SOME UTEROTONIC PROPERTIES OF AQUEOUS LEAF EXTRACT OF *SIDA CORYMBOSA* (Fries) IN LABORATORY ANIMALS

BY

John Maiganga BAKUT

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA

MAY, 2016

SOME UTEROTONIC PROPERTIES OF AQUEOUS LEAF EXTRACT OF *SIDA CORYMBOSA* (Fries) IN LABORATORY ANIMALS

BY

John Maiganga BAKUT
M.B.B.S (A.B.U, 2008)
M.Sc / PHARM-SCI / 07785 / 2010 - 2011

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MAY, 2016

DECLARATION

I declare that the work in this dissertation entitled "Some uterotonic properties of aqueous leaf extract of *Sida corymbosa* (fries) in laboratory animals" has been carried out by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

John Maiganga BAKUT	……	……
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "Some uterotonic properties of aqueous leaf extract of *Sida corymbosa* (fries) in laboratory animals" by John Maiganga BAKUT meets the regulations governing the award of the degree of Master of Science in Pharmacology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Dr. N. M. Danjuma (Signature)……
Chairman, Supervisory Committee Date ……

Dr. A. U. Zezi (Signature)……
Member, Supervisory Committee Date……..……...

Dr. N. M. Danjuma (Signature)……
Head of Department Date…….……

Prof. K. Bala (Signature)……..……
Dean, School of Postgraduate Studies Date…….

ACKNOWLEDGEMENT

God Almighty, My source of inspiration, Support, Supply and Maker of all.

To Dr. N. M. Danjuma for the guidance, leadership, instructions, corrections and mentorship that made this possible. I am truly grateful and I love pharmacology even more because of the things I learnt from you.

To Dr. A. U. Zezi, thank you for the encouragement, it made me believe this was possible and for fine tuning this work. You are indeed a resource person.

To Dr (Mrs) Odengele, thank you for simplifying statistics.

To all technicians in the Pharmacology Laboratory especially Mr. John Kono, Mallam Muazu and Nasiru, thank you for simplifying the beautiful subject of Pharmacology.

To Mrs Mary Bakut, my mum, thank you for showing me the plant.

My family, my roots, home, ever welcoming arms, thank you for the encouragement and understanding. May the Lord increase you in every good dimension.

DEDICATION

This dissertation is dedicated to my Wife (Shalini) and Daughter (Aviya).

ABSTRACT

Uterotonic property is the characteristic innate ability of a substance to spontaneously initiate and maintain uterine contractions due to its chemical composition. Over centuries, sub-saharan Africans have been using *Sida corymbosa* leaf extract during labour with the claims that it aids and facilitates childbirth. This study was carried out to validate the folkloric claim that *Sida corymbosa* leaf extract possesses uterotonic property. Acute toxicity test following single oral administration of the extract was done in accordance with Lorke's method. Preliminary phytochemical screening was carried out in accordance with Odebiyi and sofowora (1978) and of Trease and Evans (1996) methods. *In-vitro* uterototonic test was conducted on isolated pregnant and non pregnant uterine tissues. The extract, acetylcholine and oxytocin were independently instilled into the organ bath and the responses they elicited were measured on a microdynanometre. Interaction studies were also conducteed using varying concentrations of the extract and single low doses of acetylcholine, oxytocin, salbutamol, atropine and indomethacine. The LD_{50} of the extract was found to be 471.17 mg/kg and phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, saponins, cardiac glycosides, sterol, triterpenes and anthraquinones in the extract. The extract demonstrated uterotonic activity more on the pregnant than the non pregnant uterine tissue. The mean response amplitude of the pregnant uterus was 58.815 mm while that of non pregnant uterine tissue was 40.328 mm with a mean difference of 18.5 mm. The concentration that is required to elicit fifty percent of uterotonic activity (EC_{50}) of the extract was lower for the pregnant uterine tissue (1.58 mg/ml of final bath concentration) than the EC_{50} (2.09 mg/ml) of the non pregnant uterus. It indicates increased sensitivity of the pregnant uterine tissue to the extract than the non pregnant uterine tissue. When interaction studies were done (between the extract + acetylcholine and extract + oxytocin), synergy of the uterotonic activity was observed. The percentage increase of 22.3% and 26.4% were observed for the interactions of (extract + acetylcholine) and

(extract + oxytocin) respectively on non pregnant uterine tissue. The percentage increase of 9.28% and 2.79% respectively was observed in the case of the pregnant tissue. However, when interaction studies were done between (extract + salbutamol, extract + atropine and extract + indomethacine), the drugs were found to antagonise the uterotonic activity of the extract with percentage decrease of 80.7%, 88.3% and 79.7% respectively on the non pregnant uterine tissue and 64.4%, 9.3% and 98.5% respectively on the pregnant uterine tissue. The extract may be acting via adrenoceptors or prostaglandin receptor because antagonism by salbutamol and indomethacine on the uterotonic activity of the extract were apparent on both pregnant and non pregnant uterine tissues. In conclusion, the aqueous leaf extract of *Sida corymbosa* has demonstrated uterotonic activity on both pregnant and non pregnant uterine tissues. The extract has a wide variety of phytochemicals, it is slightly toxic and may be utilizing adrenoceptors or prostaglandin receptors for its uterotonic activities.

TABLE OF CONTENTS

CONTENTS PAGE

DECLARATION ii

CERTIFICATION iii

ACKNOWLEDGEMENT iv

DEDICATION v

ABSTRACT vi

TABLE OF CONTENTS viii

LIST OF TABLES xiii

LIST OF FIGURES xiv

LIST OF PLATES xvi

LIST OF APPENDIXES xvii

ABBREVIATIONS xxi

CHAPTER ONE

1.0 INTRODUCTION 1

1.1 Reproduction, Labour and Uterotonicity 1

1.2 Statement of the Problem 3

1.3 Justification 3

1.4 Aim 4

1.5 Hypothesis 4

CHAPTER TWO

2.0 LITERATURE REVIEW 5

2.1 Introduction 5

2.2 Sida corymbosa 5

2.2.1 Common names 8

2.2.2 Ethnomedicinal uses of *Sida corymbosa* 8

2.2.3 Phytochemical constituents [*Sida corymbosa*] 9

2.3 Anatomy of Uterus 9

2.4 Physiology of Uterine Muscle Contraction 11

2.5 Calcium Dependent Contraction of Uterine Muscle 11

2.6 Calcium Sensitization Mechanism and Contraction of Uterine Muscle 12

2.7 Uterine Muscle Relaxation 13

2.8 Uterine Muscle Activity, Receptors and the Role of Hormones. 14

2.8.1 The role of the posterior pituitary hormones on the uterus 15

2.9 Oxytocin 16

2.10 Prostaglandin 18

2.11 Ergot Alkaloids 20

2.12 Noradrenline 21

2.13 Angiotensin 21

2.14 Tocolytic Agents 22

2.14.1 Atropine 22

2.14.2 Salbutamol 22

2.14.3 Indomethacine 23

2.14.4 Calcium Antagonists....23

2.14.5 Adrenaline....24

CHAPTER THREE

3.0 MATERIALS AND METHOD....25

3.1 Plant Material....25

3.2 Animals....25

3.3 Drugs....25

3.4 Equipment....26

3.5 Plant Extraction....26

3.6 Preliminary Phytochemical Screening....26

3.6.1 Test for carbohydrates....27

3.6.1.1 *Molisch test*....27

3.6.1.2 *Fehlings test*....27

3.6.2 Test for anthracene derivatives (Bontrager's test)....27

3.6.3 Test for unsaturated steroid and triterpene (Liebermann-Bucchard test)....27

3.6.4 Test for unsaturated sterols (Salkowski test)....27

3.6.5 Test for cardiac glycosides (Keller-kiliani test)....28

3.6.6 Test for saponin (Frothing test)....28

3.6.7 Test for tannins....28

3.6.8 Test for flavonoids (Sodium hydroxide test)....28

3.6.9 Test for alkaloids (Dragendoff's test)....28

3.7 Acute Toxicity Studies (LD_{50} Determination) 28

3.8 Preparation of Non Pregnant Rat Uterine Tissues 29

3.9 Preparation of Pregnant Rat Uterus 30

3.10 Addition of Drugs 30

3.11 Drug Interaction Studies 31

3.12 Data Analysis 31

CHAPTER FOUR

4.0 RESULTS 32

4.1 Plant yield 32

4.2 Results of Phytochemical Screening 32

4.3 Acute Toxicity Testing of the Aqueous Leaf Extract of *Sida corymbosa* 33

4.4 Results of Uterine tissue Experiment 33

4.4.1 Addition of single agent on pretreated non-pregnant rat uterine strip 33

4.4.2 Interactions of *S. corymbosa*, acetylcholine and oxytocin on pretreated non pregnant uterine strip 35

4.4.3 Interactions of *S. corymbosa*, salbutamol, atropine and indomethacine on pretreated non pregnant uterine strip 37

4.4.4 The log Concentration - response curve of S. corymbosa aqueous extract on pretreated non pregnant uterus 39

4.5 Result of Effects of the Extract and other Drugs on Pregnant Uterine Strip 42

4.5.1 Addition of single agent on pregnant rat uterine strip 42

4.5.2 Interactions between varying concentrations of *S. corymbosa* and single low concentrations of acetylcholine and oxytocin on pregnant uterine strip 44

4.5.3 Interactions between varying concentrations of *S. corymbosa* and single low concentration of salbutamol, atropine and indomethacine on pregnant uterine strip 46

4.5.4 The log Concentration – response curve of S. corymbosa aqueous leaf extract on pregnant uterus 48

4.6 Results of Interaction Study 52

4.6.1 On non pregnant uterine strip (Synergistic and Antagonistic Study) 52

4.6.2 Interaction studies on pregnant uterine strip 56

CHAPTER FIVE

5.0 DISCUSSION 60

CHAPTER SIX

6.0 Summary 67

6.1 Conclusion.....68

6.2 Recommendation 68

References.....69

LIST OF TABLES

Table 4.1 Phytochemical Constituents of the Aqueous Leaf Extract of *Sida corymbosa* 32

Table 4.2: EC_{50} and Emax of *S. corymbosa* Aqueous Leaf Extract and that of Interaction Studies between the Extract and other Agonist on Pretreated Non Pregnant Uterine Tissue 41

Table 4.3: EC_{50} and Emax of the *S. corymbosa* Aqueous Leaf Extract and that of Interaction studies between the Extract and other Agonist on Pregnant Uterus 51

LIST OF FIGURES

Figure 4.1: Comparison of Various Effects Elicited by Varying Concentrations of Acetylcholine, Oxytocin and *S. corymbosa* Aqueous Leaf Extract on Non Pregnant Uterus.....34

Figure 4.2: Comparison of Various Effects Elicited by Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and the Interaction Studies with Single Low Concentrations of Acetylcholine and Oxytocin on Non Pregnant Uterus 36

Figure 4.3: Comparison of Various Effects Elicited by Varying Concentrations of *S. corymbosa* Aqueous Extract and Interaction studies between the Extract and Single Low Concentrations of Salbutamol, Atropine and Indomethacine on Non Pregnant Uterus 38

Figure 4.4: Log concentration - Response Curve of the *S. corymbosa* Aqueous Leaf Extract on Pretreated Non Pregnant Rat Uterine Strip 39

Figure 4.5: Comparison of Various Effects Elicited by Varying Concentrations of Acetylcholine, Oxytocin and *S. corymbosa* Aqueous Leaf Extract on Pregnant Uterine Strip 43

Figure 4.6: Comparison of Various Effects Elicited by Various Concentrations of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Single Low Concentrations of Acetylcholine and Oxytocin on Pregnant Uterus.....45

Figure 4.7: Comparison of Various Effects Elicited by Various Concentrations of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Single Low

Concentrations of Salbutamol, Atropine and Indomethacine on Pregnant Uterus 47

Figure 4.8: Log concentration - Response Curve of the *S. corymbosa* Aqueous Leaf Extract on Pregnant Rat Uterus.....49

Figure 4.9: Log concentration – Response Curve of the Effect of *S. corymbosa* Aqueous Leaf Extract by Acetylcholine and Oxytocin on Non Pregnant Uterus 53

Figure 4.10: Log concentration – Response Curve of *S. corymbosa* Aqueous Leaf Extract and that of the Extracts Interactions with Salbutamol, Atropine and Indomethacine on Pretreated Non Pregnant Uterus 55

Figure 4.11: Log Concentration – Response Curve of the Effect of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Acetylcholine and Oxytocin on Pregnant Uterus 57

Figure 4.12: Log Concentration – Response Curve of the Effect of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Salbutamol, Atropine and Indomethacine on Pregnant Uterus.....59

LIST OF PLATES

Plate 1: *Sida corymbosa* Plant in its Natural Habitat. 6

Plate 2: *Sida corymbosa* Flower in its Natural Habitat. 7

LIST OF APPENDIXES

Appendix 1(A): Effect of Aqueous Leaf Extract of S. corymbosa, Acetylcholine, Oxytocin, Salbutamol, Atropine and Indomethacine and their Interactions with the Extract on Pretreated Non Pregnant Rat Uterine Strip Expressed as Amplitude of Response in Millimeters.x 75

Appendix 1(B): Effect of Aqueous Leaf Extract of *S. corymbosa*, Acetylcholine, Oxytocin, Salbutamol, Atropine and Indomethacine and their Interactions with the Extract on Pregnant Rat Uterine Strip Expressed as Amplitude of Response in Millimeters. 76

Appendix 2 (A1): Effect of Acetylcholine on Pretreated Non Pregnant Uterine Strip .77

Appendix 2 (A2): Effect of *S. corymbosa* Aqueous Leaf Extract on Pretreated Non Pregnant Uterine Strip 78

Appendix 2 (A3): Effect of Oxytocin on Pretreated Non Pregnant Uterine Strip.....79

Appendix 2 (A4): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Acetylcholine on Pretreated Non Pregnant Uterine Strip 80

Appendix 2 (A5): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Oxytocin on Pretreated Non Pregnant Uterine Strip 81

Appendix 2 (A6): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Salbutamol on Pretreated Non Pregnant Uterine Strip 82

Appendix 2 (A7): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low concentration of Atropin on Pretreated Non Pregnant Uterine Strip 83

Appendix 2 (A8): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Indomethacine on Pretreated Non Pregnant Uterine Strip 84

Appendix 2 (B1): Effect Oxytocin on Pregnant Rat Uterine Strip 85

Appendix 2 (B2): Effect of *S.corymbosa* Aqueous Leaf Extract on Pregnant Rat Uterine Strip 86

Appendix 2 (B3): Effect of Acetylcholine on Pregnant Rat Uterine Strip 87

Appendix 2 (B4): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Acetylcholine on Pregnant Uterine Strip 88

Appendix 2 (B5): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Oxytocin on Pregnant Uterine Strip 89

Appendix 2 (B6): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Salbutamol on Pregnant Uterine Strip. 90

Appendix 2 (B7): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Atropin on Pregnant Uterine Strip 91

Appendix 2 (B8): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low concentration of Indomethacine on Pregnant Uterine Strip 92

APPENDIX 3(A): Pretreated Non Pregnant Uterine Tissue. 93

Appendix 3 (A1): EC_{50} of *S. corymbosa* on Pretreated Non Pregnant Uterine Tissue .93

Appendix 3 (A2): EC_{50} of the Interaction of *S. corymbosa* and Acetylcholine on Pretreated Non Pregnant Uterine Tissue. 94

Appendix 3 (A3): EC_{50} of the Interaction of *S. corymbosa* and Oxytocin on Pretreated Non Pregnant Uterine Tissue. 95

Appendix 3 (A4): EC_{50} of the Interaction of *S. corymbosa* and Salbutamol on Pretreated Non Pregnant Uterine Tissue. 96

Appendix 3 (A5): EC_{50} of the Interaction of *S. corymbosa* and Atropin on Pretreated Non Pregnant Uterine Tissue. 97

Appendix 3 (A6): EC_{50} of the Interaction of *S. corymbosa* and Indomethacine on Pretreated Non pregnant Uterine Tissue. 98

APPENDIX 3(B): Pregnant Uterine Tissue. 99

Appendix 3 (B1): EC_{50} of *S. corymbosa* on Pregnant Uterine Tissue. 99

Appendix 3 (B2): EC_{50} of the Interaction of *S. corymbosa* and Acetylcholine on Pregnant Uterine Tissue. 100

Appendix 3 (B3): EC_{50} of the Interaction of *S. corymbosa* and Oxytocin on Pregnant Uterine Tissue. 101

Appendix 3 (B4): EC_{50} of the Interaction of *S. corymbosa* and Salbutamol on Pregnant Uterine Tissue. 102

Appendix 3 (B5): EC_{50} of the Interaction of *S. corymbosa* and Atropin on Pregnant Uterine Tissue. 103

Appendix 3 (B6): EC_{50} of the Interaction of *S. corymbosa* and Indomethacine on Pregnant Uterine Tissue. 104

Appendix 4 (A1): Effect on Pregnant and Non-Pregnant Uterine Strips.....105

Appendix 4 (A2): Bonferroni for Multiple Comparisons on Both Pregnant and Non Pregnant Uterine Tissues.....106

Appendix 4 (A3): Analysis of Response Amplitudes of the Non Pregnant Uterine Tissue.....107

Appendix 4 (A4): Bonferroni Test for Multiple Comparisons of the Effect of Drugs Non Pregnant Uterine Tissues.....108

Appendix 4 (A5): Analysis of Response Amplitude of Pregnant Uterine Strip.....109

Appendix 4 (A6): Bonferroni Test for Multiple Comparisons of the Effect of Drugs Pregnant Uterine Tissues. 110

ABBREVIATIONS

%	Percentage
µg	Microgram
Ach	Acetylcholine
Aq	Aqueous
Atrp	Atropine
Cacl	Calcium chloride
Cm	Centimetres
CO_2	Carbon dioxide
Conc	Concentration
Ec	Effective concentration
E_{max}	Maximum effective concentration
Ext	Extract
G	Grams
H_2O	Water
Indo	Indomethacine
IU	International unit
Kcl	Potassium chloride
Kg	Kilogram
L	Litres
LD	Lethal dose
Log	Logarithm
Ml	millilitres
Nacl	Sodium chloride
$NaHco_3$	Sodium hydrogen bicarbonate
O_2	Oxygen

^{o}C	Degree Celsius
Oxyt	Oxytocin
Salb	Salbutamol
UK	United Kingdom
Vol	Volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Reproduction, Labour and Uterotonicity

Reproduction is the biological process by which new individual organisms are produced and is a fundamental feature of all known life forms (Ridley, 2004). It is one of the important aspects of the human experience, which is achieved through processes that leads to coitus, fertilization, development and growth of the fetus and subsequent delivery of the product of conception (Alan *et al.*, 2003).

Labour is a sequence of uterine contractions that results in effacement and dilation of the cervix and voluntary bearing-down efforts leading to the expulsion per vagina of the products of conception. Vaginal delivery is achieved through labour but for most pregnant women the uterus remains relatively quiescent during pregnancy until the last 4-8 weeks prior to delivery when there is increase in uterine activity. The uterine activities are weak contractions, usually irregular, of short duration and not associated with cervical dilation, they are referred to as Braxton Hicks contractions (Ikpeze, 2009).

Labour and delivery are normal physiologic processes that most women experience without complications. However, labour needs to be managed to foster safe birth for mothers and their newborns (Alan *et al*., 2003).

Sometimes, uterine contractions are either not frequent enough (< 3 in 10 min) or are too weak to cause cervical effacement and delivery. In such cases, labour augmentation can be done by skilled birth attendants after diagnosing “hypotonic uterine dysfunction”. In this state, uterine contraction is ineffective at producing adequate cervical dilation (WHO, 2003).

When labour is augmented with uterotonic drugs like oxytocin, the quality and frequency of uterine contractions are greatly affected. The contractions tend to be longer, stronger and with short relaxation periods in between. Augmentation with oxytocin plays a major role in shortening labour but it is associated with risks such as; uterine rupture, detachment of placenta, increase stress for mother, water intoxication, uterine fatigue, post partum hemorrhage, umbilical cord compression and fetal death. All of these are unsatisfactory outcomes of augmentation of labour with modern orthodox drug (WHO, 2003).

Therefore, the screening of plants with uterotonic bioactivity and the subsequent identification and characterization of the bioactive uterotonic principle(s) will be a useful guide towards the formulation of affordable and good uterotonic which may have less side effects. One plant that featured prominently from local ethnobotanical survey based on fokloric uterotonic use with claim to be used to "wash the uterus" is *Sida corymbosa*. The people of Kachia Local Government in Kaduna State Nigeria have used the leaves of *Sida corymbosa* for augmentation of labour for many years. The people claimed that whenever fresh leaves of *Sida corymbosa* are macerated and soaked in water, it has the ability of increasing the strength of uterine contraction when taken orally by a woman who is in labour. So the people use it as uterotonic for the augmentation of labour (Personal communication, 2012).

Rat uterus is a lot like the human uterus because it is a single simple uterus with a single chamber for accommodating developing embryos. They are similar because analysis of dysregulated gene sets on uterine myometrium indicates that in both species there are altered cell cycle regulation, increased extracellular matrix production and altered amino acid metabolism that are characteristically the same (Arslan *et al*., 2010).

1.2 Statement of the Problem

The use of modern oxytocics (misoprostol and oxytocin) in the induction and augmentation of labour has not been without catastrophic outcomes like rupture of uterus and detachment of placenta before delivery of fetus. In developing countries where skilled birth attendants are few and quality hospital services are lacking, especially in rural areas, catastrophic outcomes with the use of oxytocics are common. The obvious lack of skilled birth attendants makes diagnosis of "hypotonic uterine dysfunction" by unskilled birth attendant difficult and the use of oxytocics irrational in most cases.

In the 1960s, induction of labour with oxytocin was as high as 50 per cent of all pregnancies, due to enthusiasm for innovation and not on sound clinical judgment (WHO, 2003). Induction rate is 5.15 per cent of all pregnancies in Ghana (Kwawukume, 2005), 20.5 per cent in United States of America, 5-15 per cent in United Kingdom and 18-23 per cent in Nigeria (Kelly *et al*., 2003). According to World Health Organization (2005) about one million conceptions take place every day worldwide. About 50 per cent are unplanned and 25 per cent of them are unwanted and about 46 million of these pregnancies are terminated annually. A survey in Nigeria estimated that 610,000 induced abortions are carried out annually, using misoprostol and oxytocin (Henshaw *et al*., 2003).

The above facts buttress the magnitude of use and the dare need of identifying, verifying and validating the existing available plants that can easily be accessed and may be safely used for induction and augmentation of labour in Nigeria and indeed the whole world.

1.3 Justification

Sida corymbosa is a widely available plant in Sub Saharan Africa and has gained ethno-medical approval amongst the locals of various communities in sub-saharan Africa for its folkloric use as a good uterotonic agent in augmentation of labour.

If it is verified and gains pharmacological validation, it may serve as a source of an effective, naturally available, cheap and possibly less toxic uterotonic that will serve Nigerian teeming population. It may serve as a contribution to the progress of obstetrics practice globally.

1.4 Aim

The aim of this study is to evaluate the uterotonic activity of aqueous leaf extract of *Sida corymbosa*.

Specific Objectives:

- To determine acute toxicity of *Sida corymbosa* following single oral administration (LD_{50} determination).
- To confirm the phyto-chemical constituents of *Sida corymbosa* aqueous leaf extract.
- To determine the effect of the aqueous leaf extract of *Sida corymbosa* on pretreated (oestrogenised) isolated rat uterine tissue.
- To determine the effect of the aqueous extract of *Sida corymbosa* leaf on isolated pregnant rat uterus.
- To determine the possible mechanism of uterotonic activity of *S. corymbosa* or otherwise on both pretreated (oestrogenised) non pregnant and pregnant rat uterine tissues.

1.5 Hypothesis

The alternate hypothesis, states that, *Sida corymbosa* aqueous leaf extract possesses uterotonic activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Amongst the many elements that sustain life on earth, those that are derieved from plants play major roles and archeological evidences have shown that man has depended on plants for food and medicine from early human existence (Weiss and Kislav, 2004). Traditionally, a large percentage of Africans living in rural areas depend on natural plant products for managing menstrual problems, labour and post-partum hemorrhage. Ethno-medical surveys have shown that there are a lot of plant leaf used in Africa for facilitating labour and delivery (Hostettmann *et al*., 2013). These include, *Sida corymbosa,Calotropiz procera,Commelina africana,Auranta repens,Hyptis suaveolens,Occinum gratissimum,Saba comorensis,Sclerocarya birrea,Vernonia amygdalina* amongst others (Attah *et al.*, 2012).

2.2 Sida corymbosa

Sida corymbosa (plate 1 and 2), is a plant that grows in subtropical and tropical Africa, Southern Asia and Central America (Holm *et al*., 1977). It is a perennial herb of about 0.7m high with numerous erect branches. Leaves are oval, dentate, about 6.5 - 7.5cm long and 1-2cm broad. The flowers are solitary and axillary. Its fruits are more or less globular and covered on top with golden hairs (Ignacimuthu *et al.*, 2006). Inside the fruit are ovoid, black numerous seeds. The plant is a pan-tropical wild species, which grows around the roadside and on wastelands (Agyakwa and Akobundu 1998). Its botanical classification is thus; Class: *Equisetopsida,* Subclass: *Magnoliidae,* Order: *Malvales,* Suborder: *Rosanae,* Family: *Malvaceae,* Genus: *Sida* Specie: *Sida corymbosa* (Fries, 1965).



Plate 1: *Sida corymbosa* Plant in its Natural Habitat.



Plate 2: *Sida corymbosa* Flower in its Natural Habitat.

2.2.1 Common names

English - Broom weed (Russel, 1955)

Hausa – Miyar tsanya / Karkashin kwado (Agyakwa and Akobundu 1998)

Yoruba - Iseketu (Kayode, 2006)

Kenya (Digo tribe) - Mbundugo (Pakia, 2005)

India (Ghats tribe) - Pilla, Valatti and chedi (Malairajan *et al.*, 2006)

Burkina Faso (Mossi tribe) - Zon-raaga (Nacoulma and Ouedraogo, 1996)

2.2.2 Ethnomedicinal uses of *Sida corymbosa*

Sida corymbosa leaves have been used for medicinal purposes in some parts of sub-Saharan Africa for the treatment of malaria, gonorrhoea, augmentation of labour, breast cancer and gout. It is usually prepared as a decoction or poultice taken orally or applied topically (Jegede, 2012). *S. corymbosa* has been reported to contain aphrodisiac, analgesic and anti-inflammatory properties. It acts on the central nervous system and provides relief from anxiety. The intake of the decoction of *S. corymbosa* leaf is reported to lower blood pressure and improves cardiac irregularity, fever, seizure, ophthalmia, rheumatism, leucorrhoea, painful micturition, gonorrhoea, colic and general debility (FAO, 2002). Decoction of *S. corymbosa* leaf has been useful in improving wound healing and peptic ulcers (John *et al.*, 2012). The whole plant is said to be beneficial in management of spermatorrhoea, while the bark and stem are effective in sciatica and facial paralysis (FAO, 2002). The seed oil is used topically on sore muscles, sore joints, in arthritis and rheumatism (Kamla-Raj, 2012). The roots of the plant is has

astringentand diuretic properties, useful in management of cystitis, haematuria, bleeding piles and chronic dysentery. (Kuniata and Rapp, 2001).

A study has authenticated the anti-ulcer activity of *S. corymbosa*. The extract of *S. corymbosa* (250, 500 and 1000 mg/kg) showed a dose-dependent, significant ($p<0.05$) reduction of ulcer indices against gastric ulcers induced by both ethanol and diclofenac in mice. Similarly, topical application of a formulation prepared with the extract of *Sida corymbosa* on surgically created incision, produced an increase in the rate of healing of the wounds on the same experimental animal (John *et al.*, 2012).

2.2.3 Phytochemical constituents [*Sida corymbosa*]

Recent analysis revealed the presence of ephedrine and y-ephedrine as alkaloids found in the leaf of the *S. corymbosa*. Besides these, some other chemical compounds that have been isolated from the aerial parts of the plant are 6-phenyl ethyl amine, carboxylated tryptamines, quinazoline, hypaphorine, and vasicinol. Some species of this plant are reported to contain *cryptolepine* (Kuniata and Rapp, 2001). Besides these, traces of sitosterol, palmic, and stearic acids have also been isolated from this plant. The main alkaloid present in *sida* is asparagin. Fatty oil, phytosterol, mucin, potassium nitrate, resins and acids are also reported to be in the leaf of the plant. It is also known to contain saponins, tannins, and prostaglandin (Gill, 1992).

2.3 Anatomy of Uterus

The non pregnant human uterus is pear-shaped, about 7.5 cm in length, and comprises a fundus, body, and cervix. The uterine tubes enter at supero-lateral angle, termed the cornua, above which lies the fundus. The uterine body narrows to a waist, the isthmus, which continues as the cervix. It is clasped around the middle by vagina. This attachment defines the supravaginal band and the infravaginal parts of the cervix. The isthmus is 1.5

cm in length. Its junction with the uterine body is marked by the internal opening within the uterine cavity. It is the isthmus that becomes the lower segment of the uterus in pregnancy (Michael and Geoffrey, 1998).

The principal arterial supply is from the uterine artery which is abranch of the internal iliac artery. This runsin the base of the broad ligament and crosses immediatelyabove and at right-angles to the ureter as it passes forwardto the bladder. The artery reaches the uterus at the level ofthe uterine isthmus and the internal os (Ikpeze *et al*., 2009). It is the only structure,apart from the broad ligament, to lie superficially to the ureter. The uterine artery then ascends in a tortuous manner alongside the body of the uterus and anastomosiswith terminal branches of the ovarian artery, which arisesfrom the aorta below the level of the renal artery (Irene, 2011). The uterineartery also gives off a descending branch to the cervixand branches to the upper vagina, which anastomose withascending twigs from the vaginal artery.The uterine vein accompanies the artery and drains intothe internal iliac vein. It also communicates with the ovarianvein and with veins of the vagina and bladder via the pelvicvenous plexus (Alan *et al.,* 2003).In most mammals, the innervation of the uterus is very scanty. The nerve supply includes both excitatory and inhibitory sympathetic fibers and there are probably no ganglion cells as the nerve fibers end with free nerve endings. The uterus also receives some parasympathetic fibers from the sacral outflow, but there is no evidence that cholinergic fibers have any role in the control of uterine motility (Ivell, 2010).

Little attention has been paid to the regulating effect of the Autonomic Nervous System on myometrial activity. This may be because uterine activity is said to be normal in animals with transacted spinal cord. Destruction of the nervous supply to the uterus is not followed by muscle degeneration as occurs elsewhere in the body. The denervated

isolated uterus can still contract and respond normally to hormones and drugs (Katzung, 2006).

2.4 Physiology of Uterine Muscle Contraction

In the intact body, the process of smooth muscle cell contraction is regulated principally by receptor and mechanical (stretch) activation of the contractile proteins myosin and actin. A change in membrane potential, brought about by the firing of action potentials or by activation of stretch-dependent ion channels in the plasma membrane, can also trigger contraction (Aaronson *et al*., 2010). For contraction to occur, myosin light chain kinase (MLC kinase) must phosphorylate the 20-kDa light chain of myosin, enabling the molecular interaction of myosin with actin. Energy released from ATP by myosin ATPase activity results in the cycling of the myosin cross-bridges with actin for contraction. Thus contractile activity in smooth muscle is determined primarily by the phosphorylation state of the light chain of myosin, a highly regulated process. In some Smooth muscle cells, the phosphorylation of the light chain of myosin is maintained at a low level in the absence of external stimuli (i.e., no receptor or mechanical activation). This activity results in what is known as smooth muscle tone and its intensity can be varied (Barany, 1996).

2.5 Calcium Dependent Contraction of Uterine Muscle

Contraction of smooth muscle is initiated by a calcium mediated change in the thick filaments, whereas in striated muscle calcium mediates contraction by changes in the thin filaments. In Amplitude to specific stimuli in smooth muscle, the intracellular concentration of calcium increases and this activator calcium combines with the acidic protein calmodulin. This complex activates MLC kinase to phosphorylate the light chain of myosin (Fukata *et al*., 2011). Cytosolic calcium is increased through calcium release from intracellular stores (sarcoplasmic reticulum) as well as entry from the extracellular

space through calcium channels (receptor-operated calcium channels). Agonists (norepinephrine, angiotensin II, endothelin, etc.) binding to serpentine receptors, coupled to a heterotrimeric G protein, stimulate phospholipase C activity. This enzyme is specific for the membrane lipid phosphatidylinositol 4, 5-bisphosphate to catalyze the formation of two potent second messengers: inositol trisphosphate (IP3) and diacylglycerol (DG). The binding of IP3 to receptors on the sarcoplasmic reticulum results in the release of calcium into the cytosol. DG, along with calcium, activates protein kinase C (PKC), which phosphorylates specific target proteins (Aaronson *et al.*, 2010). There are several isozymes of PKC in smooth muscle, and each has a tissue-specific role (e.g., vascular, uterine, intestinal, etc.). In many cases, PKC has contraction-promoting effects such as phosphorylation of L-type calcium channels or other proteins that regulate cross-bridge cycling. Phorbol esters, a group of synthetic compounds known to activate PKC, mimic the action of DG and cause contraction of smooth muscle. Finally, L-type calcium channels (voltage-operated calcium channels) in the membrane also open in Amplitude to membrane depolarization brought on by stretch of the smooth muscle cell (Barany, 1996).

2.6 Calcium Sensitization Mechanism and Contraction of Uterine Muscle

In addition to the calcium dependent activation of MLC kinase, the state of myosin light chain phosphorylation is further regulated by MLC phosphatase, which removes the high-energy phosphate from the light chain of myosin to promote smooth muscle relaxation. There are three subunits of MLC phosphatase: a 37- kDa catalytic subunit, a 20-kDa variable subunit, and a 110- to 130-kDa myosin-binding subunit. The myosin binding subunit, when phosphorylated, inhibits the enzymatic activity of MLC phosphatase, allowing the light chain of myosin to remain phosphorylated, thereby promoting contraction. The small G protein RhoA and its downstream target Rho kinase

play an important role in the regulation of MLC phosphatase activity (Fukata *et al*., 2011). Rho kinase, a serine/threonine kinase, phosphorylates the myosin-binding subunit of MLC phosphatase, inhibiting its activity and thus promoting the phosphorylated state of the myosin light chain. Pharmacological inhibitors of Rho kinase, block its activity by competing with the ATP-binding site on the enzyme. Rho kinase inhibition induces relaxation of isolated segments of smooth muscle contracted to many different agonists. In the intact animal, the pharmacological inhibitors of Rho kinase have been shown to cause relaxation of smooth muscle in arteries, resulting in a blood pressure- lowering effect (Chitaley *et al*., 2001).

An important question facing the smooth-muscle physiologist is: what is the link between receptor occupation and activation of the calcium sensitizing activity of the RhoA/Rho kinase-signaling cascade. Currently, it is thought that receptors activate a heterotrimeric G protein that is coupled to RhoA/Rho kinase signaling via guanine nucleotide exchange factors (Barany, 1996).

2.7 Uterine Muscle Relaxation

Smooth muscle relaxation occurs either as a result of removal of the contractile stimulus or by the direct action of a substance that stimulates inhibition of the contractile mechanism for example atrial natriuretic factor is a vasodilator. Regardless, the process of relaxation requires a decreased intracellular calcium concentration and increased MLC phosphatase activity (Kurowicka *et al*., 2011). The mechanisms that sequester or remove intracellular calcium and/or increase MLC phosphatase activactivity may become altered, contributing to abnormal smooth muscle responsiveness. A decrease in the intracellular concentration of activator calcium elicits smooth muscle cell relaxation. Several mechanisms are implicated in the removal of cytosolic calcium and involve the sarcoplasmic reticulum and the plasma membrane. calcium uptake into the sarcoplasmic

reticulum is dependent on ATP hydrolysis. This sarcoplasmic reticular calcium and magnesium ATPase, when phosphorylated, binds two calcium ions, which are then translocated to the luminal side of the sarcoplasmic reticulum and released. Magnesium is necessary for the activity of the enzyme; it binds to the catalytic site of the ATPase to mediate the reaction. The sarcoplasmic reticular calcium and magnesium ATPase is inhibited by several different pharmacological agents: vanadate, thapsigargin, and cyclopiazonic acid (Aaronson *et al.*, 2010). Sarcoplasmic reticular Ca^{2+} binding proteins also contribute to decreased intracellular calcium levels. Recent studies have identified calsequestrin and calreticulin as sarcoplasmic reticular calcium binding proteins in smooth muscle. The plasma membrane also contains calcium and magnesium ATPases, providing an additional mechanism for reducing the concentration of activator calcium in the cell. This enzyme differs from the sarcoplasmic reticular protein in that it has an autoinhibitory domain that can be bound by calmodulin, causing stimulation of the plasma membrane calcium pump (Mackler *et al*., 2009).

Sodium and calcium exchangers are also located on the plasma membrane and aid in decreasing intracellular calcium. This low-affinity antiporter is closely coupled to intracellular calcium levels and can be inhibited by amiloride and quinidine. Receptor-operated and voltage-operated calcium channels located in the plasma membrane are important in calcium influx and smooth muscle contraction, as previously mentioned. Inhibition of these channels can elicit relaxation. Channel antagonists such as dihydropyridine, phenylalkylamines, and benzothiazepines bind to distinct receptors on the channel protein and inhibit calcium entry in smooth muscle (Barany, 1996).

2.8 Uterine Muscle Activity, Receptors and the Role of Hormones.

Uterine muscles contracts rhythmically both *in vitro* and *in vivo*. These spontaneous contractions are myogenic in origin as interference with the nerve supply does not

abolish them (Ruzycky and kulink, 1996). There is no evidence for the existence of intrinsic nerve plexus that controls the muscle as occurs in the gastrointestinal tract. The fundus seems to be the origin of these coordinated contractions. Sex hormones regulate myometrial control in the regulation of uterine function probably to provide a favorable environment for pregnancy (Batra, 1986).

The type of motility of the uterus and its threshold for response to uterotonic drugs varies greatly with the phase of the menstrual cycle and the stage of pregnancy. Both the force and frequency of the contractions are affected and this is due to the complex hormonal changes that occur during the cycle. On the 14^{th} day of the menstrual cycle, larger and more prolonged contractions can be recorded and during menstruation, strong coordinated contractions resembling what happens during parturition are observed (Smith, 2007).

Administration of oestrogen hyperpolarizes myometrial cells and suppresses spontaneous activity. Progesterone increases this effect. During pregnancy, a condition of electrical and mechanical quiescence and relative inexcitability is produced by endogenous progesterone acting locally at the site of implantation of the fetus. It has been reported that the number of receptors for drugs on the uterus are under hormonal control (Monster, 1967). The sensitivity of the uterus in experimental animals can be increased by pre-treating the animal with an oestrogen usually stilboesterol with a dose of about 1mg/kg twenty-four hours before the experiment. However, this may not be necessary in bioassay studies but the uterine status should be determined.

2.8.1 The role of the posterior pituitary hormones on the uterus

The posterior pituitary hormones, oxytocin and vasopressin (antidiuretic hormone, ADH) are important in the regulation of uterine activity. These hormones have vasoactive,

antidiuretic as well as uterotonic properties. They are synthesized in the hypothalamus and then transported to the posterior pituitary where they are stored and released into the circulation. Oxytocin for clinical use is prepared synthetically (Soloff, 2000).

Both hormones are polypeptides containing nine amino acids with a cys-cys linkage and a six amino acid disulfide ring. Oxytocin resembles vasopressin in having seven identical amino acids but contains leucine and isoleucine instead of arginine and phenylalanine. Oxytocin is released by certain peripheral stimuli such as suckling and cervical dilation while release of vasopressin is influenced by osmolarity of the extracellular fluid and by many drugs (Zeemen *et al*., 1997). The non-pregnant uterus and in the early pregnancy has greater sensitivity to vasopressin than to oxytocin. As pregnancy progresses the uterus becomes more sensitive to oxytocin and this has been found to correspond to increase in number of oxytocin receptors and oestrogen levels (Batra, 1986).

2.9 Oxytocin

Is a potent uterotonic nanopeptide homone (Ivell, 2010) which acts on both pregnant and pretreated or oestrogenized uterus to cause contraction. It acts both directly and indirectly to stimulate uterine smooth muscle contraction and is widely used for artificial induction of labour (Owen and Henth, 1992). It circulates as free peptide in the bloodstream and is released in a pulsatile fashion (Chard, 1989). It mediates its effect through oxytocin receptors (Mitchell and schmid, 2001), which are members of oxytocin / vasopressin family of receptors. Its subfamily is Gαq and it stimulates phospholipase C activity to induce uterine contraction by elevating intracellular calcium concentration. At the beginning of the process, phospholipase C mediated D-myoinositol 1,4,5-triphosphate (IP_3) induced calcium release from intracellular stores (Batra, 1986) leads to influx of extracellular calcium via voltage and receptor gated calcium channels (Anwer,

1989), or capacitve calcium entry (Monga *et al*, 1999). Oxytocin also stimulates an increase in cytoplasmic phospholipase A activity and induces cyclooxygenase 2(COX-2), the enzyme involved in prostaglandin synthesis, thereby having an increased effect on prostaglandin synthesis (Soloff *et al.*, 2000). Oxytocin receptor $_{m}$RNA expression is influenced by several factors including hormones, cytokines and transcription factors leading to increased expression of the receptor in pregnancy (Mitchell and Schimid, 2001).

Oxytocin is usually given by intravenous injection for the induction of labour. The onset of action is 1-2 minutes and the duration is 3-5 minutes. It can also be given intramuscularly but the onset is 5-10 minutes and the duration about 2 hours. It can also be given intranasally as a spray but the absorption may be erratic. It is bound to plasma proteins. Synthetic oxytocin has a plasma half-life of about 5 minutes but this may be less in late pregnancy and lactation. It is inactivated in the stomach, intestine and the liver by the enzyme oxytocinase and therefore inactive orally. It is eliminated through the liver, kidneys and functional mammary gland. Receptors for oxytocin have been reported to be present in the myometrium. The sensitivity of the uterus to oxytocin increases gradually during pregnancy and sharply before parturition. Oxytocin induced uterine contractions can be inhibited by B-adrenoceptor agonists, magnesium sulphate or inhalational anaesthetics.

Oxytocin has a weak vasopressin-like antidiuretic action which can result in water intoxication if large doses are infused. This may constitute an unwanted effect if oxytocin is used in patients with cardiac or renal disease, or in pre-eclampsia.

2.10 Prostaglandin

They are eicosanoids which are comprised of unsaturated fatty acids with a 20-carbon structure. They are found in almost every tissue of the body, are bioactive, short-lived and extremely potent. Various types of prostaglandins are synthesized by different cells. The endometrium and myometrium of the uterus have significant capacity of synthesizing prostaglandins. In addition to their vasoactive properties, the E and F type prostaglndins cause contractions of both non-pregnant and pregnant uterus (Matsumoto *et al*., 2007). They are lipid mediators produced by fetal membranes, uterus and placenta and are potent uterotonics (Mitchell *et al.*, 1995). When prostaglandins are bound to their receptor – specific ligands, intracellular pathways are activated. Those linked to contraction are EP_1, EP_3, FP and Thromboxane while those linked to relaxation are EP_2, EP_4, IP, DP receptor isoforms (Brodt-Eppley and Myatt, 1999).

PGE_2 and $PGF_{2\alpha}$ are uterotonics and also play a major role in cervical ripening and ultimately initiation of partubation in humans (Lopez, 2003). The contractile effect of prostaglandins is based on their ability to mobilize calcium and inhibit adenylyl cyclase activity. PGE_1 and PGF2α stimulates calcium release from the sarcoplasmic reticulum of myometrial cells (Carsten and Miller, 1977). The binding of PGF2α to its receptor stimulates G-protein activation of phospholipase C and A2. These stimulate the production of IP3 and intracellular calcium release (Molnar and Herfelendy, 1990). The released IP3 mobilizes calcium from the sarcoplasmic reticulum resulting in increased uterine contractility. PGE2 exerts dose dependent increase in calcium production (Asboth, 1996). The effects of PGE2 and PGF2α are mediated mainly through EP and FP receptor subtypes respectively (Matsumoto *et al*, 1997). Prostaglandins also have indirect effect on uterine contraction via increase of uterine sensitivity to uterotonic agents

(Garfield *et al*, 1980). It is worthy of note however, that EP2 and EP4 are responsible for smooth muscle relaxation and uterine quiescence (Brodt-Eppley and Myatt, 1999).

Prostaglandins play a significant role in two of main disorders of menstruation, dysmenorrhoea (painful menstruation) and menorrhagia (excessive blood loss). It is possible to reduce the excessive blood loss of menorrhagia with a combined oestrogen-preparation or with danazol, but the first is likely to have side effects and the second is very expensive. Some types of dysmenorrhoea are associated with increased production of the spasmogenic prostaglandins, PGE_2 and $PGF_{2\alpha}$ and can be successfully treated with non-steroidal anti-inflammatory drugs (Soloff *et al*., 2000).

Prostaglandins, given by slow intravenous infusion, have been employed for the induction of labour at term, but they are not the drugs of choice since they may produce prolonged uterine contractions which can lead to foetal distress. The prostaglandins used in obstetrics are dinoprostone (PGE_2) used to terminate pregnancy in missed or partial abortion, dinoprost ($PGF_{2\alpha}$) used to induce labour and carboprost (15-methyl PGF_2). They can be administered intravenously, but this result in a high incidence of side effects. They are preferably given by extra-amniotic insertions which require special skill. The latter route is only feasible after 14-16 weeks gestation (Matsumoto *et al*., 2007).

Since they are able to activate coordinated contractions and cause the uterus to expel its contents even in the early stages of pregnancy, the prostaglandins specified above can be used in the second trimester of pregnancy for therapeutic abortion. They may also be used for evacuation of the contents of the uterus in cases of missed abortion or hydatiform mole (a type of tumour within the uterus). However, they may fail to produce complete expulsion of uterine contents and subsequent surgical evacuation may

be necessary. Gemeprost is a prostaglandin E_1 analogue given as vaginal pessary, which is used to soften the cervix and dilate the cervical os prior to vacuum aspirations of uterine contents in the termination of first-trimester pregnancy (Iwe and Akubue, 2006).

2.11 Ergot Alkaloids

Ergot alkaloids are produced by a fungus known as *Claviceps purpurea* which grows on grasses rye and other grains. Rye is the grain most easily affected by the fungus especially when grown or stored under damp conditions. Outbreaks of ergot poisoning (ergotism) due to accidental eating of bread made from ergot-infected rye occurred frequently in Europe over 2000 years. The manifestations of these epidemics were mainly prolonged vasospasm which sometimes resulted in gangrene of the extremities and convulsions. Abortions were frequent in pregnant women and it was clear that ergot contained an active principle which had powerful effects on the uterus. The severity of each effect varied among reported epidemics and may represent different mixtures of individual alkaloids in the different crops of the fungus. Ergot was first used as an uterotonic agent over 400 years ago (Iwe and Akubue, 2006).

In 1906, Barger *et al*., isolated an alkaloid from ergot which was name ergotoxine, and later found to be a mixture of three substance – ergocristine, ergocryptine and ergotamine. The first pure alkaloid was isolated by Stoll in 1920, and was named ergotamine. None of these alkaloids, however, produced on the uterus the powerful effect of the crude extracts of ergot. It was not until 1935 that, ergometrine was recognized as the oxytocic principle in ergot.

The ergot alkaloids affect α - adrenoceptors, dopamine receptors, 5-HT receptors and probably other receptors. They were the first adrenoceptor blocking agents to be discovered. The complexicity of their actions limits their therapeutic use.

The stimulation of the uterus by ergot alkaloids also appears to involve a combination of α and 5-HT receptors as is the case in the vascular smooth muscle. Ergometrine has a relatively selective action on the uterus, though this is not marked when the muscle is tested in-vitro (Ikpeze, 2009). On the post-partum human uterus in-vivo, ergometrine has a rapid stimulant effect. Its action depends partly on the state of the organ, thus on a normally contracting uterus, ergometrine has little effects, but if the uterus is quiescent, it initiates a prolonged series of strong contractions. Ergometrine has a moderate degree of vasoconstrictor action.

The mechanism of action of ergometrine on the uterus is not fully understood. It is possible that it acts on α-adrenoceptors, like ergotamine. It has also been described as a partial agonist on 5-HT receptors (Hollingsworth *et al*., 1990). The sensitivity of the uterus to ergometrine varies dramatically with the hormonal status. Like oxytocin, the uterus at term is far more sensitive to ergometrine than earlier in pregnancy. This may also be due to the fact that there is increasing dominance of receptors as pregnancy progresses (Hollingsworth *et al*., 1990).

2.12 Noradrenline

Noradrenaline stimulates the uterus in both pregnant and non pregnant women, by action on α -adrenoceptors. With intravenous infusion, there is an immediate increase in the tone, the force and the frequency of the uterine contractions. These contractions are not coordinated and thus noradrenaline is not useful as uterotonic agent (Iwe and Akubue, 2006).

2.13 Angiotensin

Several findings have led to the conclusion that the uterus is a physioIological target of angiotensin II. Angiotensin receptors have been found in high concentrations in uterine tissue of rats and rabbits. During human labour there is an increase in circulating

angiotensin II, plasma rennin contractions and rennin activity. Vasopressin receptors mediating uterine contractions have also been found on the myometrium (Pennefather and O'neil, 1992).

2.14 Tocolytic Agents

2.14.1 Atropine

Atropine is an alkaloid from *Atropa belladonna*, but also found in other plants, mainly solanaceae family. Atropine is commonly classified as an anticholinergic or antiparasympathetic drug; however, it is termed antimuscarinic agent because it antagonises the muscarinic-like action of acetylcholine and other choline esters. Atropine competes with acetylcholine and other muscarinic agonist for common binding site on the muscarinic receptor (Hardman and Limbird, 2001). Atropine is a selective antagonist of muscarinic agents at corresponding receptors for smooth muscle, cardiac muscle and exocrine gland cells. This antagonism is so selective that atropin blockade of action of non-cholinomimetic drug is taken as evidence that the drug acts indirectly through acetylcholine release or some other cholinergic mechanism (Imming *et al*, 2006). The muscarinic acetylcholine receptor mediates various cellular amplitudes including inhibition of adenylate cyclase break down of phosphoinositol and modulation of potassium channels through the action of G-proteins. Primary transducing effect is P_i turnover (Imming *et al*., 2006).

2.14.2 Salbutamol

Salbutamol is a short acting, selective β_2 – adrenergic receptor agonist used in the treatment of asthma and COPD. It is 29 times more selective for β_2 than β_1 receptor giving it higher specificity for pulmonary β_2 receptor versus β_1 adrenergic receptor located in the heart. The R- isomer has 150 times greater affinity than S- isomer and also

it has lesser toxicity (Thompson, 2004). Activation of β_2 - adrenergic receptors on airway muscle leads to the activation of adenyl cyclase and to an increase in intracellular concentration of cyclic $3^1,5^1$- adenosine monophosphate (cyclic AMP). This increase of cAMP leads to the activation of protein kinase A, which inhibits the phosphorylation of myosin and lower intracellular ionic calcium concentration resulting in relaxation (Thompson, 2004).

2.14.3 Indomethacine

Indomethacine is a synthetic indole derivative and a non-steriodal anti-inflammatory drug (NSAID) that inhibits the enzyme cyclo-oxygenase (COX) necessary for the formation of prostaglandin and other autocoids. It also inhibits the motility of polymorphonuclear leukocytes. Indomethacine is known to inhibit two well characterized isoforms of COX, COX-1 and COX-2 with greater selectivity for COX-1. COX-1 is a constitutively expressed enzyme that is involved in gastric mucosal protection, platelet and kidney function. While COX-2 is constitutively expressed and highly inducible by inflammatory stimuli. It is found in the CNS, kidneys, uterus and other organs (Hardman and Limbird, 2001). Indomethacine prevents release of lysosomal enzyme and stabilizes liver lysosomes when exposed to a labializing condition. Inhibition of calcium ion accumulation in injured tissue and calcium ion influx into stimulated smooth muscle by indomethacine have been reported (Klaasen *et al.*, 1995).

2.14.4 Calcium Antagonists

The calcium antagonists are also under test for the above purpose. Preliminary studies have found nifedipine to be a safe and effective uterine relaxant. It has been reported to

be quite successful in stopping premature labour. It acts by blocking intracellular accumulation of calcium which is necessary for contraction (Iwe and Akubue, 2006).

2.14.5 Adrenaline

Adrenaline, given systemically, inhibits the uterus in both pregnant and non pregnant women by the action on β-adrenaceptors. This effect cannot be used clinically, since after the end of an adrenaline infusion there is a rebound increase in the uterine action and tone (Iwe and Akubue, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Plant Material

Fresh *Sida corymbosa* leaves were harvested in the morning sometime in August 2013 from the premises of Technical College, Zaria. It was presented to the Herbarium in the Department of Biological Sciences, Ahmadu Bello University Zaria for identification. The plant specimen was identified by Mallam Musa Muhammed, a taxonomist in the herbarium. The plant family was identified as malvaceae, while the specie is *Sida corymbosa* with local names; "broom weed in english" and "karkashin kwado in hausa". The identification voucher number is 1049.

3.2 Animals

Adult wistar rats weighing 160-200g bred in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the experiments. Thirteen were used for LD_{50} determination, three female non pregnant and three female pregnant rats were used for uterine tissue experiments. The animals were kept and maintained under standard conditions (spacious clean cage with vent for fresh air) and were given free access to standard diet (Vital Feeds Ltd, Jos, Nigeria) and water. They were handled according to standard guidelines for use of laboratory animals (National Institute of Health, Public Health Service Policy on Humane Care and Use of Laboratory animals, 2002).

3.3 Drugs

Diethylstilboesterol, atropine and acetylcholine were obtained from Sigma (UK), salbutamol and indomethacine from Glaxo-Smithkline (England) and oxytocin from Roche (Germany).The drugs were prepared fresh on the day of the experiment by dissolving in distilled water. De-jalon's physiological salt solution (composition in 5 g L^{-}

[1]: NaCl 45.0, $NaHCO_3$ 2.5, D-Glucose 2.5, KCl 2.1 and $CaCl_2.2H_2O$ 1.32) was used in the presence of 95% oxygen to maintain tissue viability.

3.4 Equipment

Materials other than drugs that were used in this study include syringes and needles from Dana Pharmaceuticals (Nigeria), measuring pipettes, measuring cylinders, cotton wool, weighing balance, animal cages, feeders, wire loop, microdynamometre, water bath and dissecting set.

3.5 Plant Extraction

The fresh leaves were manually harvested and rendered free of contaminants by rinsing in clean water and then shade-dried for 10 days to allow all moisture to evaporate. It was taken to the Department of Pharmacognosy, Ahmadu Bello University Zaria where the plant extraction was carried out. The aqueous leaf extract was obtained by grinding the leaves and soaking in distilled water. Six hundred grams (600g) of ground dried leaves was soaked in two litres (2L) of distilled water for 36 hours under constant shaking and agitation using the GFL shaker (No. 3017 MbH, Germany) . The resultant mixture was decanted, filtered using Whatman's filter paper (No.1, Cat No. 1001 125) and concentrated under pressure in a rotary evaporator (R110 Buchi, Switzerland) at 60°C and further dried to a constant weight in a water bath set at 40°C.

3.6 Preliminary Phytochemical Screening

Phytochemical analysis of the extract was performed according to the method of Odebiyi and Sofowora (1978) and of Trease and Evans (1996). The extract was screened for carbohydrate, anthraquinones, triterpenes, sterol, cardiac glycosides, saponins, tanins, flavonoids and alkaloids. The screening was done at the Department of Pharmacognosy, Ahmadu Bello University, Zaria.

The extract was dissolved in distilled water to obtain 1mg/ml concentration and distributed into ten test tubes for the analysis of nine phytochemicals.

3.6.1 Test for carbohydrates

3.6.1.1 *Molisch test*

To 5 mls of the extract 4 mls of molisch reagent was added in a test tube. This was followed by addition of 3 mls concentrated sulphuric acid by allowing it to run gently on the side of the test tube (Evans, 1996).

3.6.1.2 *Fehlings test*

To 2mls of extract, 5mls of a mixture of fehling solution A and B in the ratio of 1:1 was added and the mixture boiled for a few minutes.

3.6.2 Test for anthracene derivatives (Bontrager's test)

To another test tube containing the extract, 5ml of chloroform was added and shaken for 5mins. It was then filtered and the filtrate shaken with equal volume of 10% ammonia solution.

3.6.3 Test for unsaturated steroid and triterpene (Liebermann-Bucchard test)

To a test tube contain 5mls of the aqueous extract of *S. corymbosa*, 5mls of acetic acid anhydrate was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer.

3.6.4 Test for unsaturated sterols (Salkowski test)

To 3 mls of the diluted aqueous extract of *S. corymbosa*. 2-3 drops of concentrated sulphuric acid was added to side of test tube.

3.6.5 Test for cardiac glycosides (Keller-kiliani test)

About 1mg of the aqueous extract of *S. corymbosa* was dissolved in 1ml of glacial acetic acid containing traces of ferric chloric solution. This was then transferred in to a dry test tube and 1ml of concentrated sulphuric acid added down the side of the test tube to form a lower layer.

3.6.6 Test for saponin (Frothing test)

About 10ml of distilled water was added to 3 mls of the aqueous extract of *S. corymbosa*. It was then shaken vigorously for about 1min. The tube was allowed to stand for about 30mins and observed for honey comb froth (Evans, 1996).

3.6.7 Test for tannins

To 5 mls of aqueous extract is added 3-5 drops of ferric chloride solution.

3.6.8 Test for flavonoids (Sodium hydroxide test)

To 5 mls of the aqueous extract a few drops of 10% sodium hydroxide was added. If a yellow coloration is noticed it indicates the presence of flavoniods.

3.6.9 Test for alkaloids (Dragendoff's test)

To 5 mls of the aqueous extract few drops of dragendoff's reagent was added. A reddish brown precipitate indicates the presence of alkaloid (Evans, 1996).

3.7 Acute Toxicity Studies (LD_{50} Determination)

Acute toxicity studies were carried out using the method described by Lorke (1983). The experiment was done in two phases. In the first phase, the rats were divided into 3 groups of 3 rats each and were treated with 10 mg/kg, 100 mg/kg and 1000 mg/kg of the

aqueous extract of *sida corymbosa* leaves orally. They were observed for signs of toxicity and death within the first 24 hours of administration.

In the second phase, 4 groups each containing one rat were treated with 140 mg/kg, 225 mg/kg, 370 mg/kg and 600 mg/kg of aqueous extract of *sida corymbosa* leaves based on the result of the first phase. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e. the geometric mean of the consecutive doses in which 0 and 100% survival rates were recorded.

3.8 Preparation of Non Pregnant Rat Uterine Tissues

An adult female Wistar rat was treated with 0.2 mg/kg diethylstilbesterol 24 hours prior to the commencement of the non-pregnant rat uterine strip experiment (Veale *et al*, 1989). Oestrous stage was confirmed by microscopic observation of its vaginal smear (fern-like patterns) and macroscopic observation of the vulva for increased secretions. The rat was then sacrificed under chloroform anaesthesia and uterine segments, about 2 cm in length, was rapidly dissected out and freed of adherent connective tissues and fat. The segment was then mounted in a 30 mL organ bath containing physiological salt solution (De Jalon's solution). The solution contains 90g Nacl, 42ml Kcl (10%), 15g D-glucose, 5g $NaHCO_3$, 2.7ml CaCl, 95% O_2 and 5% CO_2. The lower end of a segment of the uterine tissue was attached to a tissue holder by means of silk suture and the upper end to an Ugo Basile isometric force-displacement transducer connected to a Microdynamometre. The solution was maintained at 37°C and continuously aerated with 95% oxygen gas (Eferekeya and Nworgu, 1985). The preparation was equilibrated for 30 min at resting tension of 1g before the start of the experiment. During the equilibration period, the perfusion fluid was changed every 10 minute.

3.9 Preparation of Pregnant Rat Uterus

Uterus of a pregnant rat 9 - 11 days gestation, was obtained after sacrificing the animal under chloroform anaesthesia. Myometrial strips (0.5 x 2.0 cm) were dissected out after peeling off the product of conception. It was then mounted in an organ bath containing 30 mls of continuously aerated physiologic salt solution. Bath temperature was maintained at 37 ± 0.5^{0}C.The tissues were allowed to equilibrate for 45 minutes under a constant tension of 1 g. During the equilibration period, the perfusion fluid was changed every 10 minute.

3.10 Addition of Drugs

Response of the uterine tissue to *S. corymbosa* aqueous leaf extract, standard uterotonics (acetylcholine and oxytocin) and known uterine muscle relaxants (salbutamol, atropine and indomethacine) were recorded on a microdynamometre assembly with the help of an isotonic frontal writing lever.

S.corymbosa extract, acetylcholine and oxytocin were allowed a 1minute contact period with the tissue till the maximum effect of a particular concentration is observed. A minimum wash-out frequency of 3 was used between two successive recordings. Drug interaction study was done by instilling the extract and each drug simultaneously and washing at least 3 times before the next test. Interactions were also done between the extract and acetylcholine, oxytocin, salbutamol, atropine and indomethacine. After equilibration for 45 minutes, the baseline response and frequency was recorded in the first 10 min (Perusquia and Navarette, 2005). This was followed by subsequent 1 min exposure of the tissue to 0.67 mg mL^{-1}, 1.33 mg mL^{-1}, 2.67 mg mL^{-1} and 5.33 mg mL^{-1} final organ bath concentrations. This was also done for acetylcholine (0.0167, 0.033, 0.067 and 0.133 ug mL^{-1}) and oxytocin in increasing concentration of $6.67x10^{-5}$, 1.33×10^{-4}, 2.67×10^{-4} and $5.33x10^{-4}$ IU/ml of organ bath concentrations. The selection of the

concentration of drugs that were used for the experiment were derived from literature while the concentration of the extract that was used was from preliminary experimentations.

3.11 Drug Interaction Studies

Interaction was done over a range of concentrations of the extract (0.667 mg/ml, 1.33 mg/ml, 2.67 mg/ml and 5.333 mg/ml) in the presence of low concentration of acetylcholine 0.033 µg/ml, oxytocin (1.33×10^{-4} IU/ml), salbutamol (1.0×10^{-4} mg/ml), atropine (3.33×10^{-6} mg/ml) and indomethacine (8.33×10^{-4} mg/ml) instilled simultaneously.

3.12 Data Analysis

Data was collected and presented in the form of tables, charts and graphs and interpreted accordingly. All values collated are to be expressed as Mean for the various responses recorded from the activities of the extract and other test drugs on both the pregnant and non pregnant uterine strips. Interactions are to be done between the extract and each test drug on both pregnant and non pregnant uterine strips. EC_{50} and E_{max} are to be determined using Log concentration-response curves of the drugs to be used in the experiments. Comparisons are to be done using one-way and two-way ANOVA with Bonferroni test for multiple comparisons of the dependent variable which in this case will be the amplitude of response. P value of <0.05 will indicate statistical significance in all cases.

CHAPTER FOUR

4.0 RESULTS

4.1 Plant yield

The plant extraction yielded 36.4% w/v of dark brown, sweet smelling, soft and smooth textured product.

4.2 Results of Phytochemical Screening

Table 4.1 Phytochemical Constituents of the Aqueous Leaf Extract of *Sida corymbosa*

CONSTITUENT	OBSERVATION
Carbohydrate	Present
Anthraquinones	Present
Triterpenes	Present
Sterol	Present
Cardiac glycoside	Present
Saponin	Present
Tannins	Present
Flavonoids	Present
Alkaloids	Present

4.3 Acute Toxicity Testing of the Aqueous Leaf Extract of *Sida corymbosa*

In the first phase, the result of zero percent lethality was found to be at 100 mg/kg and above while that of 100%lethality was found to be 1000 mg/kg. Then the second phase was carried out using the probit scale (Rosiello*et al*., 1977). The extract was given orally at doses of 140 mg/kg, 225 mg/kg, 370 mg/kg, and 600 mg/kg. It was found that zero lethality was recorded at a maximum dose of 370 mg/kg and 100% lethality at a minimum dose of 600 mg/kg. LD_{50} = 471.17 mg / kg

4.4 Results of Uterine tissue Experiment

4.4.1 Addition of single agent on pretreated non-pregnant rat uterine strip

When varying final bath concentrations of *S. corymbosa* aqueous leaf extract (0.67 mg/ml, 1.33 mg/ml, 2.67 mg/ml and 5.33 mg/ml) were used they elicited responses of 49 mm, 48 mm 50 mm and 50 mm respectively as shown in figure 4.1 below. When acetylcholine (0.0167 µg/ml, 0.033 µg/ml, 0.067 µg/ml and 0.133 µg/ml) final organ bath concentrations were used they elicited responses of 52 mm, 57 mm, 59 mm and 69 mm respectively. These responses superseded those elicited by the *S. corymbosa* extract on the non pregnant uterine tissue. However, when varying final bath concentrations of oxytocin (6.67×10^{-5} IU/ml, 1.33×10^{-4} IU/ml, 2.67×10^{-4} IU/ml and 5.33×10^{-4} IU/ml) were used, they all elicited uterine strip contractions amplitudes of 59 mm, 68 mm, 69 mm and 69 mm respectively which supersedes the amplitudes of both acetylcholine and the extract on the non pregnant uterine strip as shown in figure 4.1. Oxytocin elicited the highest response at low final organ bath concentration of 1.33×10^{-4} IU/ml followed by that elicited by acetylcholine before the extract. Overall, the highest final bath concentration of the extract (5.33 mg/ml) elicited response that was less than the suboptimal concentrations of both acetylcholine and oxytocin on the pretreated non pregnant rat uterine strip.

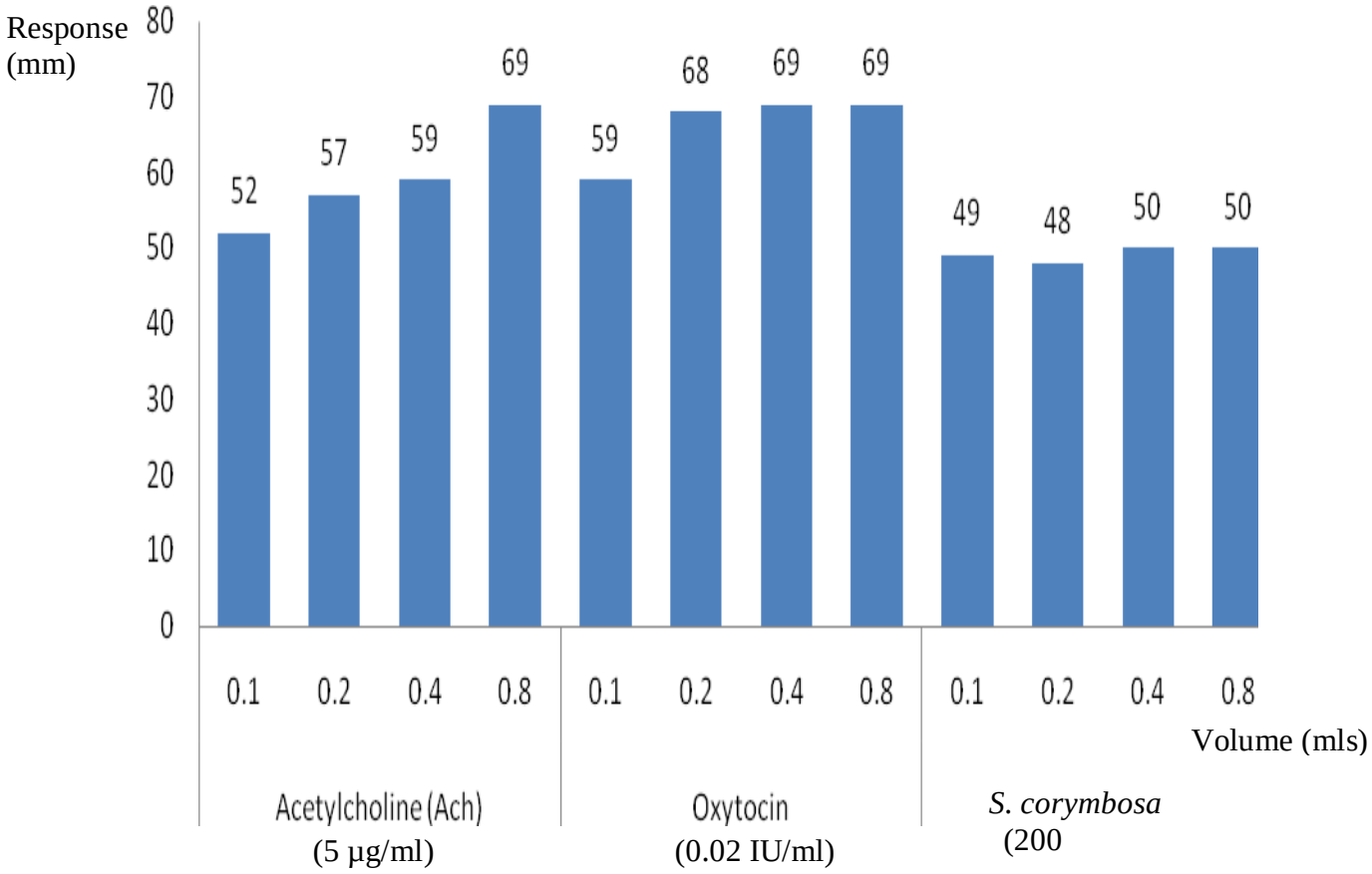


Figure 4.1: Comparison of Various Effects Elicited by Varying Concentrations of Acetylcholine, Oxytocin and *S. corymbosa* Aqueous Leaf Extract on Non Pregnant Uterus

4.4.2 Interactions of *S. corymbosa*, acetylcholine and oxytocin on pretreated non pregnant uterine strip

The effect of the extract and its interactions with acetylchyoline and oxytocin on pretreated non pregnant rat uterine strip shows increase in the uterotonic activity of the extract as noted in figure 4.2 below. When various concentrations of the extract (0.67, 1.33, 2.67 and 5.33 mg/ml) were combined with single low concentrations of actylcholine (0.033µg/ml) and oxytocin (1.33×10^{-4} IU/ml), it was observed that the Amplitude of each concentration of the extract used was increased for interactions with both oxytocin and acetylcholine. However, the increased effect of oxytocin supersedes that of acetylcholine. It was observed that when the highest concentration of the extract was interacted with oxytocin tachypylaxis occurred (figure 4.2).

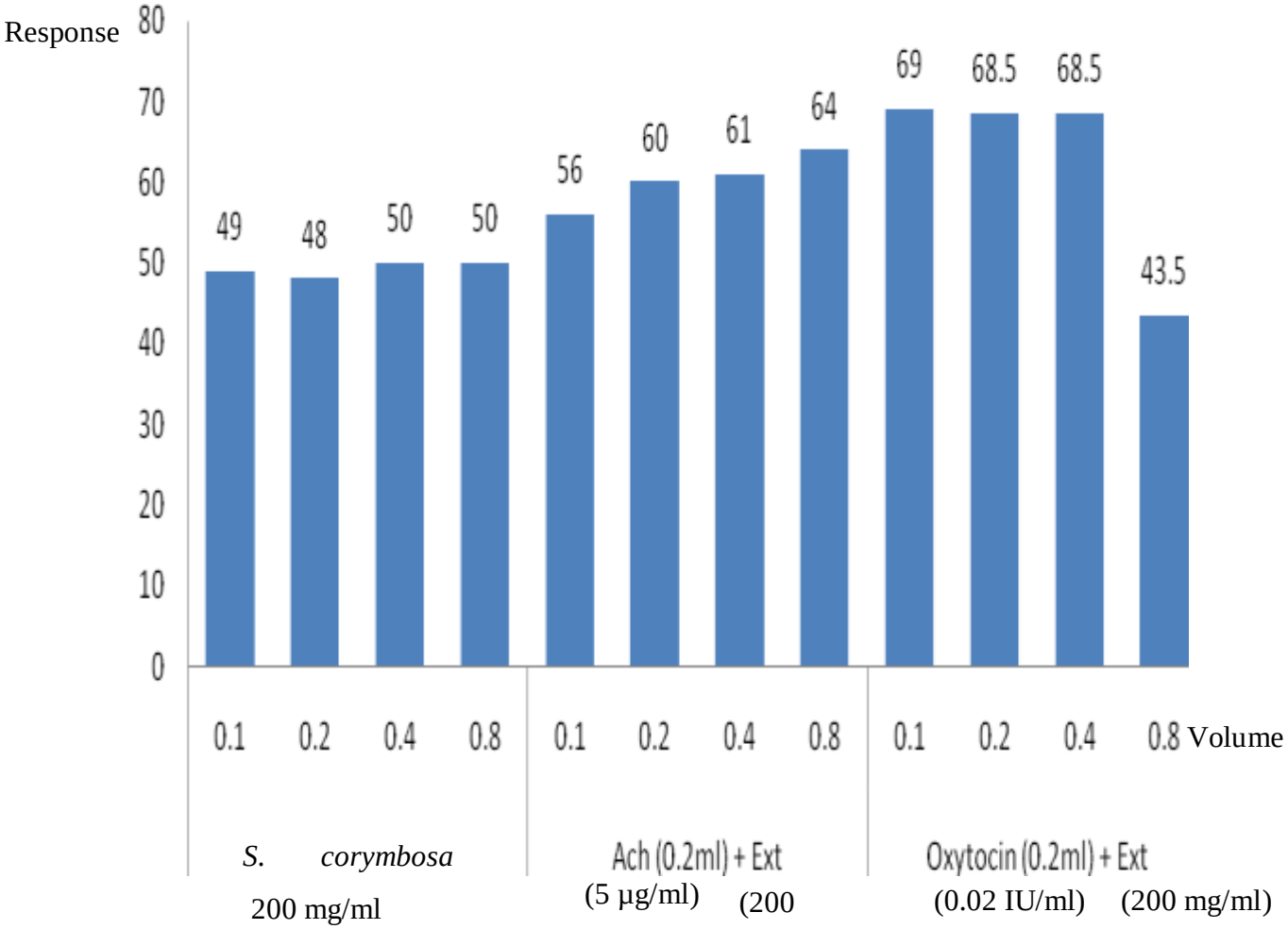


Figure 4.2: Comparison of Various Effects Elicited by Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and the Interaction Studies with Single Low Concentrations of Acetylcholine and Oxytocin on Non Pregnant Uterus

4.4.3 Interactions of *S. corymbosa*, salbutamol, atropine and indomethacine on pretreated non pregnant uterine strip

S.corymbosa aqueous leaf extract and its interactions with known tocolytics like salbutamol, atropine and indomethacine, showed inhibition of the activity of the extract by the drugs, more at lower concentration than at higher concentrations of the extract. Single low concentrations of the antagonist were used in this study; salbutamol (1.0 x 10^{-4} mg/ml, atropine 3.33 x 10^{-6} mg/ml and indomethacine 8.33 x 10^{-4} mg/ml). Salbutamol had the greatest inhibitory effect; it inhibited all concentrations of the extract except (5.33 mg/ml) which was the highest final organ bath concentration of the extract that was used in this study, as seen in figure 4.3. Atropine also inhibited the activity of the extract completely when (0.67 mg/ml and 1.33 mg/ml) were used. However, (2.67 mg/ml and 5.33 mg/ml) elicited a 12 mm and 11 mm response respectively. While indomethacine completely inhibited the activity of extract at (0.67 mg/ml and 1.33 mg/ml) final organ bath concentrations but the extract elicited a 22 mm and 18 mm response at (2.67 mg/ml and 5.33 mg/ml) final organ bath concentrations respectively. Which indicate apparent inhibition of the activity of the extract when interactions were done with salbutamol, atropine and indomethacine in decreasing order.

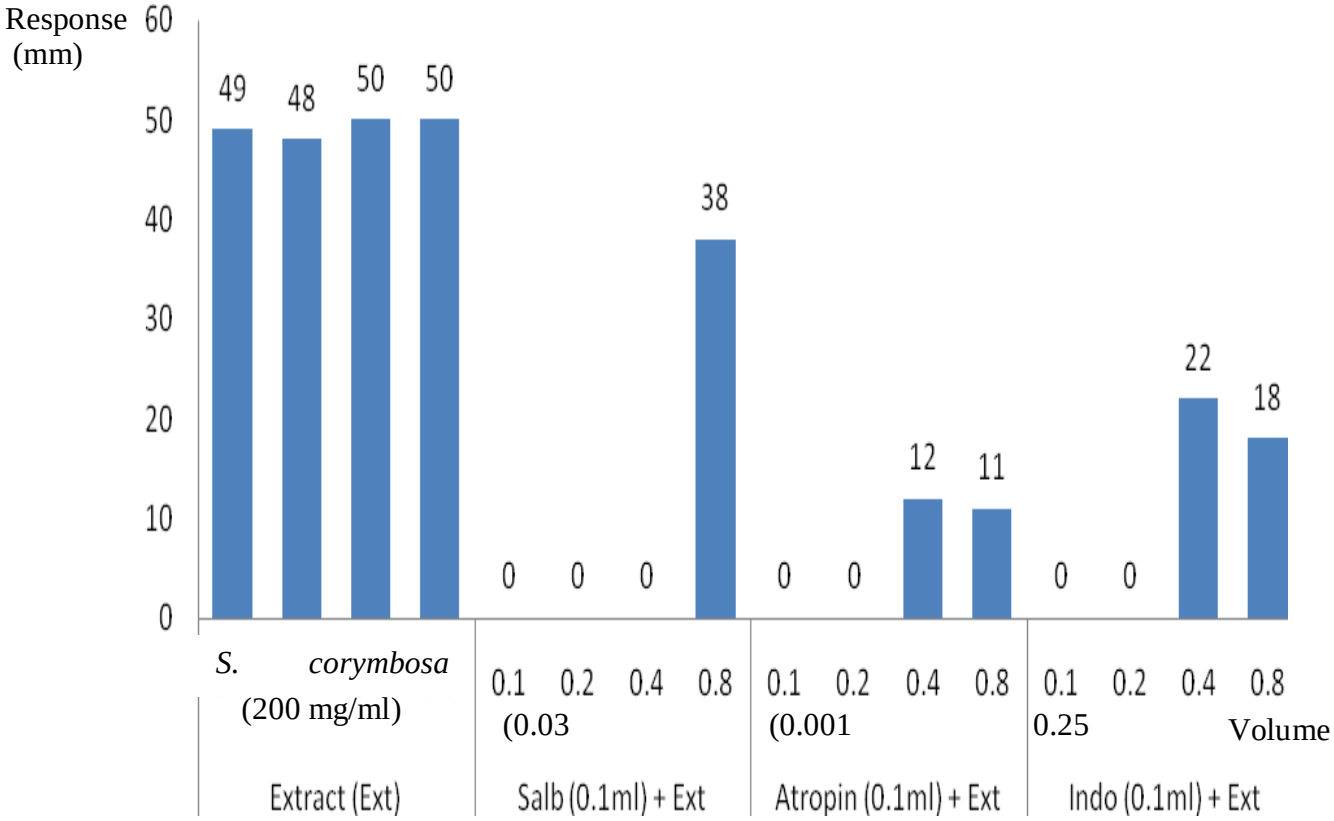


Figure 4.3: Comparison of Various Effects Elicited by Varying Concentrations of *S. corymbosa* Aqueous Extract and Interaction studies between the Extract and Single Low Concentrations of Salbutamol, Atropine and Indomethacine on Non Pregnant Uterus

4.4.4 The log Concentration - response curve of S. corymbosa aqueous extract on pretreated non pregnant uterus

The log Concentration - response curve of *S. corymbosa* aqueous extract on pretreated non pregnant uterine strip was done for the varying concentrations of the extract used in this study, for 0.67 mg/ml, 1.33 mg/ml, 2.67 mg/ml and 5.33 mg/ml. An effective concentration of (EC_{50}) of 2.09 mg/ml and maximum effect (Emax) of 5.25 mg/ml were observed after extrapolating from the tangent seen in figure 4.4 and calculating its antilog.

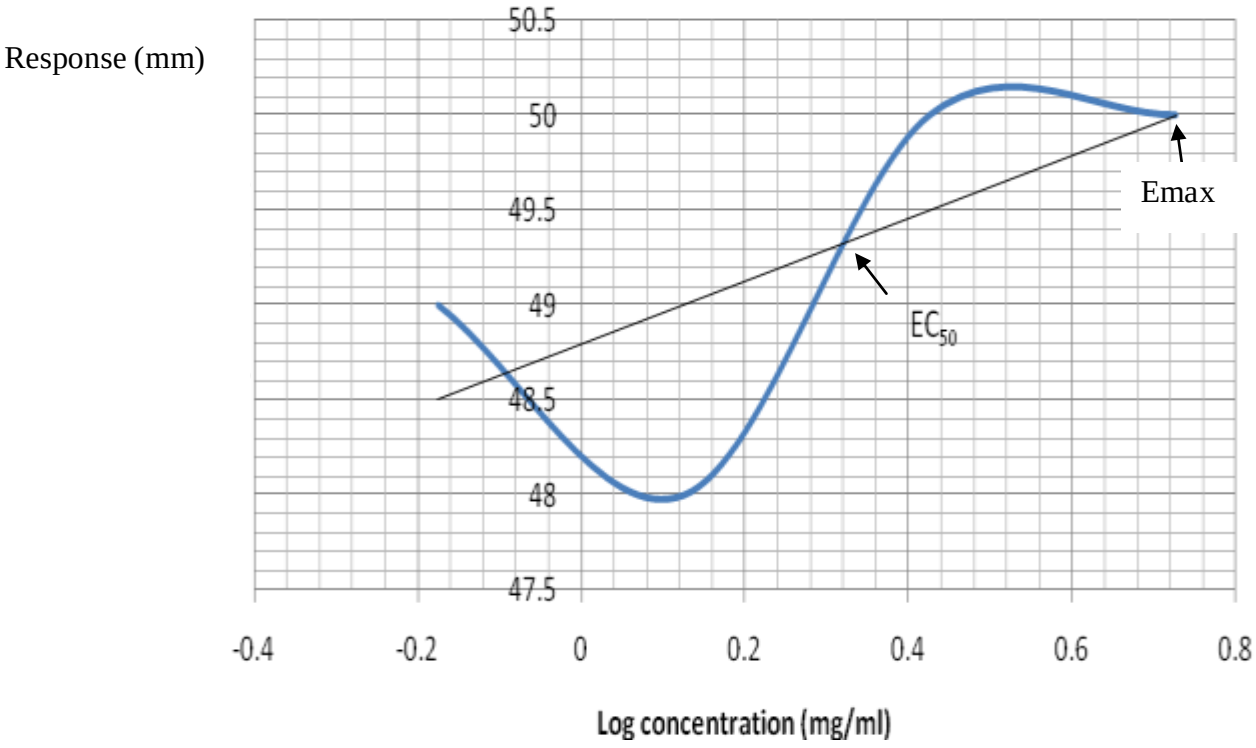


Figure 4.4: Log concentration - Response Curve of the *S. corymbosa* Aqueous Leaf Extract on Pretreated Non Pregnant Rat Uterine Strip

Effective concentration (EC_{50}) and maximum effective concentration (Emax) of *S. corymbosa* aqueous leaf extract and that of its interactions with acetylcholine, oxytocin, atropine, salbutamol and indomethacine on pretreated non pregnant uterus was extrapolated from log concentration - response curves of the agents which was plotted within the range of test concentrations used for this experiment. They were deduced as antilogs of fifty percent and hundred percent marks of the tangent line that crosses the curve. All values represent the final organ bath concentration.

Table 4.2: EC_{50} and Emax of *S. corymbosa* Aqueous Leaf Extract and that of Interaction Studies between the Extract and other Agonist on Pretreated Non Pregnant Uterine Tissue

AGENT	Log Conc (50%)	Log Conc (100%)	EC_{50} [FBC (mg/ml)]	E_{max} [FBC (mg/ml)]
Extract	0.32	0.72	2.09	5.25
Extract + Ach	0.32	0.72	2.09	5.25
Extract + Oxy	0.04	0.6	1.096	3.98
Extract + Salb	0.62	-	4.168	-
Extract + Atrp	0.28	0.66	1.905	4.57
Extract + Indo	0.26	0.66	1.819	4.57

4.5 Result of Effects of the Extract and other Drugs on Pregnant Uterine Strip

4.5.1 Addition of single agent on pregnant rat uterine strip

When varying final bath concentrations of *S. corymbosa* aqueous leaf extract (0.67 mg/ml, 1.33 mg/ml, 2.67 mg/ml and 5.33 mg/ml), acetylcholine (0.0167 µg/ml, 0.033 µg/ml, 0.067 µg/ml and 0.133 µg/ml) and oxytocin (6.67×10^{-5} IU/ml, 1.33×10^{-4} IU/ml, 2.67×10^{-4} IU/ml and 5.33×10^{-4} IU/ml) were added to the water bath containing the pregnant uterine strip. The *S. corymbosa* extract elicited responses with response amplitude of 88 mm, 89 mm, 57 mm and 89 mm respectively. Acetylcholine elicited responses with amplitudes of 29 mm, 28 mm, 47 mm and 78 mm while oxytocin elicited responses with amplitude of 68 mm, 71 mm, 78 mm and 62 mm respectively on pregnant rat uterine strip. It is apparent that the extract elicited the highest set of responses followed closely by oxytocin before acetylcholine (figure 4.5).

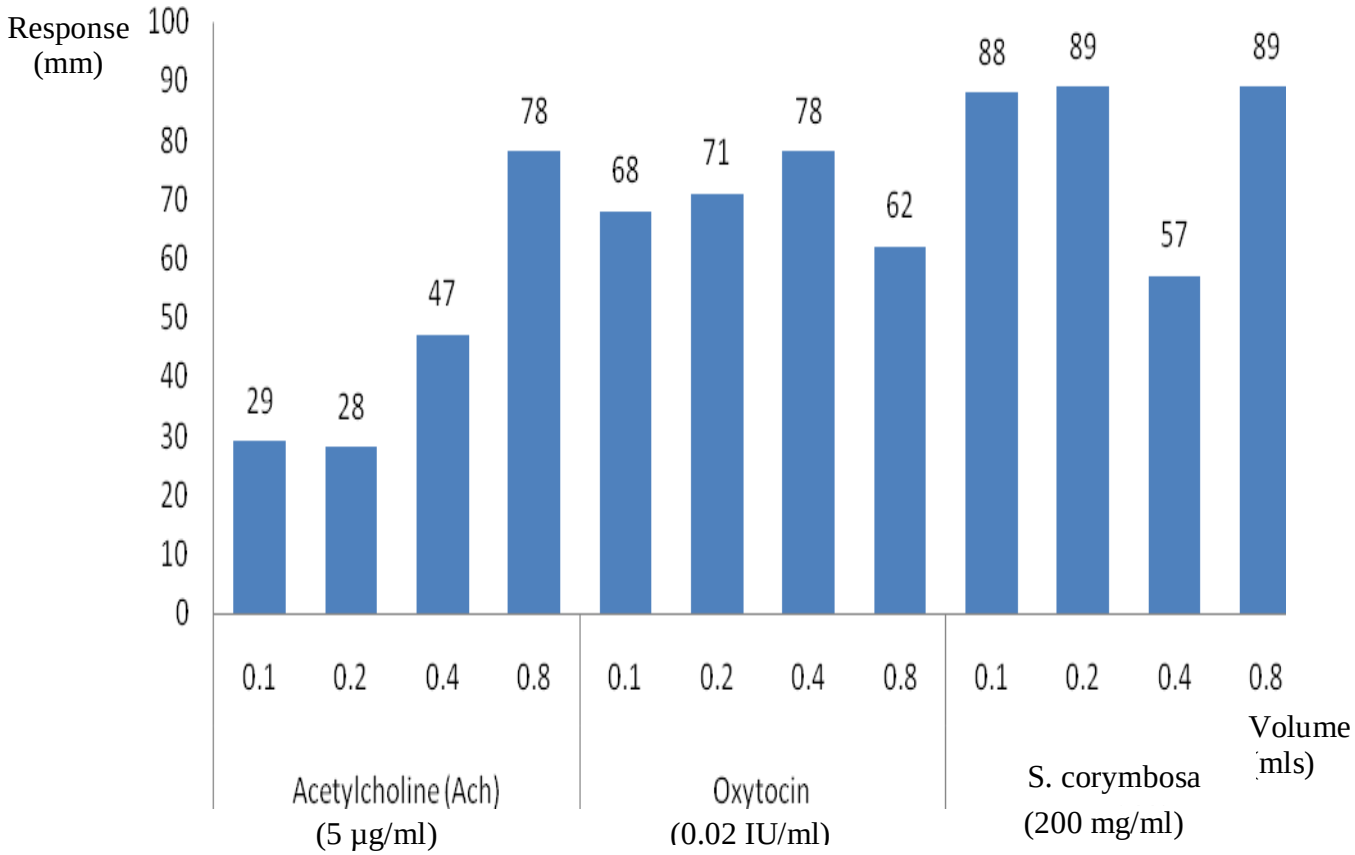


Figure 4.5: Comparison of Various Effects Elicited by Varying Concentrations of Acetylcholine, Oxytocin and *S. corymbosa* Aqueous Leaf Extract on Pregnant Uterine Strip

4.5.2 Interactions between varying concentrations of *S. corymbosa* and single low concentrations of acetylcholine and oxytocin on pregnant uterine strip

The effect of *S. corymbosa* aqueous leaf extract and its interactions with acetylchyoline and oxytocin on pregnant rat uterine strip was carried out using different concentration of the extract (0.67 mg/ml, 1.33 mg/ml, 2.67 mg/ml and 5.33 mg/ml) and single low concentration of acetylcholine (0.033 µg/ml) and oxytocin (1.33×10^{-4} IU/ml) as shown in figure 4.6. Augmentation of the extracts activity was noticed, especially when the interaction was done with oxytocin. Acetylcholine was obviously augmented by the activity of the extract as shown in figure 4.6.

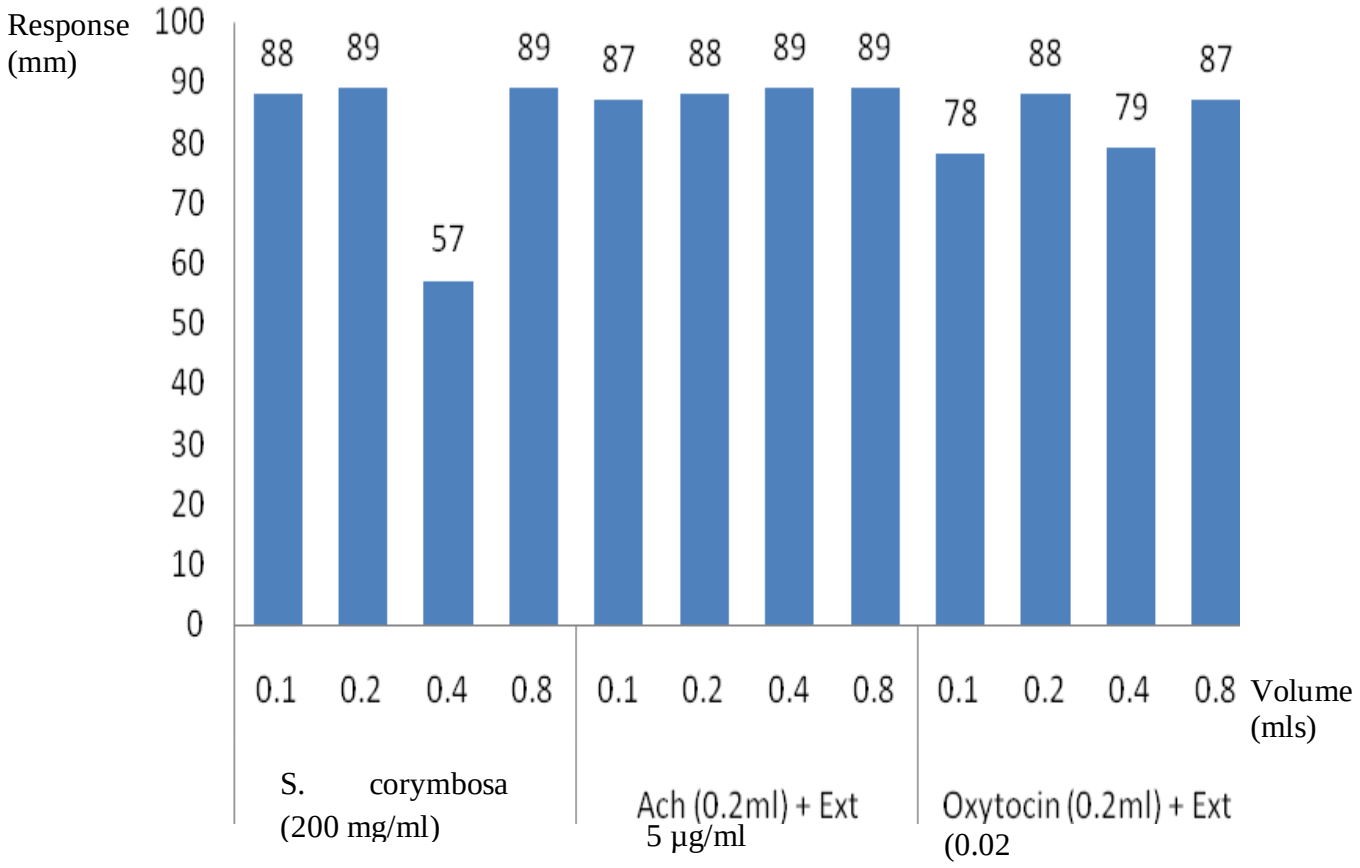


Figure 4.6: Comparison of Various Effects Elicited by Various Concentrations of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Single Low Concentrations of Acetylcholine and Oxytocin on Pregnant Uterus

4.5.3 Interactions between varying concentrations of *S. corymbosa* and single low concentration of salbutamol, atropine and indomethacine on pregnant uterine strip

The effect of different final organ bath concentrations (0.67 mg/ml, 1.33 mg/ml, 2.67 mg/ml and 5.33 mg/ml) of *S. corybosa* aqueous leaf extract was used for this interaction studies. When the interaction was done with single low concentration of salbutamol (1.0 x 10^{-4} mg/ml) there was reduction of the Amplitude of activities of the extract from 88 mm, 89 mm, 57 mm and 89 mm to 22 mm, 14 mm, 20 mm and 59 mm respectively. Also, when interaction was done with atropine (3.33 x 10^{-6} mg/ml), it was observed that reduction in activity of the extract was only achieved when atropine was combined with 5.33 mg/ml of the extract but atropine did not affect the activity of other concentration of the extract. Interaction was also done with Indomethacine (8.33 x 10^{-4} mg/ml), it was observed that indomethacine reduced the activity of the extract from 88 mm, 89 mm, 57 mm and 89 mm to 0 mm, 0 mm, 0 mm and 5 mm as shown in figure 4.6.

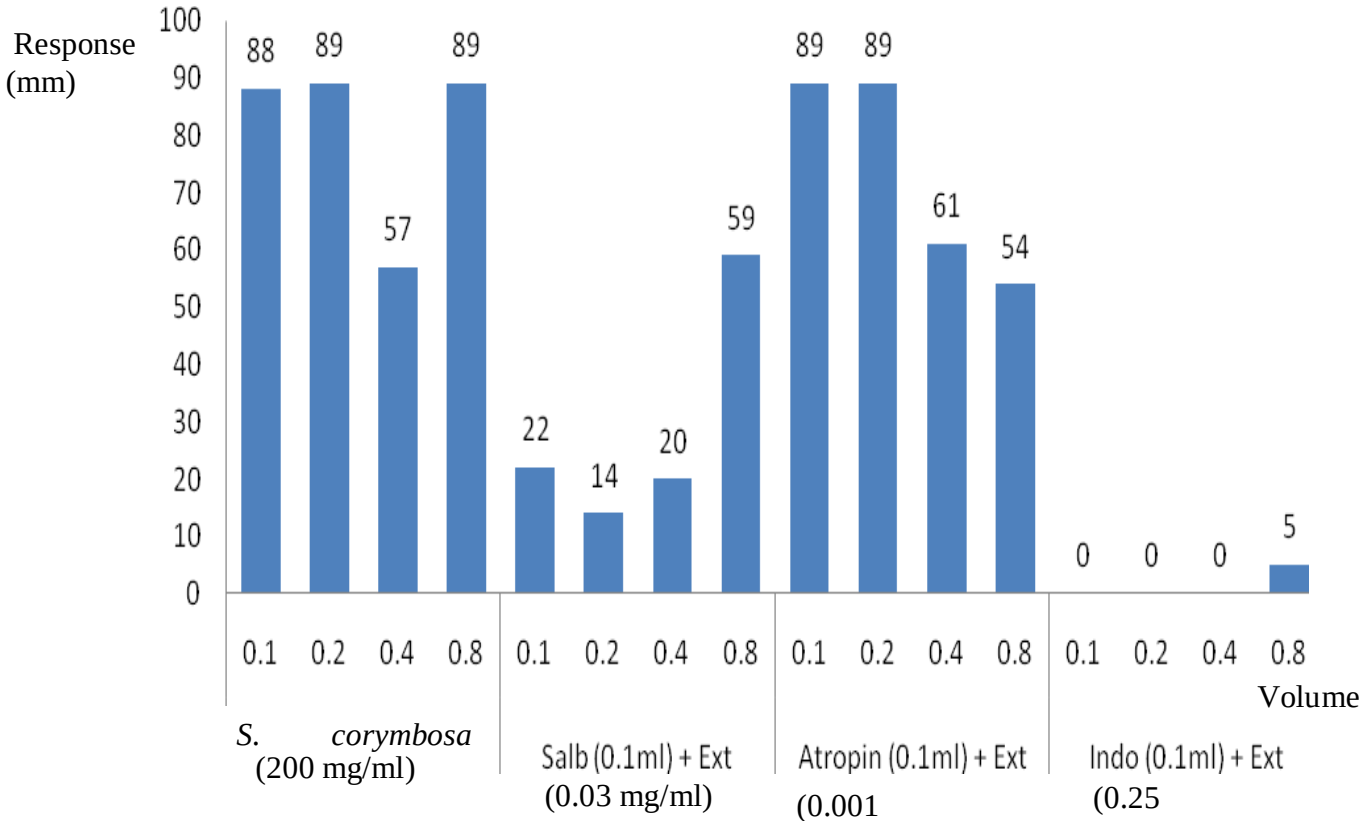


Figure 4.7: Comparison of Various Effects Elicited by Various Concentrations of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Single Low Concentrations of Salbutamol, Atropine and Indomethacine on Pregnant Uterus

4.5.4 The log Concentration – response curve of S. corymbosa aqueous leaf extract on pregnant uterus

The log Concentration – response curve of *S. corymbosa* aqueous leaf extract on pregnant uterus shown in figure 4.7 below was plotted for the range of concentrations that were used for the experiment. The effective concentration (EC_{50}) of 1.58 mg/ml and maximum effect (Emax) of 4.37 mg/ml were determined from the antilog of the logs of the points on the log concentration line were the tangent crosses fifty percent and hundred percent of the curve.

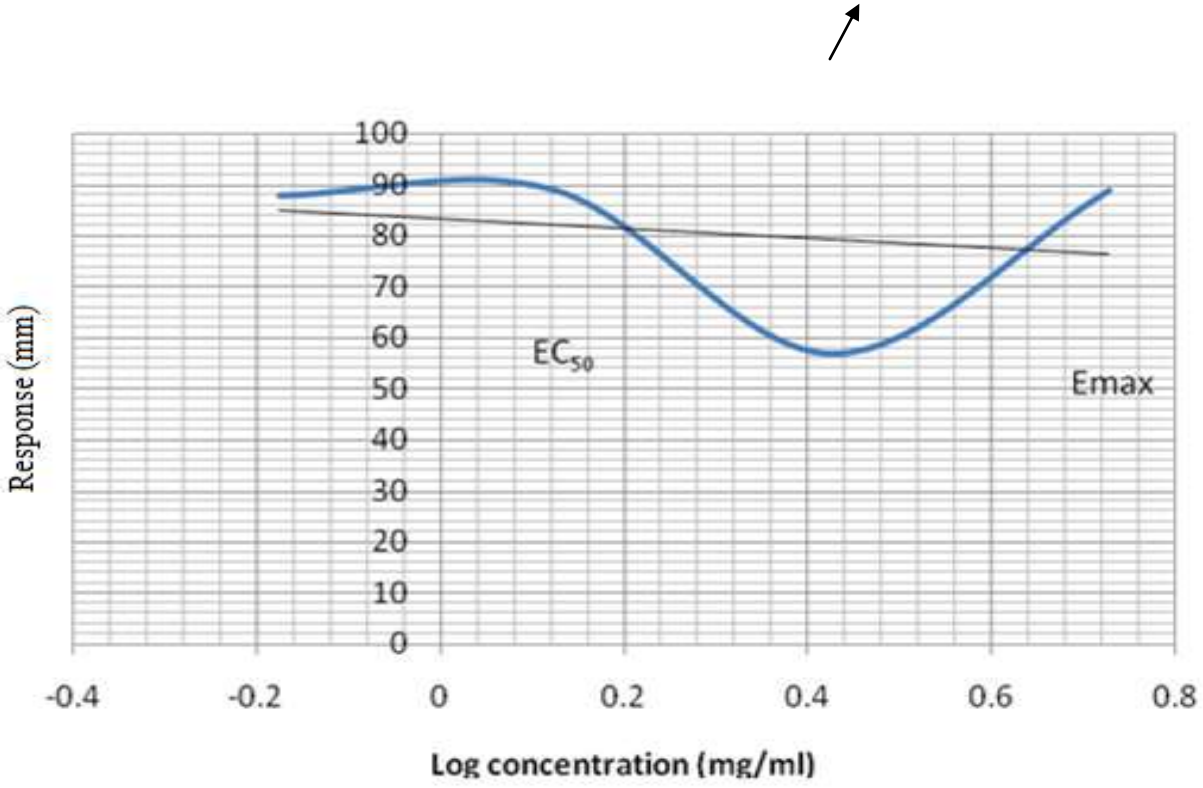


Figure 4.8: Log concentration - Response Curve of the *S. corymbosa* Aqueous Leaf Extract on Pregnant Rat Uterus

The effective concentration (EC_{50}) and maximum effective concentration (Emax) of *S. corymbosa* aqueous leaf extract and that of its interactions with actylcholine, oxytocin, salbutamol, atropine and indomethacine on pregnant uterus were extrapolated from the log concentration – response curve where the tangent crosses fifty percent and hundred percent of the curve. The antilogs of the corresponding logs on the log concentration line were determined and they are the final organ bath concentration EC_{50} and Emax (table 4.3).

Table 4.3: EC_{50} and Emax of the *S. corymbosa* Aqueous Leaf Extract and that of Interaction studies between the Extract and other Agonist on Pregnant Uterus

AGENT	Log Conc at 50%	Log Conc at 100%	EC_{50} [FBC (mg/ml)]	E_{max} [FBC (mg/ml)]
Extract	0.20	0.64	1.58	4.37
Extract + Ach	0.02	0.6	1.05	3.98
Extract + Oxy	0.28	0.68	1.91	4.79
Extract + Salb	0.6	_	3.98	_
Extract + Atrp	0.3	0.72	1.2	5.25
Extract + Indo	0.64	_	4.37	_

4.6 Results of Interaction Study

4.6.1 On non pregnant uterine strip (Synergistic and Antagonistic Study)

The effect of *S. corymbosa* aqueous leaf extract was noticed to increased response when the interactions of the extract with acetylcholine and oxytocin were done. It was observed that the interactions of the extract with acetylcholine and oxytocin elicited higher responses and the curve of the interactions lie above and to the left of the line of the extract alone on the log concentration - response curve indicating synergy of the activity of the extract by oxytocin and acetylcholine (figure 4.9).

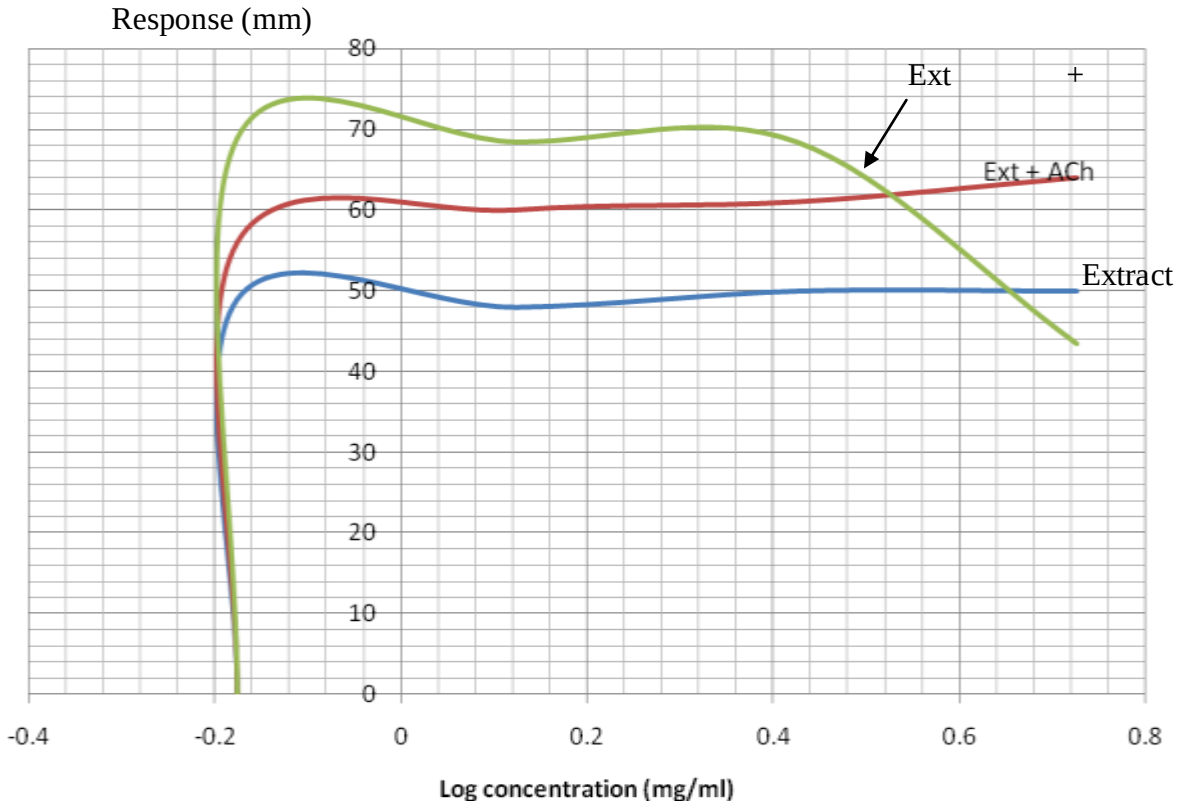


Figure 4.9: Log concentration – Response Curve of the Effect of *S. corymbosa* Aqueous Leaf Extract by Acetylcholine and Oxytocin on Non Pregnant Uterus

The interaction of *S. corymbosa* aqueous leaf extract with salbutamol, atropine and indomethacine on pretreated non pregnant uterus showed a decrease in the activity of the extract. It can be observed that the curves of the interactions of the extract with salbutamol, atropine and indomethacine lie below and to the right of the curve of the extract alone suggesting antagonism (figure 4.10).

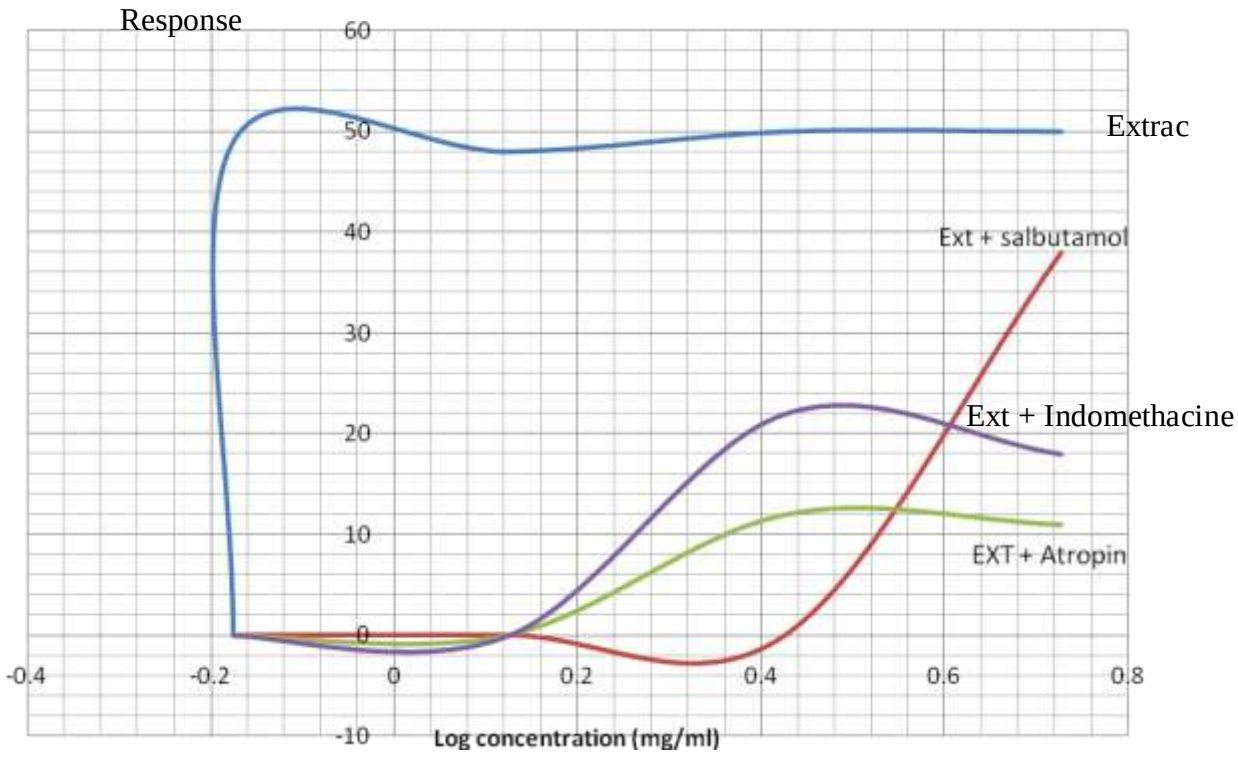


Figure 4.10: Log concentration – Response Curve of *S. corymbosa* Aqueous Leaf Extract and that of the Extracts Interactions with Salbutamol, Atropine and Indomethacine on Pretreated Non Pregnant Uterus

4.6.2 Interaction studies on pregnant uterine strip

The effect of *S. corymbosa* aqueous leaf extract on pregnant uterus was observed to be high and the interaction of the extract with acetylcholine and oxytocin showed augmentation of the activity of the extract only at high concentration (2.67 and 5.33 mg/ml) of the extract because the activity of the extract alone was observed to be higher than that of interactions with acetylcholine and oxytocin at lower concentrations (0.67 and 1.33 mg/ml) of the extract. However, at higher concentrations of the extract, it was observed that the interactions with oxytocin and acetylcholine augmented the activity of the extract as shown in figure 4.11. It can be observed that the interactions that at high concentrations, curves of the interactions with acetylcholine and oxytocin lie above the curve of the extract alone on the graph, indicating potentiation of the activity of the extract at high concentration as shown in figure 4.11.

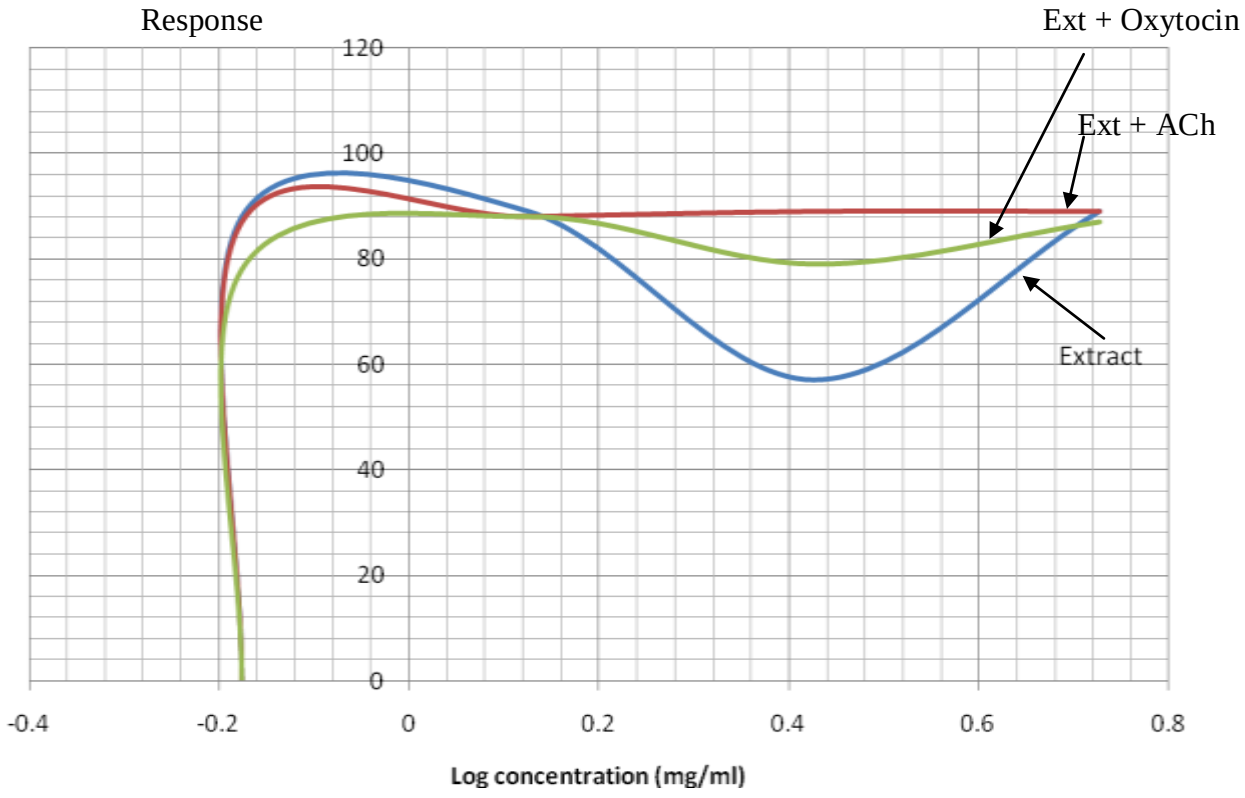


Figure 4.11: Log Concentration – Response Curve of the Effect of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Acetylcholine and Oxytocin on Pregnant Uterus

The *S. corymbosa* aqueous leaf extract alone elicited a high response on the pregnant uterus but when interactions were done with salbutamol and indomethacine, it was observed that both drugs inhibited the activity of the extract. Curves of interactions with both salbutamol and indomethacine lie below and to the left side of the graph indicating antagonism of the drugs to the extracts activity as shown in figure 4.12 below. However, the activity of the extract was not observed to be inhibited by atropine, in fact the curve of the interaction with the extract and atropine lie very close to the curve of the extract alone indicating no differences in the activity of the extract alone from the interaction of the extract and atropine as shown in figure 4.12.

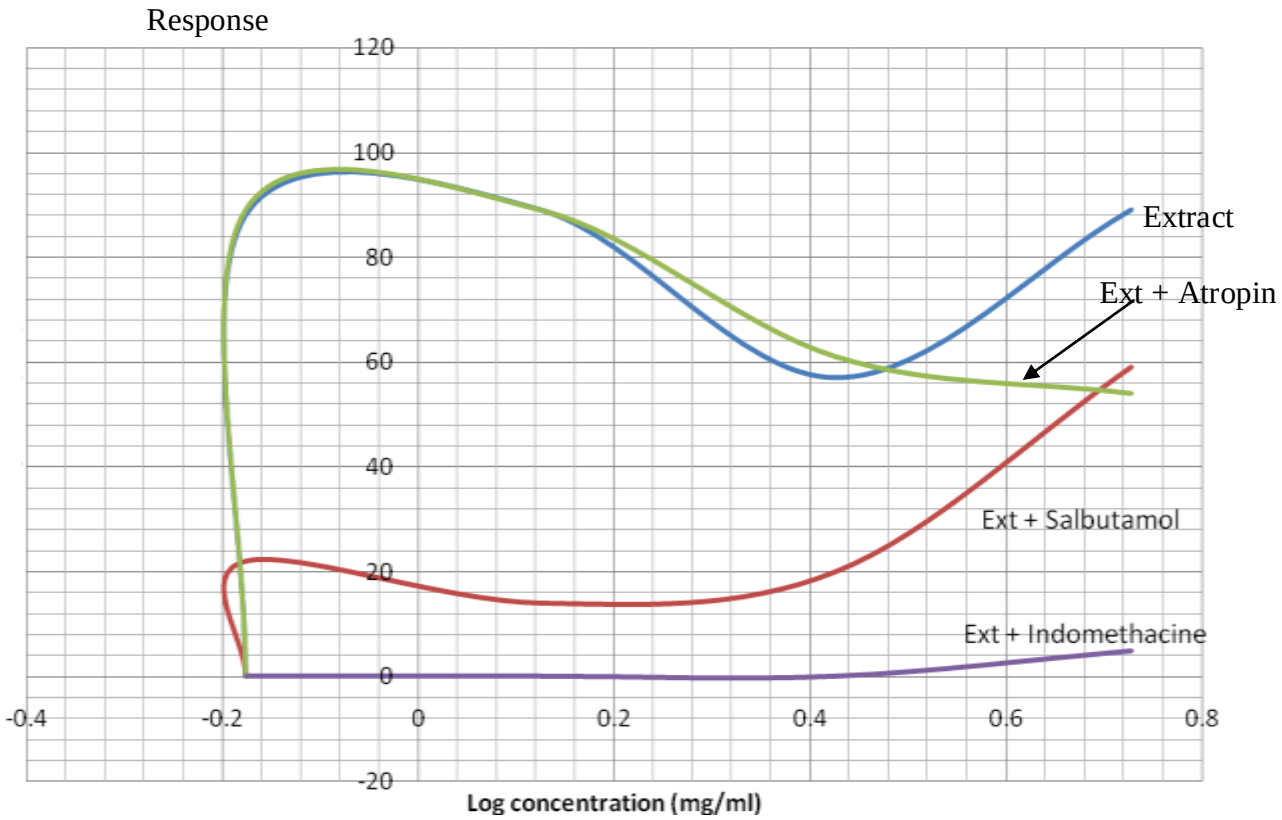


Figure 4.12: Log Concentration – Response Curve of the Effect of *S. corymbosa* Aqueous Leaf Extract and its Interactions with Salbutamol, Atropine and Indomethacine on Pregnant Uterus

CHAPTER FIVE

5.0 DISCUSSION

The acute toxicity screening of a substance is an important toxicological investigation for an unknown substance. The LD_{50} which represents the individual dose required to kill fifty percent of a population of test animals is a very useful index for grading the acute toxicity of a substance. This should not be viewed as an absolute value or as being equal to complete investigation of acute toxicity of a substance. A scale proposed by lorke (1983) roughly classifies a substance as being slightly toxic if its LD_{50} falls between 100 to 1000 mg/kg. Based on the lorke proposed scale and the value obtained from acute toxicity test of the aqueous leaf extract of *Sida corymbosa,* which was 471.17 mg/kg, it can be said that the aqueous leaf extract of *Sida corymbosa* is slightly toxic or can cause harm if consumed. This is important because the plant is currently being ingested and used among human population for various ethnomedical reasons.

From the result of the experiment carried out above, it was noticed that the mean response amplitudes elicited by the drugs used in the experiment was 58.813 mm for the pregnant uterus strip while the pretreated isolated non pregnant uterus strip had a mean activity of 40.328 mm with a mean difference of 18.5 mm (31.5%). The mean response amplitude was greater on the pregnant tissue probably due to the natural increase in the expression of receptors with increased gestational age of pregnancy (Batra, 1986) and

that naturally causes increase in the sensitivity of the pregnant tissue to drugs. Statistically, independent sample test and ANOVA revealed a p-value of 0.015 which is less than α value 0.05 at 95% confidence interval supporting the fact that there was difference in the activity of the two uterine tissues, with the pregnant being more responsive to the test drugs than the non pregnant uterine tissue.

It was also noticed that the *Sida corymbosa* aqueous leaf extract elicited responses on both pregnant and non-pregnant tissues with the EC_{50} of the extract on pregnant tissue being 1.58 mg/ml of final organ bath concentration, while that of the pretreated non pregnant uterine tissue was higher (2.09 mg/ml) of final organ bath concentration. It means that a lower concentration was required to elicit 50% of response in the pregnant uterine tissue than the non pregnant uterine tissue buttressing the fact that the pregnant tissue was more sensitive even to the extract.

The extract alone on the pregnant uterine tissue was noticed to have elicited amplitudes at different concentrations that were higher than the amplitudes of the standard uterotonic drugs used in this experiment. It was noticed to have 35.25mm mean difference from the activities elicited by acetylcholine and 11.0mm mean difference from the activities elicited by oxytocin. The non-pregnant uterine tissue, activities elicited by the extract alone were lower than those elicited by oxytocin and acetylcholine. The mean amplitudes of response elicited by acetylcholine was 10.00 mm higher than that of the extract while that of oxytocin was 17.00 mm higher than the extract alone. It means that the receptor which the extract utilizes for its activity is more expressed in pregnancy like oxytocin and acetylcholine receptors or it may be using multiple receptor types due to

the presence of a possible large variety of constituents in the crude extract of *S. corymbosa*.

However, when drug interactions were done between the extract and standard uterotonics (oxytocin and acetylcholine), potentiation of the activity of the extract by oxytocin and acetylcholine on both pregnant and pretreated non pregnant uterine tissue was observed. The potentiating effects were confirmed on the Amplitude versus log concentration graph. When the line on the graphs of interaction falls on the Left side or above the line of activity of the extract alone, it is indicative of potentiation (Rang *et al*, 2003). Also, their combined mean difference of activities was higher than that of the extract alone on both tissues. In both the pregnant and non-pregnant tissue experiments, the interactions with oxytocin and acetylcholine were observed to lie on the left and above the curve of the extract alone.

The synergistic effect of oxytocin and acetylcholine were apparently greater on the pretreated non pregnant uterine tissue than that of the synergistic effect of the same drugs on the pregnant uterine tissue. The afore mentioned findings had p-values greater than α value of 0.05 at 95 % confidence interval indicating that all the drugs had similar synergistic effect on the extract and on both pregnant and non pregnant tissue.

However, when interactions were done with standard tocolytics (salbutamol, atropine and indomethacine), it was observed that on both the pregnant and non pregnant uterine tissues the drugs decreased the amplitudes of response elicited by the extract, especially when compared to the activity of the extract alone indicating some antagonism.

Salbutamol, a β-agonist was combined with the extract and it was observed that the activity of the extract reduced significantly on the non pregnant tissue. It had a mean 39.80 mm lower than the extract alone. While, on pregnant uterine tissue the mean was

52.0 mm lower than that of the extract alone. When subjected to a multiple comparison test (Bonferroni test), the interaction of the extract and salbutamol was observed to have p – value of 0.0001 and 0.001 at 95% confidence interval on both non pregnant and pregnant tissues respectively signifying a statistically significant difference in the response of the extract alone versus response of the (extract + salbutamol) combination, confirming antagonism. This antagonism could be adduced to the fact that salbutamol, which is a known standard tocolytics (Ikpeze, 2009) acts by binding to β_2 adrenoceptor to activate adenyl cyclase which in turn increases the production of cyclic AMP to cause the activation of protein kinase which inhibits phosphorylation of myosin chain and lower intracellular calcium concentration resulting in uterine muscle relaxation (Rang *et al.*, 2003).

However, when atropine, a muscarinic receptor blocker was used in combination with the extract, the response of the extract was observed to be lower than that of the extract alone on the non pregnant uterine tissue due to the inhibitory effect of atropine, however, on pregnant uterus, it was observed to cause uterine contraction. This unusual finding was due to the adrenolytic effect of atropine (Backman and Lundberg, 1922) and mechanical depolarization it is capable of causing (Barany, 1996). It is known that at high dose or concentration or in a very sensitive uterine tissue, atropine cause contraction unlike the inhibition it causes in low concentration or in a less sensitive uterine tissue. The mean difference in reduction of activity of the extract was more on the non pregnant tissue (43.5 mm) than the pregnant tissue (7.5 mm). On the non pregnant tissue the antagonistic activity of atropine was more than that in the pregnant tissue. This could also be due to down regulation muscarinic uterine receptors in pregnancy and the fact that atropine acts to competitively inhibit only acetylcholine (Hardman and Limbird, 2001). On the pregnant uterine tissue, multiple comparison of the extract alone versus the

combination (extract + atropine) revealed a p-value of 1.0 which is higher than α – value of 0.05 at 95 % confidence interval. This means, that the combination of the extract and atropine had similar response as that of the extract alone indicating no statistical difference in response on both tissues.

Indomethacine, a cyclooxygenase 1 and 2 inhibitor (COX-1 and 2 inhibitors), the enzyme is responsible for prostaglandin synthesis and so inhibition of the enzyme prevents formation and activity of prostaglandins. On the non pregnant uterine tissue, it reduced the activity of the extract by 39.25 mm and reduced the antagonist extract on the pregnant uterine tissue by 79.5 mm. This may be due to increase expression prostaglandin receptors in pregnancy which may also translate to increase binding sites of prostaglandin and greater antagonistic effect of the indomethacine substrates (Hardman and Limbird, 2001). A multiple comparison test revealed a p- value of 0.0001 for combination (indomethacine + extract) and extract alone on both pregnant and non pregnant uterine tissues, this p-value is less than the α - value of 0.05 at 95% confidence interval and shows significant statistical difference between the tissues response to extract alone and that of (extract + indomethacine), confirming antagonism.

Statistically, significant antagonism against the *S. corymbosa* extract activity on both pregnant and pretreated non pregnant tissues occurred when salbutamol and indomethacine were interacted with the extract. That may be indicative of the possibility that the extract may be utilizing the same or similar receptor site for its activity as salbutamol and indomethacine for its activities, β – adrenoceptors or prostaglandin receptor. Also, Gill (1992) reported the presence of prostaglandin in *Sida corymbosa* extract following phytochemical screening of the plant and this may be the constituent responsible for its uterotonic activities, It could be the reason why indomethacine inhibited the extract as observed in the present study. Kuniata and Rapp (2001) screened

S. corymbosa extract for phytochemicals and they reported the presence of γ – epinephrine in the plant. Epinephrine is known to cause uterine contractions through activation of adrenoceptors and the extract may be utilizing adrenoceptors for its activity because of the epinephrine in it. That may be the reason why salbutamol inhibited the activity of the extract because salbutamol utilizes adrenoceptors for its tocolytic activity. Preliminary phytochemical screening of *Sida corymbosa* revealed the presence of alkaloids. Certain alkaloids especially Ergot alkaloids act through adrenoceptors, dopamine receptors, 5HT receptors, and probably other receptors (Hollingsworth *et al*., 1990). The alkaloid in this plant extract may be ergot alkaloids and may be the reason why an adrenoceptor antagonist, salbutamol inhibited the activity of the extract.

Phytochemical screening of aqueous leaf extract of *Sida corymbosa* during this study, revealed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycoside, sterol, triterpines, anthraquinones and carbohydrate. This corroborate with the work of Kuniata and Rapp (2001) in India, who reported the presence of carboxylated tryptomines, quinazoline, hypaphorine, and vasicinol in addition to the aforementioned constituents. Other species of the plants within the *Sida* genus are reported to contain cryptolepine , phytosterol, mucin and asparagin. This means that the plant is a rich source of phytochemicals and may be useful for development of general pharmaceutical chemicals and drugs.

Food and Agricultural Organization (2002) in its article "cultural practices" reported a wide range of folkloric uses of this plant. The oral administration of the extract of *S. corymbosa* leaf has been reported to lower blood pressure and to improve the cardiac irregularity, fevers, fits, Ophthalmia, rheumatism, leucorrhoea, painful micturition,

gonorrhea, colic, and general debility. This may also be attributable to the presence of a wide range of phytochemicals in the extract (FAO, 2002).

Tryptamine has been reported to be a phytochemical constituent of *S. corymbosa* (Kuniata and Rapp, 2001). It has molecular structure that contains an indole ring joint to an amino group and ethyl side chain. It is known to be a constituent of serotonin, an important neurotransmitter that is involved in regulation of the sleep-wake cycle (Robin, 2011) and may also be responsible for uterine contractility when administered in because it is a neurotransmitter. It may also explain why this plant is said to be successful in ethno-medicine for the treatment of nervous disorders like anxiety and fits (FAO, 2002).

Also the plant contains cardiac glycoside (Norris, 1961), Which are known to increase muscle tone and to initiate contraction mainly on cardiac muscle but this activity may cause increase in the tone of smooth muscles of which uterine muscle is a type and it is expected to elicit contractility in uterine smooth muscle. This constituent may be a cause or an important contributor to the uterotonic property of the plant.

Edward *et al*. (1986) reported that saponin, a phytochemical constituent of *Sida corymbosa* inhibits the metabolism of prostaglandin E_2 and $F_{2\alpha}$. The overall effect of these interacting phytochemical constituents may contribute to the overall uterotonic property that the plant possesses.

The cumulative effect of saponins and prostaglandins in the extract appears to be a logical cause of uterotonicity of the *Sida corymbosa* leaf extract.

CHAPTER SIX

6.0 Summary

The aqueous leaf extract of *Sida corymbosa* has demonstrated mean uterotonic activity of 75.75 mm by attenuating responses on both non-pregnant and pregnant rat uterus. Phytochemical screening of the extract revealed the presence of constituents such as alkaloids, flavonoids, tannins, saponins, cardiac glycosides, sterols, triterpenes, anthraquinones and carbohydrates. Investigation of acute toxicity using the Lorke's method showed that the LD_{50} of the extract is 471.17 mg/kg, falling in the range of slight toxicity on the lorke's classification.

The extract was found to elicit contractions and the amplitude of response was higher on the pregnant uterine tissue than the non pregnant uterine tissue. The EC_{50} was also found to be lower in the pregnant uterine strip than the non pregnant uterine strip indicating an increased sensitivity of the extract by the pregnant uterine tissue. When interactions were done between the extract and oxytocin and acetylcholine, potentiation of the activity of the extract was observed but when interactions with salbutamol, atropine and

indomethacine were done, the drugs were found to antagonize the activity of the extract. All findings of extract uterotonic activity, potentiation by agonist and antagonism by tocolytics were confirmed statistically to be significant at α – value of 0.05 and 95% confidence interval. The extract may be acting via adrenoceptors or prostaglandin receptor because antagonism by salbutamol and indomethacine were overt on both pregnant and non pregnant uterine tissues.

6.1 Conclusion

The aqueous leaf extract of *Sida corymbosa* has uterotonic property more on the pregnant than the non-pregnant uterine tissue and may be utilizing adrenoceptors or prostaglandin receptors for its uterotonic activity. The LD50 is 471.17 mg/ml, making it slightly toxic and has a wide variety of phytochemicals.

6.2 Recommendation

The aqueous leaf extract of *Sida corymbosa* has demonstrated uterotonic property on both non-pregnant uterine tissue and pregnant rat uterine tissue. Further research on the uterotonic properties of various constituents of the plant may be necessary, so as to identify the specific agent(s) that is responsible for uterotonicity. Subacute and chronic toxicity study may be necessary so as to document the toxicity profile of the crude extract. There may be need to modify clinical obstetric practice especially amongst women who use the plant extract at home before presenting to the hospital labour ward. This is because, the study has demonstrated synergistic interaction between the extract and oxytocics, such that use of another uterotonic in combination or after prior use of *Sida corymbosa* leaf extract can lead exergerated uterotonic effects leading to uterine rupture, cord compression, abruption placenta and fetal demise.

References

Aaronson, P.I., Sarwar S., Gin U., Rockenbauch M. and Connolly T.A. (2010).A role for voltage-gated but not Ca^{2+}-activated, K^{+}-channels in regulating spontaneous contractile activity in myometrium from virgin and pregnant rats, *British Journal of Pharmacology;* vol 114, pg 27.

Agyakwa, C.W., Akobundu, I.O.(1998). A handbook of West African Weeds international institute of tropical agriculture; Ibadan, Nigeria: African book builders; pg 352

Alan, H. D., Lauren N., Murphy G. and Neri L. (2003). Current Diagnosis and Treatment Obstetrics and Gynecology 10th edition Mc Graw Hill Publishing; pg 54 – 66.

Arslan, A.A, Gold, L.I and Mittal, K. (2010). Gene expression studies provide clues to the pathogenesis of uterine leimyoma: new evidence and a systematic review. Human reproduction 20: 852 – 863.

Asboth G, Phaneuf S, Finner G.N, Toth M, Bernal A.L (1996). Prostagladin E2 activates phospholiphase C and elevates intracellular calcium in cultured myometrial cells: involvement of EPI and EP3 receptor subtypes, Endocrinology: 137: 2572- 2579

Attah, A.F., O'Brien M., Koehbach J., Sonibare M.A., Moody J.O., Smith T.J. and Gruber C.W. (2012).Uterine contractility of plants used to facilitate child birth in Nigerian Ethnomedicine, *J. Ethnopharmacol*; 143 (1): 377-82

Backman, E. L. and Lundberg H. (1922).Acetylcholine antagonism in different tissues, Soc Biol. Paris 89, 475.

Barany, M. (1996).*Biochemistry of Smooth Muscle Contraction*. San Diego, CA: Academic.

Batra, S. (1986). Effect of oxytocin on calcium influx and efflux in the rat myometrium, *European Journal of Phamacology*: 120 : 57 – 61

Brodt-Eppley J. and Myatt L. (1999).Prostaglandin receptors in lower segment myometrium during gestation and labor. Obstet Gynecol: 93 : 89 – 93

Carsten, M.E., Miller J.D., effects of prostaglandins and oxytocin on calcium release from a uterine microsomal fraction. J Biol chem. 1977: 252 : 1576- 1581

Chard, T. (1989). Fetal and material oxytocin in human parturition, *American journal perinatology*; 6 : 145 – 152

Chitaley, K., Weber D.S. and Webb R.C. (2001).RhoA/Rho-kinase, vascular changes and hypertension.*Current Hypertension Rep* 3: 139–144, 2001

Dubin, N.H, and Ghodgaonkar, R.B. (1980). Angiotensin II and its effect on contractile activity and prostaglandin production in vitro pregnant uteri endocrinology: 107, 185 – 186.

Edwards, C.R., Benediktsson R.S., Lindsay and Seckl J. (1996).11 beta-Hydroxysteroid dehydrogenases: Key enzymes in determining tissue specific glucocorticoid effects. Ster, 4: 263-269.

Eferekeya, A. and Nworgu Z (1985). Inhibitory effects of gallopamil and hydralazine on early and late pregnant uteri.*Nig. J. Pharm. Sci.*, 1: 67-71.

Evans, W.C. (2002). Pharmacopoieal and Related Drugs of Biological Origin. In: Pharmacognosy, Evans, W.C. and D. Evans (Eds.). WB Saunders Co. Ltd., London; pp: 374.

Food and Agriculture Organisation, (2002). Cultural practices http://www.fao.org/ag/AGP/AGPC/doc/publicat/FAOGUL2/B204.htm.2p.

Fries, R.E. (1965). Botanical Classification of Plants, Missouri botanical garden: Accessed on 31st may 2012 <http://www.tropicos.org/sida/ 2874.

Fukata, Y., Mutsuki A. and Kaibuchi (2011).Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non muscle cells; *Trends Physiol Sci* 22: 32–39

Garfield R.E., Kannan M.S. and Daniel E.E. (1980). Gap junction formation in myometrium: Control by estrogenes , progesterone , and prostaglandin. American *Journal of physiology:* 238: C81-C89

Gill, R.M.A (2001). The impact of deer on woodland: the effect of browsing and seed dispersal on vegetation structure and composition. Forest research, alice holt lodge, wrecclesham, surrey 104th, England

Hardman J.G. and Limbird L.E. (2001). Gilman Goodman and Gilman's, The pharmacological basis of therapeutics. 10thed. New York, NY: Mc GrawHill; Pg.164.

Henshaw, S.K., Singh, S., Oye-Adeniran, B.A., Adewole, I.F., Iwere, I.V., Cusa, V.P. (2003). The incidence of induced abortion in Nigeria. Internation family planning perspective; vol 24(4): pg 156 – 164.

Hollingsworth, M., Edwards D. and Miller M., (1990). Effects of ergometrine in the rat uterus and involvement of 5HI receptors, *British Journal of Pharmacology:* 95, 362.

Holm, L.G., Plucknett D.L., Pancho J.V. and Herberger J.P. (1977). The Worlds Worst Weeds: distribution and biology.- University Press of Hawaii, Honolulu, USA.

Hostettmann, K., Marston A., Ndjoko K. and Wolfender J.L. (2013).The potential of African plants as a source of drugs, Cur. Organic Chem., 4: 973-1010.http://www.nt.gov.au/dpif/pubcat/agnotes/542.htm.4p.

Ignacimuthu, S., Ayyanar M. and Sankara-Sivaramann K. (2006).Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India).*J. Ethnobiol. Ethnomed*.2: 25.

Ikpeze, O.C. (2009). Fundamentals of Obstetrics and Gynaecology; 1st edition, Africana First Publishers: 207 - 297

Imming, P., Sinning C. and Meyer A. (2006). Drugs, their targets and the nature and number of drug targets. Nat Rev Drug Discovery; 5 (10): 821 – 834.

Irene, I.I., Iheanacho U.A. (2011). Acute effect of administration of ethanol extracts of *Ficus exasperata* vahl on kidney function in albino rats. *J. Med.* Plant Res., 1: 27-29.

Ivell, R, Richter D (1998). Structure and comparison of the oxytocin and vassopresin genes from rat .proc Nati Acid sci USA; 2006 – 2010

Ivell, R. and Richter D. (2010). Structure and comparison of the oxytocin and vassopresin genes from rat .proc Nati Acid sci USA; 2006 – 2010

Iwe, P., Akubue U. (2006). Textbook of pharmacology; 1st Ed, African first publishers limited pg. 305 – 314.

Jegede, C.O. (2012). Indigenous methods of processing *Materia Medica* among selected traditional medical practitioners in Ibadan, Nigeria. *Journal of Ethno-medicine*, 6(3): 187-195

John, L.B., Yahaya T.A., Isimi C.Y. (2012). Antiulcer and wound healing activities of *Sida corymbosa,African Journal of Traditional medicine, Pubmed, accessed* 8/8/2013.

Kamla-raj, (2012). Phytochemical screening of sida, *Journal of Human Ecology* (1): 229 – 230.

Katzung, G. B. (2006). Basic and clinical pharmacology; 10th edition, Lange, Mc GrawHill; pg 501 – 508, 768 -770

Kayode, J. (2006). Conservation of indigenous medicinal botanicals in Ekiti State, Nigeria. *J. Zhejiang University Sci.* B 7: 713-718.

Kelly, A.J., Kavanagh J., Thomas J. Vaginal prostaglandin [PGE_2 and PGF_{2a}]: for induction of labour at term (2003). *Cochrane database system*; Rev. 4 CD 003101

Klaasen C. D, Amdur M. O, Doull J (1995). The basic science of poisons. Ed. New York, NY: Mc GrawHill; pg. 601.

Klaasen, C.D., Amdur M.O. and Doull J. (1995).The basic science of poisons. Ed. New York, NY: Mc GrawHill; pg. 601.

Kuniata, L. and G. Rapp (2001).Biocontrol of Sida rhombifolia in Papua New Guinea.

Kurowicka, B., Franczak A., Oponowicz G. and Kotwicka (2011).*In vitro* contractile activity of porcine myometrium during luteolysis and early pregnancy: Effect of oxytocin and progesterone. Reprod. Biol., 2: 151-169.

Kwawukume E.Y and Emuveyan E.E. (2005).Comprehensive obstetrics in the tropics, Graphic packaging limited, Ghana. Pg 129 – 134.

Lopez, B.A. (2003). Mechanisms of labour, biochemical aspects.*BJOG*: 110 (SUPPL 20) : 39-45

Lorke, D. (1983). A new approach to practical acute toxicity testing. Archieves of toxicology; 5: 275 – 287

Mackler, A.M., Ducsay J.D., Vudhuis S.M. Yellon. (1999). Maturation of spontaneous and agonist-induced uterine contractions in the peripartum mouse uterus, Biol. Reprod., 61: 873-878

Malairajam, M.K, Sayya, A. Chopra, A., Rupasinghe, M. higher molecular IVF steroids and triterpenoids showed analgesic properties. *Journal of biological sciences, Pakistan* ; vol (4): pg 45-89

Matsumoto, T., Sagawa N., Yoshida M., Mori T., Tanaka I., Mmukoyama M., Kotani M. and Nakao K. (1997). The prostaglandin E_2 and $F_{2\alpha}$ receptor genes are expressed in human myometrium and are down regulates during pregnancy. Biochem Biophys Res commun: 238: 838-841

Micheal, D. and Geoffery C. (1998). Basic sciences in obstetrics and gynaecology .Textbook for MRCOG part 1, ChurchHill Livingstone. Pg 84 – 85.

Mitchell, B.F. and Schmid B. (2001). Oxytocin and its receptor in the process of parturition J Soc Gynecological Investigation 8: 122 – 133

Mitchell, M.D., Romero R.J., Edwin S.S. and Trautman M.S. (1995). Prostaglandins and parturition, Reproductive Fertility Dev 7: 623 – 632

Molnar, M. and Hertelendy F. (1990). PGF2 alpha and PGE2 binding to rat myometrium during gestation, parturition and postpartum. *American Journal of Physiology:* 258 : E740- E747

Monga, M., Campbell D.F. and Sanborn B.M. (1999). Oxytocin – stimulated capacitative calcium entry in human myometrial cells, *American Journal of Obstetric and Gynecoloy:*181 : 424 – 429

Monster, K.H (1967). Scientific Basis of Obstetrics and Gynaecology; Publi. Churchhill livingstone, London, 59 – 86

Nacoulma/Ouédraogo O.G. (1996). Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso. Cas du plateau central Mossi Thèse d'Etat. Université de Ouagadougou.

Otero, R., Nunez V., Barona J., Fonnegra R., Jimenez S.L., Osorio R.G., Saldarriaga M. and Diaz A. (2000). Snakebites and ethnobotany in the northwest region of Colombia. Part III: neutralization of the haemorrhagic effect of *Bothrops atrox venom. J. Ethnopharmacol.* 73: 233-241.

Owen J, Hauth J.C (1992). Oxytocin for the induction or augumentation of labor, Clinical Obstetrics and gynecology; 35 : 468 – 475

Owen, J. and Hauth J.C. (1992). Oxytocin for the induction or augumentation of labor, Clinical Obstetrics and gynecology; 35 : 468 – 475

Pakia M. (2005). African traditional plant knowledge today: an ethnobotanica study of the Digo at Kenya coast. *Journal of plant resoueces of tropical Africa*.Vol 9 pg 20-21.

Pannefather O'neil J. N. (1992). Dry used in labour; Mechanisms of Action, Medicine digest,18, no 3: 19 – 24.

Rang, H. P.(2013). Textbook of Pharmacology, 6th Ed; Edinburgh, churchchill living stone p: 234.

Ridley, M. (20 04).Evolution, 3rd edition. Blackwell publishing, pg. 314.

Russell, H. (1955). Useful plants of Weat Africa; *Journal of Ecology*: vol 43.

Ruzycky, A.L. and Kulink A. (1996). Estrogen increases the expression of uterine protein Kinase C isozymes in a tissue specific manner. *European Journal pharmacology:* 313: 257-263

Smith, R. (2007). Parturition, New England Journal of Medicine: 356: 271-283

Sofowora, A. (1996). Medicinal Plants and Traditional Medicine in Africa: Spectrum Books Ltd, Ibadan, Nigeria.100-107.

Sollof, O. and Alenandrova M. (1980). Oxytocin receptors and parturition, control of oxytocin receptor concentration in the rat myometrrium at term .endocrinology , 1980 106 , 730 – 735

Soloff, M.S., Jeng Y.J., Copland J.A., Strakovo Z. and Hoare S. (2000). Signal pathways mediating oxytocin stimulation of prostaglandin synthesis in select target cells Exp physiology 85: 51S-58S

Stall, A (1920). zurkenntnis der mutterkorn alkaloids. Verh nature ges basel, 101 , 190 – 191.

Thompson Montvale (2004). Physician's Desk Reference, 58th ed. NJ, pg. 1656.

Veale, D.J.H., Oliver D.W., Arangics N.S. and Furman K.J. (1989). Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. *Journal of Ethnopharmacology*. 37:341-346.

Weiss, E., Kislev M. (2004). Plant remains as indicators of economic activity: a case of Ashkelon, *Journal of Archeological Science:* 31, 1 - 13

WHO, (2003). Safe abortion services: The public health challenge, In safe abortion: Technical and policy guidance for health systems; Geneva.

WHO, (2005). "Managing complications in pregnancy and child birth; A guide for midwives and doctors"

Wright, C.W., Addae – kyereme J., Breen A.G., Brown J.E., Cox M.F. (2001). "Synthesis and evaluation of cyptolepine analogue for their potential as new antimalarial agents" .*Journal of medical chemistry* 44 (19): 3187 – 3194.

Zeeman, G.G., Khan – Dawood F.S. and Dawood M.Y. (1997). Oytocin and its receptor in pregnancy and parturition: current concepts and clinical implications. Obstetrics and Gynaecology: 89: 873-883

APPENDIX

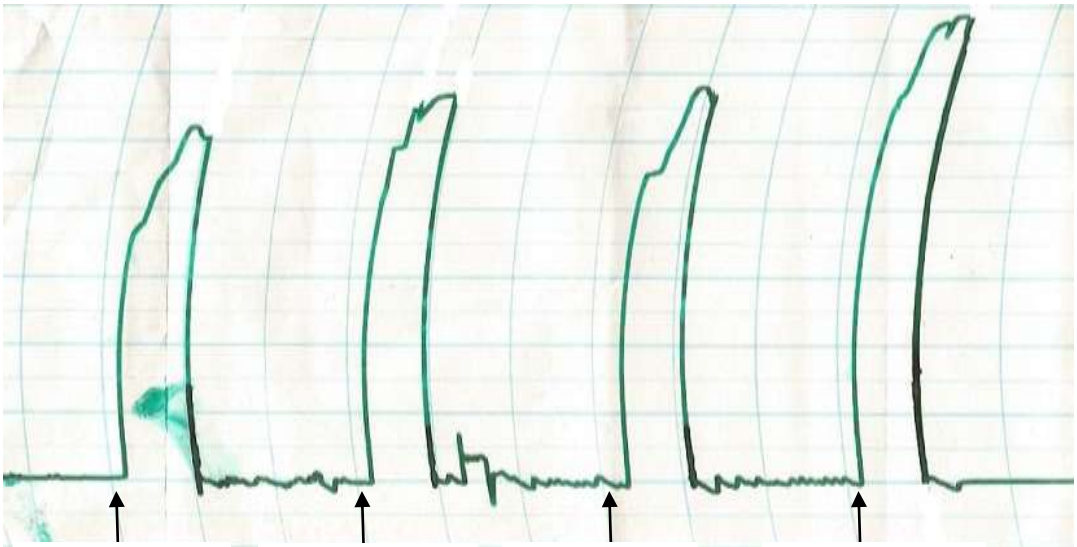
Appendix 1(A): Effect of Aqueous Leaf Extract of S. corymbosa, Acetylcholine, Oxytocin, Salbutamol, Atropine and Indomethacine and their Interactions with the Extract on Pretreated Non Pregnant Rat Uterine Strip Expressed as Amplitude of Response in Millimeters.

AGENT	FINAL BATH CONCENTRATION	AMPLITUDE (mm)
Acetylcholine (Ach)	0.0167 µg/ml	52
	0.033 µg/ml	57
	0.067 µg/ml	59
	0.133 µg/ml	69
Oxytocin	6.67 x 10-5 IU/ml	59
	1.33 x 10-4 IU/ml	68
	2.67 x 10-4 IU/ml	69
	5.33 x 10-4 IU/ml	69
Extract (Ext)	0.667 mg/ml	49
	1.333 mg/ml	48
	2.667 mg/ml	50
	5.333 mg/ml	50
Ach (0.2ml) + Ext	0.033 µg/ml + 0.667 mg/ml	56
	0.033 µg/ml + 1.333 mg/ml	60
	0.033 µg/ml + 2.667 mg/ml	61
	0.033 µg/ml + 5.333 mg/ml	64
Oxytocin (0.2ml) + Ext	1.33 x 10-4 IU/ml + 0.667 mg/ml	69
	1.33 x 10-4 IU/ml + 1.333 mg/ml	68.5
	1.33 x 10-4 IU/ml + 2.667 mg/ml	68.5
	1.33 x 10-4 IU/ml + 5.333 mg/ml	43.5
Salbutamol (Salb)	1.0 x 10-4 mg/ml	0
Salb (0.1ml) + Ext	1.0 x 10-4 mg/ml + 0.667 mg/ml	0
	1.0 x 10-4 mg/ml + 1.333 mg/ ml	0
	1.0 x 10-4 mg/ml + 2.667 mg/ml	0
	1.0 x 10-4 mg/ml + 5.333 mg/ml	38
Atropin	3.33 x 10-6 mg/ml	0
Atropin (0.1ml) + Ext	3.33 x 10-6 mg/ml + 0.667 mg/ml	0
	3.33 x 10-6 mg/ml + 1.333 mg/ml	0
	3.33 x 10-6 mg/ml + 2.667 mg/ml	12
	3.33 x 10-6 mg/ml + 5.333 mg/ml	11
Indomethacine (Indo)	8.33 x 10-4 mg/ml	0
Indo (0.1ml) + Ext	8.33 x 10-4 mg/ml + 0.667 mg/ml	0
	8.33 x 10-4 mg/ml + 1.333 mg/ml	0
	8.33 x 10-4 mg/ml + 2.667 mg/ml	22
	8.33 x 10-4 mg/ml + 5.333 mg/ml	18

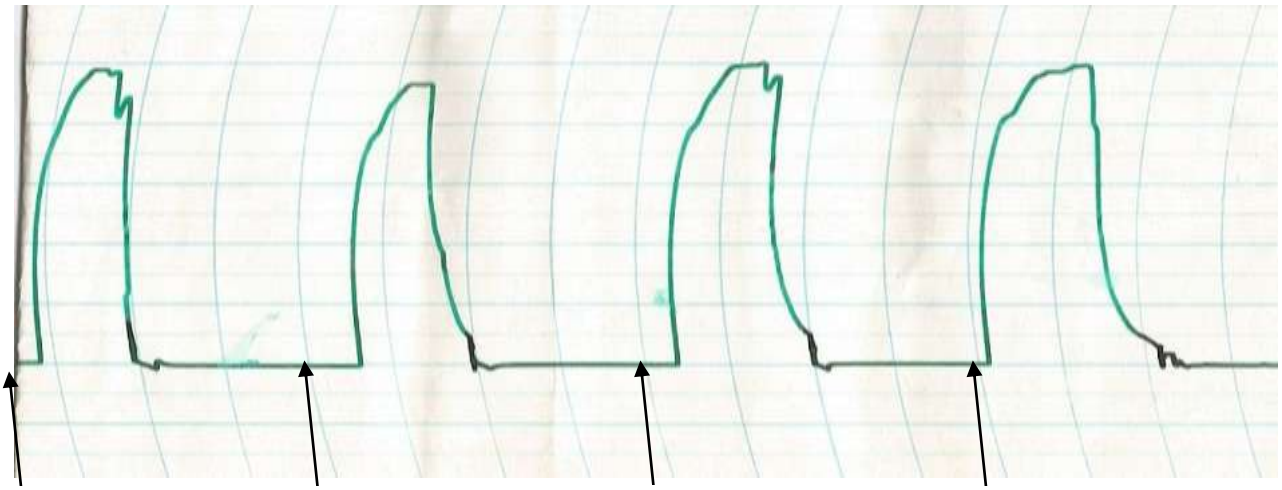
Appendix 1(B): Effect of Aqueous Leaf Extract of *S. corymbosa*, Acetylcholine, Oxytocin, Salbutamol, Atropine and Indomethacine and their Interactions with the Extract on Pregnant Rat Uterine Strip Expressed as Amplitude of Response in Millimeters.

AGENT	FINAL BATH CONCENTRATION	AMPLITUDE (mm)
Acetylcholine (Ach)	0.0167 µg/ml	29
	0.033 µg/ml	28
	0.067 µg/ml	47
	0.133 µg/ml	78
Oxytocin	6.67 x 10-5 IU/ml	68
	1.33 x 10-4 IU/ml	71
	2.67 x 10-4 IU/ml	78
	5.33 x 10-4 IU/ml	62
Extract	0.667 mg/ml	88
	1.333 mg/ml	89
	2.667 mg/ml	57
	5.333 mg/ml	89
Ach (0.2ml) + Ext	0.033 µg/ml + 0.667 mg/ml	87
	0.033 µg/ml + 1.333 mg/ml	88
	0.033 µg/ml + 2.667 mg/ml	89
	0.033 µg/ml + 5.333 mg/ml	89
Oxytocin (0.2ml) + Ext	1.33 x 10-4 IU/ml + 0.667 mg/ml	78
	1.33 x 10-4 IU/ml + 1.333 mg/ml	88
	1.33 x 10-4 IU/ml + 2.667 mg/ml	79
	1.33 x 10-4 IU/ml + 5.333 mg/ml	87
Salbutamol	1.0 x 10-4 mg/ml	0
Salb (0.1ml) + Ext	1.0 x 10-4 mg/ml + 0.667 mg/ml	22
	1.0 x 10-4 mg/ml + 1.333 mg/ ml	14
	1.0 x 10-4 mg/ml + 2.667 mg/ml	20
	1.0 x 10-4 mg/ml + 5.333 mg/ml	59
Atropin	3.33 x 10-6 mg/ml	34
Atropin (0.1ml) + Ext	3.33 x 10-6 mg/ml + 0.667 mg/ml	89
	3.33 x 10-6 mg/ml + 1.333 mg/ml	89
	3.33 x 10-6 mg/ml + 2.667 mg/ml	61
	3.33 x 10-6 mg/ml + 5.333 mg/ml	54
Indomethacine	8.33 x 10-4 mg/ml	29
Indo (0.1ml) + Ext	8.33 x 10-4 mg/ml + 0.667 mg/ml	0
	8.33 x 10-4 mg/ml + 1.333 mg/ml	0
	8.33 x 10-4 mg/ml + 2.667 mg/ml	0
	8.33 x 10-4 mg/ml + 5.333 mg/ml	5

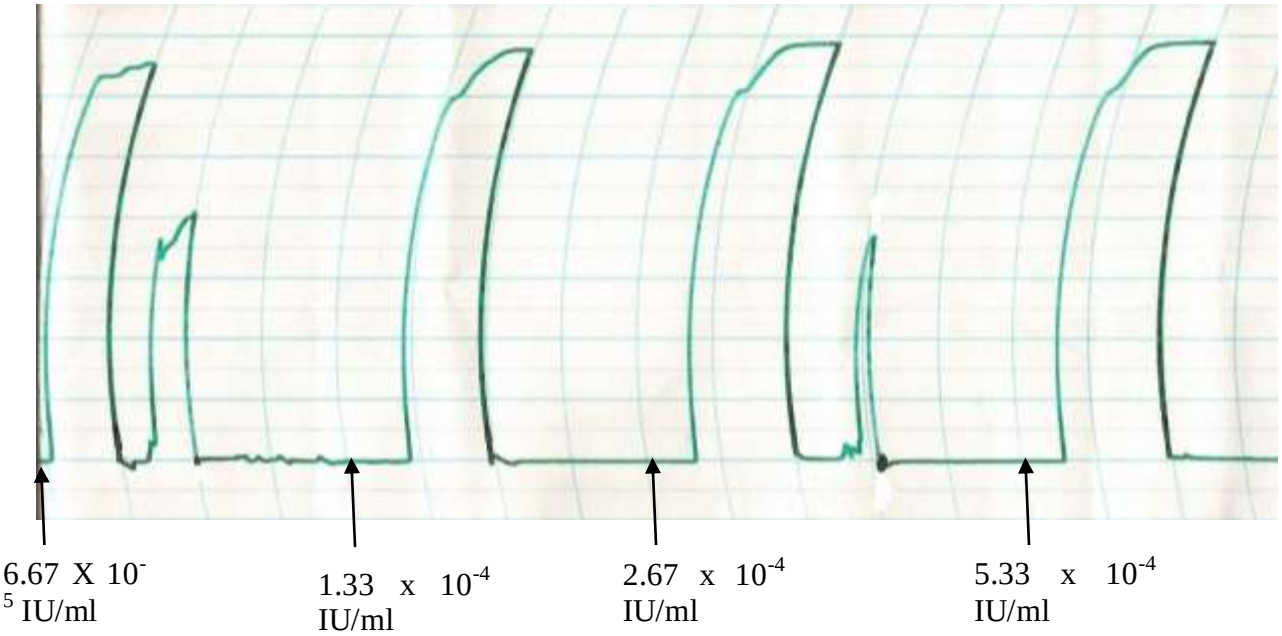
Appendix 2 (A1): Effect of Acetylcholine on Pretreated Non Pregnant Uterine Strip



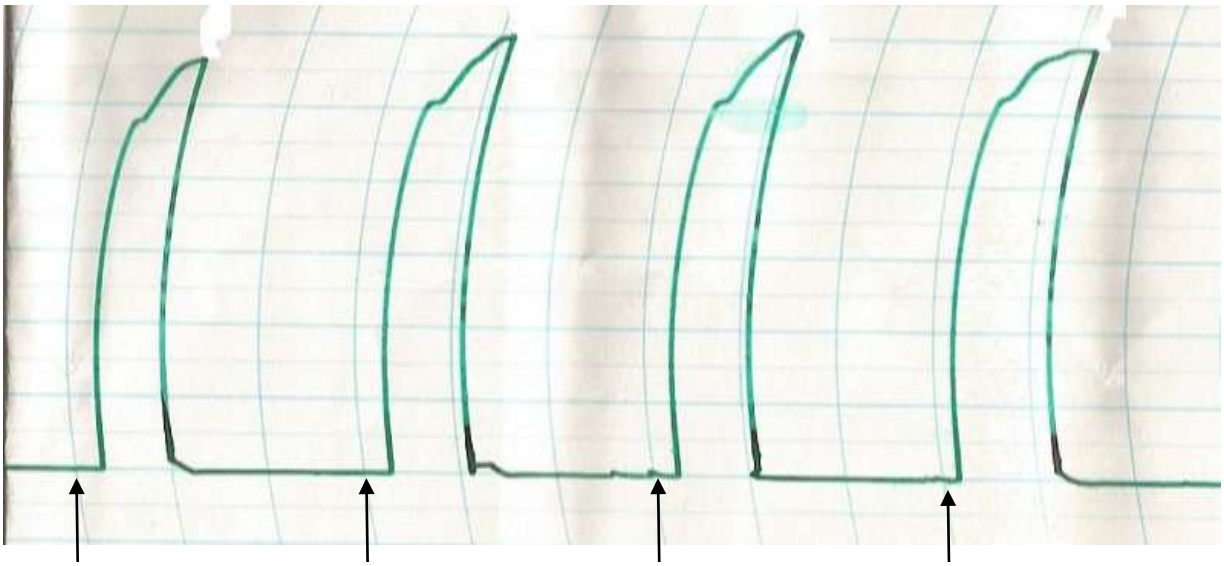
Appendix 2 (A2): Effect of *S. corymbosa* Aqueous Leaf Extract on Pretreated Non Pregnant Uterine Strip



Appendix 2 (A3): Effect of Oxytocin on Pretreated Non Pregnant Uterine Strip



Appendix 2 (A4): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Acetylcholine on Pretreated Non Pregnant Uterine Strip



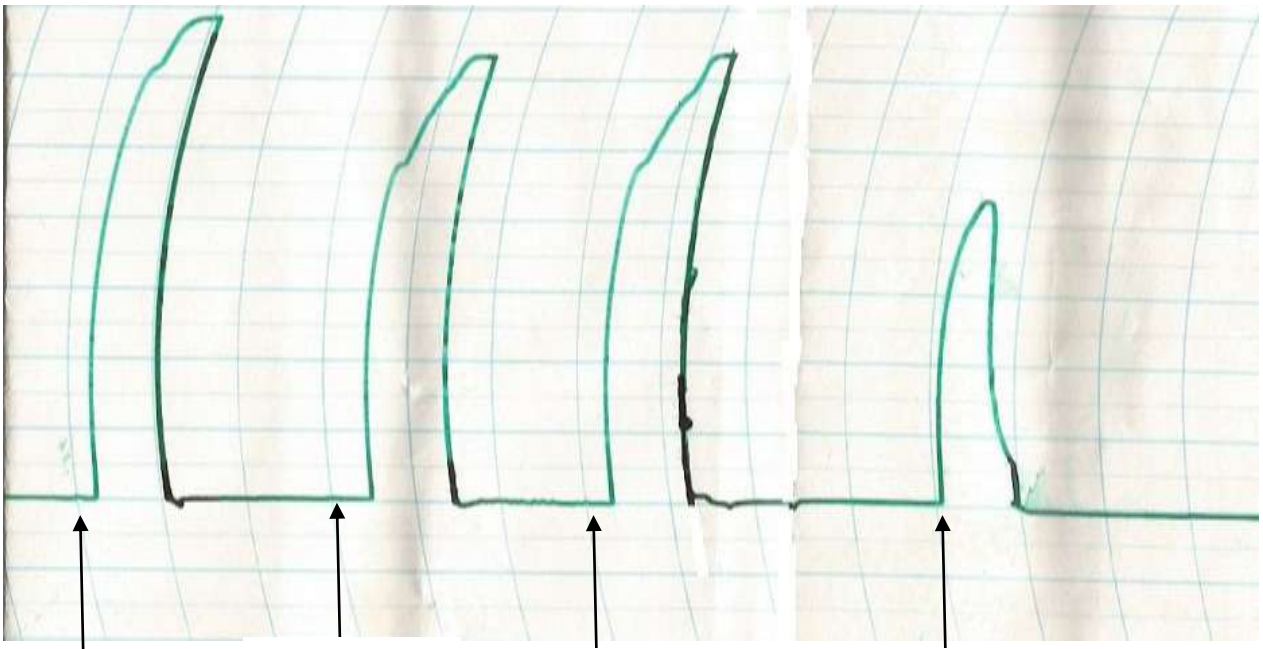
Ext (0.67 mg/ml) + Ach (0.033 µg/ml)

Ext (1.33 mg/ml) + Ach (0.033 µg/ml)

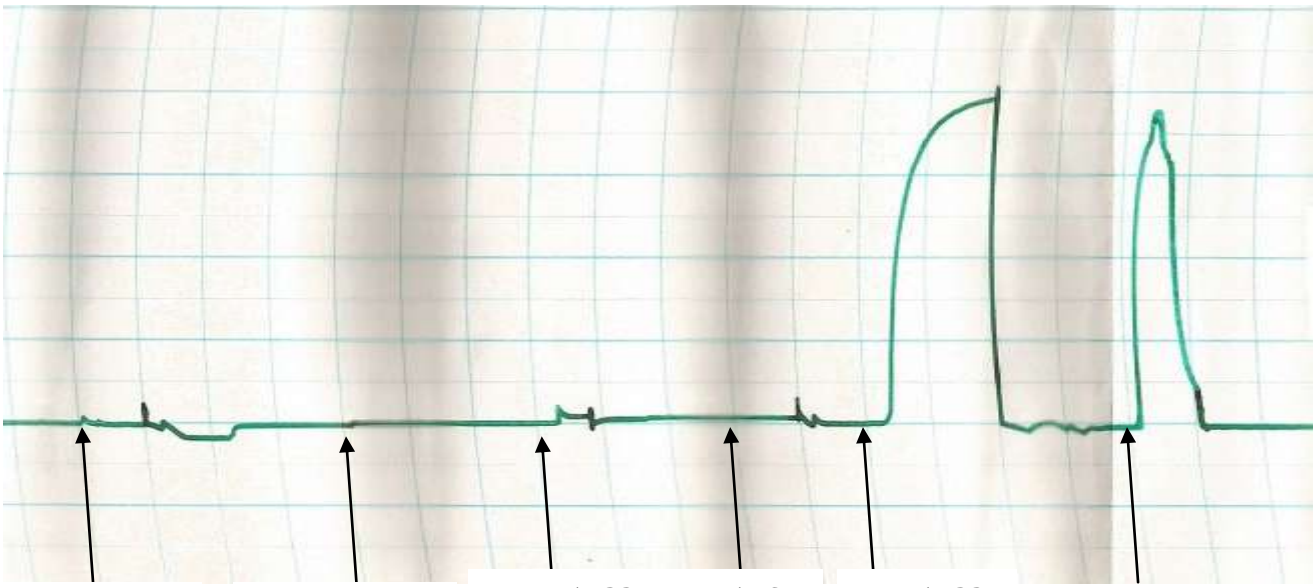
Ext (2.67 mg/ml) + Ach (0.033 µg/ml)

Ext (5.33 mg/ml) + Ach (0.033 µg/ml)

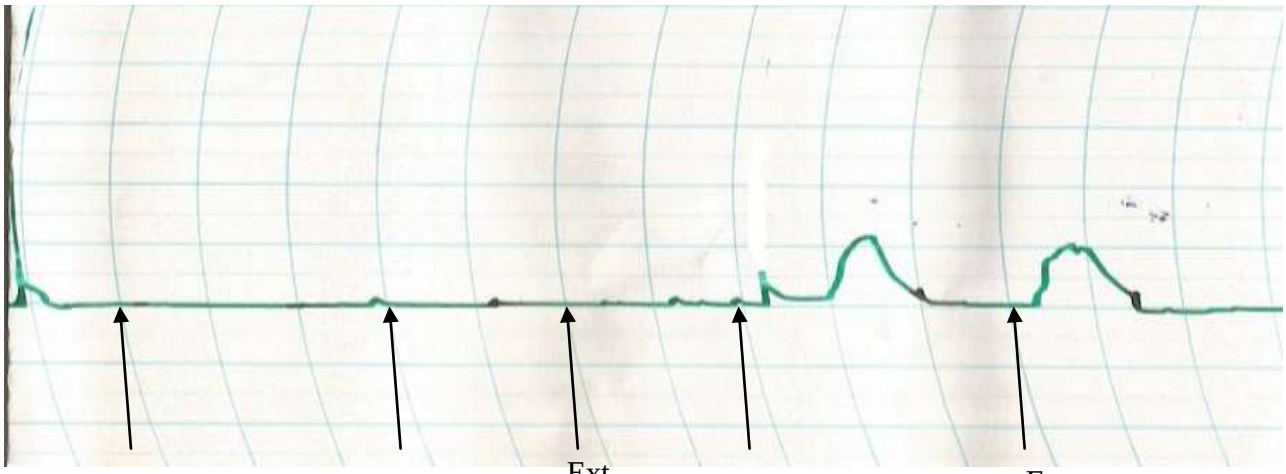
Appendix 2 (A5): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Oxytocin on Pretreated Non Pregnant Uterine Strip



Appendix 2 (A6): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Salbutamol on Pretreated Non Pregnant Uterine Strip



Appendix 2 (A7): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low concentration of Atropin on Pretreated Non Pregnant Uterine Strip



Atropin (3.33 x 10^{-6}mg/ml)

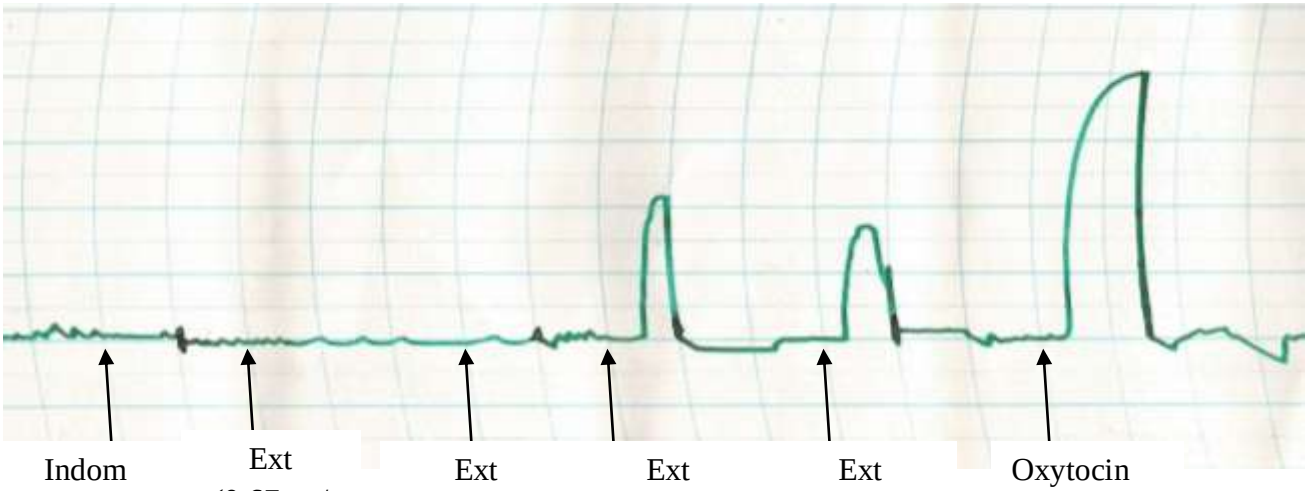
Ext (0.67mg/ml) + Atropin (3.33 x 10^{-6}mg/ml)

Ext (1.33mg/ml) + Atropin (3.33 x 10^{-6}mg/ml)

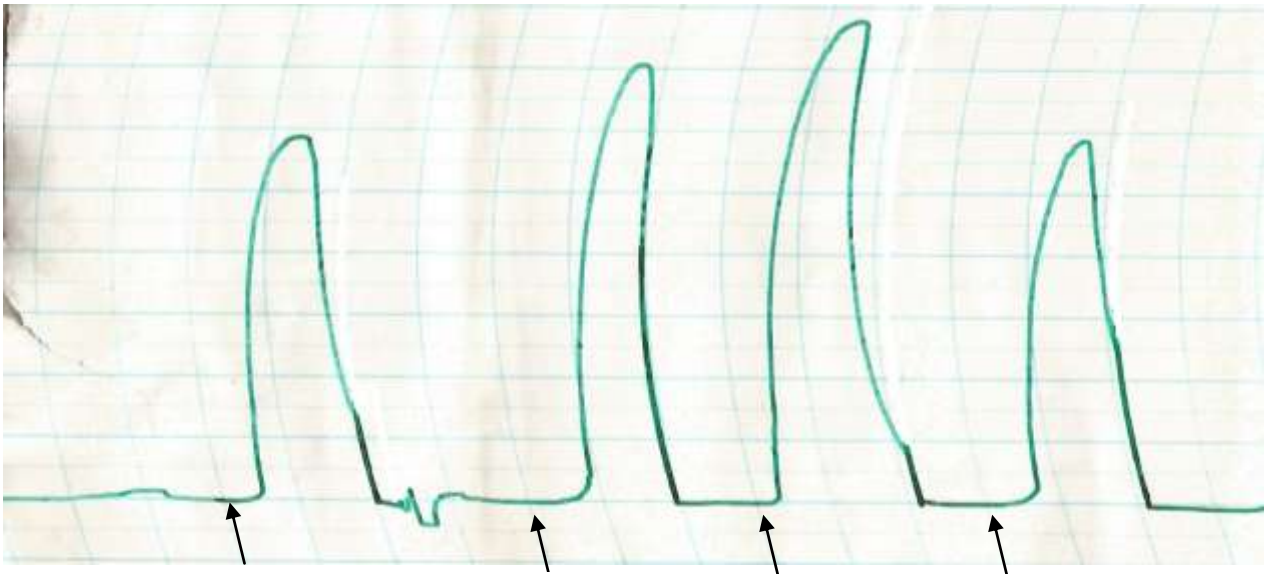
Ext (2.67mg/ml) + Atropin (3.33 x 10^{-6}mg/ml)

Ext (5.33mg/ml) + Atropin (3.33 x 10^{-6}mg/ml)

Appendix 2 (A8): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Indomethacine on Pretreated Non Pregnant Uterine Strip

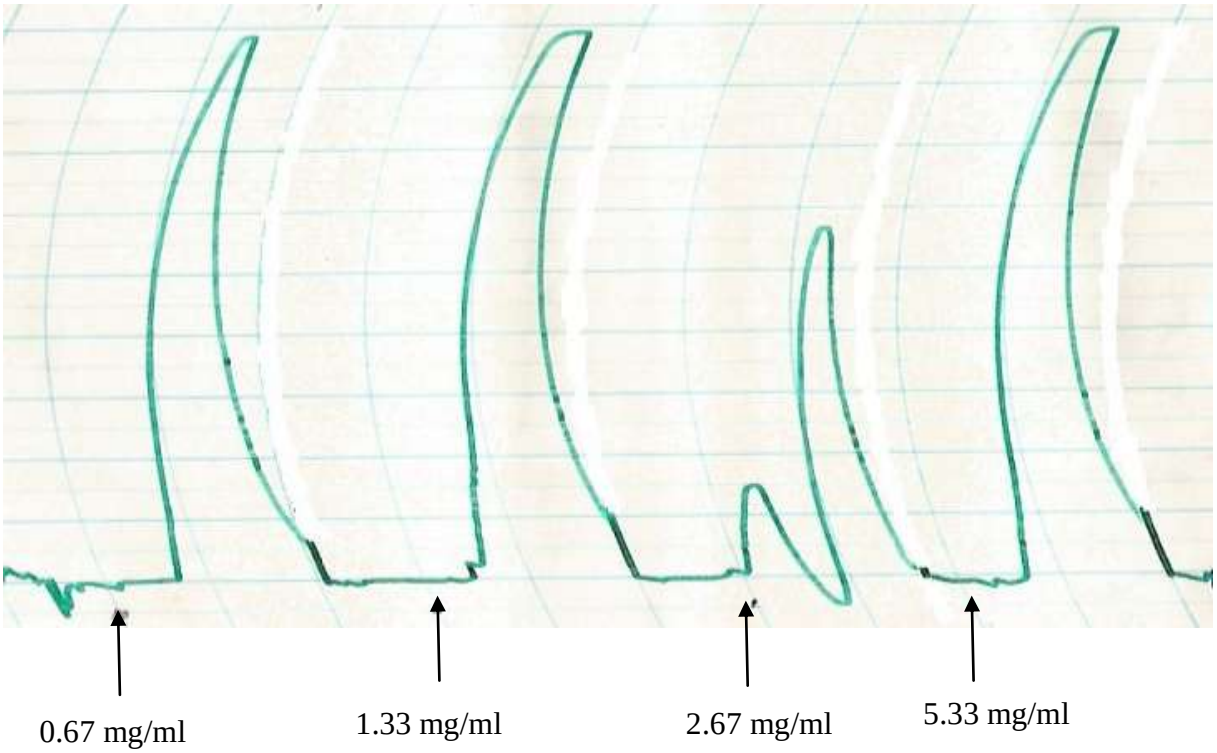


Appendix 2 (B1): Effect Oxytocin on Pregnant Rat Uterine Strip

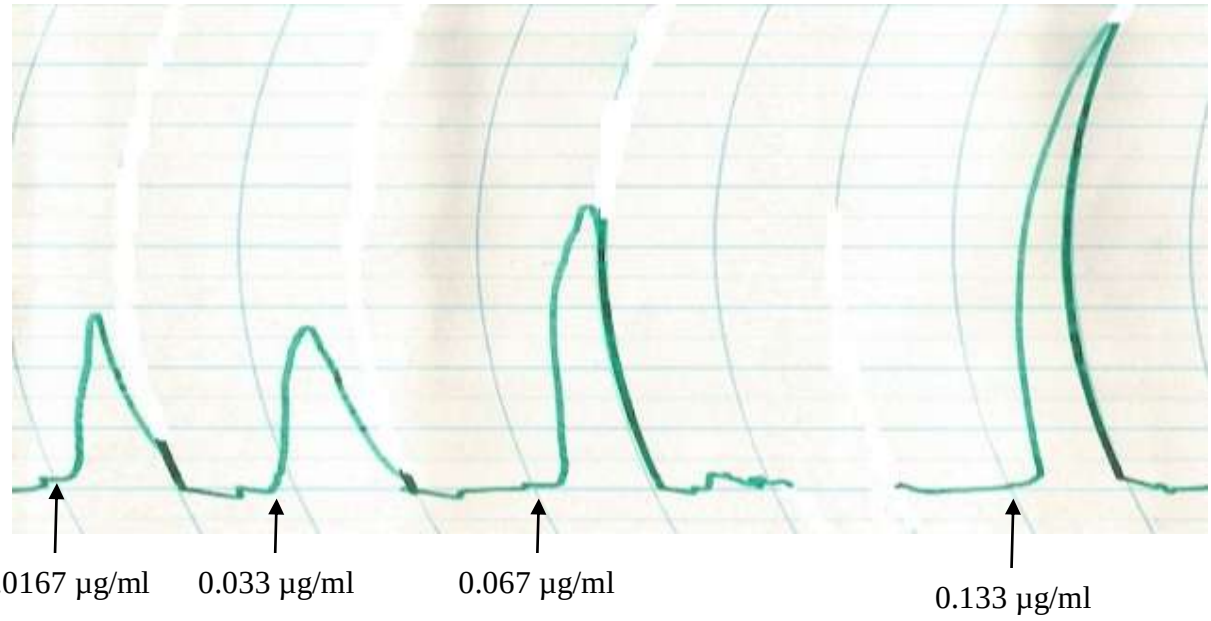


$6.67X10^{-5}$IU/ml $1.33x10^{-4}$IU/ml $2.67x10^{-4}$IU/ml 5.33×10^{-4} IU/ml

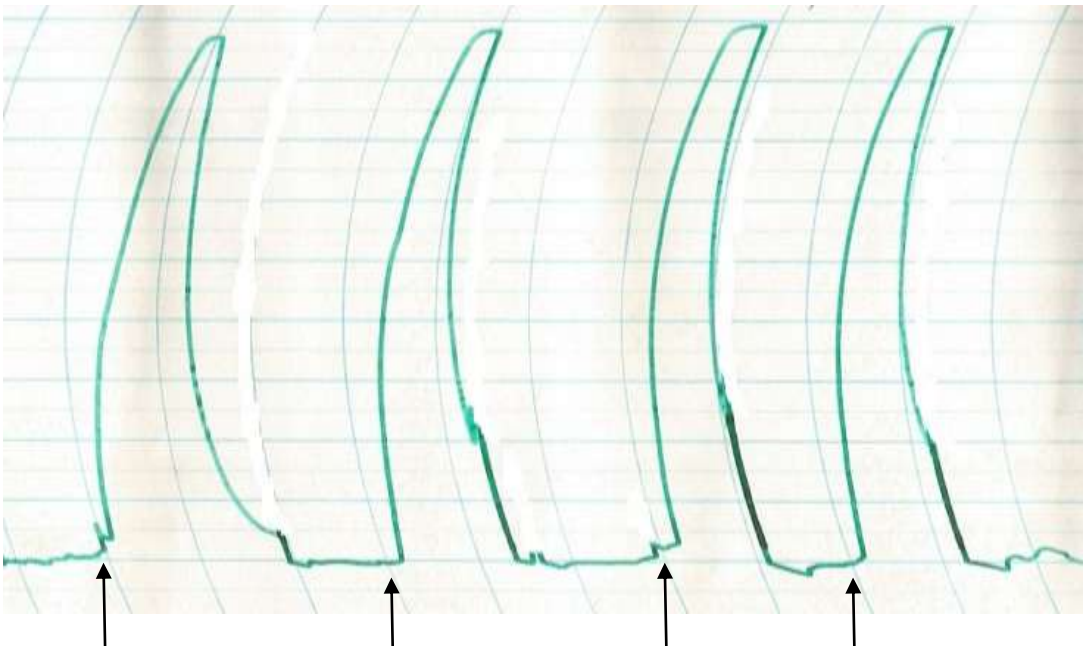
Appendix 2 (B2): Effect of *S.corymbosa* Aqueous Leaf Extract on Pregnant Rat Uterine Strip



Appendix 2 (B3): Effect of Acetylcholine on Pregnant Rat Uterine Strip



Appendix 2 (B4): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Acetylcholine on Pregnant Uterine Strip



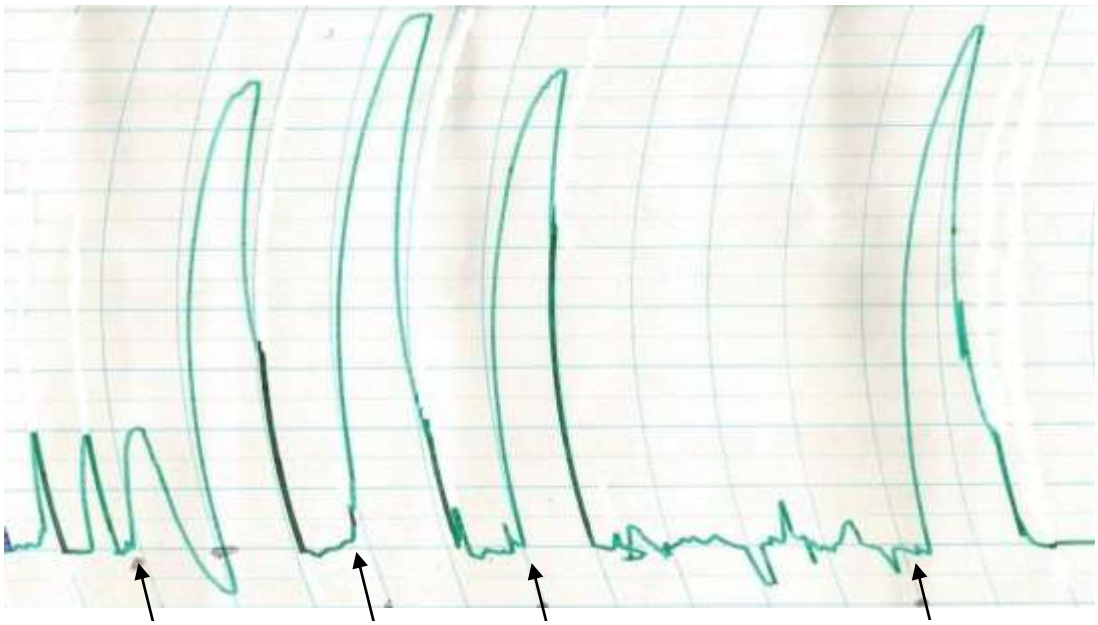
Ext(0.67mg/ml) + Ach (0.033 µg/ml)

Ext (1.33 mg/ml) + Ach (0.033 µg/ml)

Ext (2.67 mg/ml) + Ach (0.033 µg/ml)

Ext (5.33 mg/ml) + Ach (0.033 µg/ml)

Appendix 2 (B5): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Oxytocin on Pregnant Uterine Strip



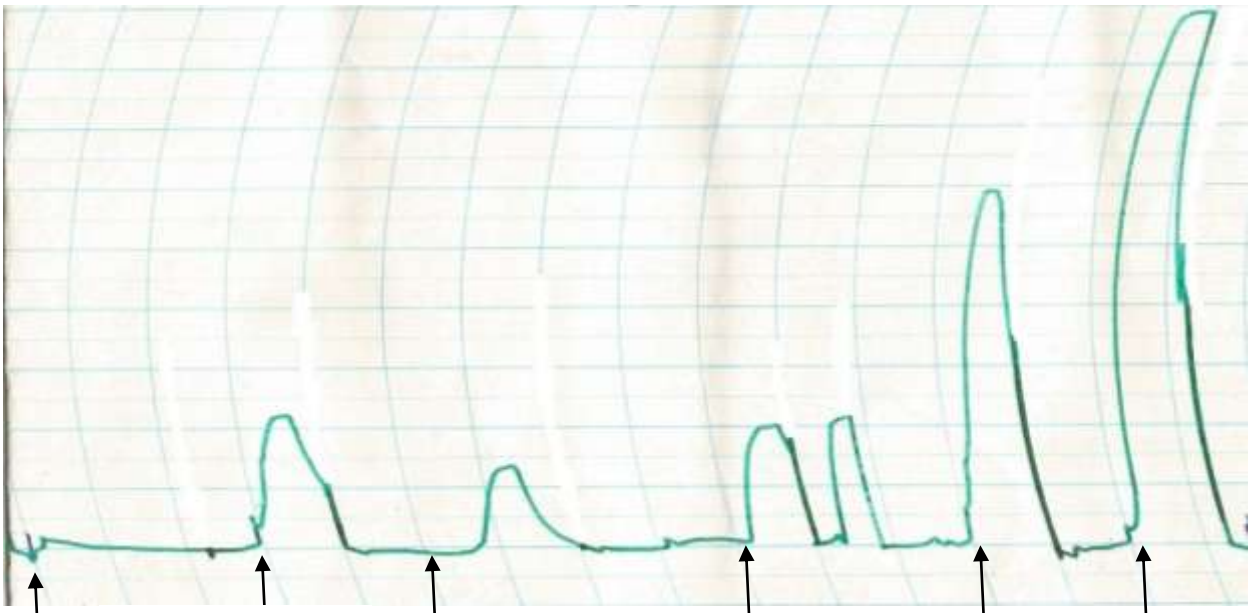
Ext (0.67 mg/ml) + Oxy (1.33 x 10^{-4} IU/ml)

Ext (1.33 mg/ml) + Oxy (1.33 x 10^{-4} IU/ml)

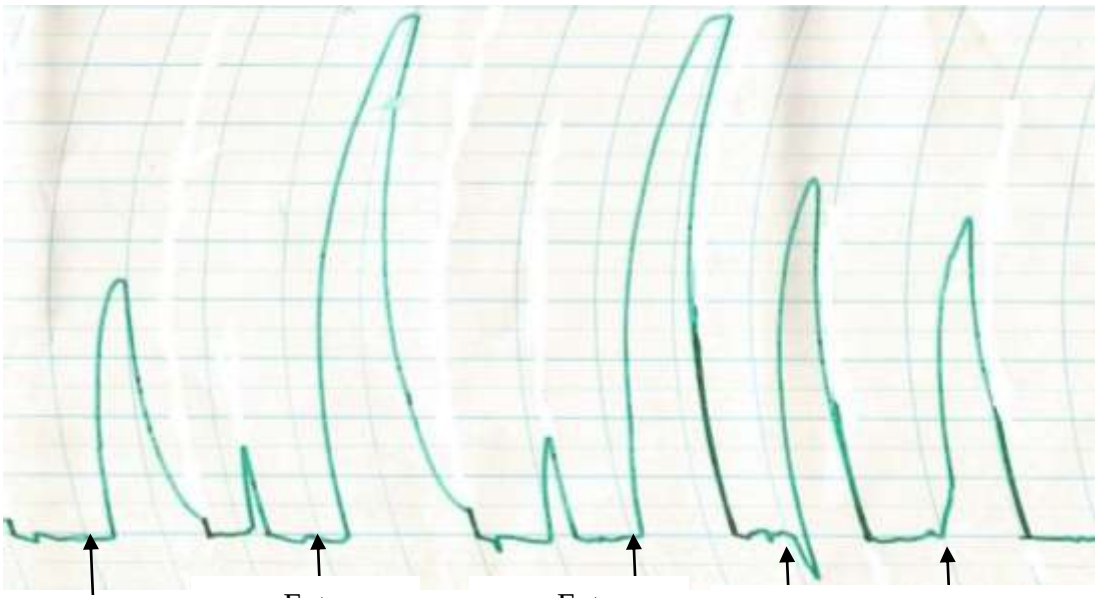
Ext (2.67 mg/ml) + Oxy (1.33 x 10^{-4} IU/ml)

Ext (5.33 mg/ml) + Oxy (1.33 x 10^{-4} IU/ml)

Appendix 2 (B6): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Salbutamol on Pregnant Uterine Strip.



Appendix 2 (B7): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low Concentration of Atropin on Pregnant Uterine Strip



Atropin (3.33×10^{-6}mg/ml)

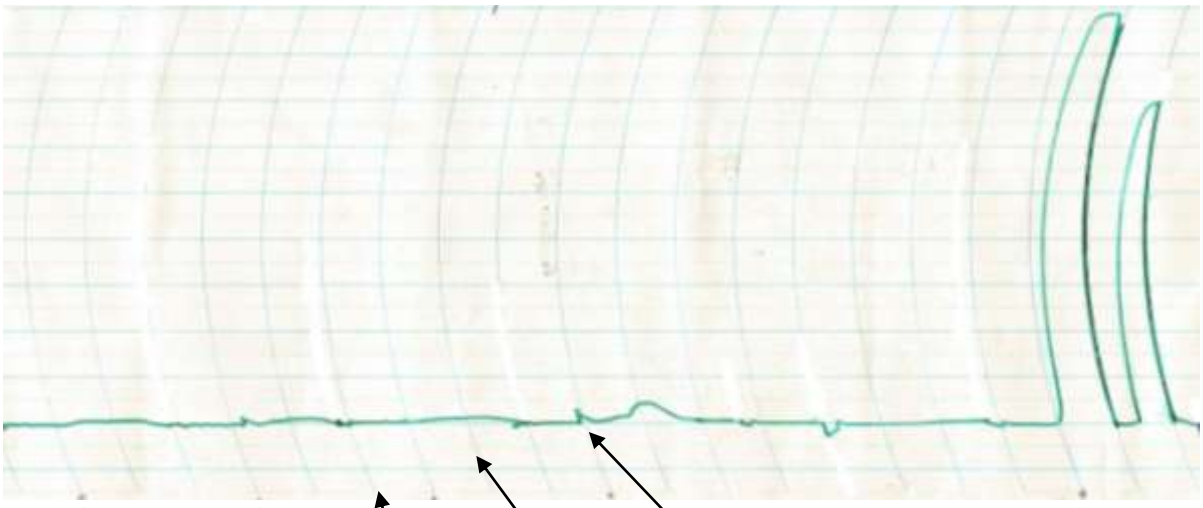
Ext (0.67mg/ml) + Atropin (3.33×10^{-6}mg/ml)

Ext (1.33mg/ml) + Atropin (3.33×10^{-6}mg/ml)

Ext (2.67mg/ml) + Atropin (3.33×10^{-6}mg/ml)

Ext (5.33mg/ml) + Atropin (3.33×10^{-6}mg/ml)

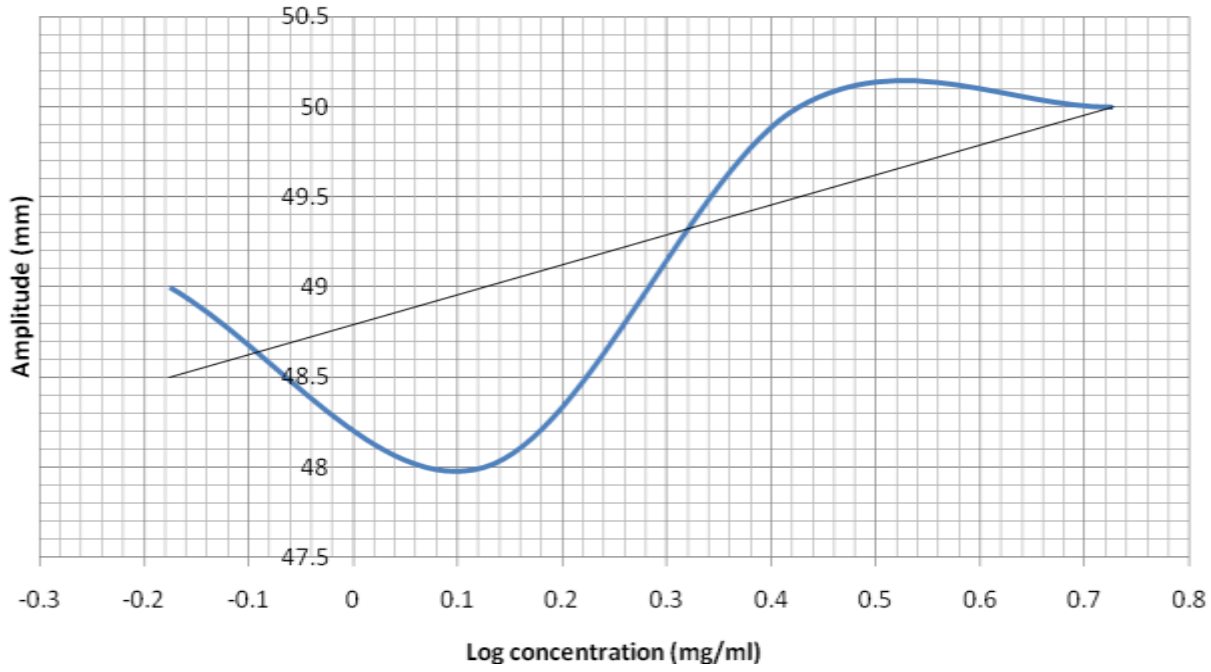
Appendix 2 (B8): Effect of Varying Concentrations of *S. corymbosa* Aqueous Leaf Extract and Single Low concentration of Indomethacine on Pregnant Uterine Strip



APPENDIX 3(A): PRETREATED NON PREGNANT UTERINE TISSUE.

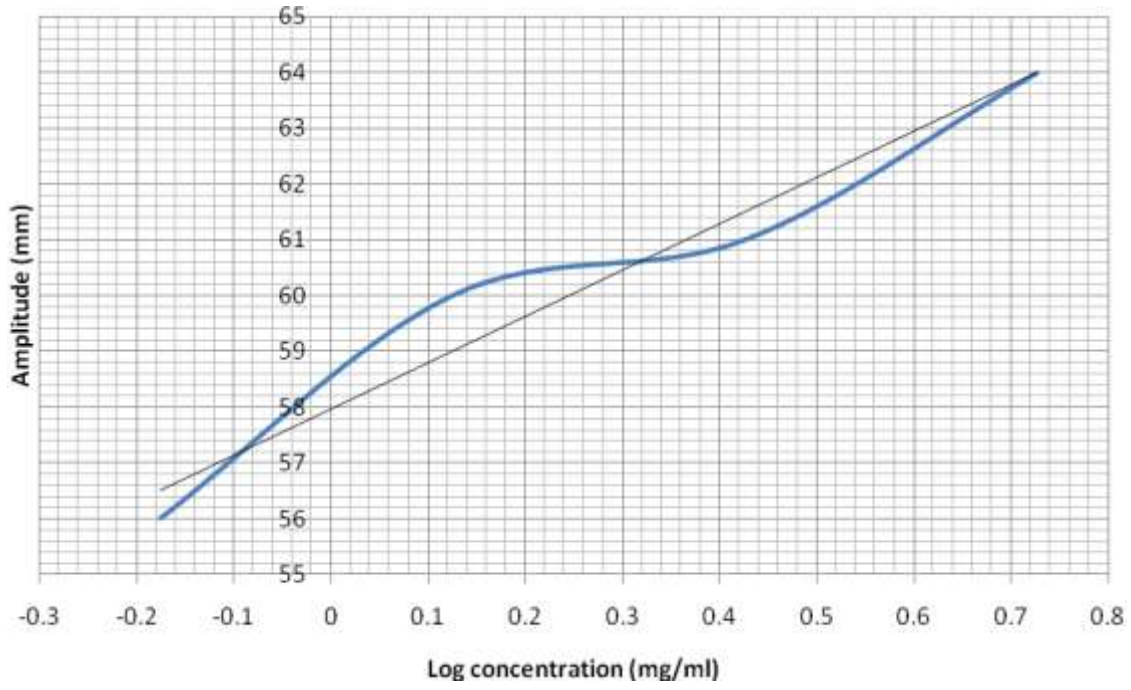
Appendix 3 (A1): EC_{50} of *S. corymbosa* on Pretreated Non Pregnant Uterine Tissue

The log Concentration curve of ***S. corymbosa*** aqueous leaf extract on pretreated non pregnant uterine strip shows effective concentration (EC_{50}) of 2.09 mg/ml and maximum effect (Emax) of 5.25 mg/ml.



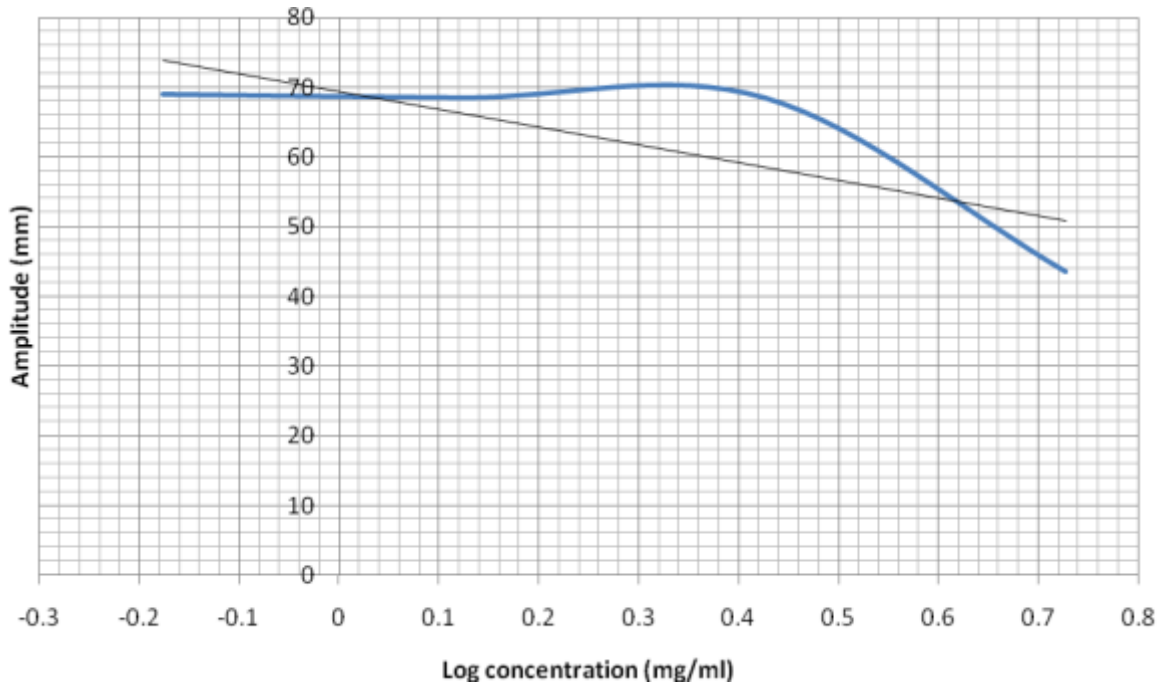
Appendix 3 (A2): EC_{50} of the Interaction of *S. corymbosa* and Acetylcholine on Pretreated Non Pregnant Uterine Tissue.

Similarly, the log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **acetylcholine** on pretreated non pregnant uterine strip also revealed an effective concentration (EC_{50}) of 2.09 mg/ml and maximum effect (Emax) of 5.25 mg/ml, same as the extract alone on pretreated non pregnant rat uterine strip.



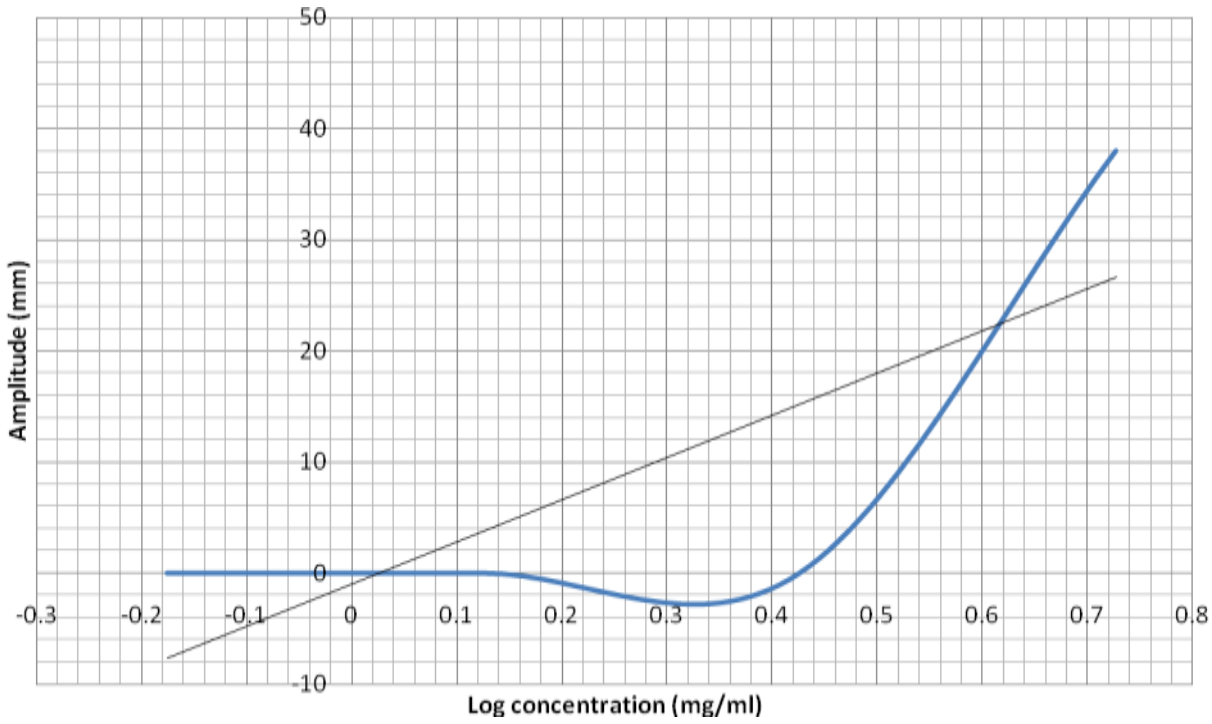
Appendix 3 (A3): EC_{50} of the Interaction of *S. corymbosa* and Oxytocin on Pretreated Non Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **oxytocin** on pretreated non pregnant uterine strip revealed an effective concentration (EC_{50}) of 1.096 mg/ml and maximum effect (Emax) of 3.98 mg/ml. Both were lower than those of the extract alone.



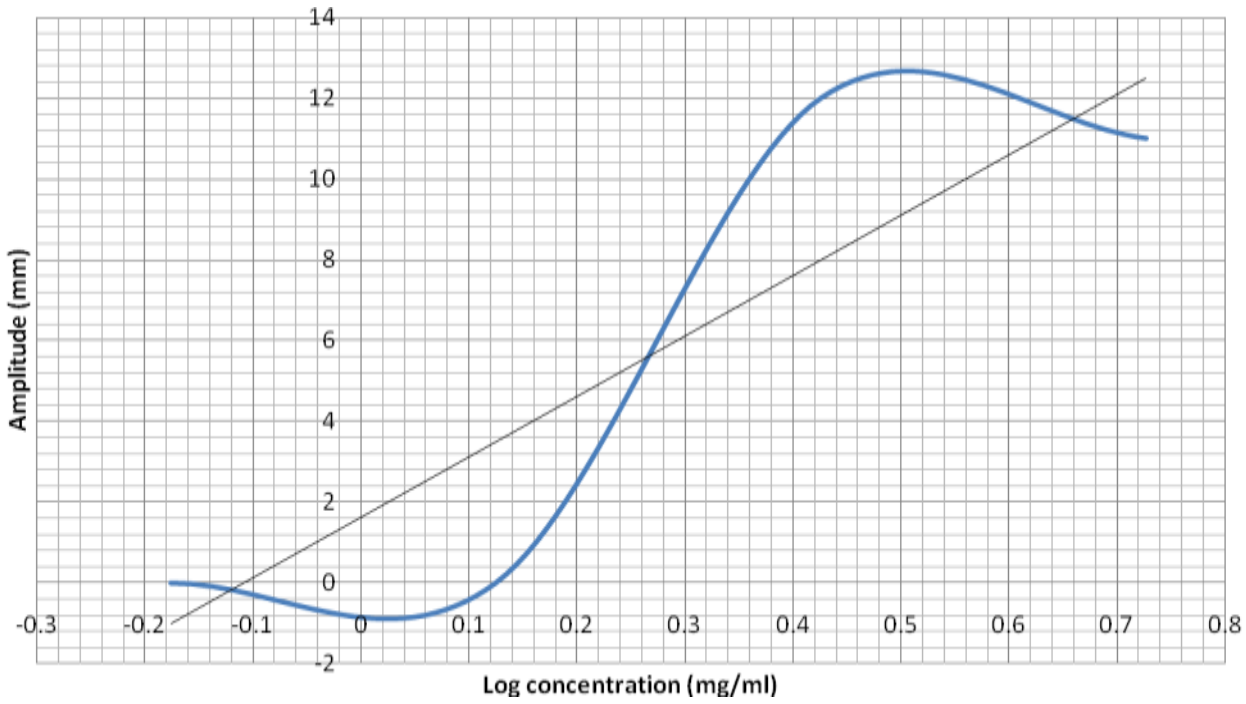
Appendix 3 (A4): EC_{50} of the Interaction of *S. corymbosa* and Salbutamol on Pretreated Non Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **salbutamol** on pretreated non pregnant uterine strip revealed an effective concentration (EC_{50}) of 4.168 mg/ml and maximum effect (Emax) was beyond the range of concentrations used for the study.



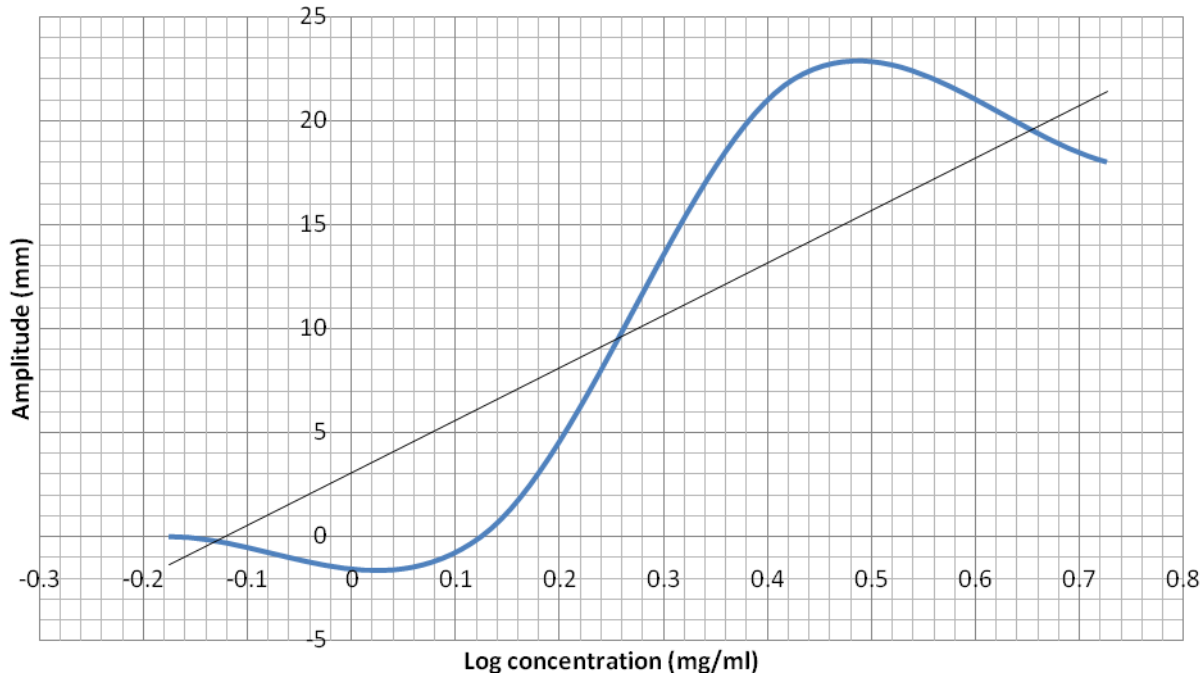
Appendix 3 (A5): EC_{50} of the Interaction of *S. corymbosa* and Atropin on Pretreated Non Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **atropin** on pretreated non pregnant uterine strip revealed an effective concentration (EC_{50}) of 1.906 mg/ml and maximum effect (Emax) of 4.57 mg/ml. Both were noticed to be slightly lower than the concentrations of the extract.



Appendix 3 (A6): EC_{50} of the Interaction of *S. corymbosa* and Indomethacine on Pretreated Non pregnant Uterine Tissue.

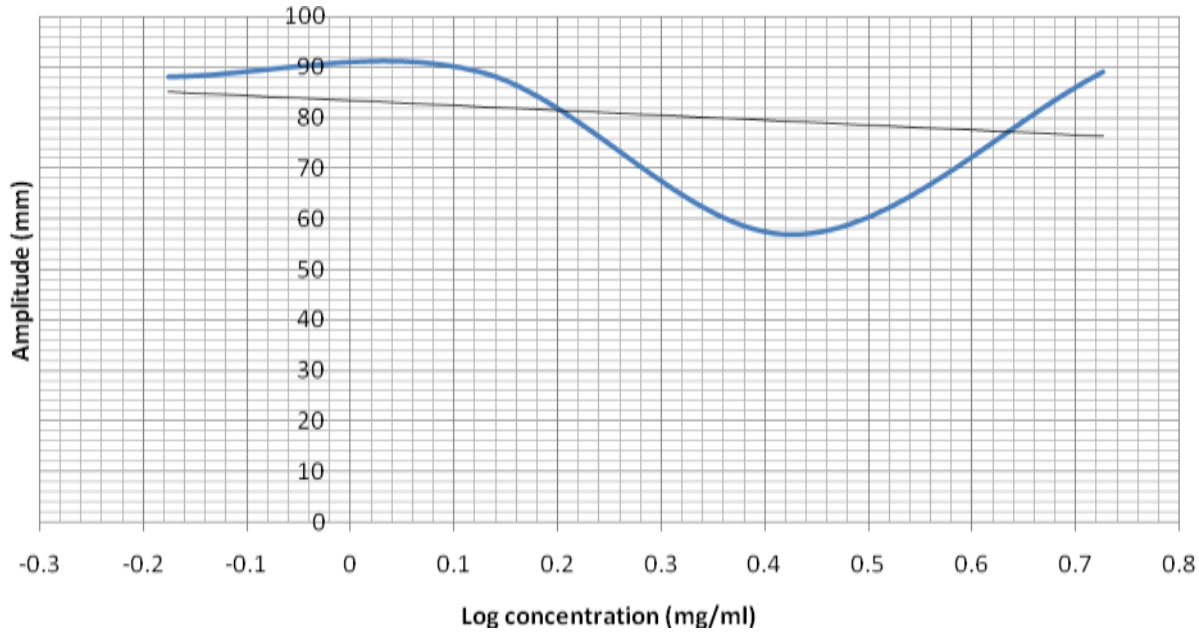
The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **indomethacine** on pretreated non pregnant uterine strip revealed an effective concentration (EC_{50}) of 2.09 mg/ml and maximum effect (Emax) of 5.25 mg/ml. On the pretreated non pregnant uterine tissue the EC_{50} and Emax were unaffected by the presence indomethacine.



APPENDIX 3(B): PREGNANT UTERINE TISSUE.

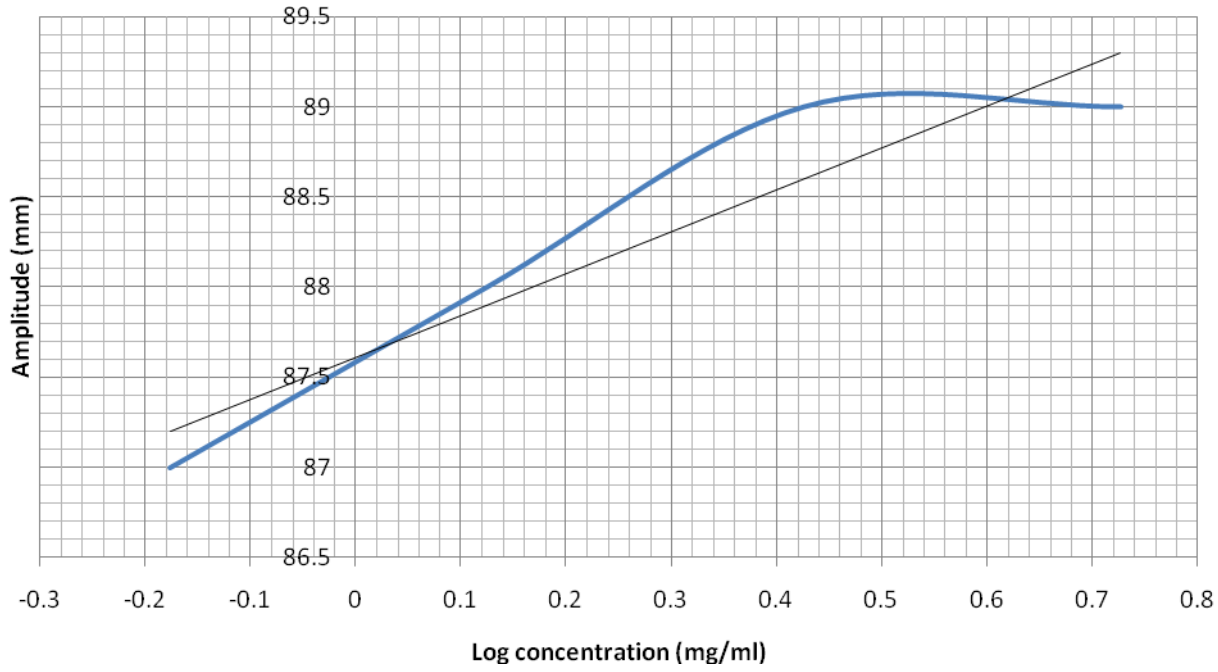
Appendix 3 (B1): EC_{50} of *S. corymbosa* on Pregnant Uterine Tissue.

The log Concentration curve of ***S. corymbosa*** aqueous leaf extract on pregnant uterine strip shows effective concentration (EC_{50}) of 1.58 mg/ml and maximum effect (Emax) of 4.37 mg/ml. It was noticed to be lower than the EC_{50} and Emax of the extract on pretreated non pregnant rat uterine strip.



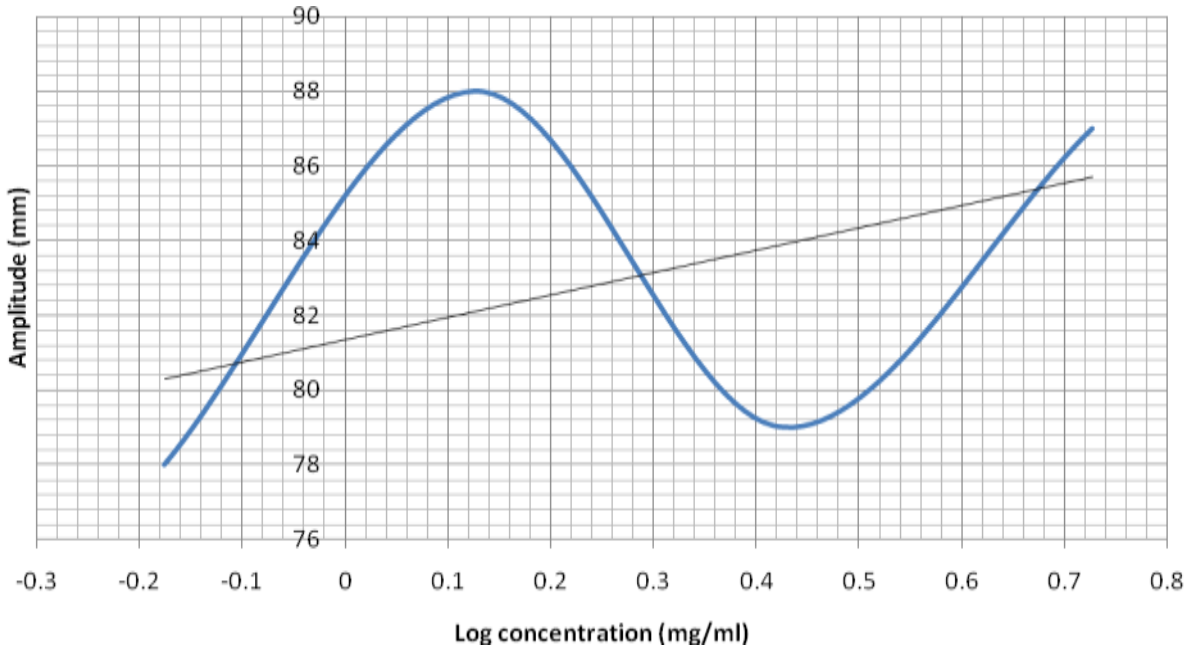
Appendix 3 (B2): EC_{50} of the Interaction of *S. corymbosa* and Acetylcholine on Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **acetylcholine** on pregnant uterine strip revealed an effective concentration (EC_{50}) of 1.05 mg/ml and maximum effective concentration of 3.98 mg/ml. Both concentrations were lower than those of the extract alone on the pregnant and pretreated non pregnant uterine strips.



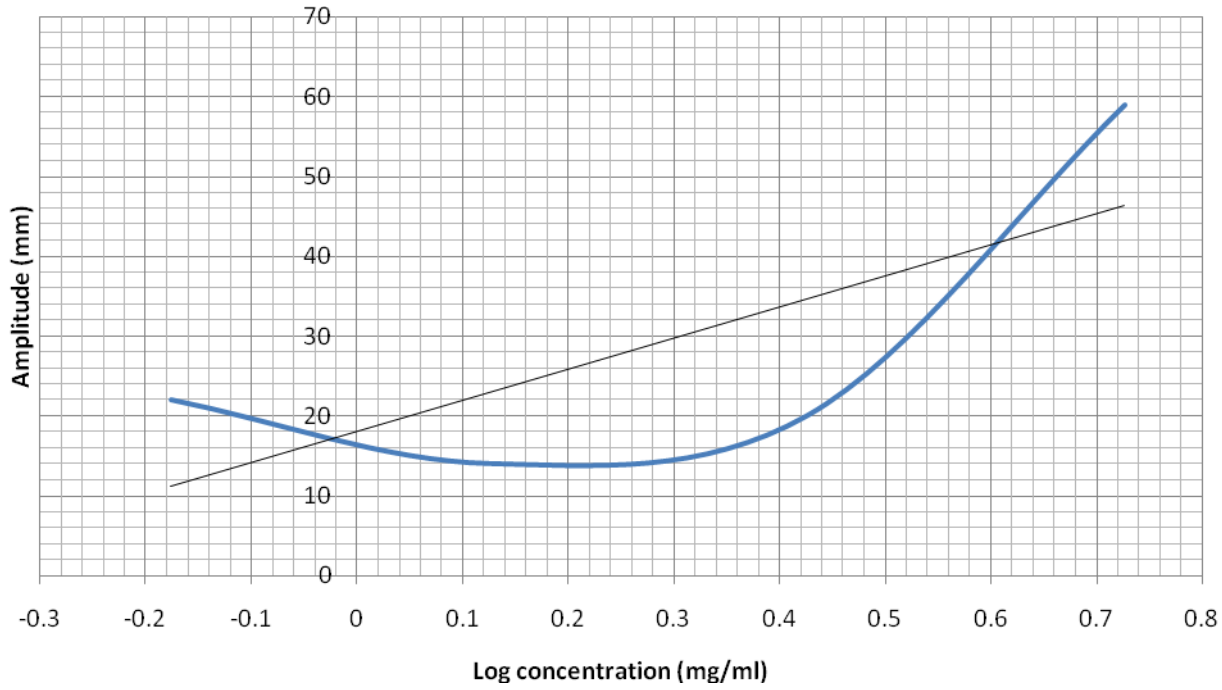
Appendix 3 (B3): EC_{50} of the Interaction of *S. corymbosa* and Oxytocin on Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **oxytocin** on pregnant uterine strip revealed an effective concentration (EC_{50}) of 1.91 mg/ml and maximum effect (Emax) of 4.79 mg/ml. Both were higher than those of the extract alone.



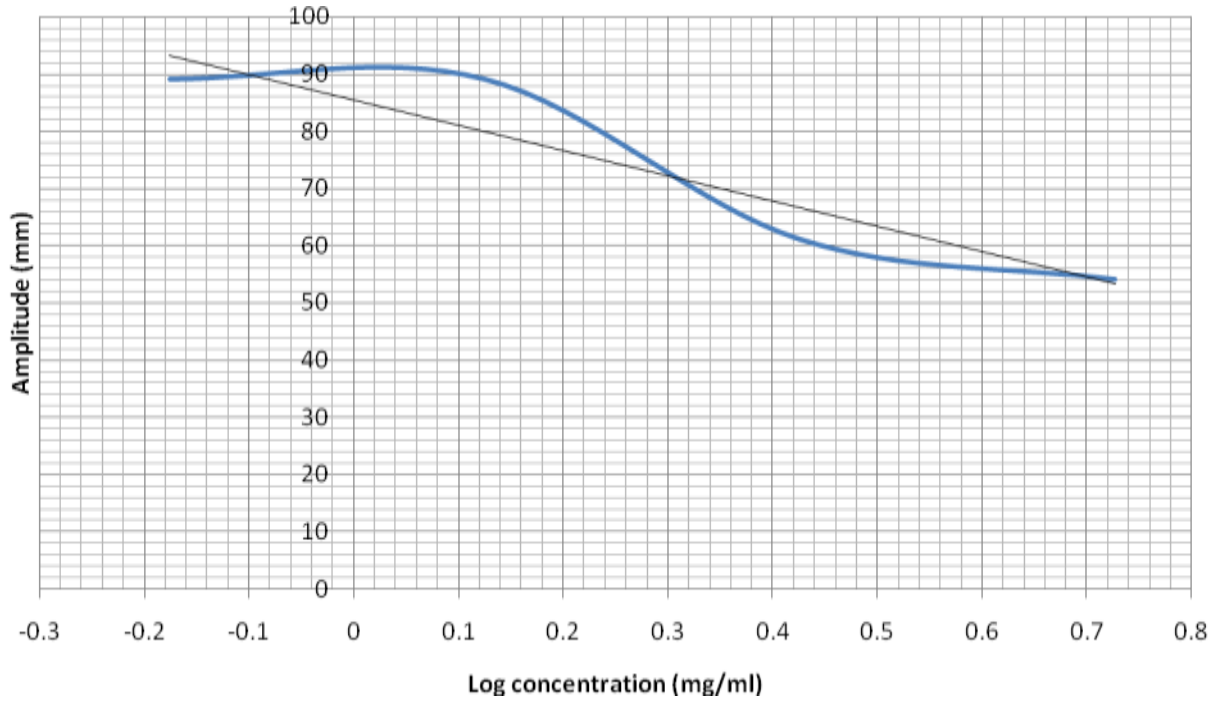
Appendix 3 (B4): EC_{50} of the Interaction of *S. corymbosa* and Salbutamol on Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **salbutamol** on pregnant uterine strip revealed an effective concentration (EC_{50}) of 3.98 mg/ml and maximum effect (Emax) was beyond the range of concentrations used for the study. The EC50 was more than two times that of extract alone on pregnant uterine strip.



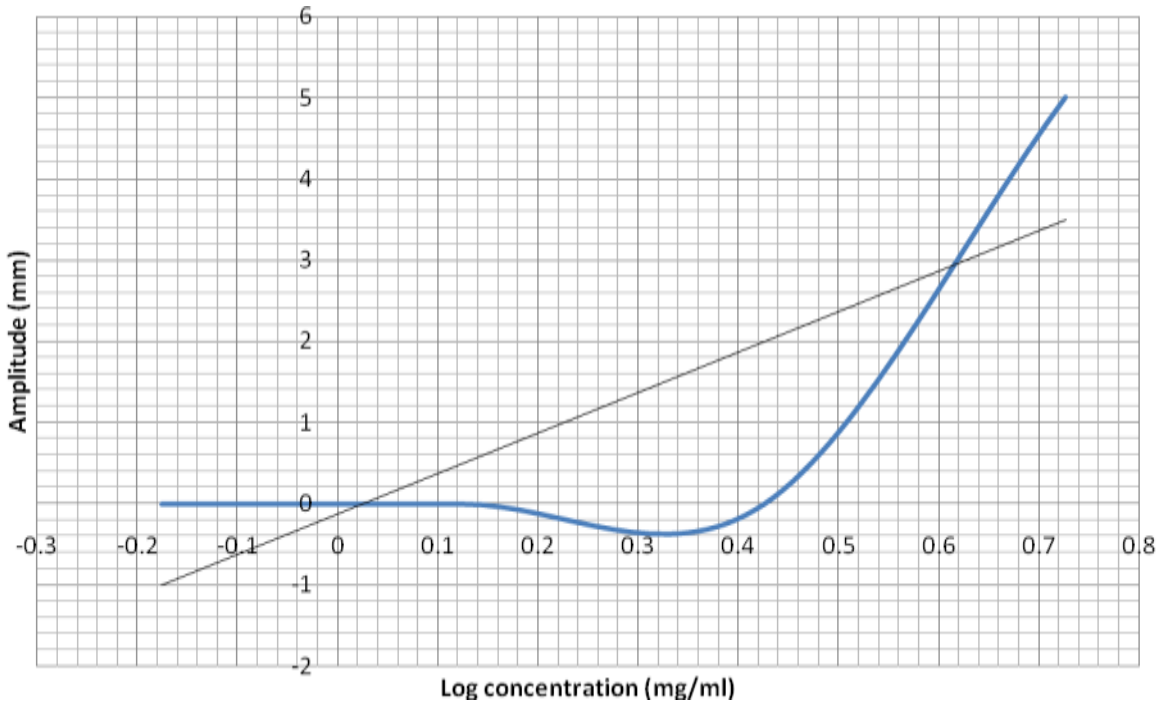
Appendix 3 (B5): EC_{50} of the Interaction of *S. corymbosa* and Atropin on Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **atropin** on pregnant uterine strip revealed an effective concentration (EC_{50}) of 1.20 mg/ml and maximum effect (Emax) of 5.25 mg/ml. Both were noticed to be slightly lower than the EC_{50} and Emax of the extract.



Appendix 3 (B6): EC_{50} of the Interaction of *S. corymbosa* and Indomethacine on Pregnant Uterine Tissue.

The log Concentration curve of varying concentrations of ***S. corymbosa*** aqueous leaf extract and their interactions with single suboptimal concentration of **indomethacine** on pregnant uterine strip revealed an effective concentration (EC_{50}) of 4.37 mg/ml.



Appendix 4 (A1): Effect on Pregnant and Non-Pregnant Uterine Strips

Status of tissues (Amplitude)	Number	Mean (mm)
Non pregnant	32	40.328
Pregnant	32	58.813

The mean of activity elicited was greater on the pregnant uterine tissue than the pretreated non pregnant uterine tissue. The independent sample test p - value was 0.015 and less than α – value of 0.05 at 95% confidence interval can be interpreted to infer that the mean difference in amplitude for pregnant and non pregnant tissues are not the same. Also student t – test value was -2.503.

Levene statistic for test of homogeneity of variances for both groups was found to be 0.417 which is > α-value of 0.05 indicating equality of variance and necessitating the use of parametric test (ANOVA) for further statistical analysis.

ANOVA revealed a p-value of 0.00 which is < 0.05 α-value at 95% confidence interval and inferring that the distribution of amplitude is not the same across categories of drugs used on both tissues.

While, multiple comparisons for the dependent variable (amplitude) was computed using **Bonferroni** test

Appendix 4 (A2):Bonferroni for Multiple Comparisons on Both Pregnant and Non Pregnant Uterine Tissues

(I) Drugs	(J) Drugs	Mean Difference (I-J)	Std. Error	Sig.	95%Confidence Interval	
					Lower Bound	Upper Bound
Extract	Ach	12.6250	9.9256	1.000	-19.937	45.187
	Oxyt	-3.0000	9.9256	1.000	-35.562	29.562
	ext+ach	-9.2500	9.9256	1.000	-41.812	23.312
	ext+oxyt	-7.6875	9.9256	1.000	-40.249	24.874
	ext+salbut	45.8750*	9.9256	.001	13.313	78.437
	ext+atropin	25.5000	9.9256	.361	-7.062	58.062
	ext+indometh	59.3750*	9.9256	.000	26.813	91.937

At α–value 0.05 and 95% confidence interval, there was no significant difference between the activity of the extract, acetylcholine, oxytocin, extract + acetylcholine, extract + oxytocin, and extract + atropine because their P-values were > 0.05. However, there was significant difference between the activity of the extract and extract + salbutamol and extract + indomethacine because their P-values were <0.05.

Appendix 4 (A3): Analysis of Response Amplitudes of the Non Pregnant Uterine Tissue

Amplitude	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ach	4	59.250	7.1356	3.5678	47.896	70.604	52.0	69.0
Oxyt	4	66.250	4.8563	2.4281	58.523	73.977	59.0	69.0
Extract	4	49.250	.9574	.4787	47.727	50.773	48.0	50.0
ext+ach	4	60.250	3.3040	1.6520	54.993	65.507	56.0	64.0
ext+oxyt	4	62.375	12.5855	6.2928	42.349	82.401	43.5	69.0
ext+salbut	4	9.500	19.0000	9.5000	-20.733	39.733	.0	38.0
ext+atropin	4	5.750	6.6521	3.3260	-4.835	16.335	.0	12.0
ext+indometh	4	10.000	11.6619	5.8310	-8.557	28.557	.0	22.0
Total	32	40.328	26.9954	4.7722	30.595	50.061	.0	69.0

The mean activity of the extract on pretreated non-pregnant uterine strip is 49.250; oxytocin had the highest mean activity while the interaction of the extract + atropine had the least mean activity.

One way ANOVA revealed a p-value of 0.00 which is < 0.05 α-value at 95% confidence interval and inferring that the distribution of amplitude was not the same across categories of drugs used on the pretreated non pregnant uterine strip. Multiple comparisons for the dependent variable was computed using **Bonferroni** test.

Appendix 4 (A4): Bonferroni Test for Multiple Comparisons of the Effect of Drugs Non Pregnant Uterine Tissues

(I) Drugs	(J) Drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract	Ach	-10.0000	7.0088	1.000	-34.619	14.619
	Oxyt	-17.0000	7.0088	.649	-41.619	7.619
	ext+ach	-11.0000	7.0088	1.000	-35.619	13.619
	ext+oxyt	-13.1250	7.0088	1.000	-37.744	11.494
	ext+salbut	39.7500*	7.0088	.000	15.131	64.369
	ext+atropin	43.5000*	7.0088	.000	18.881	68.119
	ext+indomethacine	39.2500*	7.0088	.000	14.631	63.869

At α–value 0.05 and 95% confidence interval, there was no significant difference between the activity of the extract and acetylcholine, oxytocin, extract + acetylcholine and extract + oxytocin because their P-values were > 0.05. However, there is significant difference between the activity of the extract and extract + salbutamol, extract + atropin and extract + indomethacine because their P-values were <0.05.

Appendix 4 (A5): Analysis of Response Amplitude of Pregnant Uterine Strip

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ach	4	45.500	23.3595	11.6798	8.330	82.670	28.0	78.0
Oxyt	4	69.750	6.6521	3.3260	59.165	80.335	62.0	78.0
Extract	4	80.750	15.8403	7.9202	55.544	105.956	57.0	89.0
ext+ach	4	88.250	.9574	.4787	86.727	89.773	87.0	89.0
ext+oxyt	4	83.000	5.2281	2.6141	74.681	91.319	78.0	88.0
ext+salbut	4	28.750	20.4512	10.2256	-3.792	61.292	14.0	59.0
ext+atropin	4	73.250	18.4097	9.2048	43.956	102.544	54.0	89.0
ext+indometh	4	1.250	2.5000	1.2500	-2.728	5.228	.0	5.0
Total	32	58.813	31.8863	5.6368	47.316	70.309	.0	89.0

The mean activity of the extract pregnant uterine strip is 80.750; interaction between extract + acetylcholine had the highest mean activity while the interaction of the extract + indomethacine had the least mean activity.

One way ANOVA revealed a p-value of 0.00 which is < 0.05 α-value at 95% confidence interval and inferring that the distribution of amplitude was not the same across categories of drugs used on the pretreated non pregnant uterine strip. Multiple comparisons for the dependent variable was computed using **Bonferroni** test.

Appendix 4 (A6): Bonferroni Test for Multiple Comparisons of the Effect of Drugs Pregnant Uterine Tissues.

(I) Drugs	(J) Drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract	Ach	35.2500	10.1011	.053	-.231	70.731
	Oxyt	11.0000	10.1011	1.000	-24.481	46.481
	ext+ach	-7.5000	10.1011	1.000	-42.981	27.981
	ext+oxyt	-2.2500	10.1011	1.000	-37.731	33.231
	ext+salbut	52.0000*	10.1011	.001	16.519	87.481
	ext+atropin	7.5000	10.1011	1.000	-27.981	42.981
	ext+indomethacine	79.5000*	10.1011	.000	44.019	114.981

At α–value 0.05 and 95% confidence interval, there was no significant difference between the activity of the extract and acetylcholine, oxytocin, extract + acetylcholine, extract + oxytocin and extract + atropin because their P-values were > 0.05. However, there is significant difference between the activity of the extract and extract + salbutamol, and extract + indomethacine because their P-values were <0.05.