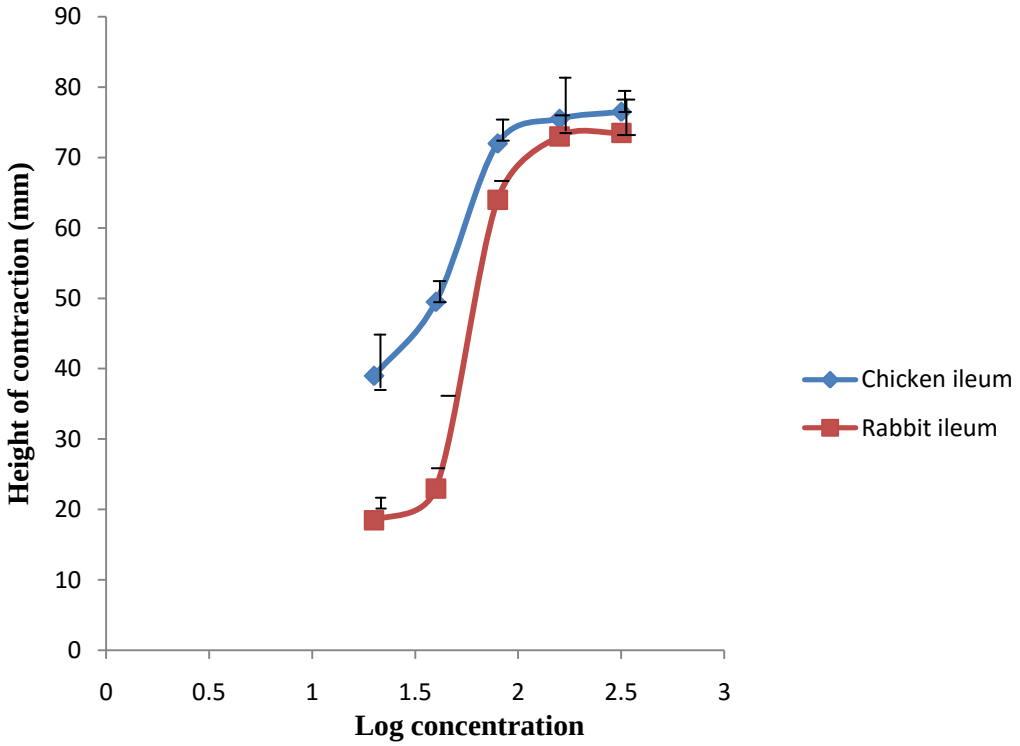


Figure 4.2Log Concentration-response Relationship Using Acetylcholine-induced Contraction on IlealSmooth Muscles of Chicken and Rabbit

4.3 Demonstration of Drug Antagonism and Synergism in Chicks

This study shows the antagonistic effect of chlorpromazine on apomorphine-induced hyperactivity in 4-day old chicks. In both groups, they were administered apomorphine 0.5 mg/kg and became hyperactive. After 20 minutes, group I were administered normal saline 10 ml/kg and they were still hyperactive because it has no effect on the apomorphine and recovered at 168.20±3.34 minutes while group II which also received chlorpromazine 20 minutes later became calm because of its antagonistic effect on apomorphine and also recovered at 182.60±2.62 minutes.

The synergistic effects of chlorpromazine on diazepam-induced sleep in 4-day old chicks were observed. Both groups received diazepam 15 mg/kg, 20 minutes later, group I were administered normal saline 10 ml/kg while group II were given chlorpromazine 2 mg/kg. Both groups slept and recovered at 102.40 ± 3.22 minutes and 146.80 ± 2.40 minutes for groups I and II respectively.

Table 4.1a Antagonistic Effect of Chlorpromazine on Apomorphine-induced Hyperactivity in Chicks

Treatments (mg/kg)	Onset of hyperactivity(seconds)	Mean recovery (minutes)
Apomorphine 0.5 + Normal saline 10 ml/kg	79.87±1.11	168.20±3.34
Apomorphine 0.5 + CPZ 2	76.67±0.79	182.60±2.62***

Data are expressed as mean± SEM (n=5 per group) and was analysed using Student T-test.
*** $p \leq 0.001$ significant difference when compared to control.
CPZ= Chlorpromazine

Table 4.1b Synergistic Effect of Chlorpromazine on Diazepam-induced Sleep in Chicks

Treatments (mg/kg)	Onset of sleep (seconds)	Duration of sleep (minutes)
Diazepam 15 + Normal saline 10 ml/kg	66.87±1.11	102.40±3.22
Diazepam 15 + CPZ 2	63.47±0.87	146.80±2.40*

Data are expressed as mean± SEM (n=5 per group) and was analysed using Student T-test.
*$p \leq 0.05$ significant difference when compared to control.

4.4.Microsomal Enzyme Induction and Inhibition Experiment in Chicks

From this experiment, phenobarbitone (20 mg/kg) significantly decreased diazepam (15 mg/kg) induced sleeping time to 73.20minutes as compared to the control group 134.10minutes being a typical microsomal enzyme inducer(Table 4.1). On the other hand, chloramphenicol, (15 mg/kg) and cimetidine (200 and 400 mg/kg) which are enzyme inhibitors, showed significant ($p \leq 0.05$) increases in sleeping times to 191.60, 180.90and 195.60minutes respectively. These were all statistically significant compared to the control.

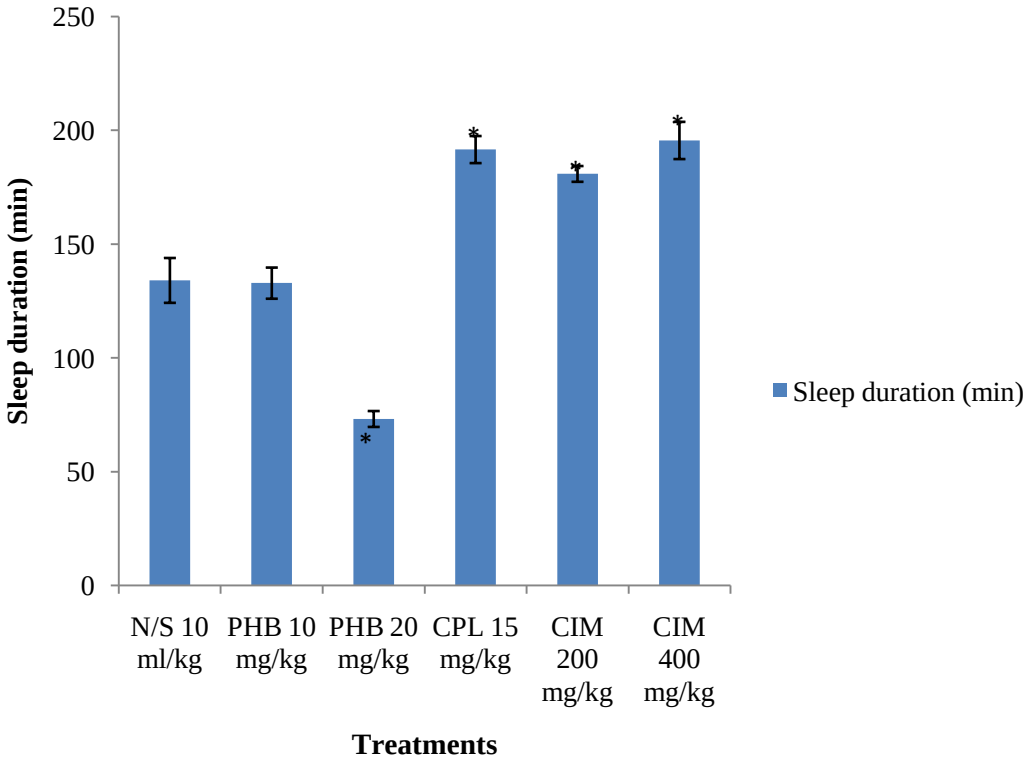


Figure 4.3 Microsomal enzymes induction and inhibition on sleep time of Diazepam in 4-day old chicks

N/S= Normal saline, PHB= Phenobarbitone, CPL= Chloramphenicol, CIM= Cimetidine
Data are expressed as mean± SEM (n=10 per group) and analysed using one way ANOVA followed by Bonferroni*post hoc* test.
* *P*< 0.05 statistical significant difference from the control

4.5 Demonstration of Parasympathomimetic and Sympathomimetic Effects in IlealTissue

In this experiment, the parasympathomimetic and sympathomimetic effects of some drugs on chicken and rabbit ileal smooth muscles were studied and recorded. The parasympathomimetic drugs (Acetylcholine, Carbachol,Methacholine and Nicotine) produced contraction on both ileal smooth muscles while the sympathomimetic drugs (Adrenaline, Noradrenaline, Isoprenaline and Tyramine) produced relaxation also on both ileal smooth muscles. This is shown in Figures 4.4a and 4.4b below.

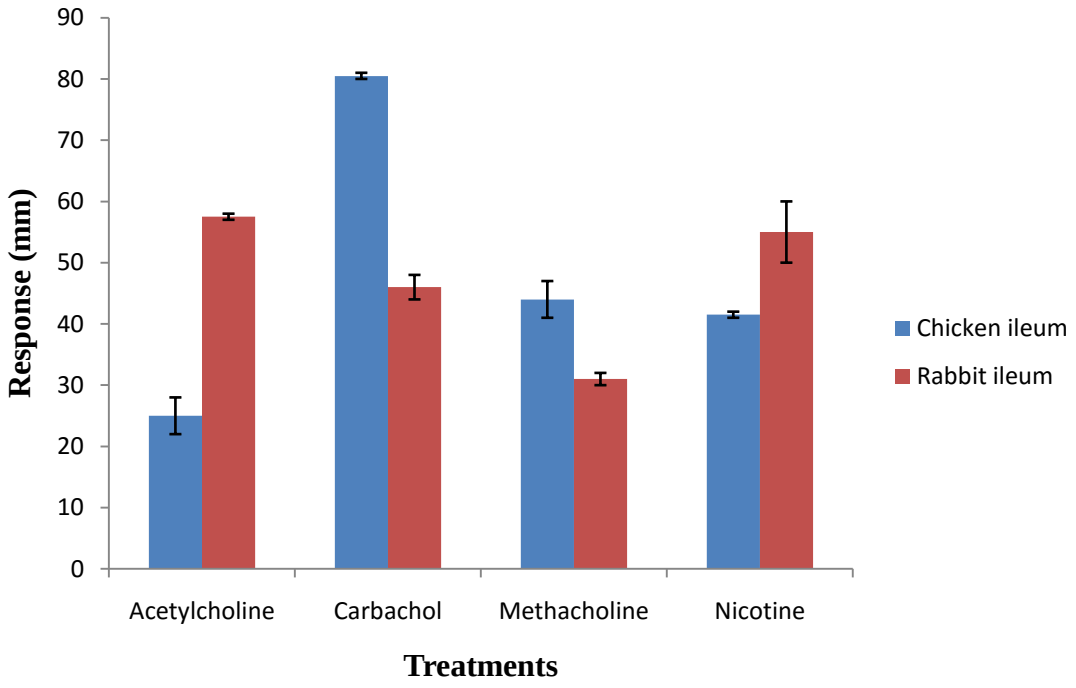


Figure 4.4a Effect of parasympathomimetics drugs on chicken and rabbit ileal smooth muscles

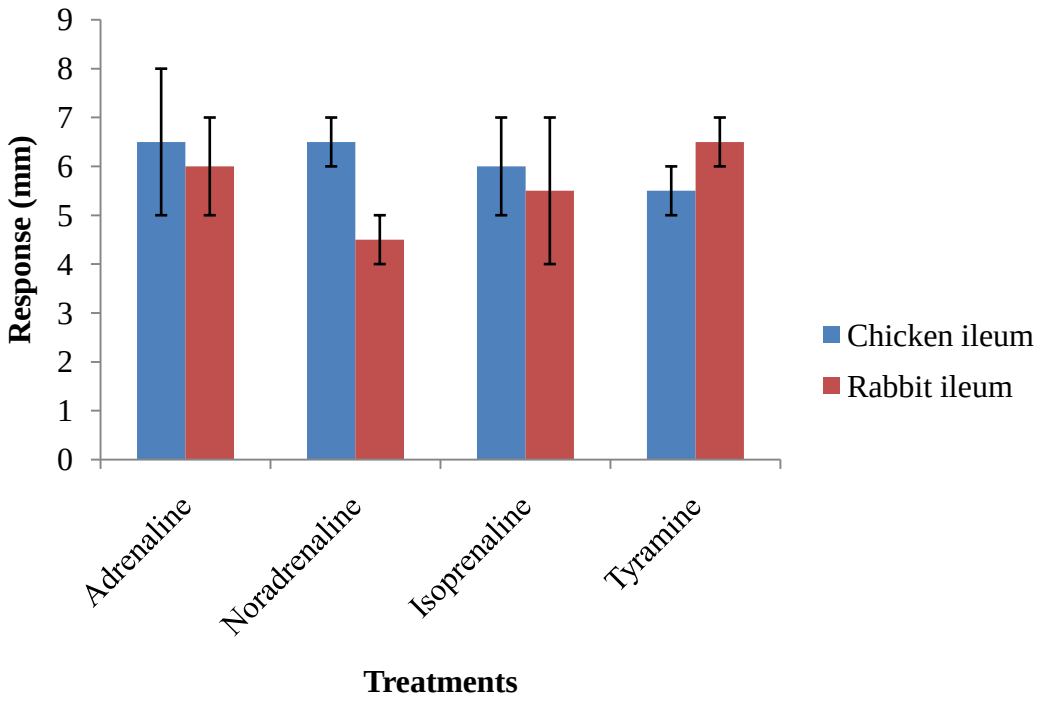


Figure 4.4b Effect of sympathomimetic drugs on chicken and rabbit ileal smooth muscles

4.6 Analgesic Studies

4.6.1 Hot plate method

Figure 4.5a shows that pentazocine and morphine at the same doses of 10 and 20 mg/kg showed a significant increase in latency time compared to the control group (Normal saline 10 ml/kg), using hot plate induced-pain in 4-day old chicks. The peak analgesic effects were observed at 60 minutes (10.2, 10.0, 10.0 and 10.4 minutes for pentazocines (10 and 20 mg/kg) and morphines (10 and 20 mg/kg) respectively compared to the normal saline which is 1.8 minutes) whereas at 120 minutes, the analgesic effect was present but tending towards the control (4.2, 4.6, 4.2, and 3.8 minutes for pentazocines (10 and 20 mg/kg) and morphines (10 and 20 mg/kg) respectively compared to the normal saline which is 2.6 minutes).

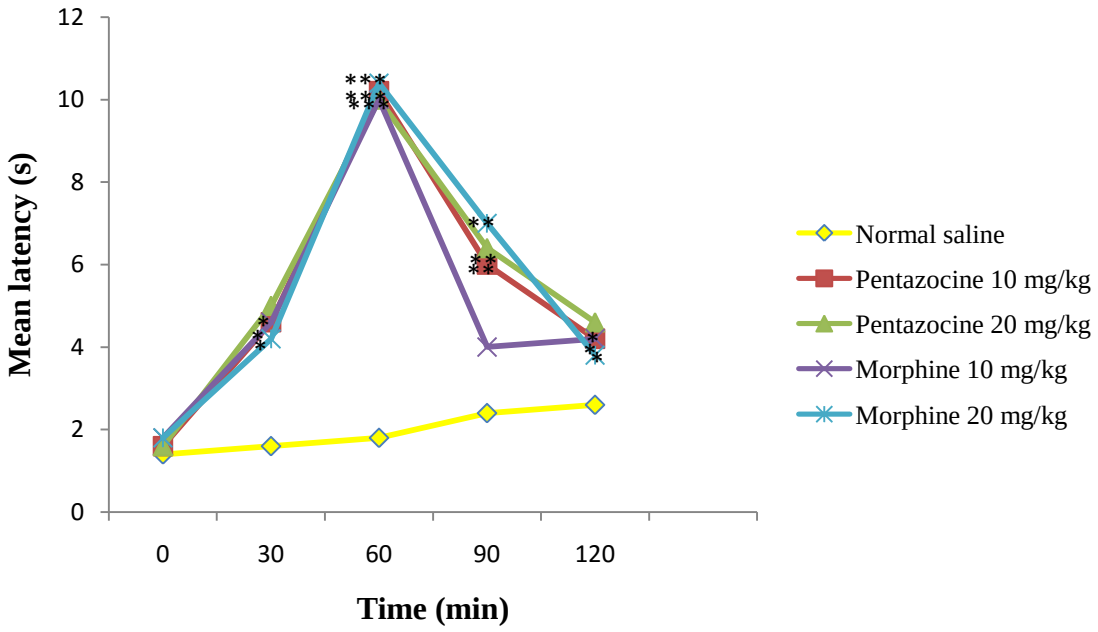


Figure 4.5aAnalgesic Actions of Pentazocineand Morphine usingHot PlateMethodin Chicks

Data are expressed as means± SEM in seconds (n=5 per group). Data were analyzed using repeated measures ANOVA followed by Bonferroni*post hoc* test.

*$p \leq 0.05$ (30 minutes), **$p \leq 0.01$ (90 minutes), ***$p \leq 0.001$ (60 minutes) significant difference from the control (Normal saline 10 ml/kg).

4.6.2 Acetic acid-induced writhing test.

Administration of 0.1 ml of 0.6 % acetic acid solution in mice produced writhes suitable for analgesic studies.This was not the case in chicks (Figure 4.6). There wereno writhes observed in the chicks even with increasingthe strength of acetic acid to 0.9 % and 1.2 %. Fresh experiment did not produce any writhes rather it was observed that the chicks became calm hence, the analgesic effects could not be investigated further in chicks.

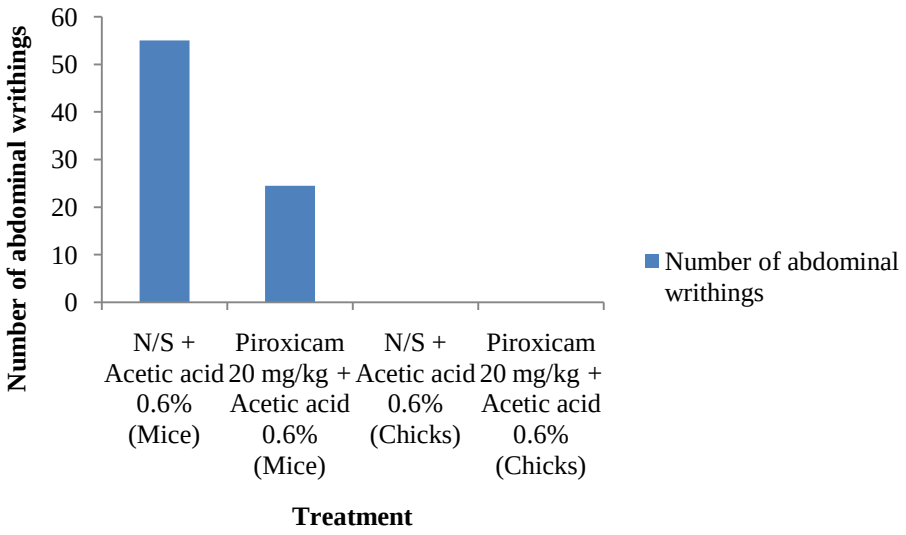


Figure 4.5b Acetic Acid-induced Writhing in Mice and Chicks

4.7Pentylenetetrazol-induced Convulsion in Chicks

This result shows that phenobarbitone at the doses of 20 and 30 mg/kg in a 4-day old chicks exhibit 100% protection against PTZ 80 mg/kg induced seizure as it does in rats and mice traditionally used. Normal saline 10 ml/kg showed 0% protection against PTZ 80 mg/kg induced seizure in chicks.

Table 4.2 Effect of Phenobarbitone on PTZ-induced Convulsion in chicks

Treatments (mg/kg)	Onset of Seizure (min)	Recovery (min)	% Protection	% Mortality
Normal saline 10 ml/kg	4.38±12.24	12.72±1.24	0	80
Phenobarbitone 20	-	-	100	0
Phenobarbitone 30	-	-	100	0

Onset of seizures and Recovery are expressed as mean± SEM, n=10.

4.8 Demonstration of Parasympathomimetic and Sympathomimetic Effects on Isolated Heart Atria (*in vitro)*

The *in vitro* isolated heart atria experiment is traditionally carried out on guinea pigs. The contraction and relaxation of the heart is brought about by the stimulation of the sympathetic and parasympathetic nervous system as such drugs that mimic the sympathetic and parasympathetic nervous system should produce the same effects. This was seen in the guinea pig heart atria but on the part of the chicken heart atria, there were no contraction and relaxation seen different from that of the baseline (see appendices I and II) even with doubling the strength of the drugs in a fresh experiment.

4.9 Determination of Median Lethal Dose (LD_{50}) in Conventional Medicine,Herbal Medicine and Pesticide

LD_{50} valueof Artemether, Dichlorvos and *Ficuscapensis* in 7-day old chicks (43-65 g) and adult male mice (22-25 g) were found to be 470 mg/kg, 1.41 mg/kg and >5000 mg/kg respectively (Table 4.3). Both the chicks and mice intoxicated with Dichlorvos showed signs of acute poisoning such as pecking, ataxia, closing of eyes, gasping, crouching and convulsion before death within the period of 24 h of observation. It was also observed that in each of the groups, the mice usually show the signs of toxicity and death before the chicks.

Table 4.3 LD_{50} of Artemether, Dichlorvos and *Ficuscapensis* in Chicks, Mice and Rats

	Chicks (7-day old cockerels 43-65 g)	Mice (adult male 22-25 g)	Rats (adult male wistar 190-220 g)
Drug/route of administration	LD_{50} (mg/kg)	LD_{50} (mg/kg)	LD_{50} (mg/kg)
Artemether (i.p)	470	470	-
Dichlorvos (i.p)	1.41	1.41	-
Ficuscapensis (methanol stem bark) (p.o)	>5000	>5000	>5000

CHAPTER FIVE

5.0 DISCUSSION

Diazepam is a benzodiazepine (BZ) with anticonvulsant, anxiolytic, sedative, muscle relaxant, and amnesic properties; and it has a long duration of action. Like other benzodiazepines, it acts by enhancing GABA-ergic neurotransmission through an allosteric interaction at the benzodiazepine-$GABA_A$ -barbiturate-chloride ionophore receptor complex (Carrasco and Vande-Kar, 2003). It is used in the treatment of severe anxiety disorders, as a hypnotic agent in the short-term management of insomnia, as a sedative and general anaesthetic pre-medication, as an anticonvulsant, and in the management of alcohol withdrawal syndrome. In the laboratory, diazepam-induced sleep is frequently deployed for teaching and research purposes as in the demonstration of *in vivo* dose-response relationships, usually in mice and rats. Acetylcholine is a chemical substance that is found between the nerve synapses, or gaps, between nerve cells. It was the first neurotransmitter to be discovered, as well as the most abundant in the body. It plays an important role in the signal of muscle movement, sensation of pain, learning and memory formation, the regulation of the endocrine system and rapid eye movement (REM) sleep cycles (Karczmar, 1993).Acetylcholine is the most common neurotransmitter to induce gastrointestinal smooth muscle contractions in the enteric nervous system, others include 5HT, PGs etc. This is mediated by muscarinic acetylcholine receptors on the surface of smooth muscle cells. Thereare five different muscarinic receptor subtypes (M_1-M_5) all of which belong to the superfamily of the G-protein-coupled receptors. The muscarinic M_2 acetylcholine receptor is the major muscarinic receptor sub-type expressed by smooth muscle tissues in the gastrointestinal tract, where it is co-expressed with a smaller population of M_3 receptor (Lino and Nojyo, 2006).

Similarly, in the *in vitro* set up, acetylcholine induced concentration-dependent contractions of both the rabbit and chicken ileal smooth muscle in the same manner. Ach has been shown to increase the amplitude of spontaneous contractions in the rabbit small intestine and the frequency of these contractions in the circular but not longitudinal muscle layers (Grasa*et al.,* 2004). The muscarinic subtype of receptor that directly mediates smooth muscle contraction in the GI tract is the m_3 subtype (Uchiyama and Chess-Williams, 2004), this subtype is coupled to G_q and the activity of PKC (Caulfield and Birdsall, 1998) Thus, chicken may be used as replacement for rabbit in this experiment.In this study using chicks, diazepam showed dose-dependent sleeping time as it does in mice and rats, implying that chicks can be used as alternative animal model in this experiment.

The dose-response or concentration-response and log dose-response or log concentration-response curves were shaped concave hyperbola and sigmoid, respectively. This is a classical Pharmacological phenomenon and indeed the sigmoidal log dose-response curve is the hsymbol of Pharmacology. It was PhilippusAureolus Theophrastus Bombastus von Hohenheim using the pseudoname, Paracelsus who in 16th century first described this truth in these famous words: “All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing not a poison.”

Results that illustrate this have been reported severally. For example Morris *et al.* (1988) Using cows and follicle stimulating hormone (FSH) demonstrated *in vivo* dose-response relationship. Also Stevenson and Tumbull (1974), using rats and pentobarbitone demonstrated *in vivo* dose-response relationship. Similarly, concentration-response relationship have been shown *in vitro* for guinea pig ileum and acetylcholine (Bukhari*et al.,* 2013), guinea pig ileum and crude extract of *Conyzabonariensis* (Bukhari*et al.,* 2013) and guinea pig ileum and ethanol leaf extracts of *Amaranthuscaudatus* (Saba and Oridupa, 2012). Taken together, the implication is that one could utilize the readily available and affordable

chicks and chickens as a replacement for mice, rats, rabbits and guinea pigs in demonstrating dose/concentration-response relationships *in vivo* and *in vitro*. This is beside the point that the adult chicken carcass is edible.

Drug antagonism is a mechanism by which one drug inhibits the action of another drug. It may block or reduce the effectiveness of one or more of the drugs. On the other hand, an interaction between two or more drugs that causes the total effect of the drugs to be greater than the sum of the individual effects of each drug is termed drug synergism. It can be beneficial or harmful (Greco *et. al.*, 1995). Synergistic drug combinations have been shown to be highly efficacious and therapeutically more specific (Lehar *et. al.*, 2009). Drug antagonism, in contrast, is often undesirable, but could be useful in selecting against drug resistant mutations (Chait*et. al*, 2007).Apomorphine is a $D_1$and D_2 receptor agonist with a CNS stimulatory effect used for the treatment of Parkinson's disease (Carlos *et al*., 2006).It is a subcutaneously administered dopamine receptor agonist used predominantly in the therapy of hypomobility of advanced Parkinson's disease.

Chlorpromazine as a classical neuroleptic drug produces both therapeutic effects as well as unwanted side effects. These unwanted effects include sedation, haematological, autonomic, endocrine and neurological effects (Krupp and Barnes, 1989). It is thought that blockade of dopamine D_2 receptors in the basal ganglia caused by chlorpromazine induces these untoward effects (Den Boer *et al.*, 1991). Chlorpromazine blocks certain stereotypic behaviours in animals induced by dopamine agonists like apomorphine and amphetamine, such as circling, chewing and hyperactivity (Chiodo and Bunney, 1983; White and Wang, 1983). When neuroleptics are administered acutely to animals, these drugs block the effects of dopamine agonists (Rupiniak*et al.*, 1983). These proves are all in line with this finding as chlorpromazine antagonizes the hyperactivity induced by apomorphine in the chicks whereas, the control chicks administered with normal saline remained hyperactive. On the other hand,

in the synergistic group, diazepam and chlorpromazine both being CNS depressan and sedative agents (Davis *et al.,* 1986), in synergism caused an increase in the sleeping time of the chicks as compared to the control which is diazepam and normal saline.

From this, one could conclude that the easily accessible and affordable chicks can be used as replacement for mice and rats in this experiment for pharmacological education and research.

Enzyme induction is the process by which the rate of synthesis of an enzyme is increased relative to the un-induced organism. It is the phenomenon of increased drug metabolising ability of the enzymes by several drugs and chemicals. Examples of enzyme inducers are; phenobarbitone, phenytoin, rifampicin, ethanol etc. On the other hand, enzyme inhibition is the process of decreased drug metabolising ability of the enzymes by several drugs and chemicals. Examples of enzyme inhibitors are; chloramphenicol, cimetidine, Valproic acid, etc (Barry and Feely, 1990). The consequences of enzyme induction and inhibition will be determined by the relative activity of the parent drug and the formed metabolite. Usually the metabolite will be less active than the original compound and therefore enzyme induction will result in a reduction in the pharmacological effect due to the increased drug metabolism (Barry and Feely, 1990). Enzyme inhibition would result in an increased concentration of parent drug at the receptor site and hence an increase in drug action (Bolt *et al.,* 1975).The effects of enzyme induction and enzyme inhibition are reversible on withdrawal of the inducing or inhibiting compound although the rate of onset and offset of these processes may differ (MacDonald Robinson, 1968).It has been noted that treatment of rats and other animals with phenobarbitonewill cause an increased activity in a number of enzyme systems. A study by Conney*et al.* (1960)suggested that tolerance and cross-toleranceto barbiturates might result from increased activity of the liver microsomalenzymes that metabolize these compounds.Piel*et al.* (1969), reported that rats pretreated with phenobarbitone have shortened sleeping time compared to those not pretreated. These and similar studies portray

phenobarbitone and barbiturates in general as microsomal enzyme inducers. Conversely, chloramphenicol and more recently, cimetidine are typical microsomal enzyme inhibitors, resulting in increased diazepam-sleeping times as observed in the current study. Therefore, microsomal enzyme induction and inhibition is so profoundly important, that it is routinely demonstrated in pharmacology programmes. This is usually done in mice and rats, the present data shows that the exact same results obtainable with mice and rats can equally be gotten using the more readily available and affordable chicks.

Motility in the small intestine, as in all parts of the digestive tract is controlled predominantly by excitatory and inhibitory signals from the enteric nervous system. This motility is however modulated by inputs fromthe central nervous system (sympathetic nerve andparasympathetic nerve) (Hansen, 2003). Generally the reactivity of smooth muscles depends on their structure and the presence of calcium ions, although they are modulated by various factors which activate specific signaling pathways leading to either contraction or relaxation (Sumimoto and Kuriyama, 1986).

Usually, this experiment is been carried out in rabbits and guinea pigs ileal smooth muscles, but it was designed to see if the same results will be obtainable using chicken ileum. The results in the study showed stimulation by cholinoceptor (Acetylcholine, Carbachol, Methacholine and Nicotine) and adrenoceptor (Adrenaline, Noradrenaline, Isoprenaline and Tyramine) leading to contraction and relaxation of both the rabbit and chicken ileal smooth muscles. This therefore means that, chicken ileum can be used in this experiment as alternative to rabbits and guinea pigs ileum.

The hot plate method of analgesic testing is one of the most common tests of nociception that is based on a phasic stimulus of higher intensity (Mandegary*et al.,* 2004). Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Parkhouse

and Plaury, 1979). Using the hot plate method, Manjunatha and Ratnakar (2015) showed that pentazocine had significant difference in analgesic activity when compared to saline control. Sikka and Kaushik (2011); Kurlekar and Bhatt (2004) also showed that morphine had similar significant difference in analgesic activity when compared to the control. From this study, since similar effects for pentazocine and morphine were demonstrated in chicks at tested doses of 10 and 20 mg/kg for each drug in a 4-day old cockerel and showed a significant difference when compared to the saline control, it implies that, chicks can be used as substitute in hot plate test.

On the other hand, it would appear that chicks would be unsuitable for acetic acid-induced writhing test for analgesic effect (peripherally mediated nociception), as even 100% increase in the concentration of acetic acid produced no writhes rather it was observed that the chicks became calm. This would be an example of species difference, a well-recognised phenomenon in biological systems. Other examples of such are; Tamoxifen, a therapeutic agent used in the treatment of breast cancer, is a known carcinogen in rodents and other experimental animal species, but it is generally regarded as being largely safe in humans (Wiseman and Lewis, 1996);Coumarin is metabolized in rat via 3,4-epoxidation, which represents an activating pathway because it is carcinogenic in this species whereas, in mice it undergoes 7-hydroxylation, which is considered to be a detoxifying pathway making it a non-carcinogen in mice (Lewis and Lake, 1995); Indomethacin is a non-specific anti-inflammatory drug (NSAID) that was developed specifically to subside the inflammatory responses to the indolic hormones, serotonin and tryptophan (Boynton *et al.,* 1988; Brandt, 1991). It was introduced in 1963 for the treatment of rheumatoid arthritis, degenerative joint diseases, ankylosingspondilitis, gout, acute musculoskeletal disorders, inflammation and oedema following surgical techniques and pain associated with primary dysmenorrhoea (Hardman *et al.,* 2001) whereas it is a potent rodenticide (Taiwo and Conteh, 2008);

Butadiene in mice is carcinogenic via P450-mediated activation to the mono- and diepoxide, whereas, in rat, it is noncarcinogenic because the diepoxide is not formed in them (Lewis *et al.,* 1997).

From this study, a number of possibilities present themselves. One would be to use > 1.2% acetic acid solution for the experiment and also to use 1-2 day old chicks.

The pentylenetetrazole (PTZ) test represents a valid model for human generalized and absence seizures (Loscher and Schmidt, 1988). It has been used experimentally to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. The exact mechanism of the epileptogenic action of PTZ at the cellular neuronal level is still unclear but it has been generally reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) neurotransmission (De Sarro*et al.,* 2003). Kubova and Mares (1991) has shown that phenobarbitone in rats inhibits PTZ-induced seizure in them. Also Akula*et al.,* (2009); Ishola*et al.,* (2013) has shown that phenobarbitone inhibits PTZ-induced seizure in mice. This also is in line with the results obtained here, at the doses of 20 and 30 mg/kg phenobarbitone shows 100% protection against 80 mg/kg PTZ-induced seizure in chicks meaning that chicks can be used as replacement in this experiment.

The autonomic nervous system is one of the most important regulators of the mammalian heart. It is known that all regions of the heart are innervated by sympathetic nerves, while parasympathetic innervation of the heart is present mainly in the supraventricular tissues (Loffelholz&Pappano, 1985). The postganglionic release of acetylcholine (ACh) from parasympathetic nerve terminals activates postsynaptic muscarinic ACh receptors, and the effects of ACh on atria and ventricles are quite different (Endoh, 1999; Dhein*et al.,* 2001). The contraction and relaxation of the heart atria is brought about by sympathetic and parasympathetic nervous system. The results in this study showed contraction by adrenergic

agents (Adrenaline, Noradrenaline, Isoprenaline and Tyramine) and relaxation by cholinergic agent (Acetylcholine) only with the guinea pig heart atria traditionally used for this experiment. On the part of the chicken heart atria, there were no responserelative tothat of the baseline even with repeated experiment. This could be as a result of environmental changes or species variation as discussed above. This suggests that chicken heart atria are not suitable as alternative to guinea pig heart atria.

Acute toxicity is defined specificallyas adverse effects occurring following oral or dermaladministration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours (MSDS HyperGlossary, 2006). Determination of median lethal dose (LD_{50}) (the dose which will cause death to 50% of the tested group of animals) is usually an initial step in the assessment and evaluation of toxic characteristics of a substance and also the initial screening experiments performed with all compounds. It provides information on the health hazards likely to arise from short-term exposure and the mode of toxic action of a substance (Akhila*et al.*, 2007). Theintraperitoneal and oral LD_{50} valueof Artemether, Dichlorvos and *Ficuscapensis* in 7-day old chicks (39±45 g) and adult male mice (22±34 g) were found to be the same. The value for the intraperitoneal LD_{50} of Artemether in chicks and mice was found to be 470 mg/kg body weight while their value in Dichlorvos was found to be 1.41 mg/kg body weight. This suggests that Artemether is moderately toxic while Dichlorvos is highly toxic both in chicks and mice according to the lethal dose classification of toxic levels of chemicals by Lorke (1983).Also, oral LD_{50} value of *Ficuscapensis*in chicks, mice and adult male rats was also found to be >5000 mg/kg meaning it is practically non-toxic in all the three models. This implies that, chicks can be used as alternative animal model in the determination of lethal dose since the result showed that chicks have the same value with both rats and mice in all the three tested substances.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

In summary, chickens/chicks were found to be suitable in, the dose/concentration-response relationship both *in vivo* and *in vitro.* Also in the demonstration of drug antagonism and synergism, microsomal enzymes induction and inhibition, and PTZ-induced seizurechicks were found to be a good alternative to rats and mice. In the *in vitro*demonstration of parasympathomimetic and sympathomimetic drugs on ileal tissue, similar results were obtained from the rabbit and chicken ileal smooth muscles. In the analgesic studies, the hot plate method produced the same results as in rats and mice while in the acetic acid-induced abdominal writhing there was no writhes observed in the chicks even with increasing the dose of the acetic acid. In the isolated heart atria, there was no response observed with the chicken heart atria different from that of the baseline.

In the determination of median lethal dose (LD_{50}) in conventional medicine, herbal medicine and pesticide, the results are similar for rats, mice and chicks.

6.2 Conclusion

Suitability of chicks as experimental animals in pharmacological researchwere performed and based on the results obtained from the experiments chickens/chicks can be used as alternative in dose/concentration-response relationship both *in vivo* and *in vitro,* demonstration of drug antagonism and synergism, microsomal enzymes induction and inhibition, PTZ-induced seizure,*in vitro*demonstration of parasympathomimetic and sympathomimetic drugs, analgesic study using hot plate method and determination of median lethal dose (LD_{50}) whereas they are not suitable for isolated heart atria and acetic acid-induced abdominal writhing. For now, the conclusion one can draw remains that chicks would be suitable replacement for mice and rats in the hot plate method of screening for centrally-acting

analgesic; but would not be suitable in acetic acid-induced writhe screening for peripherally acting analgesics.

6.3 Recommendations

The recommendations for further research on this study include:

1. Further studies on acetic acid-induced writhing using chicks (*in vivo*) by increasing the percentage of acetic acid administered.
2. Further studies on isolated chicken heart atria (*in vitro*).

6.4 Contributionto Knowledge

1. In the study for dose-response relationship, diazepam produced maximal sleep duration of 1400 minutes at a dose of 40 mg/kg in chicks.
2. Pentazocine and morphine at 10 and 20 mg/kg respectively shows peak latency period of 10 s after 60 minutes of administration in chicks.

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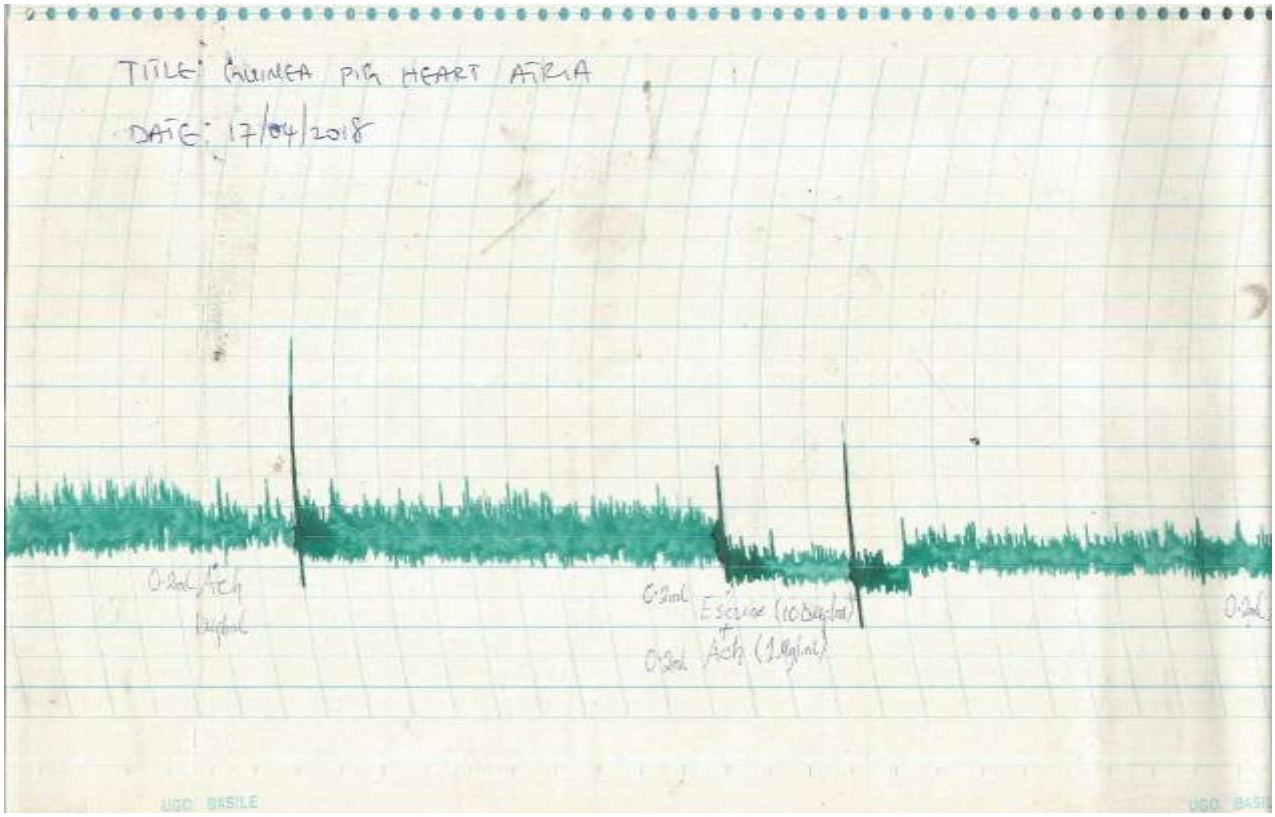
APPENDIX I


TITLE: GUINEA PIG HEART AORTA
DATE: 17/04/2018
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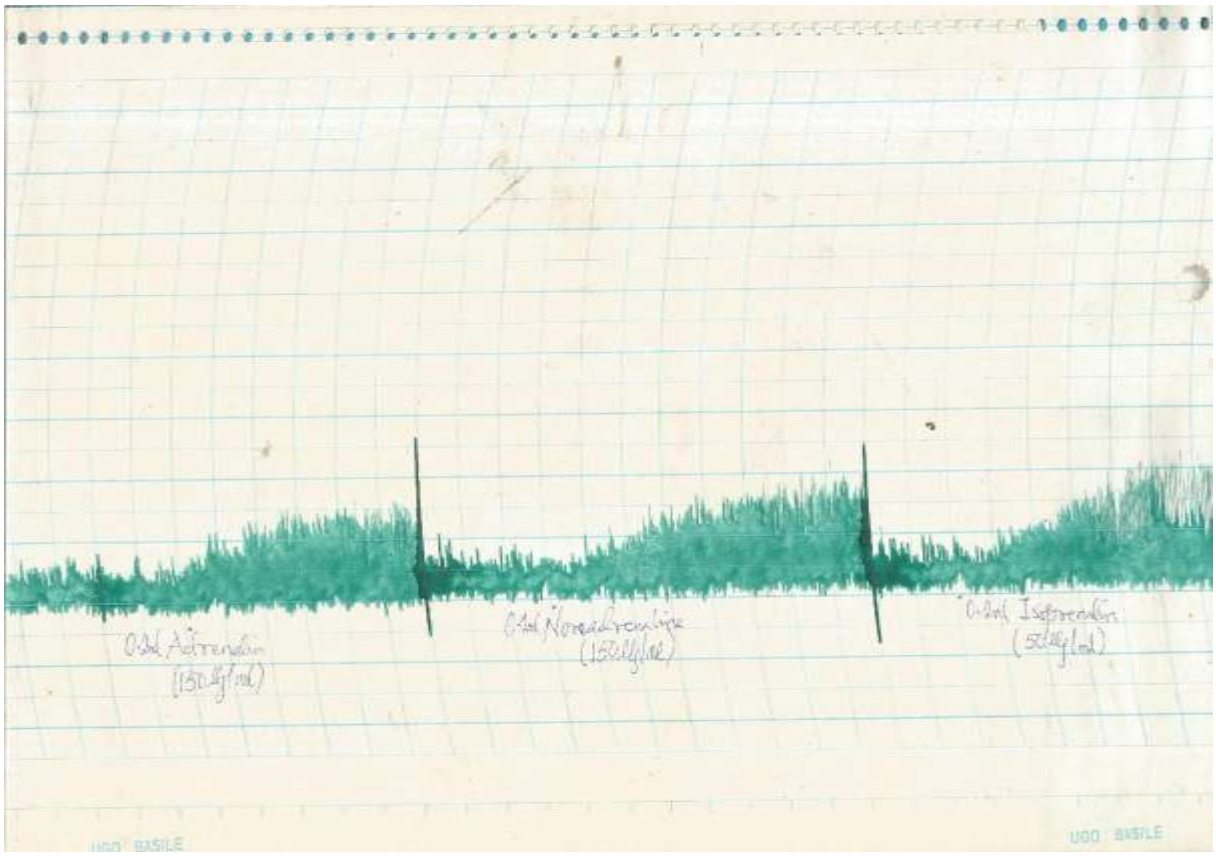
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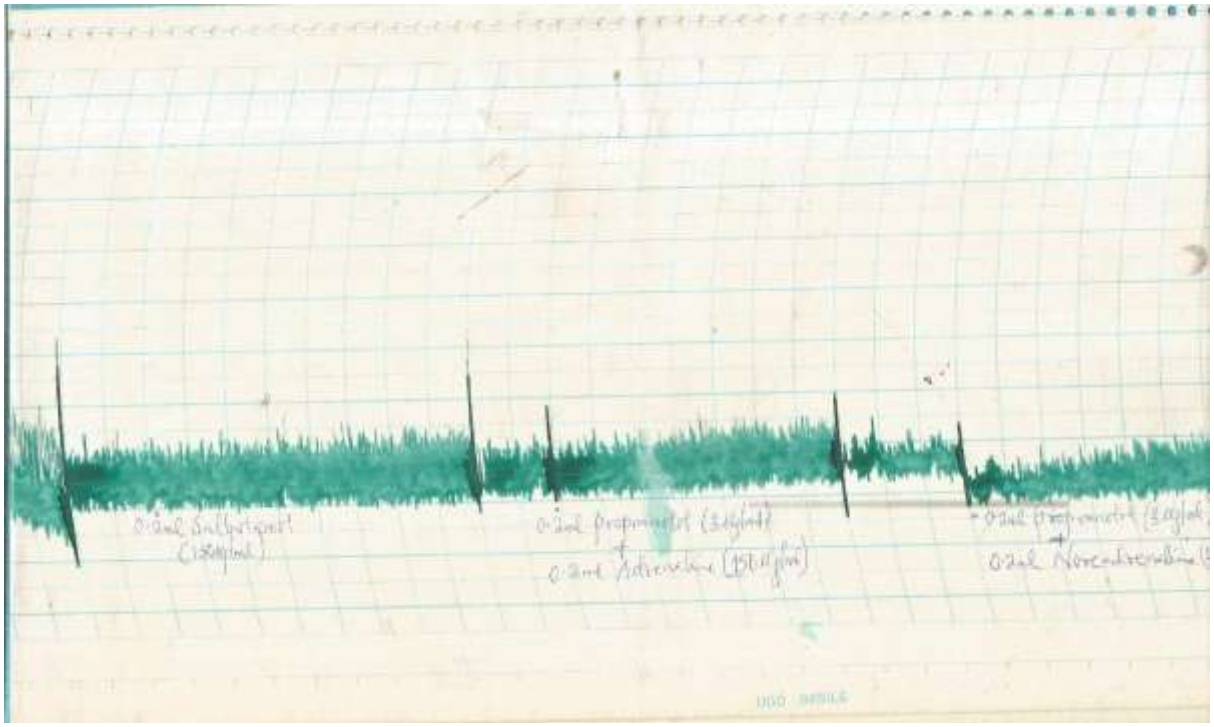
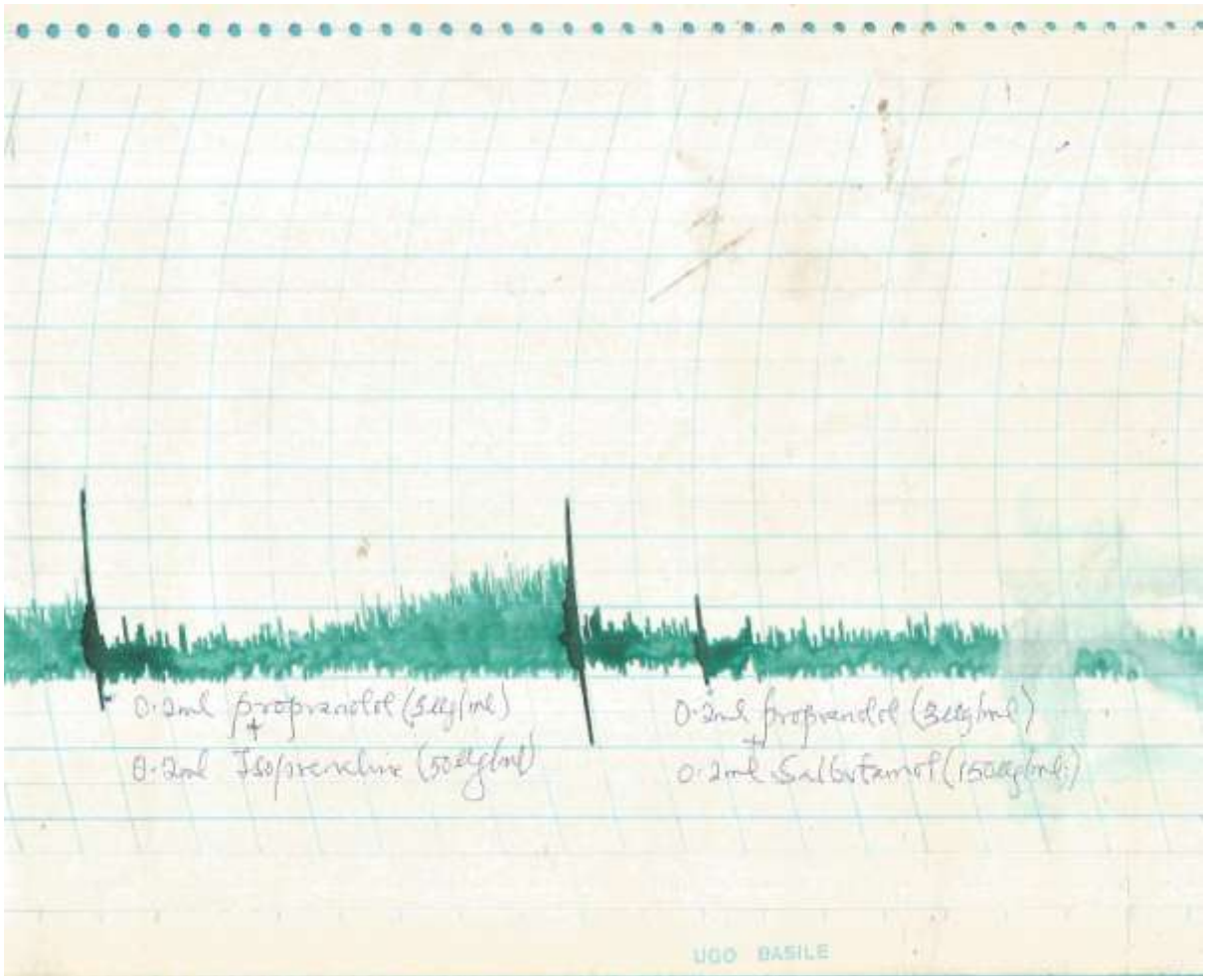
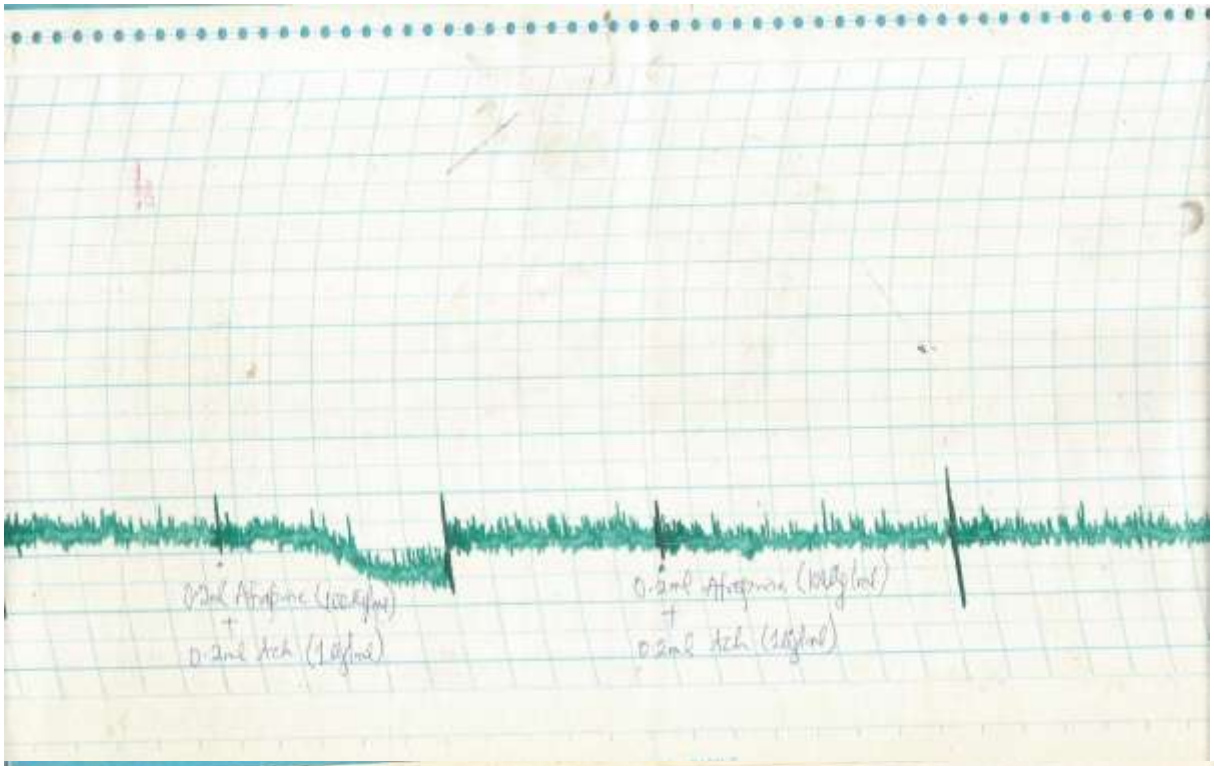
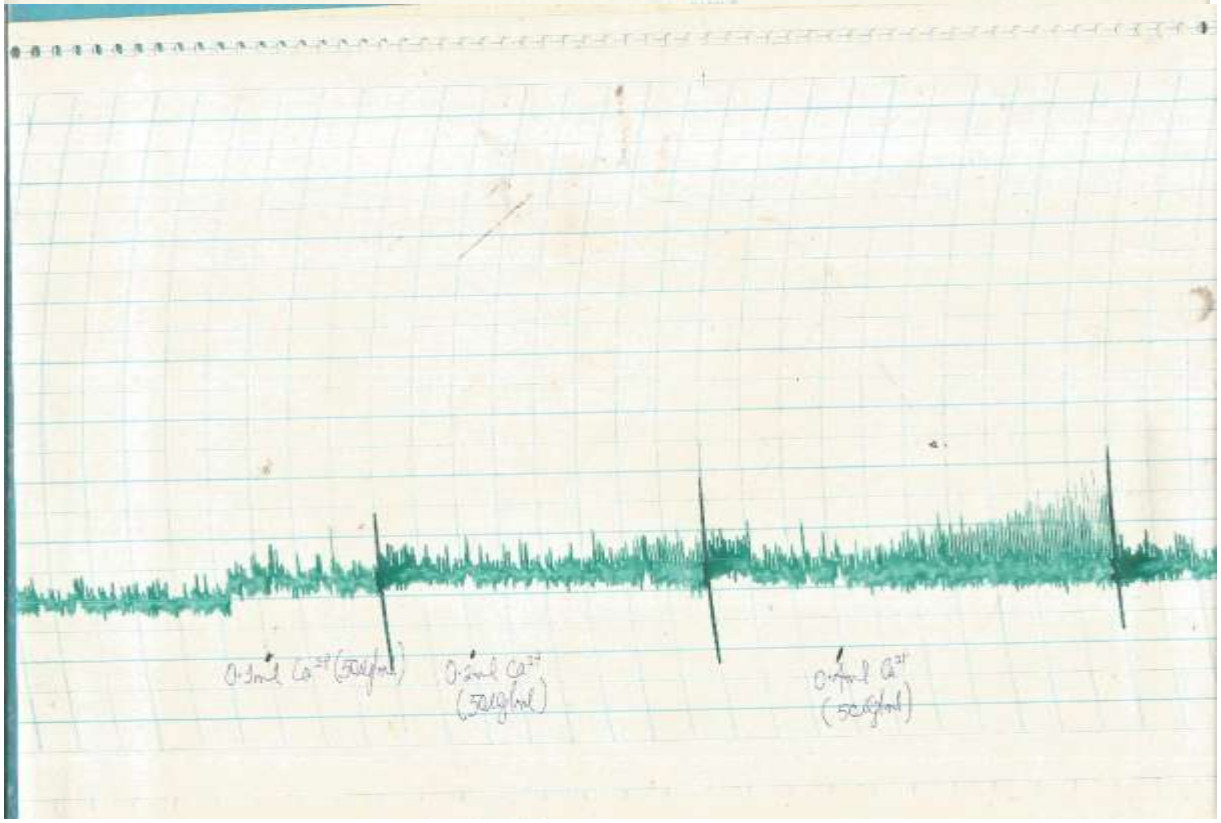
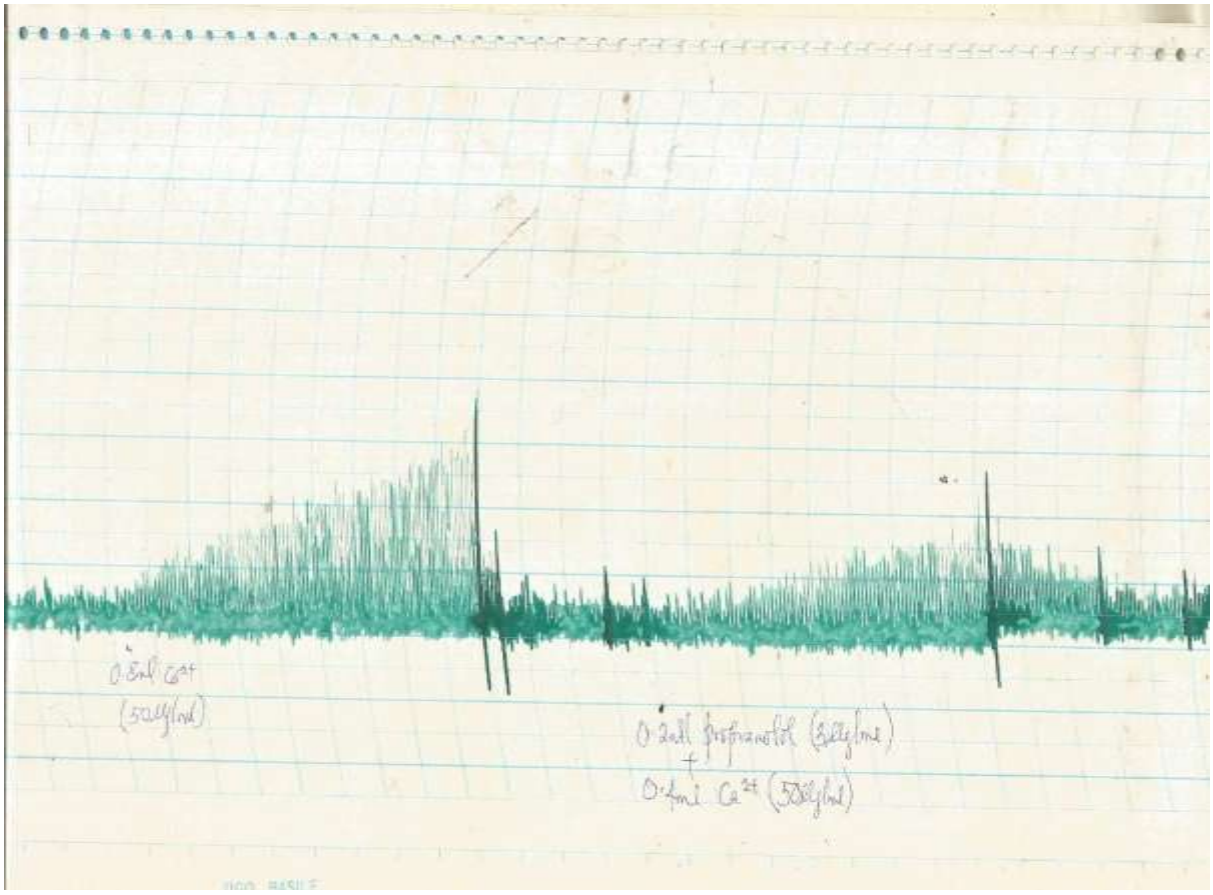

0·2ml Salbutamol
(150μg/ml)
0·2ml Propranolol (5μg/ml)
+
0·2ml Adrenaline (150μg/ml)
+ 0·2ml Propranolol (5μg/ml)
+
0·2ml Noradrenaline
UGO BASILE


0·2ml Propranolol (5μg/ml)
+
0·2ml Isoprenaline (50μg/ml)
0·2ml Propranolol (5μg/ml)
+
0·2ml Salbutamol (150μg/ml)
UGO BASILE


0.2ml Atropine (1000ng/ml)
+
0.2ml Ach (1ng/ml)
0.2ml Atropine (100ng/ml)
+
0.2ml Ach (1ng/ml)


0.2ml Ca++ (50ng/ml)
0.2ml Ca++ (50ng/ml)
0.2ml Ca++ (50ng/ml)


0.8ml Ca++
(50µg/ml)
0.2ml propranolol (3µg/ml)
+
0.4ml Ca++ (50µg/ml)

APPENDIX II