STUDIES ON THE NEUROMUSCULAR EFFECTS OF THE EXTRACTS OF ABRUS PRECATORIUS LINN

BY

•SEYI-'LADELE AMOSUN

A thesis submitted to the Postgraduate School, Ahmadu Bello University, in partial fulfillment of the requirements for the degree M.Sc. Pharmacology.

Department of Pharmacology
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria.

September, 1982.

THESIS APPROVAL

This thesis by 'Seyi-ladele Amosun meets the requirements governing the degree of Master of Science (MSc) of Ahmadu Bello University and it is approved for its contribution to scientific knowledge and literary presentation.

External Examiner:

Prof. P. I. Akubue, B.Pharm., Ph.D, M.I. Biol., F.P.S.N.
Professor of Pharmacology and Dean, Faculty of Pharmaceutical Sciences
University of Nigeria
Nsukka.

KASHIM IBRAHIM LIBRARY
RECEIVED
03 JAN 19
ZARIA
AHMADU BELLO UNIVERSITY

Internal Examiners:

1. Dr. Charles Wambebe, B. Pharm, Ph.D.

2. Dr. Duro Adegboye, B.Sc, Ph.D

3. Dr. N. Nwude, Tierarzt, Dr. Med. Vet., Ph.D.

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated by quotation marks and the sources of information are specifically acknowledged by means of references.

.....
SEYI LADELE AMOSUN

COPYRIGHT STATEMENT

"Attention is drawn to the fact that copyright of this thesis rests with the author. This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the prior written consent of the author.

This thesis may be made available for consultation by the University Library and may be photocopied or lent to other libraries for the purposes of consultation".

.....

Dedicated

to the Glory of the Lord

as seen in

'Funmi, 'Toyin, 'Toye & 'Tomi.

ACKNOWLEDGEMENTS

I am very grateful to the Lord who gave me the opportunity to increase in "wisdom and knowledge, and in the fear of the Lord". This opportunity was made available through the help of Prof. G. E. Osuide, Dean, Postgraduate School, as well as the Dean, Faculty of Pharmaceutical Sciences and the Head of Pharmacology Department.

My supervisors also played major roles in making sure that I successfully utilise this opportunity. I am especially grateful to Drs. Charles Wambebe, Duro Adegboye and N. Nwude.

To make a complete honour list is to write another thesis, for I should mention men and women who encouraged me along the way - the technical staff of Pharmacology Department, Malam Jibrin, who collected some of the plant (Abrus precatorius Linn.), my parents in-law, brothers and sisters, Maxwell Kusi-Boateng, who typed this thesis, my wife and kids, plus a lot more who prayed for me.

To everyone, I say the Lord bless and keep you.

ABSTRACT

Abrus precatorius Linn. was reported to be a medicinal plant (Ghosal & Dutta, 1971). The seeds of the plant have been reported to cause paralysis in horses (Nwude, 1979). In order to determine if this plant contributes to paralysis observed in humans at the Physiotherapy Department, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, an investigation was carried out on the neuromuscular effects of the crude water, petroleum ether and ethanol extracts of the seeds, leaves and root of Abrus precatorius Linn. Isolated toad's rectus abdominis, rat's phrenic nerve-diaphragm muscle preparation, as well as chicks were used in this study. Isometric contractions of the muscle preparations were recorded by Ugo Basile recording microdynamometer (model No. 7050) .

Only the ethanol extracts antagonised the effect of acetylcholine on the isolated tissues.. The ethanol extract of the seeds (0.1 - 1.6 mg/ml) inhibited (32.00 - 84.33%) acetylcholine-induced contractions of toad's rectus abdominis mucole. Similarly, the ethanol extract of the leaves (1.0 - 8.0 mg/ml)

inhibited (21.77 - 80.02%) the acetylcholine-induced contraction of the toad's rectus abdominis, while the ethanol extract of the roots (0.5 - 4.0 mg/ml) also inhibited (20.75 - 85.80%) the contraction of the tissue. The antagonism was reversible. On the rat's tissue, the ethanol extract of the seeds (0.1 - 0.8 mg/ml) inhibited (0.1+2 - 77.50%) the contraction of the diaphragm elicited by electrical stimulation of the phrenic nerve. Similarly, the ethanol extract of the leaves (0.5 - 4.0 mg/ml) antagonised (0.99 - 89.83%) the contraction of the diaphragm muscle via phrenic nerve stimulation, while the ethanol extract of the roots (0.3 - *2.4* mg/ml) inhibited (4.89 - 86.47%) the same contraction. The antagonism in each case was potentiated by d-tubocurare and reversed by eserine.

The inhibitory effect of the ethanol extracts on the nerve-induced contraction of the rat's diaphragm was potentiated in the presence of reduced Ca++ (1.25 mM), elevated Mg^{++} (2.50 mM) or reduced K^{+} (0.9 mM).

The hot water extracts of the seeds, leaves and roots, and the petroleum ether extract of the seeds induced spastic paralysis in five day old chick;;. On the other hand, the ethanol extracts of the seeds., leaves and roots caused flaccid paralysis when injected intravenously into chicks. The ethanol extract of the seeds was most toxic, whereas that of the leaves was least toxic on weight basis.

The ethanol extracts showed a similarity to d-tubocurare vis-a-vis the pattern of neuromuscular blockade. The present data indicate that the ethanol extracts of the seeds, leaves and roots of Abrus precatorius possess neuromuscular blocking effects.

TABLE OF CONTENTS

	Page
Title	(i)
Thesis Approval	(ii)
Declaration	(iii)
Copyright Statement	(iv)
Dedication	(v)
Acknowledgements	(vi)
Abstract	(vii)
List of Tables	(xii)
List of Figures	(xv)
Abbreviations and Symbols	(xxiv)
CHAPTER 1.0: INTRODUCTION	1
CHAPTER 2.0: LITERATURE REVIEW	3
2.1 Medicinal uses of Abrus precatorius Linn	3
2.2 Toxicology	4
2.3 Chemistry of Abrus precatorius Linn ...	6
2.4 The Neuromuscular Junction	8
2.5 Neuromuscular Blocking Agents	9
CHAPTER 3.0: MATERIALS AND METHODS	14
3.1 Test Animals	14
3.2 Plant Materials	14
3.3 Extraction Procedures	15

Page

3.4 Drug Solutions 19

3.5 Physiological Solutions 20

3.6 Variation in ion concentration 20

3.7 Isolated tissue preparations 21

3.8 Test on Chicks 26

CHAPTER 4.0: RESULTS 30

4.1 Extraction 30

4.2 Effects of the water extracts on neuromuscular transmission 32

4.3 Effects of the petroleum ether extracts on neuromuscular transmission 38

4.4 Effects of the ethanol extracts on neuromuscular transmission 44

4.5 Influence of variation in concentration of ions on neuromuscular effects of the ethanol extracts 63

4.6 Effects of the extracts on chicks 80

CHAPTER 5.0: DISCUSSION 95

CHAPTER 6.0: SUMMARY AND CONCLUSIONS 101

6.1 Summary 101

6.2 Conclusions 102

REFERENCES 104

APPENDIX 114

LIST OF TABLES

Table No.		Page
Table 3.1	Final concentrations of extracts of *Abrus precatorius* tested on isolated tissues.	25
Table 4.1	Influence of ethanol extract of the seeds of *Abrus precatorius* on Ach-induced contraction of rectus abdominis of toad.	46
Table 4.2	Influence of ethanol extract of the seeds of *Abrus precatorius* on phrenic nerve stimulation of the rat's diaphragm.	48
Table 4.3	Influence of ethanol extract of the leaves of *Abrus precatorius* on Ach-induced contraction of rectus abdominis of toad.	53
Table 4.4	Influence of ethanol extract of the leaves of *Abrus precatorius* on phrenic nerve stimulation of the rat's diaphragm.	55
Table 4.5	Influence of ethanol extract of the roots of *Abrus precatorius* on Ach-induced contraction of rectus abdominis of toad.	59
Table 4.6	Influence of ethanol extract of the roots of *Abrus precatorius* on phrenic nerve stimulation of the rat's diaphragm.	61

Page

Table 4.7 Influence of Ca^{++}, Mg^{++} and K^{+} on the effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm using Kreb's solution. 69

Table 4.8 Influence of Ca^{++}, Mg^{++} and K^{+} on the effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm, using Kreb's solution. 74

Table 4.9 Influence of Ca^{++}, Mg^{++} and K^{+} on the effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm, using Kreb's solution. 79

Table 4.10 Lethal effects of ethanol extract of the seeds of Abrus precatorius on chicks. 86

Table 4.11 Lethal effects of ethanol extract of the leaves of Abrus precatorius on chicks. 87

Table 4.12 Lethal effects of ethanol extract of the roots of Abrus precatorius on chicks. 88

Table 4.13 Relationship between dose and observed % mortality after injecting iv the ethanol extract of the seeds of Abrus precatorius into chicks. 89

Page

Table 4.14 Relationship between dose and observed % mortality after injecting iv the ethanol extract of the leaves of Abrus precatorius into chicks. 90

Table 4.15 Relationship between dose and observed % mortality after injecting iv the ethanol extract of the roots of Abrus precatorius into chicks. 91

LIST OF FIGURES

		Page
Figure 3.1:	Abrus precatorius Linn.	16
Figure 4.1:	The influence of the cold and hot water extracts of the seeds of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis.	33
Figure 4.2:	The influence of cold water extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm.	34
Figure 4.3:	The influence of hot water extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm.	34
Figure 4.4:	The influence of the cold and hot water extracts of the leaves of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis.	36
Figure 4.5:	The influence of cold water extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm.	37

Page

Figure 4.6: The influence of the hot water extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm. 37

Figure 4.7: The influence of the cold and hot water extracts of the roots of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis. 39

Figure 4.8: The influence of cold water extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm. 40

Figure 4.9: The influence of hot water extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm. 40

Figure 4.10: The influence of the petroleum ether extracts of the seeds, leaves and roots of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis muscle. 42

Figure 4.11: The influence of the petroleum ether extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm. 42

Page

Figure 4.12: The influence of the petroleum ether extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm. 43

Figure 4.13: The influence of the petroleum ether extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm. 43

Figure 4.14: The influence of the ethanol extract of the seeds of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis. 45

Figure 4.15: The influence of the ethanol extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm via phrenic nerve stimulation. 47

Figure 4.16: The effect of d-tubocurare (dt; 0.5 µg/ml) and eserine (es; 1.0 ug/ml) on the influence of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of the rat's diaphragm (upper row). The influence of the same extract on the contraction of the diaphragm via direct muscle stimulation (lower row). 50

Page

Figure 4.17: The logarithm (log) concentration response graph for the inhibitory effect of the ethanol extract of the seeds of Abrus precatorius on toad's rectus abdominis and rat's phrenic nerve diaphragm. 50

Figure 4.18: The influence of the ethanol extract of the leaves of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis muscle. 52

Figure 4.19: The influence of the ethanol extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm muscle via phrenic nerve stimulation. 54

Figure 4.20: The effect of d-tubocurare (dt; 0.5 µg/ml) and eserine (es; 1.0 µg/ml) on the influence of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of the rat's diaphragm (upper row). The influence of the same extract on the contraction of the diaphragm via direct muscle stimulation (lower row). 56

Page

Figure 4.21: The logarithm (log) concentration response graph for the inhibitory effect of ethanol extract of the leaves of Abrus precatorius on toad's rectus abdominis and rat's phrenic nerve-diaphragm. 56

Figure 4.22: The influence of the ethanol extract of the roots of Abrus precatorius on Ach (0.5 µg/ml)-induced contraction of toad's rectus abdominis muscle. 58

Figure 4.23: The influence of the ethanol extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm muscle via phrenic nerve stimulation. 60

Figure 4.24: The effect of d-tubocurare (dt; 0.5 µg/ml) and eserine (es; 1.0 µg/ml) on the influence of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of the rat's diaphragm (upper row). The influence of the same extract on the contraction of the diaphragm via direct muscle stimulation (lower row). 62

Page

Figure 4.25: The logarithm (log) concentration response graph for the inhibitory effect of the ethanol extract of the roots of Abrus precatorius on toad's rectus abdominis and rat's phrenic nerve-diaphragm. 62

Figure 4.26: The influence of hypo-Ca^{++} concentration (1.25 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 65

Figure 4.27: The influence of hyper-Ca^{++} concentration (5.0 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 65

Figure 4.28: The influence of hypo-Mg^{++} concentration (0.63 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 67

Figure 4.29: The influence of hyper-Mg^{++} concentration (2.5 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 67

Page

Figure 4.30: The influence of hypo-K^{+} concentration (0.9 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 68

Figure 4.31: The influence of hypo-Ca^{++} concentration (1.25 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 71

Figure 4.32: The influence of hyper-Ca^{++} concentration (5.0 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 71

Figure 4.33: The influence of hypo-Mg^{++} concentration (0.63 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 72

Figure 4.34: The influence of hyper-Mg^{++} concentration (2.5 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 72

Page

Figure 4.35: The influence of hypo-K^+ concentration (0.) mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 73

Figure 4.36: The influence of hypo-Ca^{++} concentration (1.25 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 76

Figure 4.37: The influence of hyper-Ca^{++} concentration (5.0 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 76

Figure 4.38: The influence of hypo-Mg^{++} concentration (0.63 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 77

Figure 4.39: The influence of hyper-Mg^{++} concentration (2.5 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 77

Page

Figure 4.40: The influence of hypo-K^+ concentration (0.9 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm. 78

Figure 4.41: 50% lethal dose of the ethanol extract of the seeds of Abrus precatorius. 92

Figure 4.42: 50% lethal dose of the ethanol extract of the leaves of Abrus precatorius. 93

Figure 4.43: 50% lethal dose of the ethanol extract of the roots of Abrus precatorius. 94

ABBREVIATIONS AND SYMBOLS

Ach	acetylcholine
$\overset{o}{A}$	angstrong unit
Ca^{++}	calcium ion
$CaCl_2$	calcium chloride
CO_2	carbon dioxide
Cm	centimetre
dt	d-tubocurare
^oC	degree centigrade
es	eserine
g	gram
>	greater than
ip	intraperitoneal
iv	intravenous
<	less than
l	litre
Mg^{++}	magnesium ion
$MgSO_4 7H_2O$	magnesium sulphate
µg	microgram
mg	milligram
mg/kg	milligram per kilogram
mg/ml	milligram per millilitre

ml	millilitre
mm	millimetre
mm/min	millimetre per minute
mM	millimole
M	molar
O_2	oxygen
%	percent
K^+	potassium ion
KCl	potassium chloride
KH_2PO_4	potassium hydrogen phosphate
p	propability
Na^+	sodium ion
NaCl	sodium chloride
Na HCO_3	sodium bicarbonate
Na $H_2PO_4.2H_2O$	sodium hydrogen phosphate
v/v	volume per volume
w/v	weight per volume

CHAPTER 1.0

INTRODUCTION

Cases of skeletal muscle paralysis probably as a result of taking medicinal plants or feeding on some local plants have been observed by the author at the Physiotherapy Department, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. It was therefore necessary to investigate some of the more common medicinal plants for their neuromuscular activities.

Nwude (1979) had reviewed the poisonous plants in Nigeria. He reported that the plant Abrus precatorius caused paralysis in horses. It was also reported by Dalziel (1937) as a blood and nerve poison. Since parts of this plant are used for medicinal purposes (Ghosal & Dutta, 1971) and the plant is common in Zaria area, it was decided to study the neuromuscular effects of the plant.

The main objective of this study therefore is to examine the neuromuscular effects of the water, petroleum ether and ethanol extracts of the seeds, leaves and roots of Abrus precatorius. It will be determined if the extracts of the plant block or enhance transmission of nerve impulses across the neuromuscular junction using isolated rectus abdominis of toad and isolated phrenic nerve-diaphragm preparation of the rat. It will also be determined

if the plant produces its intoxication via direct skeletal muscle paralysis or indirectly via a central mechanism using chicks. In the case of the former, the nature of the paralysis will be examined.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Medicinal Uses of *Abrus precatorius* Linn.

According to Ghosal and Dutta (1971), almost every part of *Abrus precatorius* (Leguminosae) has been used in indigenous medicine. For example, the leaves have been used in the treatment of biliousness, leucoderma, itching and skin diseases. The plant is also used in treatment of cough (Akinloye & Adalumo, 1980). Watt and Breyer-Brandwijk (1962) quoted the report by Gilges (1953) that the Luvale tribe of East Africa have used the vapour from the crushed leaf in boiling water as a steam bath for inflammed eyes. The leaves have also been used in East Africa as a vegetable and as medicine for stomach complaints (Watt & Brayer-Brandwijk, 1962). According to information obtained by the author, the Yorubas in Nigeria add the ground leaves to pap, as a sweetener.

The seeds have been used in the treatment of opthalmia, snake bite, ulcers and intestinal worms (Ghosal & Dutta, 1971). The powdered seed was used among various tribes in Central Africa as an oral contraceptive, the dose being approximately 200 mg.

The effect of a single dose was reported to continue for thirteen menstrual cycles (Watt & Brayer-Brandwijk, 1962). Carboneschi (1947) reported that the infusion of the seeds was used in Brazil for treatment of conjuctivitis granulosa. In addition, the seeds were used in East Africa for treating veneral diseases (Watt & Breyer-Brandwijk, 1962). Information collected by the author revealed that the black spot on the seed resemble the pupil of the eye and are therefore ingredients in charms to ensure invisibility among the Yorubas of Nigeria.

The root was reportedly used as a remedy against snake bite in East Africa, while the fresh roots are chewed to induce aphrodisiac effect. The roots also possess emetic properties. A decoction of the whole plant with three or four pods was reportedly drunk by the Yao tribe of East Africa in the treatment of gonorrhoea (Watt & Breyer-Brandwijk, 1962).

2.2 Toxicology

The seeds have been known to be highly poisonous and were reported to be encountered in cases of criminal poisoning (Subramania et al, 1973). If the seeds are swallowed whole, poisoning is unlikely,

since the hard outer coat is unaffected by digestive secretions. If chewed, the core of the seed comes in contact with the intestinal secretions resulting in disintegration, absorption and possible poisoning (Arena, 1974). Reported symptoms of human poisoning include nausea, vomiting, severe diarrhoea, weakness, cold perspiration and trembling of the hands. In horses, violent purging, incoordination and paralysis were reported (Nwude, 1979).

According to Desai and Rupawala (1967), the steroidal oil fraction obtained from the petroleum ether extract of the seeds of Abrus precatorius indicated antifertility activity. Oral feeding with 150 mg of the oil daily for 20 consecutive days, prior to mating, induced 100% sterility. A single dose of 150 mg of the oil daily for one to five days in the post coital period, also produced 80% sterility in rats. Similarly, marked antifertility effects were also observed in mice.

Anti microbial activity of the seeds was also reported (Desai & Sirsi, 1966). The ethanol extract of the total seed was reported to inhibit the growth of a number of bacteria including Staphylococcus aureus and Escherichia coli. Dalziel (1937) suggested that

the seed is poisonous to blood and nerve cells.

Akinloye and Adalumo (1980) described the leaves of Abrus precatorius as almost as sweet as sucrose but advised against its use as a liquorice substitute until it is ascertained that the toxic principles of the seeds are absent in the leaves.

Antifertility activity was also reportedly exhibited by the roots of Abrus precatorius. The petroleum ether and alcoholic extracts of the roots, given orally to rats at 100 mg/kg per day, one to five days post coitus, prevented nidation. The alcoholic extract also showed antiestrogenic activity when given orally. The oral LD50 of the alcoholic extract was 2 g/kg (Agarwal et al, 1970). The plant was also used as arrow poison in hunting (Watt & Breyer-Brandwijk, 1962).

2.3 Chemistry of Abrus precatorius Linn.

Toxic principles that have been identified in the seeds include abric acid and abrin, a protein toxin (Watt & Breyer-Brandwijk, 1962). Olsnes and Pihl (1973) reported that abrin was made up of two constituent peptide chains. As little as 1.0 ng of the smaller peptide was reported to strongly inhibit protein

synthesis in a cell-free system from rabbit reticulocytes. On the other hand, 10.0 ng of the larger peptide gave no significant inhibition of protein synthesis, and 100.0 ng gave only a very slight inhibition.

Ghosal and Dutta (1971) have shown that the major alkaloidal constituents of the seeds are abrine (N-methyltryptophan), precatorine and hypaphorine. The occurence of some other alkaloids were also reported, namely trigonelline, choline and methyl ester of N, N-dimethyltryptophan metho cation. Apparently, these alkaloids are also present in the leaves and roots of Abrus precatorius (Ghosal & Dutta, 1971).

The leaves and the roots are reported to contain glycyrrhizin, a natural sweetener (Watt & Breyer-Brandwijk, 1962). In 1980, Akinloye and Adalumo put the yield of glycyrrhizin in the leaves at 9% w/w. Morris (1976) reported that glycyrrhizin is about 50 times sweeter than sucrose, and that it has long been used as a flavouring agent. The major component of glycyrrhizin is reported to be an acidic triterpene glycoside, having a structural similarity to the corticosteroids. High doses of glycyrrhizin have been reported to exert steroidal effects on humans (Morris, 1976).

2.4 The Neuromuscular Junction

The first demonstration of the nerve-muscle junction as a distinct entity by Claude Bernard (1856) was reported by Hubbard (1972). Claude Bernard used the South American Indian arrow poison, curare. He found that curarised muscle contracted normally when stimulated directly, but did not contract when its nerve was stimulated. Since the nerve appeared to conduct impulses towards the region of the nerve-muscle junction, it was deduced that there was a block of transmission in the most distal part of the nerve where contact was made with the muscle. This region is the neuromuscular junction.

The neuromuscular junction is characterised by a short distance separating the membrane of the nerve from the muscle membrane. The nerve endings occupy a gutter hollowed out of the muscle surface, with an extracellular space between the nerve endings and the muscle membrane (Couteaux, 1972). This space is the synaptic cleft, and its width is about 100 - 500 $\mathring{A}$. The axon terminal contains many mitochondria, and numerous bodies, about 200 - 600 $\mathring{A}$ in diameter, called synaptic vesicles (Couteaux, 1972).

The main activity at the neuromuscular junction is the transmission of impulses from the nerve terminal, across the synaptic cleft, to the outer surface of the muscle membrane, resulting in muscular activity. It is universally agreed that transmission at the neuromuscular junction of skeletal muscles is chemical and is mediated by acetylcholine (Dale, 1936). Katz and Miledi (1968) pointed out the significant role of calcium ions in the release of acetylcholine in response to nerve membrane depolarisation. Katz and Miledi (1967b), using frog sartorius muscle, showed the release of acetylcholine in the absence of external sodium ions, when the sodium ions in the bath had been totally replaced by calcium ions.

2.5 Neuromuscular Blocking Agents

Neuromuscular blockade may be effected through a disturbance affecting either the presynaptic or the postsynaptic parts of the neuromuscular junction (Cheymol & Bourillet, 1972). Presynaptic block may be due to failure of synthesis of acetylcholine, which occurs if the nerve is poisoned by hemicholinium, or failure of release of acetylcholine due to the presence of magnesium, or absence of calcium ions (Cheymol & Bourillet, 1972).

Postsynaptic block may be competitive or non-competitive. The competitive or non-depolarising block is one in which the blocking agent competes with acetylcholine for the nicotinic receptor sites on the postsynaptic membrane without stimulating the receptors (Thesleff, 1958). The block can be overcome in the presence of excess acetylcholine or any other depolarising drug at the neuromuscular junction. Examples of drugs with competitive blocking action against acetylcholine are d-tubocurare, gallamine, pancuronium and dimethyltubocurare.

The non-competitive or depolarising blocking agent produces an initial positive effect. Such an agent depolarises the postsynaptic membrane in the same manner as acetylcholine, but its effect is more persistent. This results in a brief period of firing manifested by transient muscular fasciculation. This phase is succeeded shortly by neuromuscular paralysis. Decamethonium, suxamethonium, and hexamethonium are examples of depolarising blocking agents (Koelle, 1980).

A number of poisonous agents from both animal and plant sources have been implicated in muscle paralysis. For example, tetradotoxin which is extracted from the viscera of Japanese salt water fish belonging to the genus Fugu of the tetradontid family, blocks nerve

conduction along the axon (Cheymol & Bourillet, 1972). Katz and Miledi (1967a) reported that tetradotoxin, while blocking the excitation of the postsynaptic membrane by acetylcholine, did not interfere with acetylcholine release from nerve endings.

Botulinum toxin, a protein neurotoxin produced by the bacteria Clostridium botulinum, blocks the release of acetylcholine from the cholinergic nerve terminal (Ambache, 1949). According to Su et al. (1966), high concentrations of the crude venom from cobra snakes diminished the acetylcholine output of the rat phrenic nerve-diaphragm, but the purified neurotoxin lacked such an effect. In 1966, Chang and Lee reported that the characteristics of the neuromuscular blockade by the neurotoxin are essentially the same as those of d-tubocurare, although the neurotoxin acted much more slowly and less reversibly than d-tubocurare. Saxitoxin, obtained from the shell-fish Saxidomus giganteus, is another neurotoxin having neuromuscular effects similar to those of tetradotoxin (Cheymol & Bourillet, 1972).

Another example of the poisonous agents is the venom of the tick Ixodes damini. This tick infests humans in certain areas of the United States of America, and

have been reported to result in paralysis of the face, arms and legs, plus other manifestations. The disease-causing agent in such ticks is still a puzzle (Meyers, 1980).

The presence of certain ions in the extracellular fluid can block, inhibit or enhance neurotransmission (Debecker, 1972). The presence of sodium and potassium ions are essential in the development of action potentials in the nerve (Katz, 1952). Calcium ions are involved in the release process of acetylcholine (Katz & Miledi, 1968). When calcium ion concentration is increased, the quantal content of the end-plate potential increases in the mammalian neuromuscular junction (Hubbard et al, 1968 a). When magnesium ion is present in the extracellular fluid, its action is prejunctional in reducing the output of acetylcholine from the motor nerve ending (Engbaek, 1972). It should be noted that the effects of calcium and magnesium ions are mutually antagonistic (Hubbard et al, 1968 b). In the synapse of the giant squid, manganese ion has been reported to have acted as a strong and apparently competitive antagonist to calcium ions in the process of transmitter release (Katz & Miledi, 1969).

Some ions have been reported to influence the neuromuscular property of botulinum toxin. For example,

Simpson (1971) showed the influence of potassium, calcium and magnesium ions. Potassium ions augmented the toxin-induced paralysis whereas high magnesium ion concentrations or omission of calcium ions hindered the toxin-induced paralysis on rat phrenic nerve-diaphragm preparation (Simpson, 1971). Also, Simpson (1973) studied the effects of calcium, strontium and magnesium ions on botulinum toxin (type A) - paralysis of rat phrenic nerve-hemidiaphragm preparations. He observed that the onset of botulinum toxin activity was dependent upon the release of acetylcholine. Thus calcium ions or, to a lesser degree, strontium ions, which partake in the process of acetylcholine release, initiated the activity of the toxin. The effect of strontium ions in triggering off the activity of the toxin was antagonised by magnesium ions. High levels of calcium and strontium ions caused prolongation of the period of paralysis of the isolated tissue preparation (Simpson, 1973).

CHAPTER 3.0

MATERIALS AND METHODS

3.1 Test Animals

Adult albino rats, Wister strain (male and female), were inbred in the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. Chicks were obtained from the Agricultural Enterprises Nigeria Limited, Zaria, Nigeria, while toads (Bufo regularis) were obtained from water ponds in the university.

3.2 Plant Materials

Abrus precatorius (family: Leguminosae, Figure 3.1) can be found in various parts of Nigeria where it is known locally as "Idon Zakara" in Hausa, "Ayanwono" in Igbo, or "Oju-ologbo" in Yoruba. The seed of this plant is also referred to as crab's eye, precatory bean, rosary pea or jequirity bean. This small climbing plant is a native of India but it is found in a number of other tropical countries. It is a twining perennial vine, climbing on other plants to a height of 3.0 - 6.0 metres. The stem is green when young, but changes to a grey bark when older. The alternate and compound leaves are about 6 - 15 mm long and 4 - 8 mm broad, and are oblong in shape. The fruit is a legume pod, about 2½ cm long or

less, and is covered with fine appressed hairs, and contains many seeds. The seed is the most distinctive feature of the plant. Each seed is ovoid, having a bright glossy scarlet colour with a black spot (Kingsbury, 1964; Nwude, 1979).

The seeds, leaves and roots of Abrus precatorius were collected from a garden near the Kubani Dam, Ahmadu Bello University, Zaria, Nigeria. The parts of the plant collected were dried to constant weight in an oven (50°C) and ground . The pulverised materials were kept in the refri gerator until ready for usc.

3.3 Extractions

3.3.1 Water Extraction

40.0 g of ground seeds was extracted in 120.0 mls of cold distilled water (about 25°C) for 24 hours. Another 40.0 g of the ground seeds was extracted in 120.0 mls of boiling distilled water for one hour.

To obtain the cold and hot water extracts for the leaves, a similar procedure to the seeds was followed. 20.0 g of the dried and ground leaves was extracted in 100.0 mls of cold distilled water (about 24°C). The same weight of the leaves was extracted in 100.0 mls of boiling distilled water.



Figure 3.1 Abrus precatorius Linn.

10.0 g of dried, pulverised roots was extracted in 50.0 mls of distilled water (about 24°C). An equal weight of another portion of the roots was again extracted in 50.0 mls of boiling distilled water.

In all the cases above, the solid particles were filtered off through a filter paper, and the weight and volume of the filtrate were measured. The weight of the extract in the solution was obtained using the following process:-

Weight of raw material used = x g

Weight of filtrate = y g

Volume of filtrate = z mls

On the basis that the specific gravity of water is one (1.0),

i.e. 1 ml of water ≡ (equivalent to) 1 g of water

∴ weight of crude extract (K) in filtrate

$$\simeq (y - z)\ g$$

$$\% \text{ yield} = \frac{K}{X} \times 100\%$$

3.3.2 Petroleum Ether Extraction

100.0 g of the ground seeds was extracted in petroleum ether under reflux, in a soxhlet, for 24 hours. Following the same procedure, 400.0 g of dried and ground leaves, and 50.0 g of dried, pulverised roots were extracted separately, in petroleum ether. The different

petroleum ether solutions were left in separate dry glass containers, in a fume cupboard, allowing the petroleum ether solution to evaporate spontaneously. The weights of the extracts were measured.

3.3.3 Ethanol Extraction

Ground seeds of Abrus precatorius (400.0 g) were extracted with 90% v/v ethanol at 60° - 70°C, under reflux, in a soxhlet, for 24 hours. The alcoholic solution was concentrated by evaporation at reduced pressure in a rotatory evaporator. The now very concentrated solution was put in a crucible and kept in a desicator (containing silica gel), to evaporate to dryness.

Following similar procedure as for the seeds, 500.0 g of the dried, ground leaves, and 25.0 g of the dried, ground roots were extracted separately in ethanol (90% v/v). The weights of the extracts were measured.

The extractions were repeated in similar proportions when the extracts were required.

3.4 Drug Solutions

3.4.1 Preparation of Drug Solutions

Stock solutions of the crude ethanol extracts, and the petroleum ether extract of the leaves, were prepared by dissolving a known weight of each extract in a known volume of 0.9% w/v saline solution. Stock solutions of the petroleum ether extracts of the seeds and roots were made with 5% acacia, in a ratio of 4:2:1 of the extract: respective physiological fluid: acacia. Solutions of some reference drugs were also prepared. These were solutions of acetylcholine, d-tubocurine, and eserine (all from Sigma Chemical Co.). Stock solutions of acetylcholine, d-tubocurine and eserine were made by dissolving, in each case, a known weight of the drug in a known volume of normal saline (0.9% w/v NaCl) solution.

3.4.2 Storage of Drug Solutions

Due to the instability of some of the reference drugs used in this project, fresh drug solutions were made for each day's work. The stock solutions of the test extracts were kept in air-tight amber-coloured bottles and stored in the refri gerator until ready for use.

3.5 Physiological Solutions

The Frog Ringer's solution was used for the toad's tissue (Burn, 1952), while Kreb's solution was used for the rat's tissue (Bulbring, 1946).

The concentrations of solutes in Frog Ringer's solution (g per litre of solution) were NaCl = 6.5, KCl = 0.14, $NaH_2PO_4.2H_2O$ = 0.01, D'Glucose = 2.0, $NaHCO_3$ = 0.4, and $CaCl_2$(M) = 0.12.

Also, the concentrations of solutes in Kreb's solution (g per litre of solution) were NaCl = 6.9, KCl = 0.35, $MgSO_4.7H_2O$ = 0.29, KH_2PO_4 = 0.22, D'Glucose = 2.0, $NaHCO_3$ = 2.1, and $CaCl_2$ = 0.28.

3.6 Variation in Ion Concentration

The influence of K^+, Na^+, Ca^{++}, Mg^{++} on the effects of the extracts on neuromuscular transmission was studied. The concentrations of each ion (hypo- and hyper concentrations) were varied in the physiological solution for each of the tissues used.

To obtain the hypo-concentration of one of the ions, the weight of each salt of the particular ion is reduced to half of the weight of the salt in the normal physiological solution.

For the hyper-concentration of the same ion, the weight of each salt of the ion is increased to twice the weight of the salt in the normal physiological solution.

3.7 Isolated Tissue Preparations

3.7.1 Rectus abdominis of Toad (Burn, 1952; modified).

The toad was stunned and decapitated, and the spinal cord was destroyed with a probe. Placing the toad in supine position, the skin of the abdomen was cut to expose the whole of the abdomen. The two rectus abdominis muscles were seen. A piece was cut from one of them and transferred into a petri dish containing freshly prepared Frog Ringer's solution, and aerated with ordinary air.

After the tissue was freed of fatty materials, a thread was sewn through one end of the tissue, and attached to the aerator. To the other end of the tissue, a longer thread was sewn for attachment to the transducer (Ugo Basile). The preparation was then transferred into the tissue bath (containing 20 mls of the physiological fluid), and the longer thread was tied to the transducer. The tissue was continously aerated with air. The temperature of the tissue bath was

maintained at the room temperature of about 28°C. The preparation was left in the tissue bath to equilibrate for 30 minutes before studying the effects of drugs on it.

3.7.2 Phrenic nerve-diaphragm Preparation (Bulbring, 1946; modified).

A rat was killed by a blow on the head, its throat cut and left to bleed. The skin was removed from the middle of the chest. The muscles were freed from the chest wall, and the ribs cut alongside the base of the sternum on both sides of the animal. With the upper part of the thorax completely removed, the phrenic nerve was seen running from the diaphragm up to the thymus gland. A thread was tied to the nerve close to the thymus gland. An incision was made in the abdominal wall just below the diaphragm. A fan-shaped segment of the diaphragm was cut with the phrenic nerve attachment left intact. The nerve was then cut below the thymus, and the whole preparation was transferred into a petri dish containing Kreb's solution and aerated with 95% O_2 and 5% CO_2.

The base of the fan-shaped muscle piece was attached to the electrode with a thread. Also the nerve was

connected to the electrode. A longer thread was sewn through the apex of the tissue, to be attached to the transducer (Ugo Basile). The whole preparation was then transferred into the organ bath (containing 100 mls of the Kreb's solution), and the longer thread attached to the transducer. The temperature of the organ bath was maintained at 37°C using a thermostatically-controlled water circulator. The preparation was also left in the organ bath for 30 minutes to equilibrate before studying the effects of drugs on it.

3.7.3 Recording Apparatus

The Ugo Basile recording microdynamometer (model No. 7050) was used to record the isometric contractions of the various isolated muscle preparations. The speed of the recorder was set at 24 mm/min. The stimulating parameters for the phrenic nerve were 8 - 10 volts, a frequency of 1 pulse per second and duration of 10 milliseconds. The stimulating parameters for the diaphragm muscle was maintained as above except the voltage which was increased to 20 volts (i.e. supramaximal voltage).

3.7.4 Test Solutions

For the rectus abdominis preparation, control contractions were first obtained using acetylcholine (0.5 µg/ml), after which the influence of d-tubocurarine (0.5 µg/ml) and eserine (1.0 µg/ml) on Ach were obtained. Also, for the phrenic nerve-diaphragm preparation, the diaphragm was stimulated via the phrenic nerve and directly with supramaximal voltage, and the influence of d-tubocurare (0.5 µg/ml) and eserine (1.0 µg/ml) on the two routes of stimulation was examined. Four various concentrations of the extracts were then tested, and each concentration repeated four times, using the Latin Square randomisation process (Armitage, 1971). The solvent vehicles were also tested on the tissues.

The concentrations of the different extracts (i.e. the final concentrations after adding the extracts into the Physiological fluid) tested on the isolated tissue preparations are given in Table 3.1. After each test, the tissues were washed repeatedly and allowed to rest for up to 30 minutes. In a situation where the extract antagonised neuromuscular transmission, the student's t-test was used to evaluate the statistical significance of the antagonism (Armitage, 1971).

TABLE 3.1

FINAL CONCENTRATIONS OF EXTRACTS OF ABRUS PRECATORIUS TESTED ON ISOLATED TISSUES

Extracts	Toad rectus abdominis muscle				Rat phrenic nerve-diaphragm preparation			
	X_1	X_2	X_3	X_4 (mg/ml)	X_1	X_2	X_3	X_4 (mg/ml)
Seeds								
cold water	1.17	2.34	4.68	9.36	0.47	0.94	1.88	3.76
hot water	1.56	3.12	6.24	12.48	0.63	1.26	2.52	5.04
petroleum ether	2.5	5.0	10.0	20.0	1.0	2.0	4.0	8.0
ethanol	0.10	0.40	0.80	1.60	0.10	0.20	0.40	0.80
Leaves								
cold water	0.52	1.04	2.08	4.16	0.21	0.42	0.84	1.68
hot water	1.39	2.78	5.56	11.12	0.56	1.12	2.24	4.48
petroleum ether	2.5	5.0	10.0	20.0	1.0	2.0	4.0	8.0
ethanol	1.00	2.00	4.00	8.00	0.50	1.00	2.00	4.00
Root								
cold water	0.43	0.86	1.72	3.44	0.17	0.34	0.68	1.36
hot water	0.89	1.78	3.56	7.12	0.36	0.72	1.44	2.88
petroleum ether	1.25	2.5	5.0	10.0	0.5	1.0	2.0	4.0
ethanol	0.50	1.00	2.00	4.00	0.30	0.60	1.20	2.40

X_1, X_2, X_3 and X_4 are the four various concentrations tested (mg/ml of tissue bath).

In studying the influence of K^+, Na^+, Ca^{++} and Mg^{++} on the effects of the extracts on neuromuscular transmission, the concentrations of the mentioned ions in the physiological fluids were varied. For the toad's tissue, the variations were K^+ = 0.9 mM and 3.6 mM, Na^+ = 0.06 M and 0.24 M, Ca^{++} = 0.54 mM and 2.16 mM. in the Frog Ringer's solution. Mg^{++} are absent in Frog Ringer's solution. For the rat's tissue, the variations were K^+ = 0.9 mM and 3.6 mM, Na^+ = 0.06 M and 0.24 M, Ca^{++} = 1.25 mM and 5.0 mM, Mg^{++} = 0.63 mM and 2.5 mM in the Kreb's solution. Stimulating parameters were first obtained using the respective normal physiological solutions. The influence of the hypo- and hyper-concentrated physiological solutions on the normal tissue contraction was then examined. Where an influence was indicated, the extracts were then tested (same concentrations as in Table 3.2) in the presence of the varied ion concentration.

3.8 Test on Chicks.

5-day old chicks were used in studying the type of paralysis caused by the injected extracts, and in determining the acute toxicity of the extracts.

3.8.1 Type of Paralysis

The chicks were weighed and divided into seven groups of five chicks each. Groups 1, 2, 3, 4, 5, 6 and 7 were injected with 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 mg/kg of the particular extract respectively. The ethanol extracts were injected intravenously (iv) into the right jugular vein, while the water and petroleum ether extracts were injected intraperitoneally (ip). The solvent vehicles (distilled water, 0.9% w/v NaCl solution and 5% w/v acacia) were injected into control groups of five chicks each. 0.5 ml of saline solution was injected into the right jugular vein while the same volume of distilled water and 5% w/v acacia were injected intraperitoneally. The chicks were then observed for a period of 48 hours. The experiments were carried out in two batches.

3.8.2 Acute Toxicity

For extracts that showed the toxic effects within 24 hours, the lethal dose was determined using the Graphic Method of Miller and Tainter (1944). In 3.8.1 above, the signs of toxicity were observed, and the number of dead and survivors was recorded. The formula used to calculate the corrected percent mortality is shown below:-

$$0\% \text{ dead} = 100\ \frac{(0.25)}{n}$$

$$100\% \text{ dead} = 100\ \frac{(n - 0.25)}{n}$$

where n = number of animals in each group = 10.

A graph of the corrected % mortality against the dose was plotted on a logarithmic-probit graph paper. From the graph, the 50% lethal dose was obtained.

In plotting the above graph, the regression equation was used. In a situation in which the dose and % mortality are variables, the regression of the % mortality on the dose was considered using the equation

$$y = a + bx \text{ (Armitage, 1971)}$$

in which y = % mortality

a = intercept

b = slope

x = dose

$$\text{slope } (b) = \frac{\Sigma (x - \bar{x})(y - \bar{y})}{\Sigma (x - \bar{x})^2}$$

$$\text{intercept } (a) = \bar{y} - b\bar{x}$$

where $\bar{x}$ = mean of the doses given

$\bar{y}$ = mean of the observed % mortality

Σ = summation sign

x = dose, y = % mortality

The correlation coefficient (R) was also calculated using the formula

$$R = \frac{\Sigma(x - \bar{x})\,(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2\ \Sigma(y - \bar{y})^2}}$$

in which $\bar{x}$ = mean of the doses given

$\bar{y}$ = mean of the observed % mortality

Σ = summation sign

x = dose

y = % mortality

CHAPTER 4.0

RESULTS

4.1 Extraction

4.1.1. Seeds

A grey coloured cold water extract (25.6 mls weighing 28 g) and a greyish-red coloured hot water extract (32 mls weighing 36 g) were obtained from the water extract of 40.0 g each of the seeds of Abrus precatorius. The mean of the weights of the extracts in the cold and hot water solutions were 2.4 g and 4.0 g respectively (i.e. 6% and 10% yield respectively).

3.9 mls (2 g) of the ether soluble extract of the seed was obtained from the petroleum ether extraction of 100 g of the seeds (i.e. 2% yield). The extract was an oily yellowish liquid.

400 g of the seeds, extracted with ethanol, gave 58.8 g brown-coloured solid extract (i.e. 15% yield).

4.1.2. Leaves

38.4 mls (40 g) cold water extract, and 43.2 mls (48 g) hot water extract were obtained from the water

extraction of 20 g each of the leaves. The mean of the weights of the extracts in the cold and hot water solutions were 1.6 g and 4.8 g respectively (i.e. 8% and 24% yield respectively). The extract solutions were green in colour, although the hot water extract was darker.

400 g of the leaves, extracted with petroleum ether, gave 4.8 g solid extract, greenish in colour (i.e. 1.2% yield).

500 g of the leaves, extracted with ethanol gave 45 g solid crude extract, green in colour (i.e. 9% yield).

4.1.3. Roots

Brown coloured cold and hot water extracts were obtained from the water extraction of the roots, though the hot water extract was darker in colour. 10 g of the roots was used for each extraction. 11.6 mls (12 g) cold water extract and 11.2 mls (12 g) hot water extract were obtained from the water extractions. The mean of the weights of the extracts in the cold and hot water solutions were 0.4 g and 0.8 g respectively (i.e. 4% and 8% yield respectively).

50 g of the roots extracted with petroleum ether gave 1.5 mls (1.0 g) crude extract which was an oily fluid, very light brown in colour (i.e. 2% yield).

25 g of the roots extracted with ethanol gave 1.3 g crude extract, brown in colour (i.e. 5.2% yield).

4.2 Effects of the water extracts on neuromuscular transmission.

4.2.1. Water extracts of the Seeds.

The responses of the toad's rectus abdominis muscle to acetylcholine (0.5 ug/ml) were neither inhibited nor enhanced by the cold (1.17 - 9.36 mg/ml) and hot water extracts (1.56 - 12.48 mg/ml) of the seeds of Abrus precatorius (Figure 4.1). In addition, both the solvent vehicle and the extracts on their own had no stimulatory effect on the toad tissue. In the same pattern, the cold (0.47 - 3.76 mg/ml) and hot water extracts of seeds (0.63 - 5.04 mg/ml) had no effect on the contraction of the rat's isolated diaphragm either via phrenic nerve stimulation or direct muscle stimulation (Figure 4.2 and 4.3 respectively. The solvent vehicle also had no effect on the contraction of the diaphragm.

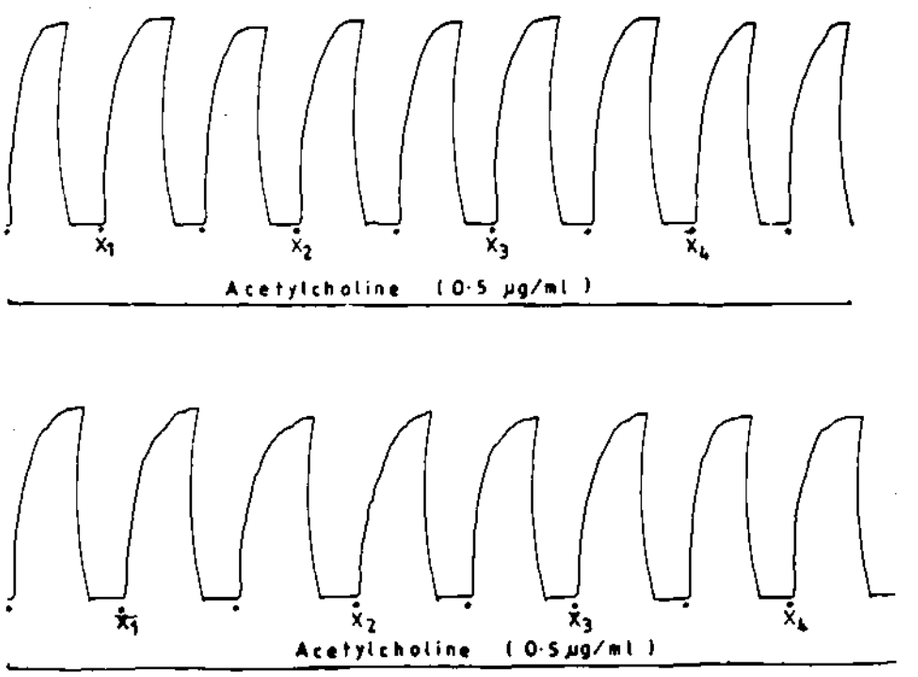


Figure 4.1: The influence of the cold and hot water extracts of the seeds of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus abdominis. Upper and lower tracings represent results obtained with cold and hot water extracts respectively.
Cold water: X_1 = 1.17, X_2 = 2.34, X_3 = 4.68, X_4 = 9.36 (mg/ml).
Hot water: X_1 = 1.56, X_2 = 3.12, X_3 = 6.24, X_4 = 12.48 (mg/ml).

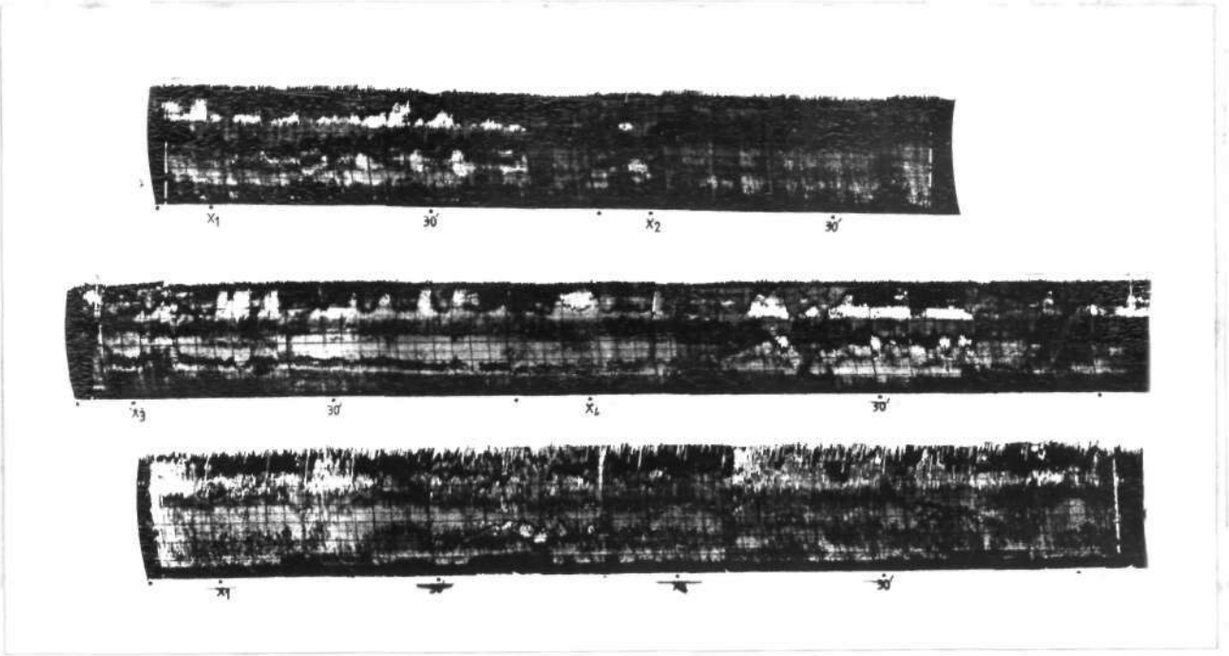


Figure 4.2: The influence of cold water extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.
$X_1 = 0.47$, $X_2 = 0.94$, $X_3 = 1.88$, $X_4 = 3.76$ (mg/ml).

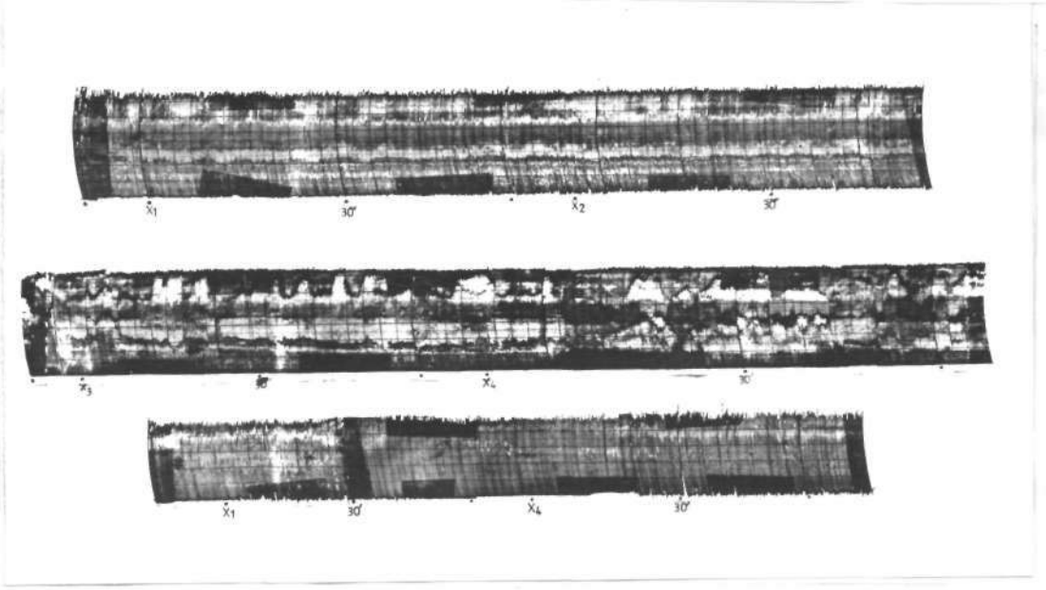


Figure 4.3: The influence of hot water extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.
$X_1 = 0.63$, $X_2 = 1.26$, $X_3 = 2.52$, $X_4 = 5.04$ (mg/ml).

4.2.2. Water Extracts of the Leaves

The cold water extract (0.52 - 4.16 mg/ml) and the hot water extract (1.39 - 11.12 mg/ml) of the leaves had no effect on the acetylcholine-induced (0.5 µg/ml) contraction of the isolated rectus abdominis of the toad (Figure 4.4). The extracts on their own and the solvent vehicle had no effect on the isolated tissue also.

Similarly, the cold (0.21 - 1.68 mg/ml) and hot water extracts (0.56 - 4.48 mg/ml) had no effect on the contraction of the isolated diaphragm of the rat neither via phrenic nerve stimulation nor direct muscle stimulation (Figures 4.5 & 4.6 respectively). The solvent vehicle did not show any effect on the contraction of the isolated diaphragm.

4.2.3. Water extracts of the Roots

The cold water extract (0.43 - 3.44 mg/ml) and the hot water extract (0.89 - 7.12 mg/ml) of the roots had no effect on acetylcholine (0.5 µg/ml) - induced contraction of the isolated rectus abdominis (Figure 4.7). Both the solvent vehicle and the extracts on their own had no effect on the isolated tissue.

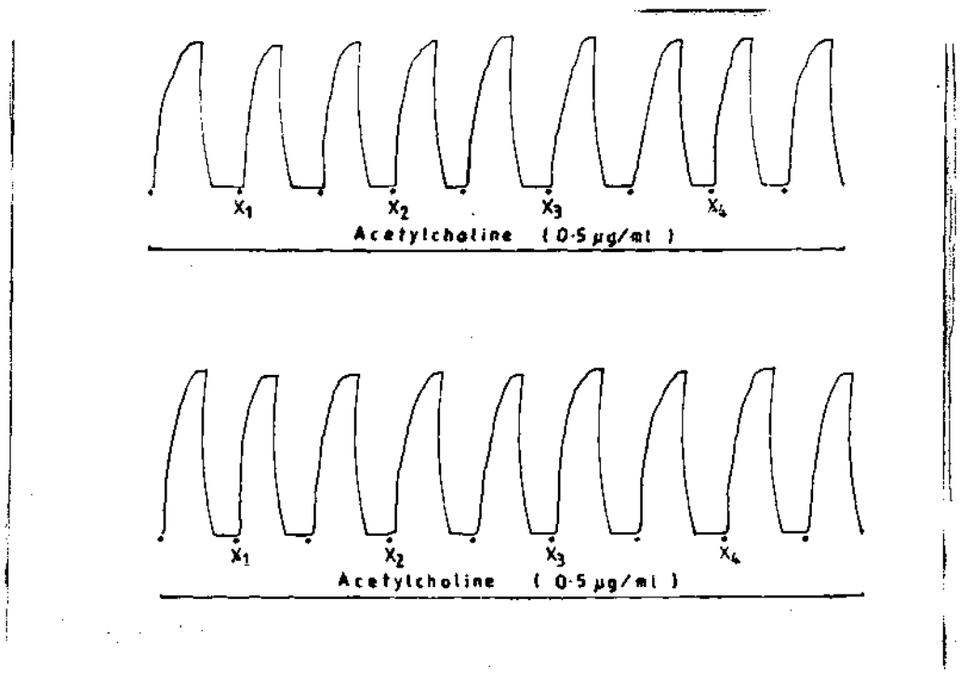


Figure 4.4: The influence of the cold and hot water extracts of the leaves of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus abdominis. Upper and lower tracings represent results obtained with cold and hot water extracts respectively.
Cold water: X_1 = 0.52, X_2 = 1.04, X_3 = 2.08, X_4 = 4.16 (mg/ml)
Hot water: X_1 = 1.39, X_2 = 2.78, X_3 = 5.56, X_4 = 11.1[illegible] (mg/ml).

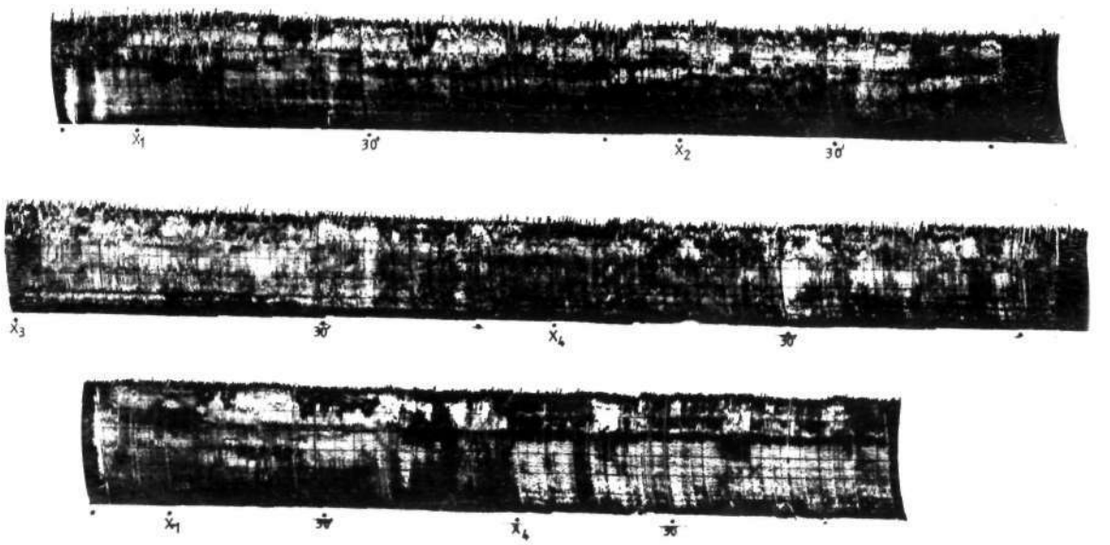


Figure 4.5: The influence of cold water extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.
$X_1 = 0.21$, $X_2 = 0.42$, $X_3 = 0.84$, $X_4 = 1.68$ (mg/ml).

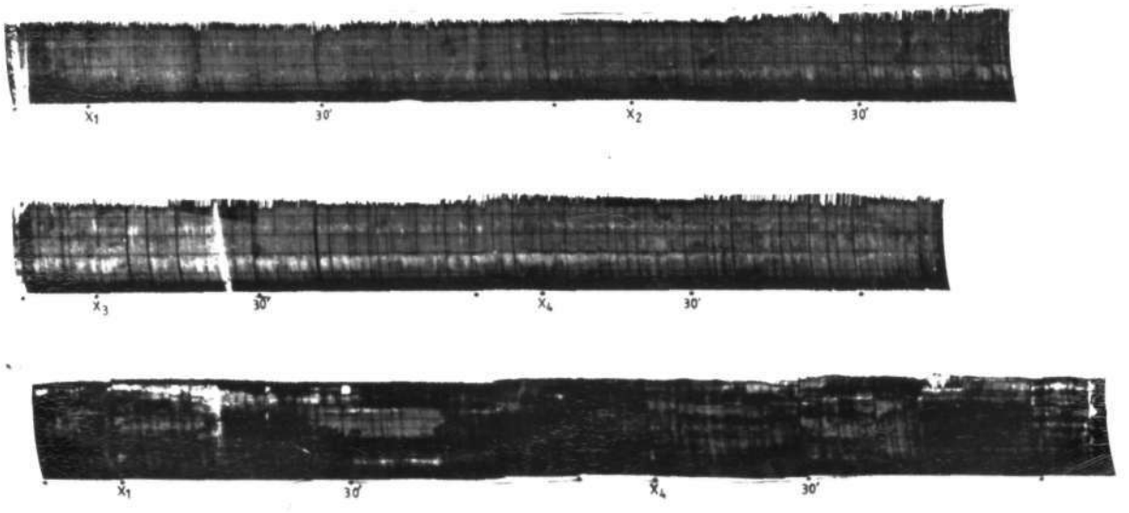


Figure 4.6: The influence of the hot water extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.
$X_1 = 0.56$, $X_2 = 1.12$, $X_3 = 2.24$, $X_4 = 4.48$ (mg/ml)

Also, the cold water extract (0.17 - 1.36 mg/ml) and the hot water extract (0.36 - 2.88 mg/ml) had no effect on the contraction of the diaphragm neither via nerve stimulation nor via direct muscle stimulation (Figures 4.8 & 4.9 respectively). The solvent vehicle had no effect on the contraction of the isolated diaphragm.

4.3 Effects of the Petroleum Ether Extracts on Neuromuscular Transmission.

4.3.1 Petroleum Ether Extracts on Rectus Abdominis of the Toad.

Using the Latin Square randomisation process, the petroleum ether extract of the seeds (2.5 - 20.0 mg/ml) did not antagonise or enhance the effect of acetylcholine (0.5 ug/ml) on the isolated rectus abdominis of the toad (Figure 4.10). Similarly, the extracts of the leaves (2.5 - 20.0 mg/ml) and the roots (1.25 - 10.0 mg/ml) had no effect on the activity of acetylcholine (0.5 ug/ml) on rectus abdominis of the toad (Figure 4.10). The extracts on their own did not show a stimulatory effect on the isolated tissue, neither did the solvent vehicle for the seeds and roots (5% w/v acacia), and that for the leaves (0.9% w/v saline solution).

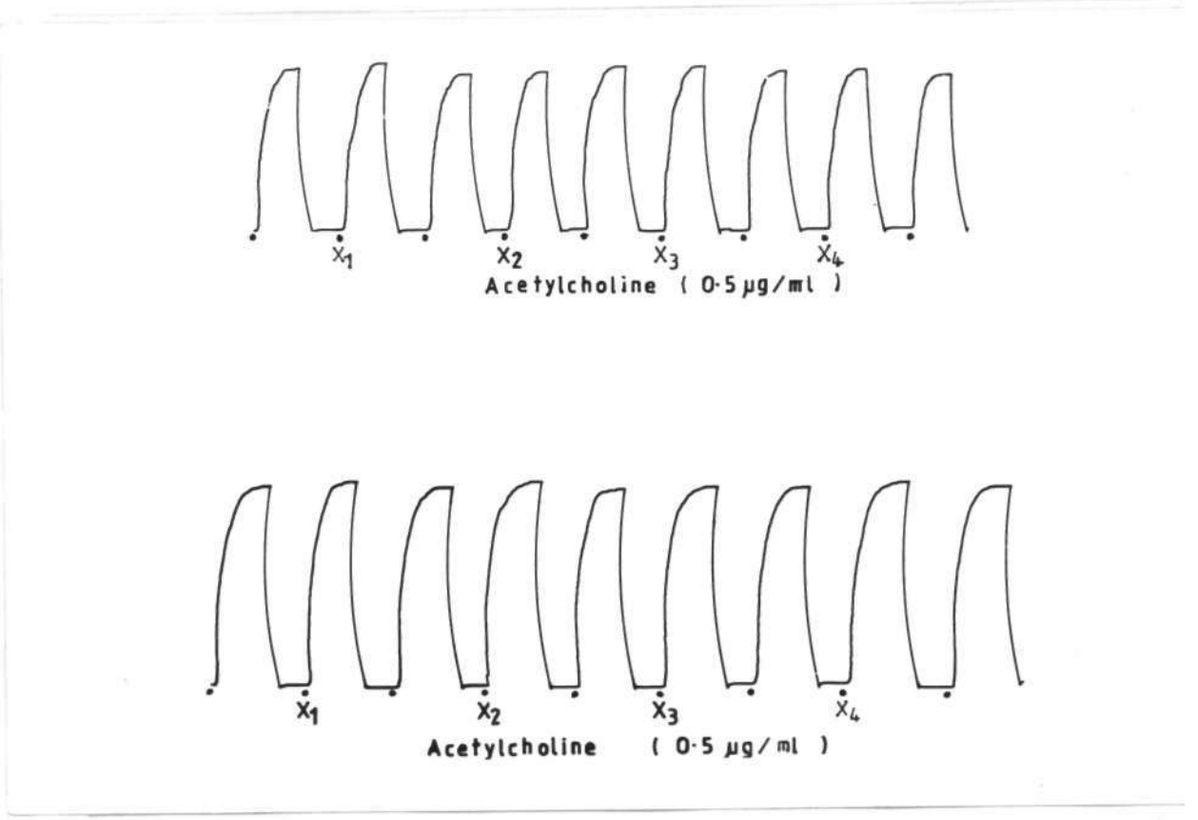


Figure 4.7: The influence of the cold and hot water extracts of the roots of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus abdominis. Upper and lower tracings represent results obtained with cold and hot water extracts respectively.
Cold water: X_1 = 0.43, X_2 = 0.86, X_3 = 1.72, X_4 = 3.44 (mg/ml)
Hot water: X_1 = 0.89, X_2 = 1.78, X_3 = 3.56, X_4 = 7.12 (mg/ml)

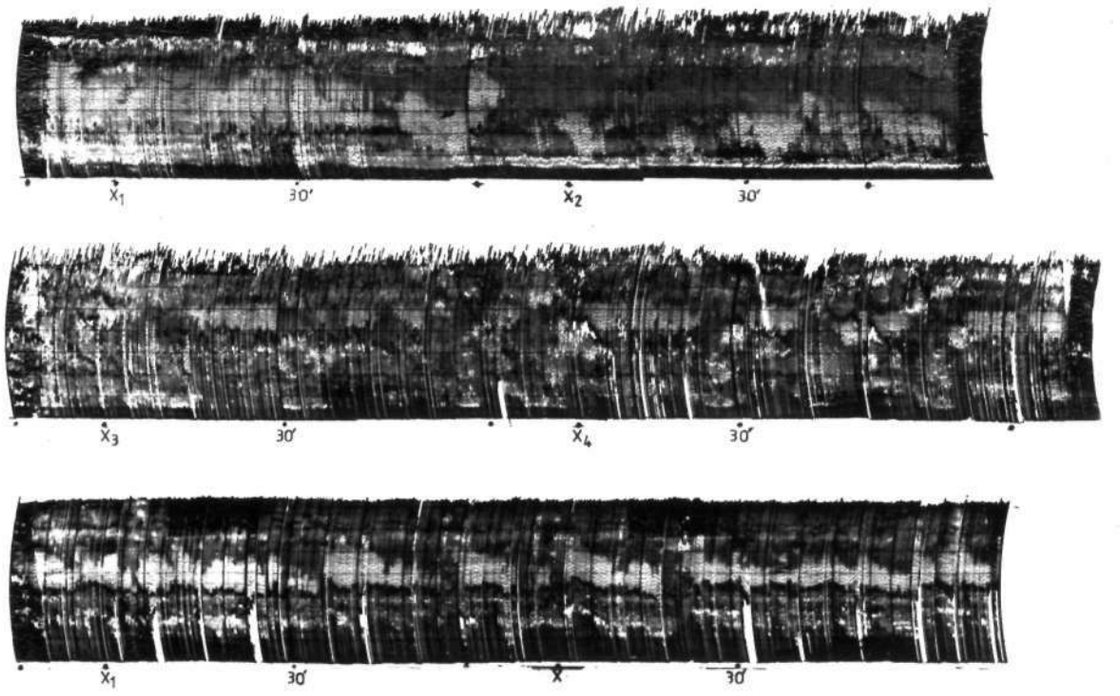


Figure 4.8: The influence of cold water extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.
X_1 = 0.17, X_2 = 0.34, X_3 = 0.68, X_4 = 1.36 (mg/ml).

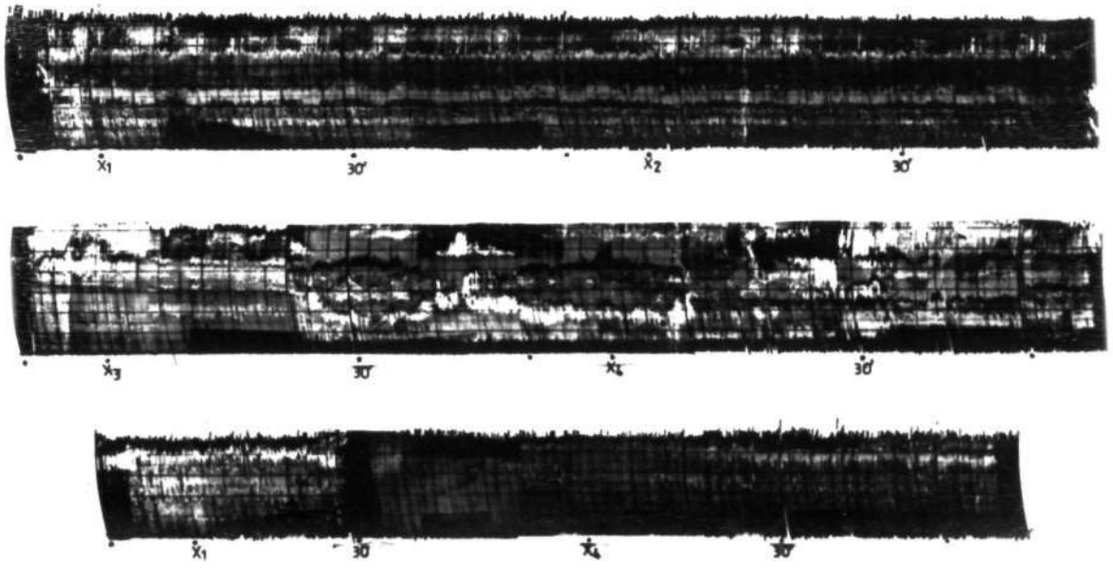


Figure 4.9: The influence of hot water extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.
X_1 = 0.36, X_2 = 0.72, X_3 = 1.44, X_4 = 2,88 (mg/ml).

4.3.2 Petroleum Ether Extracts on Phrenic Nerve-diaphragm of the rat.

The petroleum ether extract of the seeds (1.0 - 8.0 mg/ml) did not inhibit or enhance the contraction of the diaphragm muscle via nerve stimulation. Neither did the same concentrations of the extract influence the direct electrical stimulation of the diaphragm muscle (Figure 4.11). The solvent vehicle (5% w/v acacia) did not influence the direct and indirect stimulation of the diaphragm muscle. Similarly, the petroleum ether extracts of the leaves (1.0 - 8.0 mg/ml) and of the roots (0.5 - 4.0 mg/ml) did not influence the contraction of the isolated diaphragm muscle neither via nerve stimulation nor via direct electrical stimulation of the muscle (Figures 4.12 & 4.13 respectively). The solvent vehicle of the extract of the leaves (0.9% w/v saline solution) and that of the roots (5% w/v acacia) did not exhibit neuromuscular properties on the phrenic nerve-diaphragm preparation of the rat.

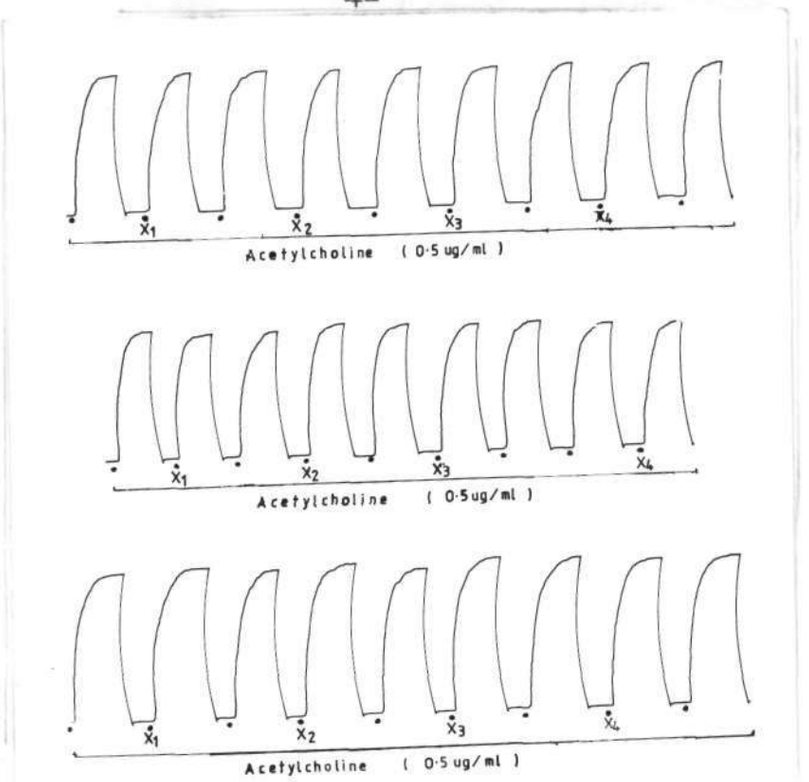


Figure 4.10: The influence of the petroleum ether extracts of the seeds (1st row), leaves (2nd row) and roots (3rd row) of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus abdominis muscle.
1st row: $X_1 = 2.5$, $X_2 = 5.0$, $X_3 = 10.0$, $X_4 = 20.0$ (mg/ml)
2nd row: $X_1 = 2.5$, $X_2 = 5.0$, $X_3 = 10.0$, $X_4 = 20.0$ (mg/ml)
2rd row: $X_1 = 1.25$, $X_2 = 2.5$, $X_3 = 5.0$, $X_4 = 10.0$ (mg/ml)

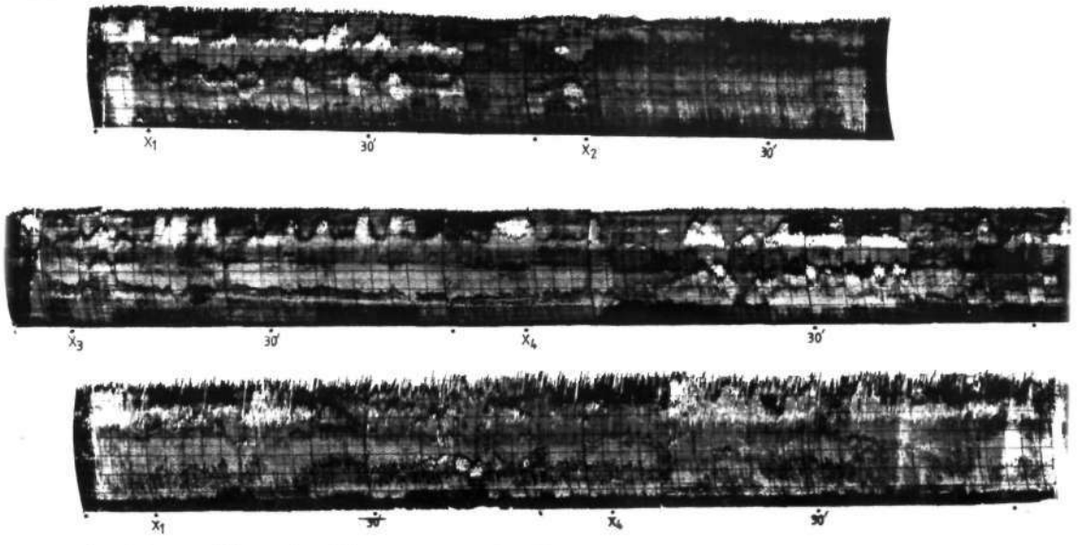


Figure 4.11: The influence of the petroleum ether extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.

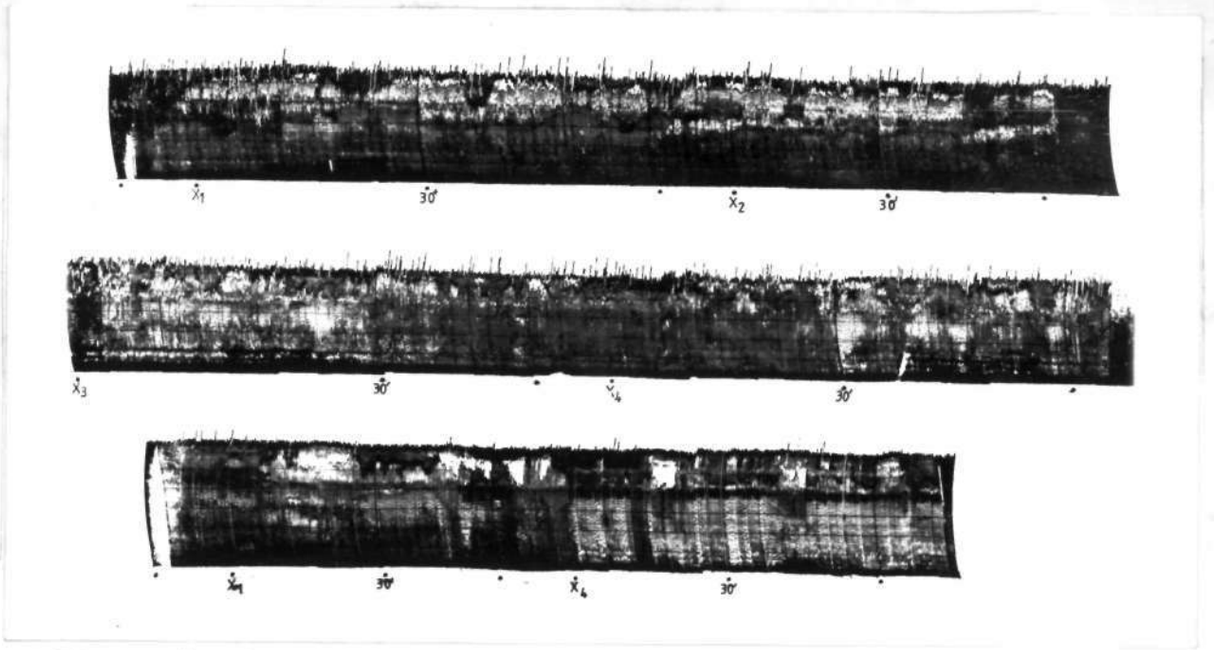


Figure 4.12: The influence of the petroleum ether extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.

$X_1 = 1.0$, $X_2 = 2.0$, $X_3 = 4.0$, $X_4 = 8.0$ (mg/ml).

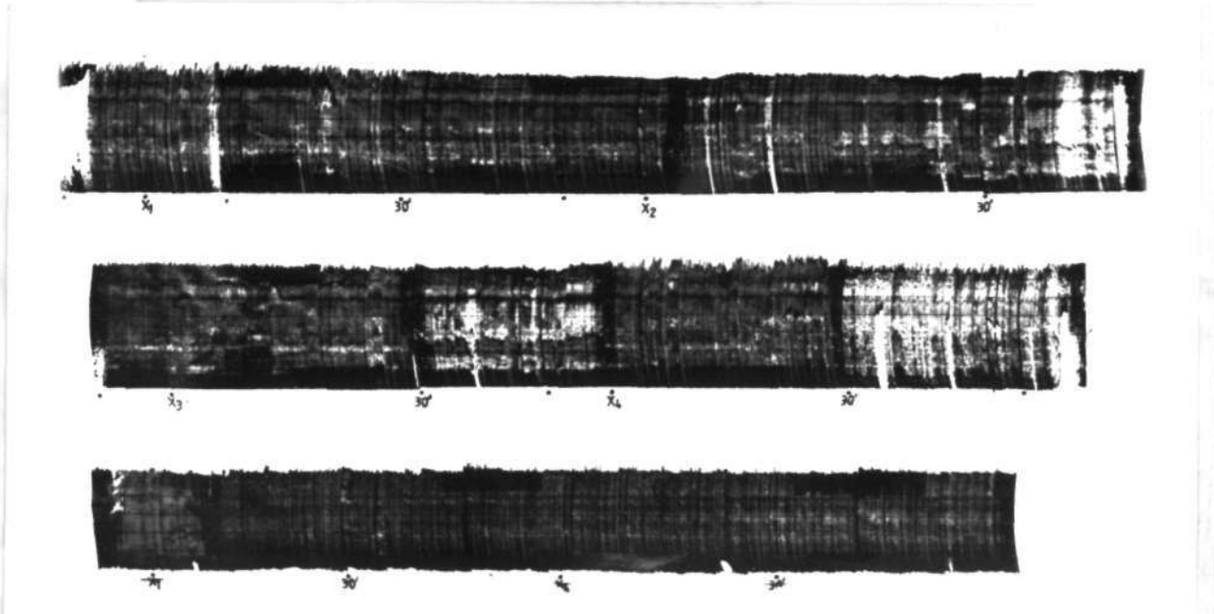


Figure 4.13: The influence of the petroleum ether extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm. The tracings on the first two rows, and the third row show results of phrenic nerve stimulation and direct muscle stimulation respectively.

$X_1 = 0.5$, $X_2 = 1.0$, $X_3 = 2.0$, $X_4 = 4.0$ (mg/ml).

4.4 Effects of the Ethanol Extracts on Neuromuscular Transmission.

4.4.1 Ethanol Extract of the Seeds

The influence of the ethanol extract of the seeds on the effect of acetylcholine (0.5 ug/ml) on isolated rectus abdominis of the toad is shown in Figure 4.14. The rectus abdominis muscle response to acetylcholine was antagonised (32.00 - 84.33%) by the ethanol extract (0.1 - 1.6 mg/ml; Table 4.1). The antagonism was reversible after repeated washings of the tissue. The antagonism was statistically significant ($p < 0.001$). The solvent vehicle (0.9% w/v NaCl solution) and the extract on its own, had no effect on the contraction of the tissue.

Figure 4.15 shows the influence of the ethanol extract of the seeds on rat's phrenic nerve-diaphragm. The extract (0.1 - 0.8 mg/ml) inhibited (0.42 - 77.50%) the contraction of the diaphragm elicited by electrical stimulation of the phrenic nerve (Table 4.2). The student's t-test indicated that the inhibition by the extract (0.2 - 0.8 mg/ml) was highly significant ($p < 0.001$). The inhibitory effect of the extract

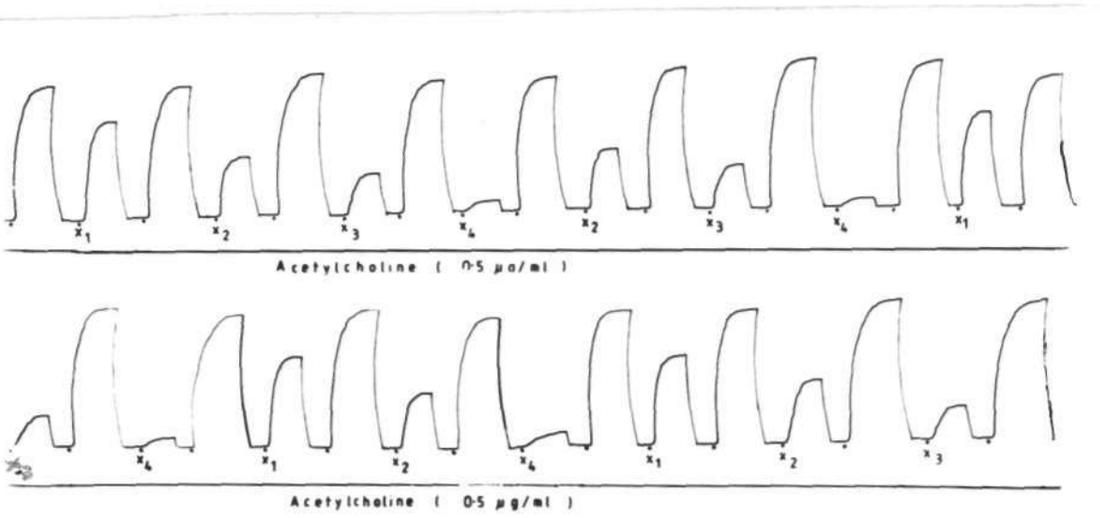


Figure 4.14: The influence of the ethanol extract of the seeds of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus abdominis.
X_1 = 0.1, X_2 = 0.4, X_3 = 0.8, X_4 = 1.6 (mg/ml).

TABLE 4.1

INFLUENCE OF ETHANOL EXTRACT OF THE SEEDS OF ABRUS PRECATORIUS ON ACH-INDUCED CONTRACTION OF RECTUS ABDOMINIS OF TOAD.

Concentration of drugs (mg/ml)	AM(mm) ±	s e m	n	%inhibition
C (Ach - 0.5 ug/ml)	67.65	0.58	4	-
X_1 (0.1)	46.00*	0.92	4	32.00
X_2 (0.4)	29.50*	0.65	4	56.39
X_3 (0.8)	19.25*	1.32	4	71.54
X_4 (1.6)	10.60*	0.23	4	84.33

C = Control

AM = Arithmetic mean

sem = standard error of mean

n = number of times each concentration was repeated

* = $p < 0.001$, student's - t - test.

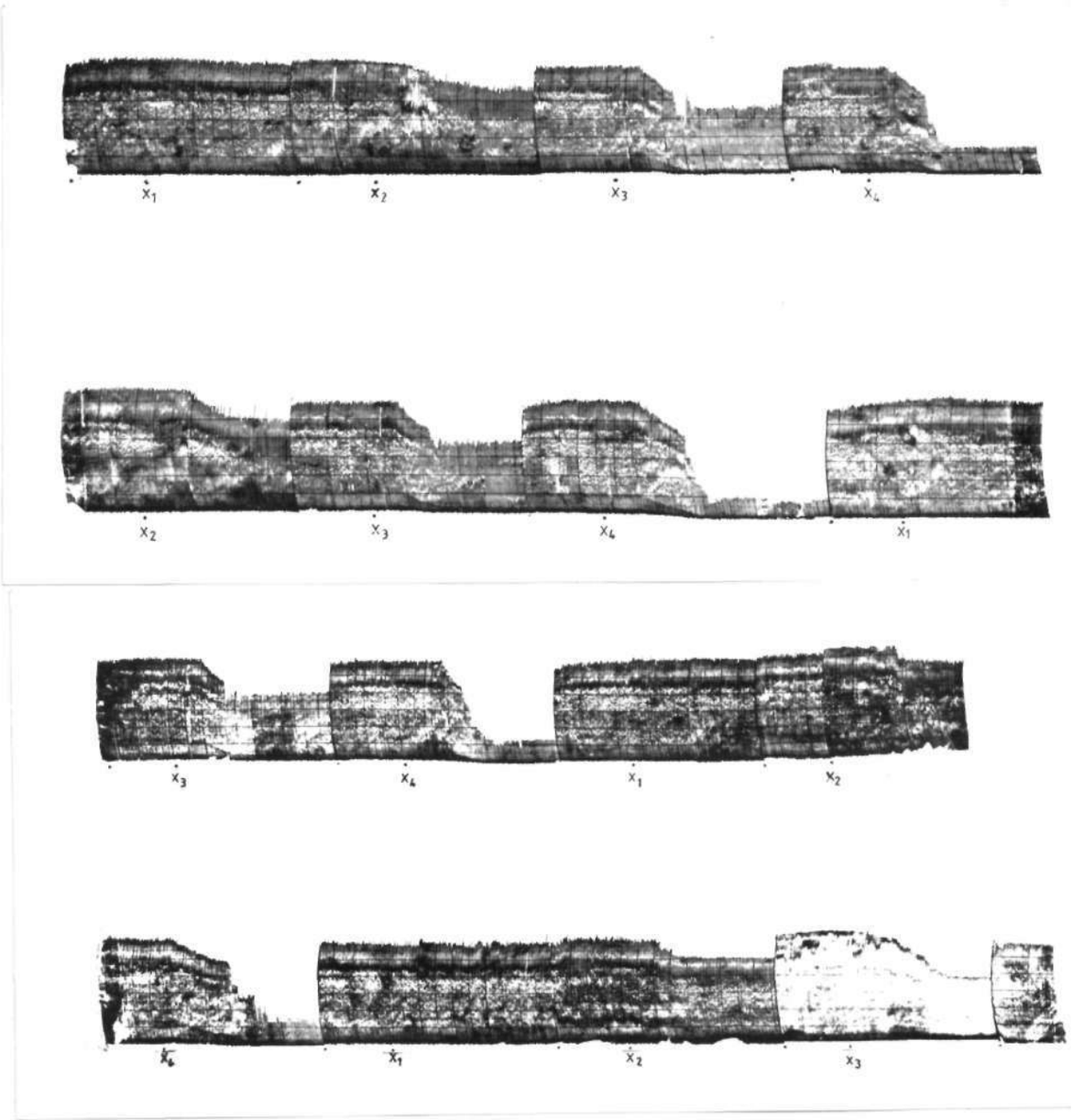


Figure 4.15: The influence of the ethanol extract of the seeds of Abrus precatorius on the contraction of the isolated rat's diaphragm via phrenic nerve stimulation.
X_1 = 0.1, X_2 = 0.2, X_3 = 0.4, X_4 = 0.8 (mg/ml).

TABLE 4.2

INFLUENCE OF ETHANOL EXTRACT OF THE SEEDS OF ABRUS PRECATORIUS ON PHRENIC NERVE STIMULATION OF THE RAT'S DIAPHRAGM.

Concentration of drugs (mg/ml)	AM (mm)±	sem	n	% inhibition
Control	60.00	0.91	4	-
X_1 (0.1)	59.75	0.48	4	0.42
X_2 (0.2)	49.25*	0.86	4	17.92
X_3 (0.4)	38.50*	0.65	4	35.83
X_4 (0.8)	13.50*	1.19	4	77.50

AM = Arithmetic mean

sem = standard error of mean

n = number of times each concentration was repeated

* = $p < 0.001$, student's t-test.

(0.4 mg/ml) was potentiated by d-tubocurine (0.5 ug/ml), but reversed by eserine (1.0 µg/ml). However, the extract (0.1 and 0.8 mg/ml) did not affect the contraction of the diaphragm muscle induced by direct electrical stimulation (Figure 4.16). The solvent vehicle (physiological saline) also had no effect on the diaphragm muscle contraction neither via nerve nor via direct muscle stimulation.

A logarithm ($\log_{10}$) concentration-response graph for the effect of the ethanol extract of the seeds on toad's rectus abdominis, and rat's phrenic nerve-diaphragm showed that the inhibition was concentration-dependent (Figure 4.17).

4.4.2 Ethanol Extract of the Leaves

The effect of the ethanol extract of the leaves on isolated rectus abdominis of toads is shown in figure 4.18. The extract (1.0 - 8.0 mg/ml) inhibited (21.77 - 80.02%) the effect of exogenous acetylcholine (0.5 µg/ml) on the isolated tissue (Table 4.3). The student's t-test indicated that the inhibition was highly significant ($p < 0.001$). The inhibitory effect was reversible after repeated washings of the tissue. The extract alone and the solvent vehicle (physiological saline)

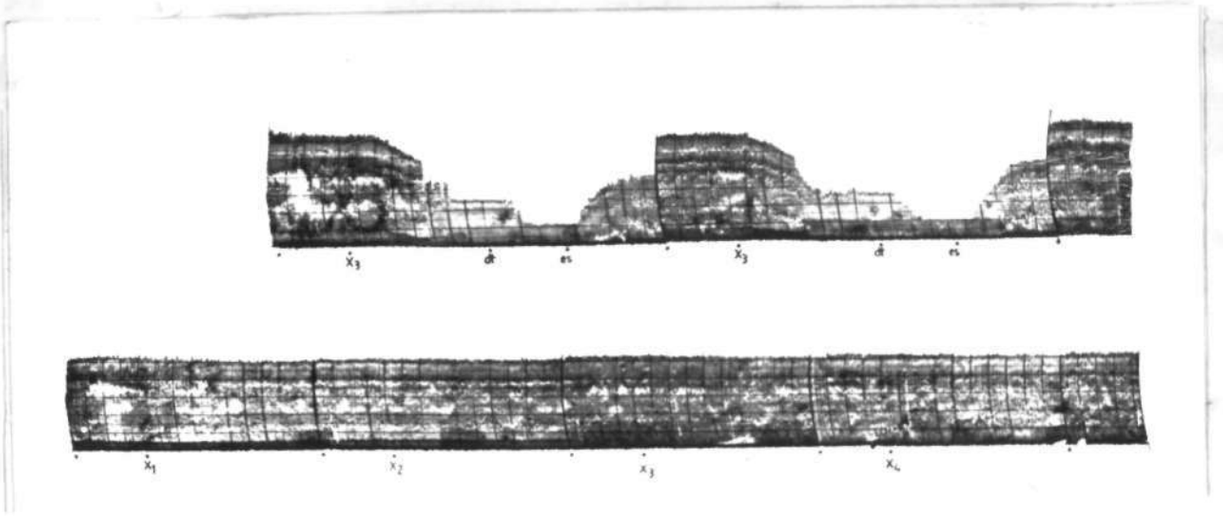


Figure 4.16: The effect of d-tubocurare (dt; 0.5 ug/ml) and eserine (es; 1.0 ug/ml) on the influence of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of the rat's diaphragm (upper row). The influence of the same extract on the contraction of the diaphragm via direct muscle stimulation (lower row).
X_1 = 0.1, X_2 = 0.2, X_3 = 0.4, X_4 = 0.8 (mg/ml).

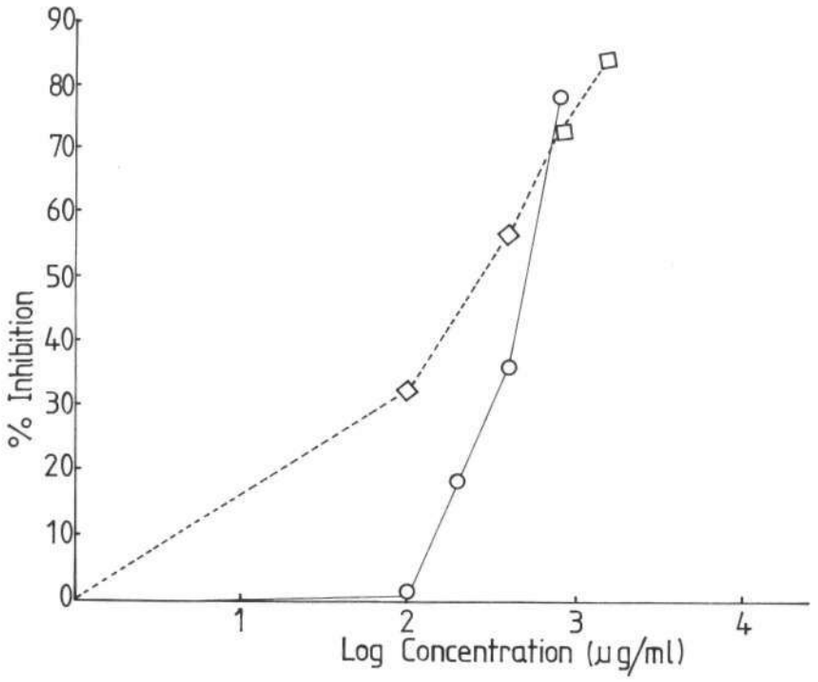


Figure 4.17: The logarithm (log) concentration response graph for the inhibitory effect of the ethanol extract of the seeds of Abrus precatorius on toad's rectus abdominis and rat's phrenic nerve diaphragm.

□---□ toad's tissue; ○——○ rat's tissue

had no effect on the tissue. The influence of the ethanol extract of the leaves on rat's phrenic nerve-diaphragm preparation is shown in figure 4.19. The extract (0.5 - 4.0 mg/ml) antagonised (0.99 - 89.83%) the effect of endogenous acetylcholine at the neuromuscular junction, during the contraction of the diaphragm muscle via phrenic nerve stimulation (Table 4.4). 1.0 - 4.0 mg/ml of the extract had significant ($p < 0.001$) inhibitory effect on the phrenic nerve-diaphragm preparation of the rat (Table 4.4).

The antagonism of the extract (2.0 mg/ml) was potentiated by d-tubocurine (0.5 ug/ml), whereas eserine (1.0 µg/ml) reversed the inhibition (Figure 4.20). However, the extract (0.5 and 4.0 mg/ml) did not affect the contraction of the diaphragm muscle via direct electrical stimulation (Figure 4.20). The solvent vehicle (physiological saline) had no effect on the phrenic nerve-diaphragm preparation.

The $\log_{10}$ concentration-response graph for the inhibitory effects of the extract on both isolated tissues used in this study, indicated that the inhibition was concentration-dependent (Figure 4.21).

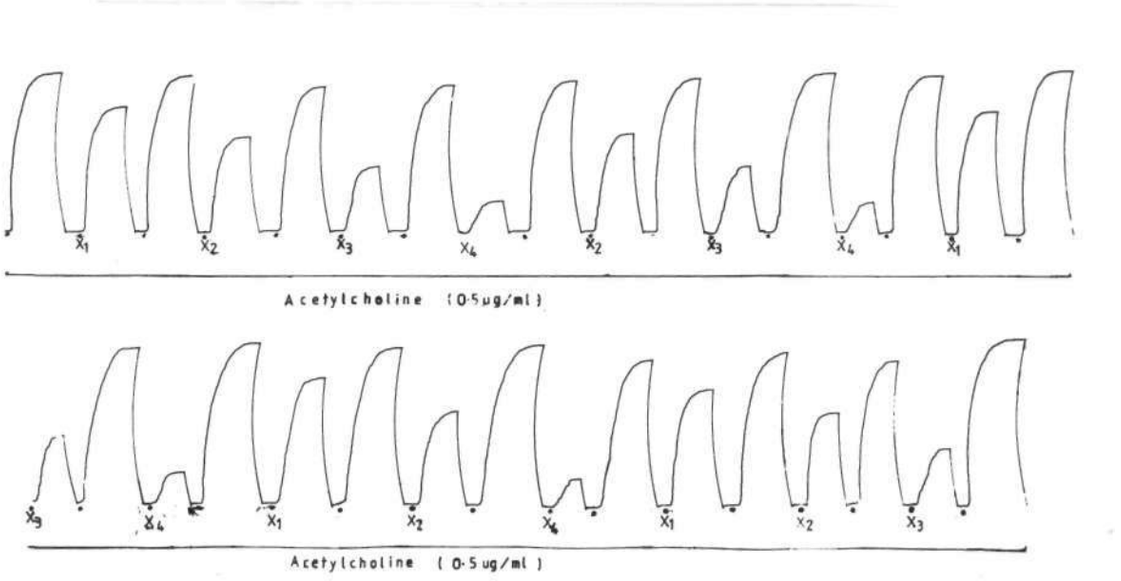


Figure 4.18: The influence of the ethanol extract of the leaves of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus muscle.
X_1 = 1.0, X_2 = 2.0, X_3 = 4.0, X_4 = 8.0 (mg/ml).

TABLE 4.3

INFLUENCE OF ETHANOL EXTRACT OF THE LEAVES OF ABRUS PRECATORIUS ON ACH-INDUCED CONTRACTION OF RECTUS ABDOMINIS OF TOAD.

Concentration of drugs (mg/ml)	AM (mm) ±	sem	n	% inhibition
C (Ach - 0.5 ug/ml)	73.82	0.74	4	-
X_1 (1.0)	57.75*	0.95	4	21.77
X_2 (2.0)	44.75*	0.48	4	39.38
X_3 (4.0)	30.25*	0.75	4	59.02
X_4 (8.0)	14.75*	0.25	4	80.02

C = control

AM = Arithmetic mean

sem = standard error of mean

n = number of times each concentration was repeated

* = $p < 0.001$, student's t-test.

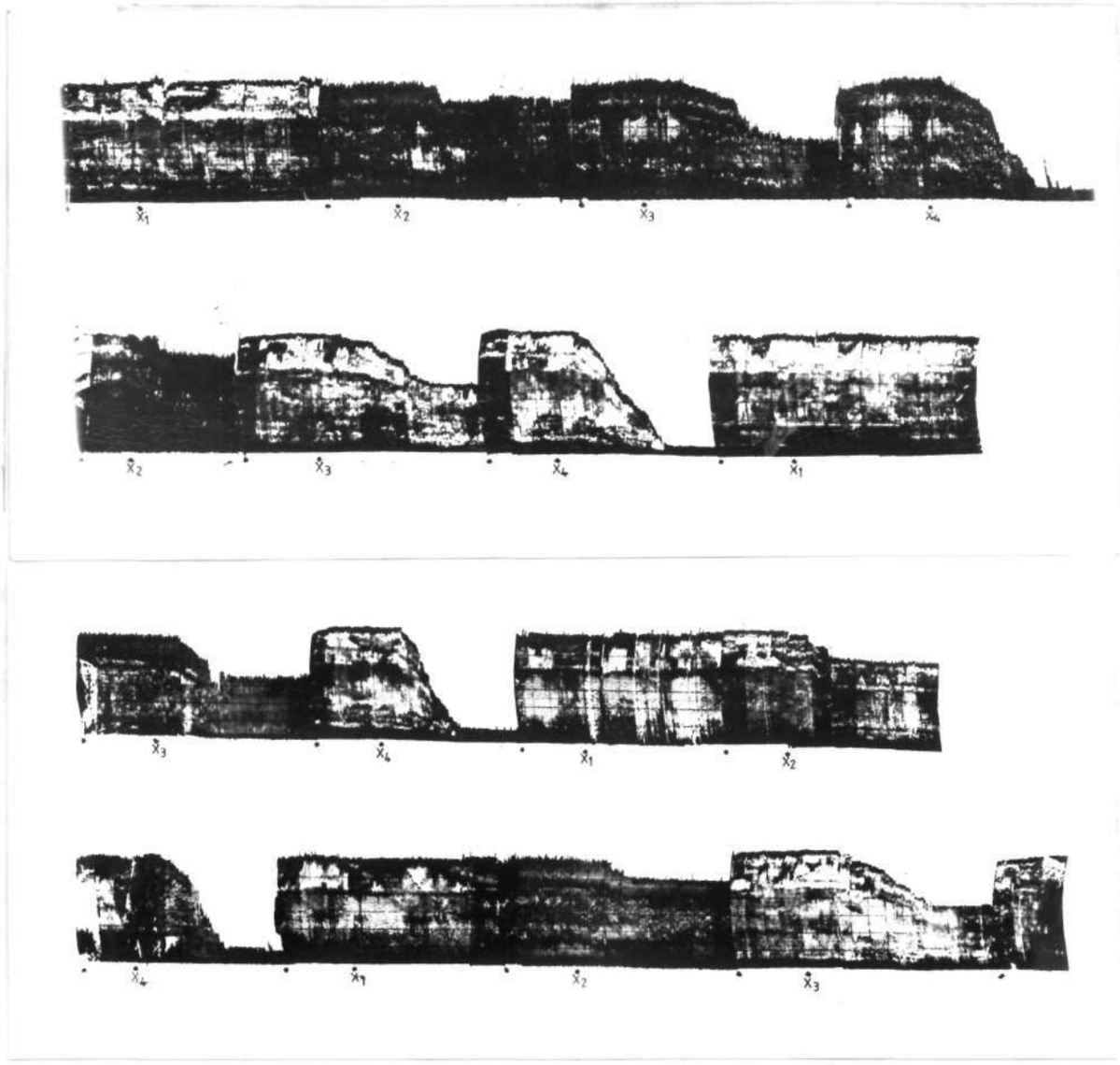


Figure 4.19: The influence of the ethanol extract of the leaves of Abrus precatorius on the contraction of the isolated rat's diaphragm muscle via phrenic nerve stimulation.
X_1 = 0.5, X_2 = 1.0, X_3 = 2.0, X_4 = 4.0 (mg/ml)

...RAHIM LIBRA...

TABLE 4.4

INFLUENCE OF ETHANOL EXTRACT OF THE LEAVES OF ABRUS PRECATORIUS ON PHRENIC NERVE-STIMULATION OF THE RAT'S DIAPHRAGM

Concentration of drugs (mg/ml)	AM(mm) ±	sem	n	% inhibition
Control	63.88		4	-
X_1 (0.5)	63,25	0.86	4	0.99
X_2 (1.0)	52.50*	1.19	4	17.82
X_3 (2.0)	35.00*	0.82	4	45.21
X_4 (4.0)	6.50*	0.29	4	89.83

AM = Arithmetic mean

sem = standard error of mean

n = number of times each concentration was repeated

* = $p < 0.001$, student's t-test

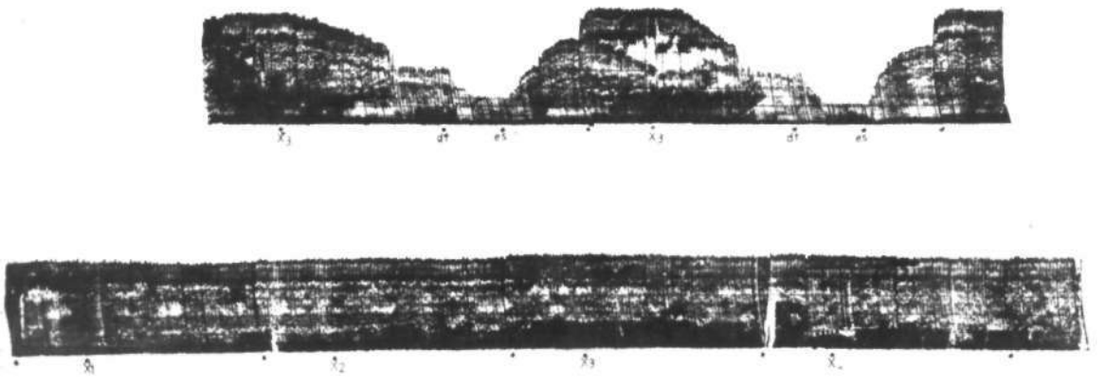


Figure 4.20: The effect of d-tubocurare (dt; 0.5 ug/ml) and eserine (es; 1.0 ug/ml) on the influence of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of the rat's diaphragm (upper row). The influence of the same extract on the contraction of the diaphragm via direct muscle stimulation (lower row). X_1 = 0.5, X_2 = 1.0, X_3 = 2.0, X_4 = 4.0 (mg/ml).

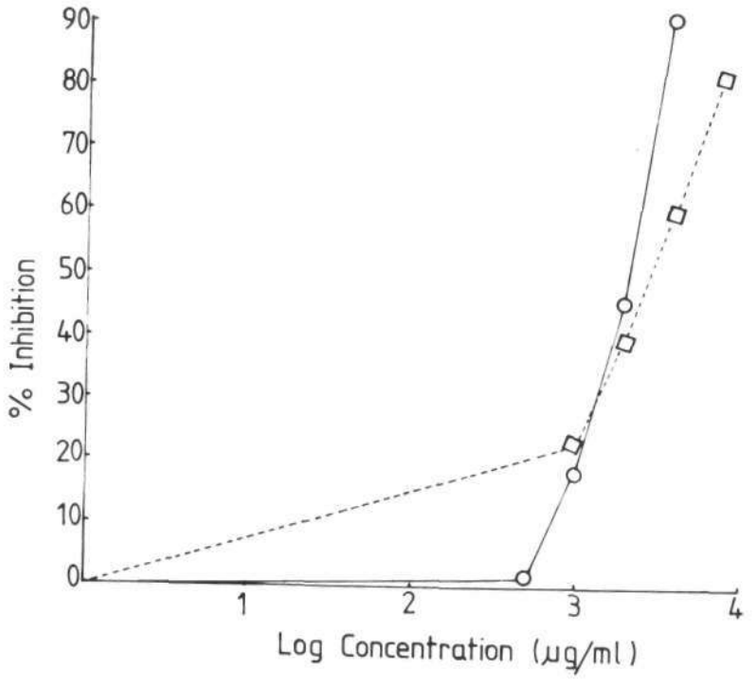


Figure 4.21: The logarithm (log) concentration response graph for the inhibitory effect of the ethanol extract of the leaves of Abrus precatorius on toad's rectus abdominis and rat's phrenic nerve-diaphragm.

□---□ toad's tissue; O———O rat's tissue.

4.4.3 Ethanol Extract of the Roots

Figure 4.22 shows the influence of the ethanol extract of the roots on isolated rectus abdominis muscle of toads. The extract (0.5 - 4.0 mg/ml) antagonised (20.75 - 85.80%) the effect of acetylcholine (0.5 µg/ml) on the isolated tissue (Table 4.5), and the antagonism was statistically significant ($p < 0.001$). The antagonism was reversed after repeated washings of the tissue. The extract alone and the solvent vehicle (physiological saline) had no effect on the tissue.

Figure 4.23 shows the influence of the ethanol extract of the roots on isolated phrenic nerve-diaphragm preparation of the rat. The contraction of the diaphragm muscle via phrenic nerve stimulation, was inhibited (4.89 - 86.47%) by the extract (0.3 - 2.4 mg/ml), and the inhibition was statistically significant ($p < 0.001$; Table 4.6). The inhibitory effect of the extract was potentiated by d-tubocurine (0.5 µg/ml), but reversed by eserine (1.0 µg/ml). The extract (0.3 and 2.4 mg/ml) did not affect the direct electrical stimulation of the diaphragm muscle (Figure 4.24). The solvent vehicle (physiological saline)

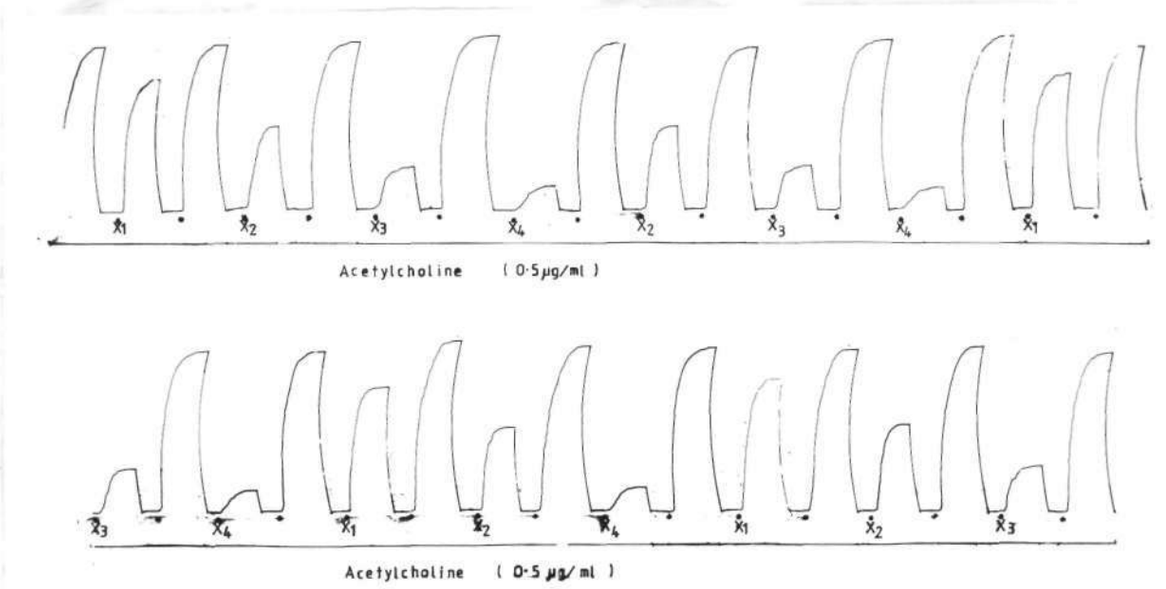


Figure 4.22: The influence of the ethanol extract of the roots of Abrus precatorius on Ach (0.5 ug/ml)-induced contraction of toad's rectus abdominis muscle.
X_1 = 0.5, X_2 = 1.0, X_3 = 2.0, X_4 = 4.0 (mg/ml).

TABLE 4.5

INFLUENCE OF ETHANOL EXTRACT OF THE ROOTS OF ABRUS PRECATORIUS ON ACH-INDUCED CONTRACTION OF RECTUS ABDOMINIS OF TOAD

Concentration of drugs (mg/ml)	AM(mm) ±	sem	n	% inhibition
C (Ach - 0.5 µg/ml)	75.71	0.42	4	-
X_1 (0.5)	60.00*	1.08	4	20.75
X_2 (1.0)	38.50*	0.50	4	49.15
X_3 (2.0)	20.25*	0.25	4	73.25
X_4 (4.0)	10.75*	0.25	4	85.80

C = control

AM = Arithmetic mean

sem = standard error of mean

n = number of times each concentration was repeated

* = $p < 0.001$, student's t-test.

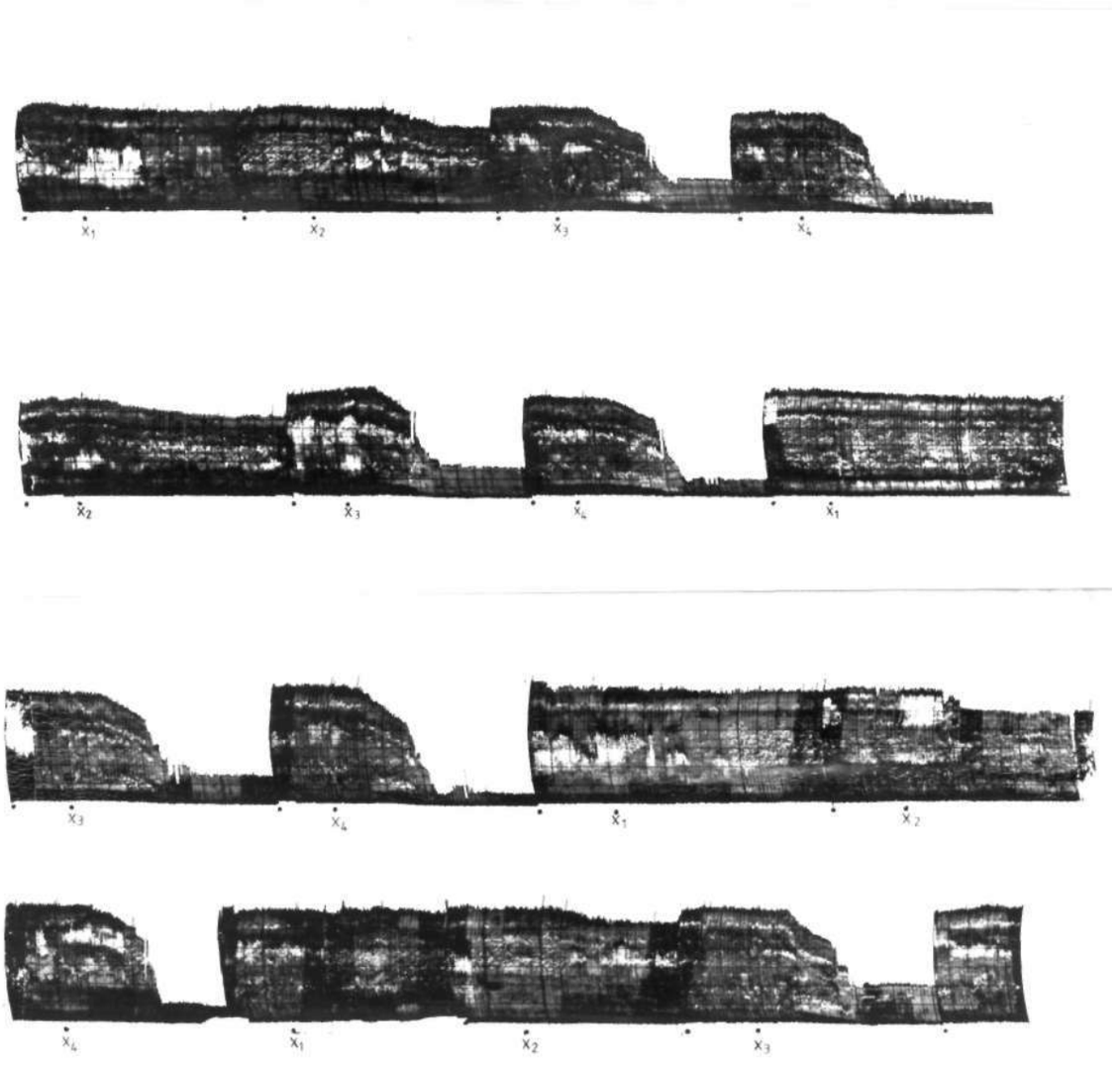


Figure 4.23: The influence of the ethanol extract of the roots of Abrus precatorius on the contraction of the isolated rat's diaphragm muscle via phrenic nerve stimulation. X_1 = 0.3, X_2 = 0.6, X_3 = 1.2, X_4 = 2.4 (mg/ml).

TABLE 4.6

INFLUENCE OF ETHANOL EXTRACT OF THE ROOTS OF ABRUS PRECATORIUS ON PHRENIC NERVE STIMULATION OF THE RAT'S DIAPHRAGM.

Concentration of drug (mg/ml)	AM(mm) ±	sem	n	% inhibition
Control	62.82	0.41	4	-
X_1 (0.3)	59.75*	0.25	4	4.89
X_2 (0.6)	50.75*	1.25	4	19.21
X_3 (1.2)	19.00*	1.29	4	67.76
X_4 (2.4)	8.50*	0.65	4	86.47

AM = Arithmetic mean

sem = standard error of mean

n = number of times each concentration was repeated

* = $p \ll 0.001$, student's t-test

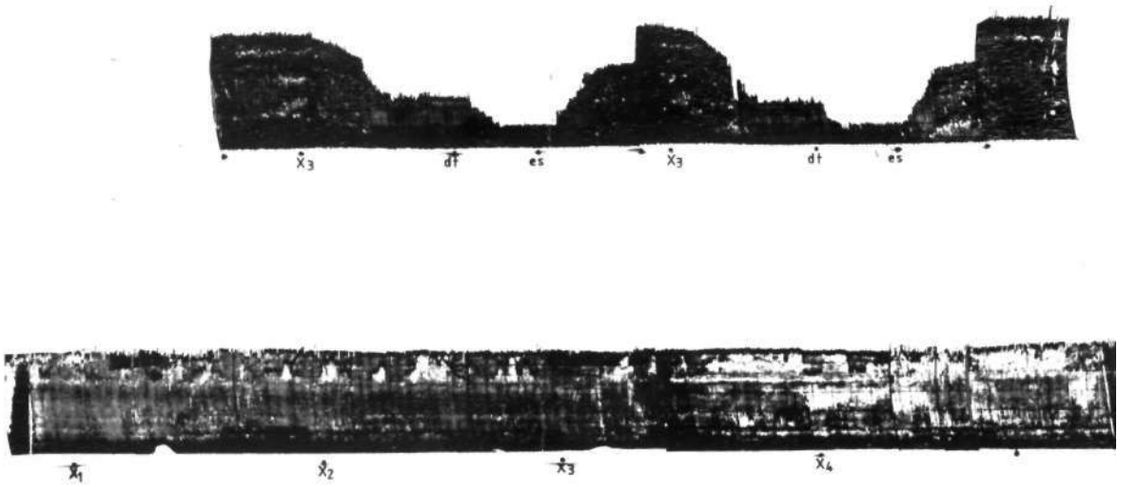


Figure 4.24: The effect of d-tubocurare (dt; 0.5 ug/ml) and eserine (es; 1.0 ug/ml) on the influence of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm (upper row). The influence of the same extract on the contraction of the diaphragm via direct muscle stimulation (lower row).

X_1 = 0.3, X_2 = 0.6, X_3 = 1.2, X_4 = 2.4 (mg/ml).

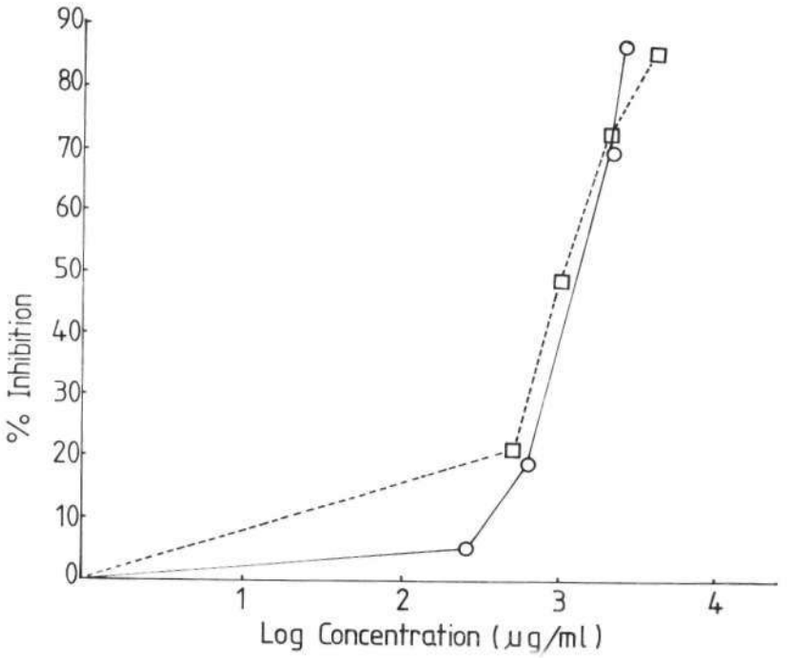


Figure 4.25: The logarithm (log) concentration response graph for the inhibitory effect of the ethanol extract of the roots of Abrus precatorius on toad's rectus abdominis and rat's phrenic nerve-diaphragm.

□---□ toad's tissue; O——O rat's tissue.

had no effect on phrenic nerve-diaphragm preparation.

The inhibitory effects of the extract on both isolated tissues are concentration-dependent, as indicated on the $\log_{10}$ concentration-response graph (Figure 4.25).

4.5 Influence of Variation in Concentration of ions on Neuromuscular Effects of the Ethanol Extracts

4.5.1 Isolated Rectus Abdominis of Toads

Variation in the concentration of Ca^{++} (0.54 mM and 2.16 mM), K^{+} (0.9 mM and 3.6 mM), and Na^{+} (0.06 M and 0.24 M) had no significant effect on the depolarisation produced by applied acetylcholine (0.5 µg/ml) on the isolated tissue. Thus, variation in concentration of the mentioned ions in Frog Ringer's solution did not influence the neuromuscular property of the ethanol extracts of the seeds, leaves and roots.

4.5.2 Isolated Phrenic nerve-diaphragm of the rat.

4.5.2.1 Effects of the ions only

1.25 mM Ca^{++} inhibited the contraction of the isolated diaphragm via phrenic nerve stimulation, while 5.0 mM Ca^{++} enhanced the contraction.

0.63 mM Mg^{++} enhanced phrenic nerve-induced contraction of the isolated diaphragm whereas 2.5 mM of Mg^{++} inhibited the contraction.

0.9 mM K^{+} inhibited the contraction of the isolated rat's diaphragm via phrenic nerve stimulation. 3.6 mM K^{+} did not influence the contraction.

Variation in Na^{+} concentration (0.06 M and 0.24M) did not influence the normal nerve-induced contraction of the isolated rat's diaphragm.

4.5.2.2 Ethanol Extract of the Seeds

The influence of hypo and hyper concentrations of Ca^{++} on the effects of the ethanol extracts of the seeds on nerve-induced contraction of the muscle, is shown in Figures 4.26 and 4.27 respectively. In the presence of reduced Ca^{++} concentration (1.25 mM), the ethanol extract of the seeds (0.1 - 0.8 mg/ml) inhibited (32.65 - 100.00%) the nerve-induced contraction of the diaphragm. The extract (0.8 mg/ml) also inhibited (25.21%) the contraction in the presence of increased Ca^{++} concentration (5.0 mM; Table 4.7).

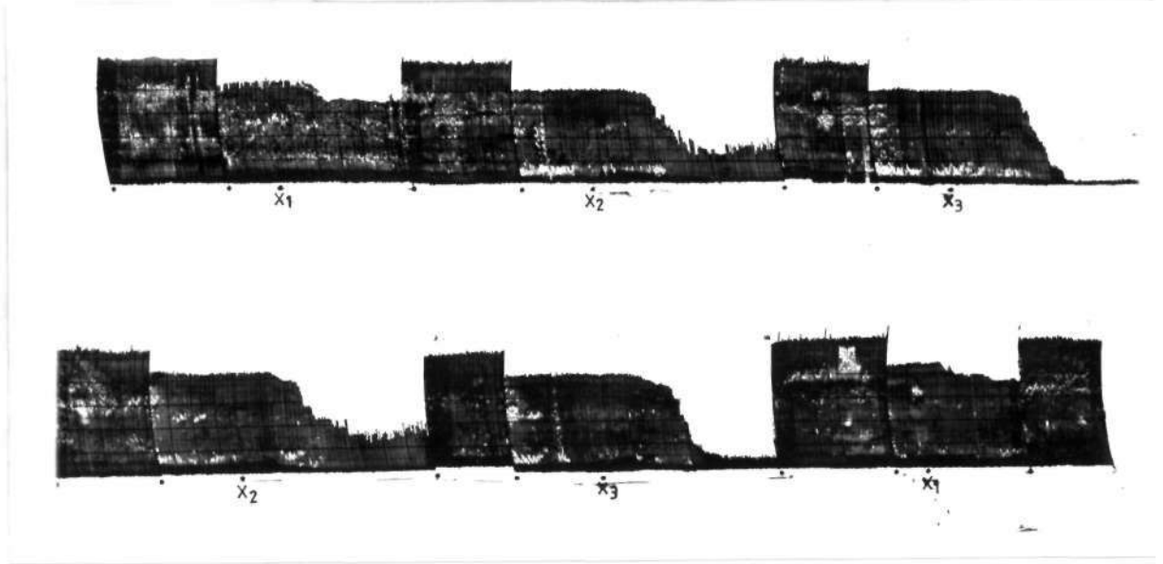


Figure 4.26: The influence of hypo-Ca^{++} concentration (1.25 mM on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.1, X_2 = 0.2, X_3 = 0.4, (mg/ml).

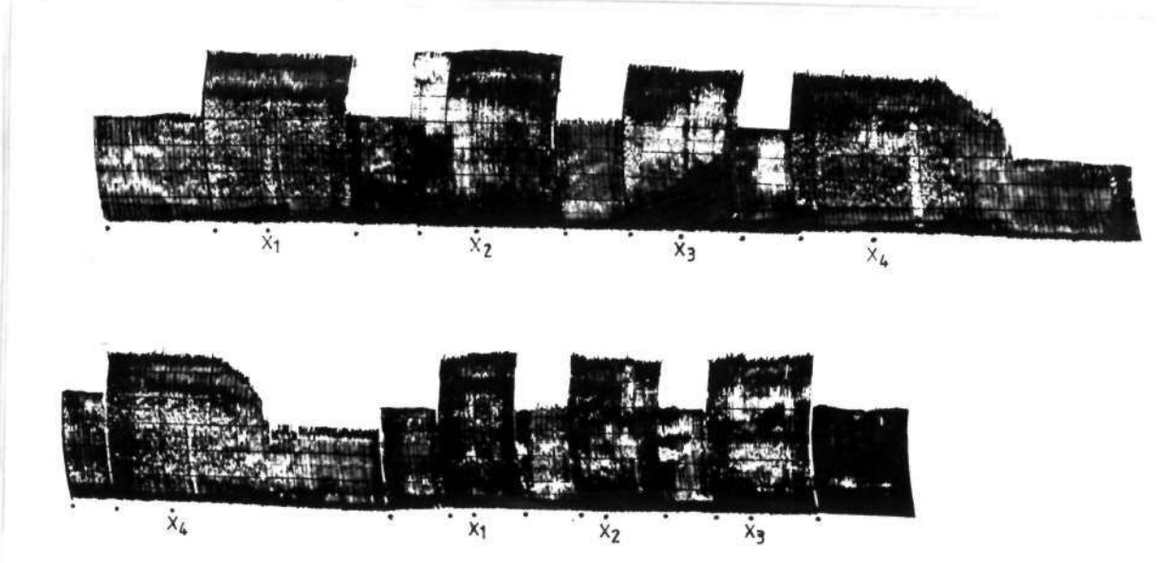


Figure 4.27: The influence of hyper - Ca^{++} concentration (5.0 on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.1, X_2 = 0.2, X_3 = 0.4, X_4 = 0.8 (mg/ml).

The influence of variation in concentration of Mg^{++} on the effect of the ethanol extract of the seeds on nerve-induced contraction of the diaphragm muscle is shown in Figures 4.28 and 4.29 (hypo and hyper concentrations respectively). In the presence of reduced Mg^{++} concentration (0.63 mM), the extract (0.4 and 0.8 mg/ml) inhibited (18.28 - 64.04%) the nerve-induced contraction (Table 4.7). The extract (0.1 - 0.8 mg/ml) also inhibited (59.16 - 100.00%) the same contraction in the presence of increased Mg^{++} concentration (2.5 mM; Table 4.7).

Figure 4.30 shows the influence of hypo concentration of K^{+} on the effect of ethanol extract of the seeds on nerve-induced contraction of the diaphragm. In the presence of reduced K^{+} concentration (0.9 mM), the extract (0.1 - 0.8 mg/ml) inhibited (57.49 - 100.00%) the contraction (Table 4.7).

4.5.2.3 Ethanol Extracts of the Leaves

The influence of variation in concentration of Ca^{++} on the effect of the ethanol extract of the leaves on nerve-induced contraction of the diaphragm muscle is shown in Figures 4.31 and 4.32 (hypo and hyper

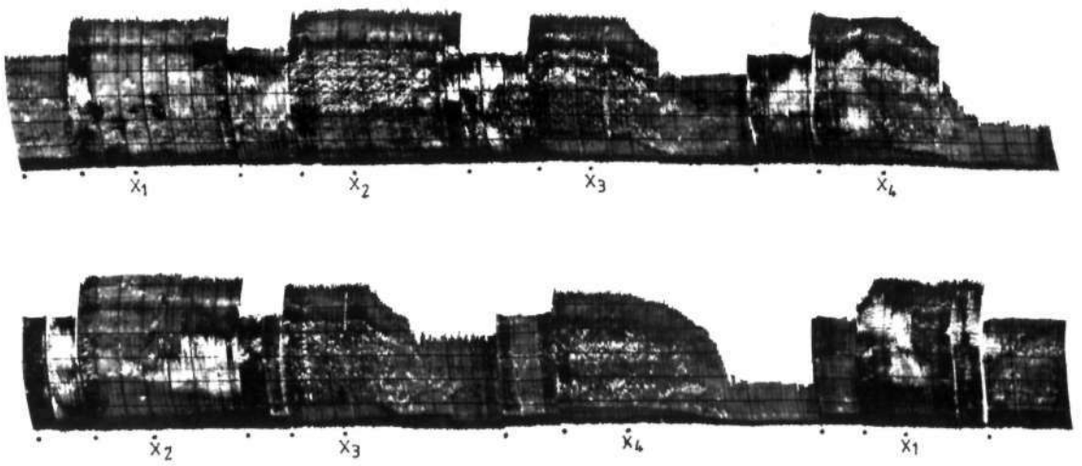


Figure 4.28: The influence of hypo - Mg^{++} concentration (0.63 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.1, X_2 = 0.2, X_3 = 0.4, X_4 = 0.8 (mg/ml).

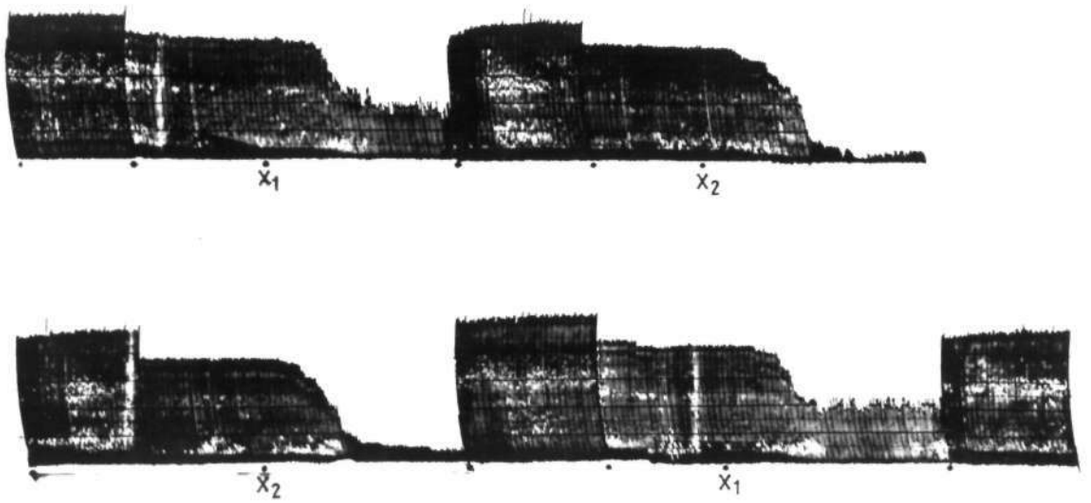


Figure 4.29: The influence of hyper - Mg^{++} concentration (1.50 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.1, X_2 = 0.2 (mg/ml).

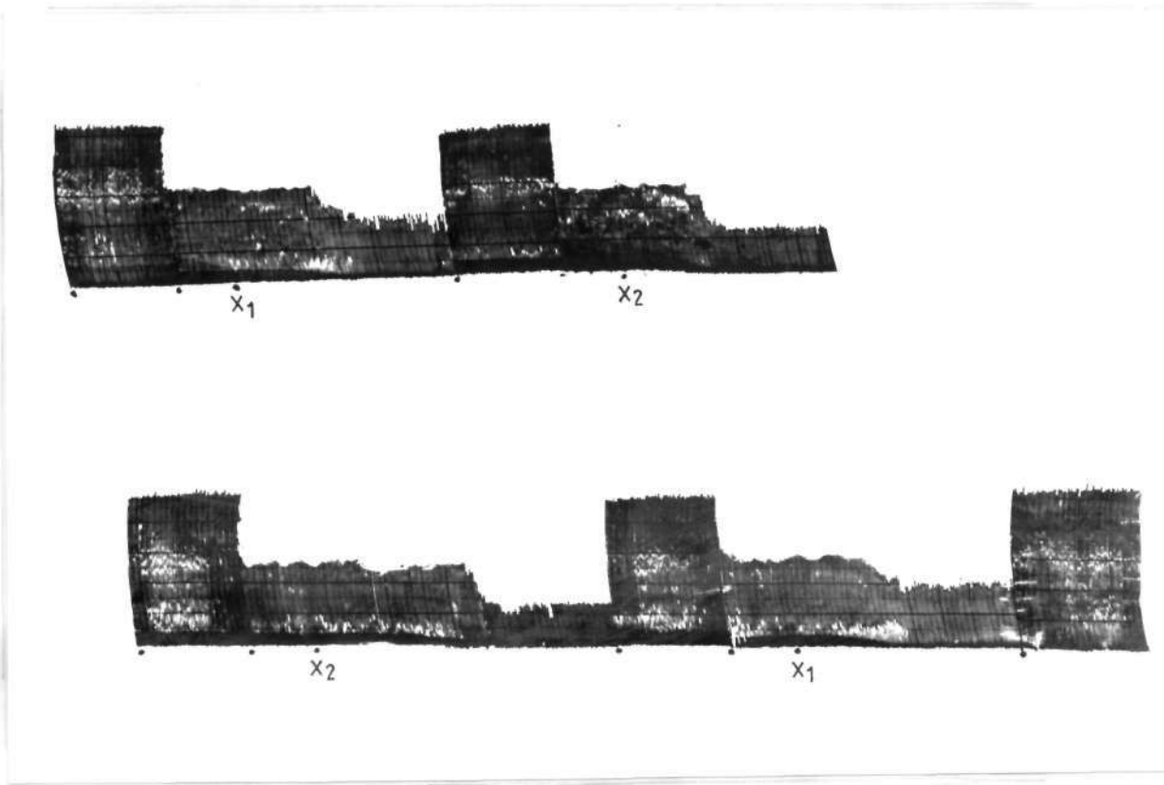


Figure 4.30: The influence of hypo - K^+ concentration (0.9 mM) on the neuromuscular effect of the ethanol extract of the seeds of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.1, X_2 = 0.2 (mg/ml).

INFLUENCE OF Ca^{++}, Mg^{++} AND K^{+} ON THE EFFECT OF THE ETHANOL EXTRACT OF THE SEEDS OF ABRUS PRECATORIUS ON NERVE-INDUCED CONTRACTION OF RAT'S DIAPHRAGM, USING KREB'S SOLUTION.

Concentration of drugs (mg/ml)	% INHIBITION					
	Normal physiological solution	Ca^{++}		Mg^{++}		K^{+}
		1.25 mM	5.0 mM	0.63 mM	2.5 mM	0.9 mM
X_1 (0.1)	0.42	32.65	0.00	0.00	59.16	57.49
X_2 (0.2)	17.92	70.59	0.00	0.00	93.03	77.73
X_3 (0.4)	35.83	93.36	0.00	18.24	100.00	100.00
X_4 (0.8)	77.50	100.00	25.21	64.04	100.00	100.00

The results obtained using the normal physiological solution is presented as the control.

concentrations respectively). In the presence of reduced Ca^{++} concentration (1.25 mM), the extract (0.5 - 4.0 mg/ml) inhibited (46.02 - 100.00%) the nerve-induced contraction of the diaphragm (Table 4.8). On the other hand, the extract (0.5 - 4.0 mg/ml) did not inhibit the nerve-induced contraction in the presence of increased Ca^{++} concentration (5.0 mM; Table 4.8).

The influence of hypo and hyper concentrations of Mg^{++} on the effects of the ethanol extracts of the leaves on nerve-induced contraction of the muscle is shown in Figures 4.33 and 4.34 respectively. In the presence of reduced Mg^{++} concentration (0.63 mM), the extract (0.5 - 4.0 mg/ml) did not inhibit the contraction of the muscle (Table 4.8). On the other hand, the same concentrations of the extract inhibited (48.43 - 100.00%) the contraction of the diaphragm in the presence of 2.5 mM Mg^{++} concentration (Table 4.8).

The influence of hypo concentration of K^{+} on the effect of ethanol extract of the leaves on nerve-induced contraction of the diaphragm is shown in Figure 4.35. The extract (0.5 - 4.0 mg/ml) in the presence of 0.9 mM K^{+} concentration, inhibited (58.06 - 100.00%) the contraction of the diaphragm (Table 4.8).

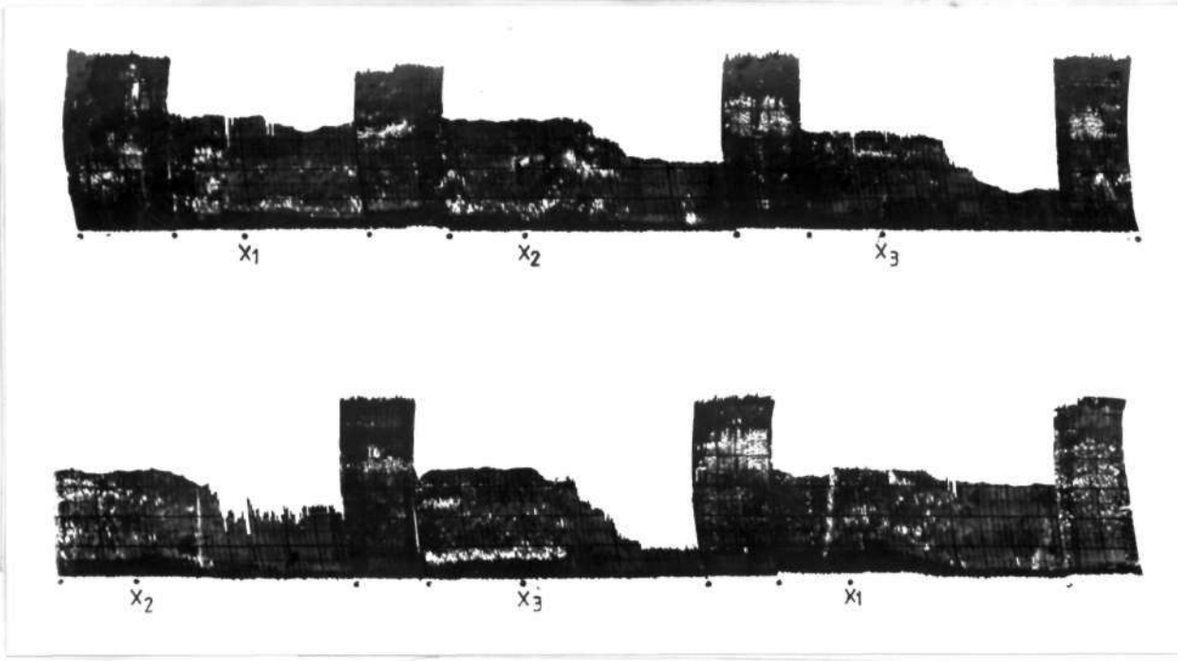


Figure 4.31: The influence of hypo - Ca^{++} concentration (1.25 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.5, X_2 = 1.0, X_3 = 2.0 (mg/ml).

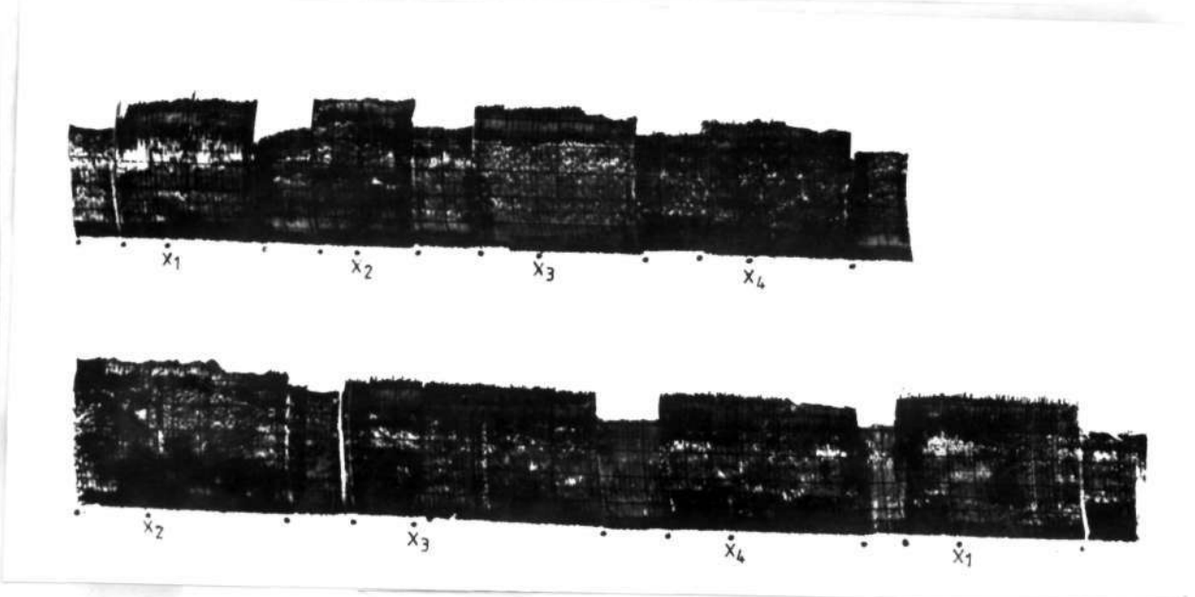


Figure 4.32: The influence of hyper - Ca^{++} concentration (5.0 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.5, X_2 = 1.0, X_3 = 2.0, X_4 = 4.0 (mg/ml).

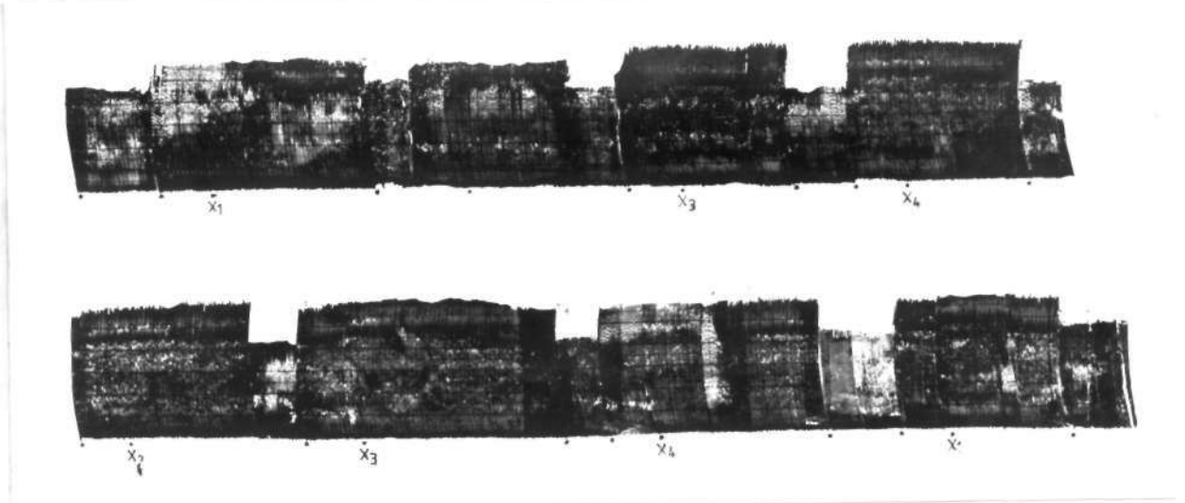


Figure 4.33: The influence of hypo - Mg^{++} concentration (0.63 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.5, X_2 = 1.0, X_3 = 2.0, X_4 = 4.0 (mg/ml).

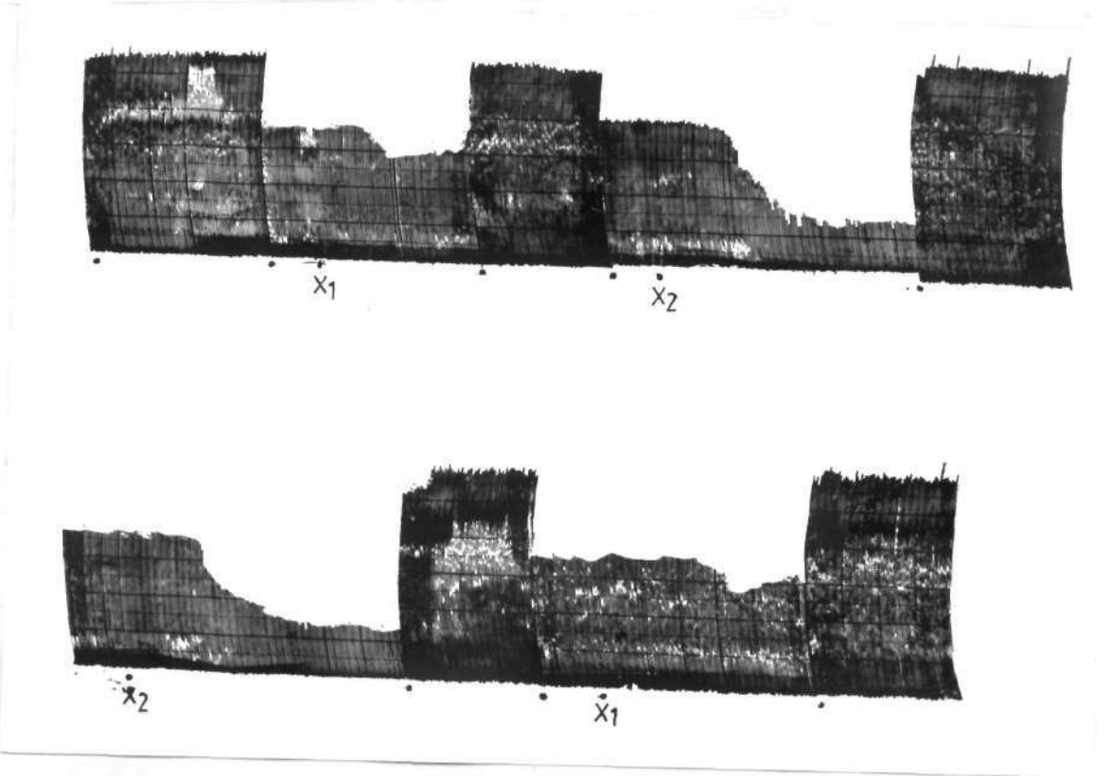


Figure 4.34: The influence of hyper - Mg^{++} concentration (2.5 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.5, X_2 = 1.0 (mg/ml).

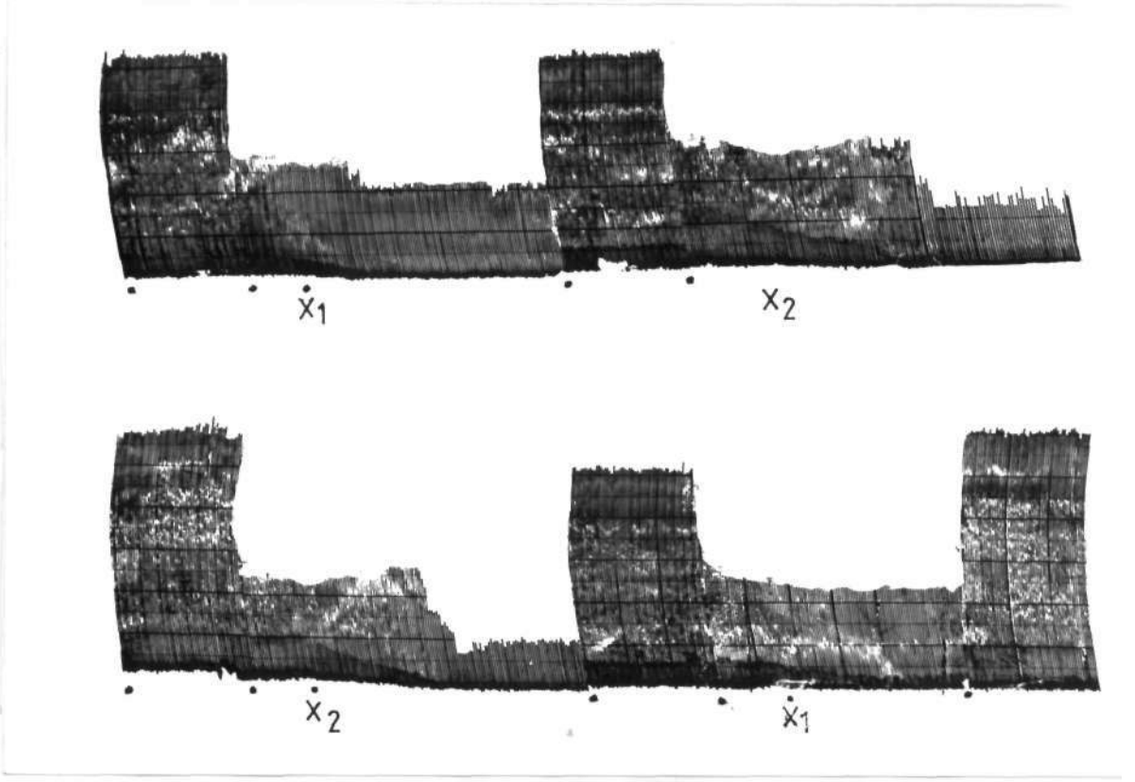


Figure 4.35: The influence of hypo - K^+ concentr tion (0.9 mM) on the neuromuscular effect of the ethanol extract of the leaves of Abrus precatorius on nerve-induced contraction of rat's diaphragm.
X_1 = 0.5, X_2 = 1.0 (mg/ml).

TABLE 4.8

INFLUENCE OF Ca^{++}, Mg^{++} AND K^{+} ON THE EFFECT OF THE ETHANOL EXTRACT OF THE LEAVES OF ABRUS PRECATORIUS ON NERVE-INDUCED CONTRACTION OF RAT'S DIAPHRAGM, USING KREB'S SOLUTION.

Concentration of drugs (mg/ml)	% INHIBITION				
	Normal physiological solution	Ca^{++}		Mg^{++}	K^{+}
		1.25 mM	5.0 mM	0.63 mM	2.5 mM	0.9 mM
X_1 (0.5)	0.99	46.02	0.00	0.00	48.43	58.06
X_2 (1.0)	17.82	63.43	0.00	0.00	77.27	82.26
X_3 (2.0)	45.21	81.72	0.00	0.00	100.00	100.00
X_4 (4.0)	89.83	100.00	0.00	0.00	100.00	100.00

The results obtained using normal physiological solution is presented as the control.

4.5.2.4 Ethanol Extract of the Roots.

The influence of reduced Ca^{++} concentration (1.24 mM) on the effects of the ethanol extract of the roots on the contraction of the diaphragm via nerve stimulation is shown in Figure 4.36. The extract (0.3 - 2.4 mg/ml) inhibited (62.62 - 100.00%) the nerve-induced contraction (Table 4.9). In the presence of increased Ca^{++} concentration (5.0 mM), the extract (1.2 - 2.4 mg/ml) inhibited (30.68 - 62.76%) the muscle contraction (Figure 4.37; Table 4.9).

The influence of variation in concentration of Mg^{++} on the effect of the ethanol extract of the roots on nerve-induced contraction of the diaphragm muscle is shown in Figures 4.38 and 4.39 (hypo and hyper concentrations respectively). 1.2 and 2.4 mg/ml of the extract inhibited (20.09 - 49.59%) the contraction of the diaphragm in the presence of 0.63 mM Mg^{++} concentration (Table 4.9). In the presence of 2.5 mM Mg^{++} concentration, the extract (0.3 - 2.4 mg/ml) inhibited (5 .39 - 100.00%) the same contraction (Table 4.9).

The influence of hypo-concentration of K^{+} on the effect of the ethanol extract of the roots on the nerve-induced contraction of the rat's diaphragm is shown in Figure 4.40. In the presence of 0.9 mM K^{+}

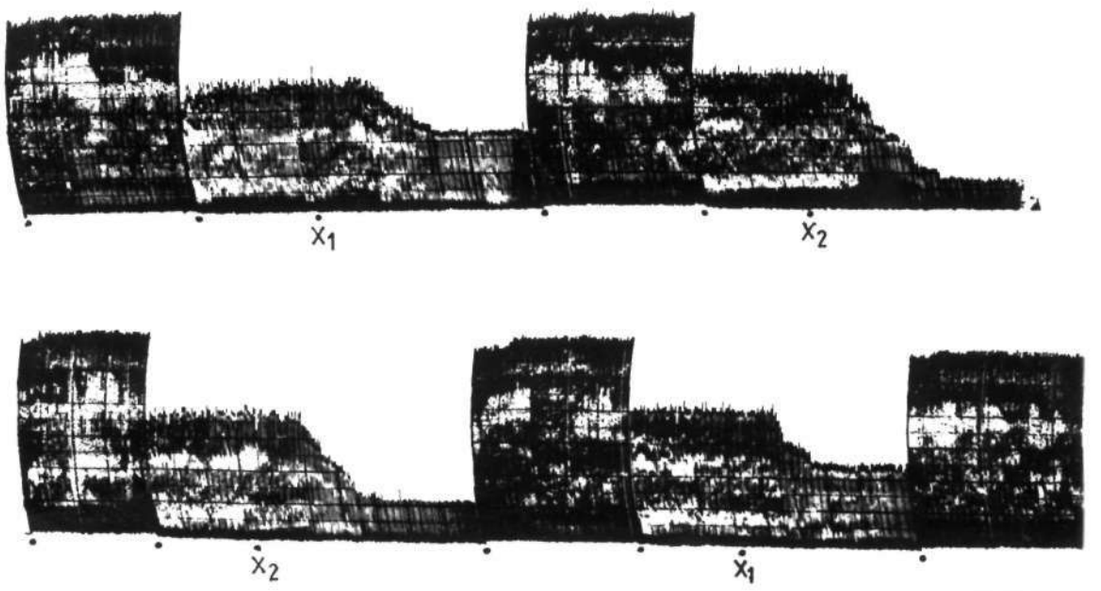


Figure 4.36: The influence of hypo - Ca^{++} concentration (1.25 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm.

X_1 = 0.3, X_2 = 0.6 (mg/ml).

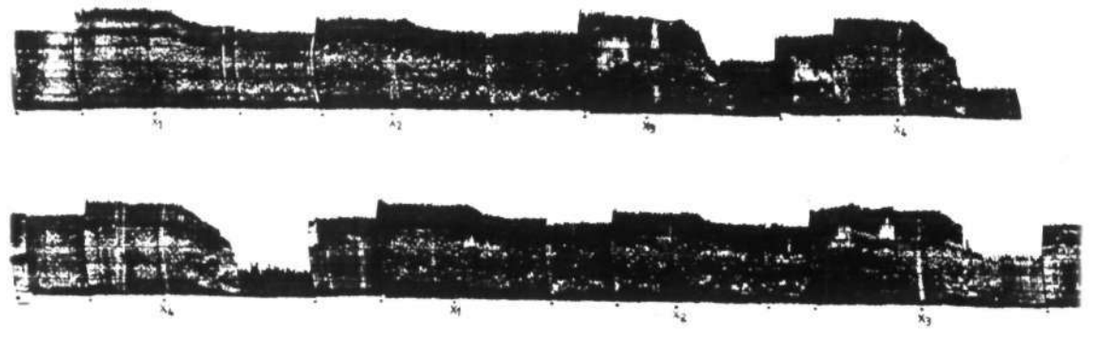


Figure 4.37: The influence of hyper - Ca^{++} concentration (5.0 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm.

X_1 = 0.3, X_2 = 0.6, X_3 = 1.2, X_4 = 2.4 (mg/ml).

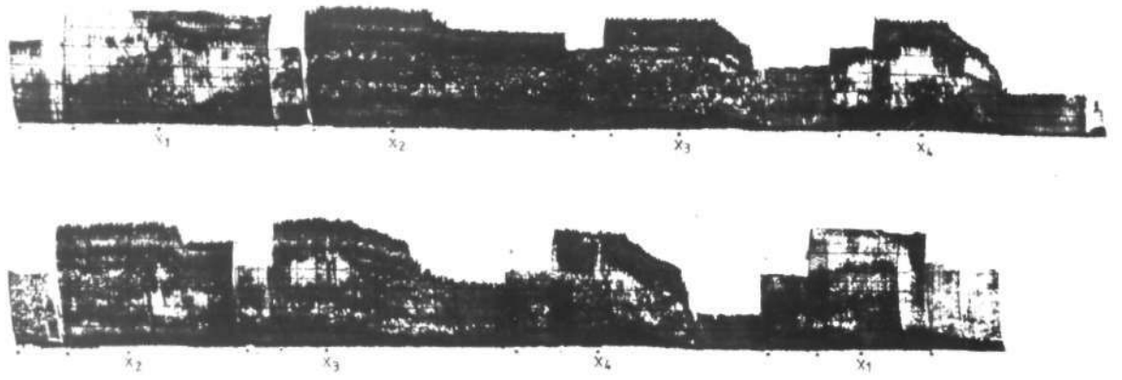


Figure 4.38: The influence of hypo - Mg^{++} concentration (0.63 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm.

X_1 = 0.3, X_2 = 0.6, X_3 = 1.2, X_4 = 2.4 (mg/ml).

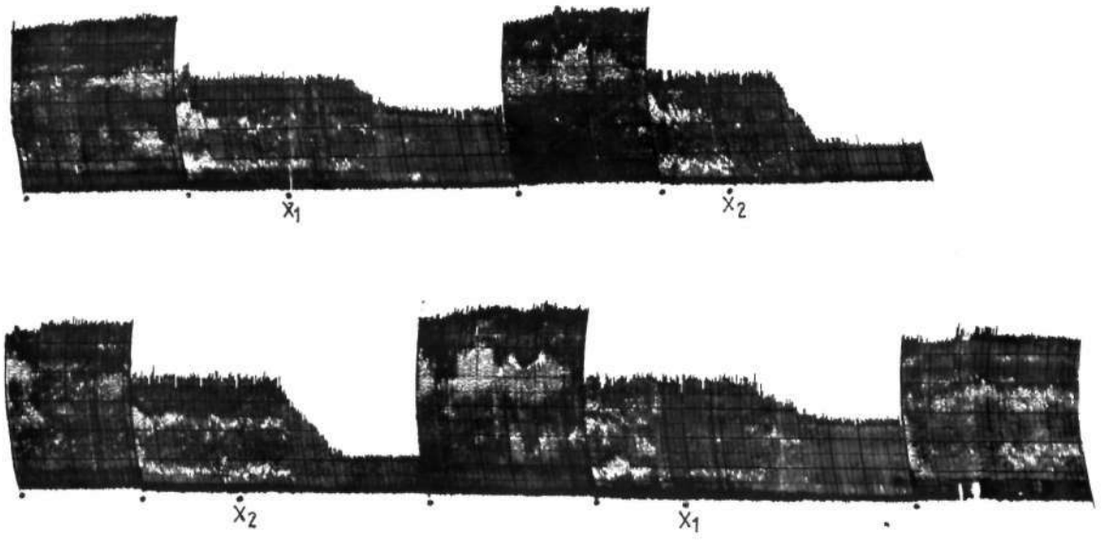


Figure 4.39: The influence of hyper - Mg^{++} concentration (2.5 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm.

X_1 = 0.3, X_2 = 0.6 (mg/ml).

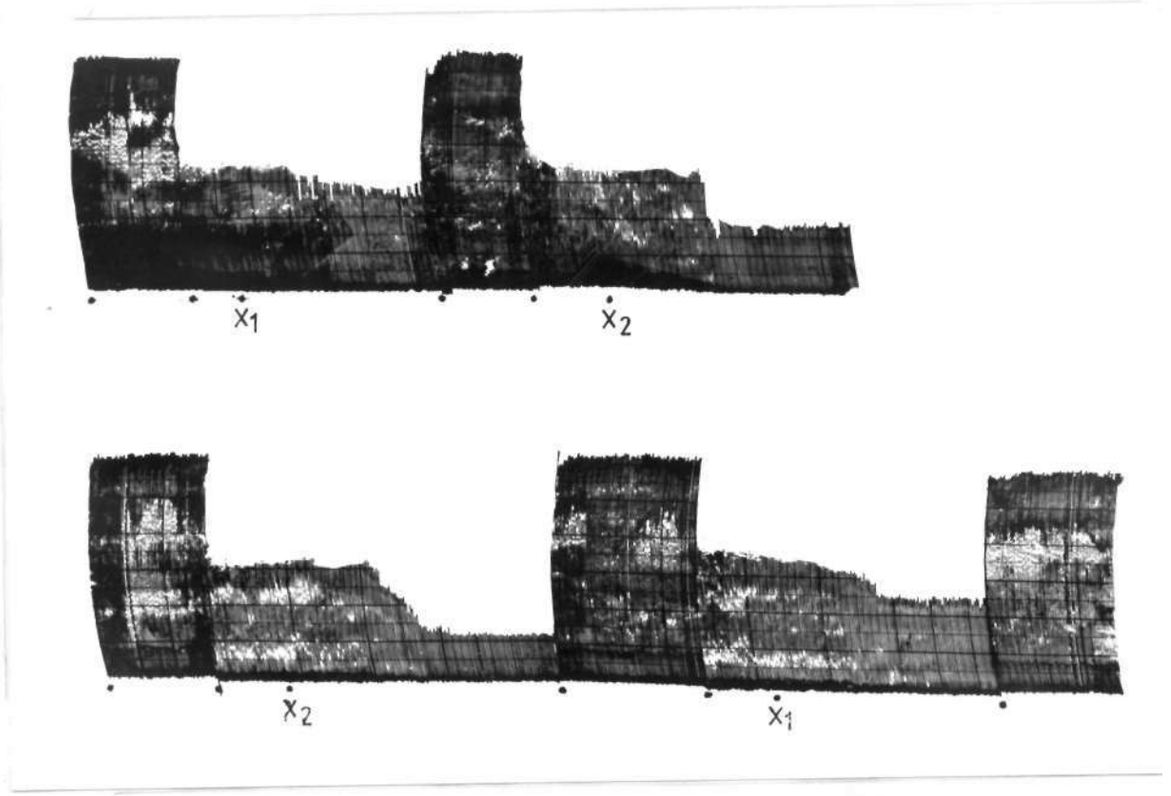


Figure 4.40: The influence of hypo - K^+ concentration (0.9 mM) on the neuromuscular effect of the ethanol extract of the roots of Abrus precatorius on nerve-induced contraction of rat's diaphragm.

X_1 = 0.3, X_2 = 0.6 (mg/ml).

TABLE 4.9

INFLUENCE OF Ca^{++}, Mg^{++} AND K^{+} ON THE EFFECT OF THE ETHANOL EXTRACT ON THE ROOTS OF ABRUS PRECATORIUS ON NERVE-INDUCED CONTRACTION OF RAT'S DIAPHRAGM, USING KREB'S SOLUTION.

Concentration of drugs (mg/ml)	% INHIBITION					
	Normal physiological solution	Ca^{++}		Mg^{++}		K^{+}
		1.25 mM	5.0 mM	0.63 mM	2.5 mM	0.9 mM
X_1 (0.3)	4.89	62.62	0.00	0.00	53.39	58.17
X_2 (0.6)	19.21	82.56	0.00	0.00	78.81	76.23
X_3 (1.2)	69.76	100.00	30.68	20.09	100.00	100.00
X_4 (2.4)	86.47	100.00	62.76	49.59	100.00	100.00

The results obtained using normal physiological solution is presented as the control.

concentration, the extract (0.3 - 2.4 mg/ml) inhibited (58.17 - 100.00%) the contraction of the diaphragm (Table 4.9).

4.6 Effects of the Extracts on Chicks

4.6.1 Type of Paralysis caused by the Water Extracts

The cold water extract of the seeds (1.0 - 20.0 mg/kg body weight) injected intraperitoneally (ip), had no effect on the chicks, while a dose of 20.0 mg/kg body weight of the hot water extract resulted in spastic paralysis and death in 40% of the chicks used. Signs observed in the affected chicks included drowsiness and violent tonic convulsions leading to death after 28 hours. Another group of chicks injected ip with the largest volume of the solvent vehicle (0.5 ml) did not show any adverse effect.

The 5-day old chicks injected ip with the cold water extract of the leaves (1.0 - 20.0 mg/kg body weight) did not show any adverse signs. The chicks (40%) injected with the hot water extract had clinical signs of drowsiness and violent tonic convulsions, leading to spastic paralysis and death after 32 hours. The largest

volume of the solvent vehicle used (0.5 ml), which was injected ip into another group of chicks, had no effect on the chicks.

The cold water extract of the roots (1.0 - 20.0 mg/kg body weight), injected ip, had no effect on the chicks, whereas the hot water extract (20.0 mg/kg body weight), injected ip, resulted in spastic paralysis and death in 40% of the chicks used. The affected chicks showed signs of drowsiness and violent tonic convulsions, leading to death after 25 hours. The group of chicks injected ip with the largest volume of the solvent vehicle used (0.5 ml) did not show any adverse effect.

4.6.2 Type of Paralysis caused by the Petroleum Ether Extracts.

The 5 day old chicks (40%) injected ip with the petroleum ether extract of the seeds (10.0 mg/kg ip) showed signs of spastic paralysis and death after 25 hours. Other signs observed included restlessness and violent tonic convulsions leading to death.

The extracts of the leaves (1.0 - 20.0 mg/kg; ip) and of the roots (1.0 - 20.0 mg/kg, ip) had no effect on 5 day old chicks.

The group of chicks injected ip with the saline solution (0.5 ml), and the group injected ip with 5% w/v acacia (0.5 ml) did not show any toxic effect.

4.6.3 Type of Paralysis caused by the Ethanol Extracts.

10 mg/kg of the ethanol extract of the seeds, injected iv into a group of 5 days old chicks, resulted in flaccid paralysis and death within approximately 20 seconds. The signs shown by the chicks (100%) included staggering, drowsiness, convulsions and death with the neck flexed anteriorly while the legs were extended.

Similarly, 20 mg/kg of the ethanol extract of the leaves injected iv into young chicks induced flaccid paralysis and death (100%) within 1½ hours. The signs observed prior to death were similar to those induced by the seeds.

10 mg/kg of the ethanol extract of the roots also resulted in flaccid paralysis and death within approximately 50 minutes, when injected iv into 5 day old chicks. Prior to death, the signs observed were similar to those induced by the extracts of the seeds and leaves.

The solvent vehicle, saline solution (0.5 ml) injected iv into the chicks in the control group, did not show any adverse effect.

4.6.4 Lethal Effects of Ethanol Extracts

4.6.1.1 Mortality

Tables 4.10, 4.11 and 4.12 show the percent mortality obtained when different doses of the ethanol extracts of the seeds, leaves and roots respectively, of Abrus precatorius, were injected iv into 5 day old chicks. In table 4.10, all the chicks injected iv with 10.0 mg/kg body weight of the ethanol extract of the seed died within 20 seconds while no mortality was reported in the group of chicks injected with 1.0 mg/kg of the extract. Also all the chicks injected iv with 20.0 mg/kg body weight of the ethanol extract of the leaves died within 1½ hours whereas no death was reported in the group of

chicks injected with 5.0 mg/kg of the extract (Table 4.11).

Similarly, Table 4.12 shows that all the chicks injected iv with 10.0 mg/kg body weight of the ethanol extract of the roots died within 50 minutes while no mortality was reported in the group of chicks injected with 2.5 mg/kg of the extract.

In the control groups of the three ethanol extracts, no mortality was observed.

4.6.4.2 Relationship between Dose and Mortality

There was an increase in % mortality as the dose injected into the chicks increased. As shown in table 4.13, the correlation coefficient (R) for the ethanol extract of the seeds was 0.9779, which was statistically significant ($p < 0.001$). Table 4.14 shows that for the ethanol extract of the leaves, R = 0.9690 ($p < 0.01$). Similarly, table 4.15 shows that for the ethanol extract of the roots, R = 0.9615 ($p < 0.01$). Appendices 1, 2 and 3 show the process of calculating slope (b), intercept (a), and correlation coefficient (R) for the ethanol extracts of the seeds, leaves and roots respectively of Abrus precatorius.

4.6.4.3 Acute Toxicity (LD 50)

Figures 4.41, 4.42 and 4.43 show the 50% lethal dose of the ethanol extracts of the seeds, leaves and roots respectively. The LD50 of the seeds was 4.15 mg/kg iv, while that of leaves was 11.0 mg/kg iv. Similarly, the LD50 of the root was 4.6 mg/kg iv.

TABLE 4.10

LETHAL EFFECTS OF ETHANOL EXTRACT OF THE SEEDS OF *ABRUS PRECATORIUS* ON CHICKS.

Group	Dose (mg/kg)	Number of Chicks				
		Initial	Dead	Survival	% dead	Corrected % mortality
1	10.0	10	10	0	100	97.5
2	7.5	10	8	2	80	80.0
3	5.0	10	6	4	60	60.0
4	2.5	10	4	6	40	40.0
5	1.0	10	0	10	0	2.5
6	C	10	0	10	0	0.0

C - control group injected iv with normal saline solution, the solvent vehicle

TABLE 4.11

LETHAL EFFECTS OF ETHANOL EXTRACTS OF THE LEAVES OF ABRUS PRECATORIUS ON CHICKS.

Group	Dose (mg/kg)	Number of chicks			% dead	Corrected % mortality
		Initial	Dead	Survival		
1	20.0	10	10	0	100	97.5
2	15.0	10	6	4	60	60.0
3	10.0	10	4	6	40	40.0
4	5.0	10	0	10	0	2.5
5	C	10	0	10	0	0.0

C - control group injected iv with normal saline solution, the solvent vehicle.

TABLE 4.12

LETHAL EFFECTS OF ETHANOL EXTRACTS OF THE ROOTS OF *ABRUS PRECATORIUS* ON CHICKS.

Group	Dose (mg/kg)	Number of Chicks			% dead	Corrected % mortality
		Initial	Dead	Survival		
1	10.0	10	10	0	100	97.5
2	7.5	10	8	2	80	80.0
3	5.0	10	6	4	60	60.0
4	2.5	10	0	10	0	2.5
5	C	10	0	10	0	0.0

C - control group injected iv with normal saline solution, the solvent vehicle.

TABLE 4.13

RELATIONSHIP BETWEEN DOSE AND OBSERVED % MORTALITY AFTER INJECTING IV THE ETHANOL EXTRACT OF THE SEEDS OF *ABRUS PRECATORIUS* INTO CHICKS.

	x dose (mg/kg)	y observed % mortality
	10.0	100.0
	7.5	80.0
	5.0	60.0
	2.5	40.0
	1.0	0.0
	0.0	0.0
Mean	$\bar{x}$ = 4.33	$\bar{y}$ = 46.67
s.d.	3.89	41.31
s.e.m.	± 1.59	± 16.86

slope = 10.37 intercept = 1.77

R = 0.9779 $p < 0.001$

LD50 = 4.15 mg/kg iv

sd = standard deviation
s.e.m. = standard error of mean.
R = correlation coefficient
p = propability
LD50 = 50% lethal dose

TABLE 4.14

RELATIONSHIP BETWEEN DOSE AND OBSERVED % MORTALITY AFTER INJECTING IV THE ETHANOL EXTRACT OF THE LEAVES OF ABRUS PRECATORIUS INTO CHICKS.

	x Dose (mg/kg)	y observed % mortality
	20.0	100.0
	15.0	60.0
	10.0	40.0
	5.0	0.0
	0.0	0.0
Mean	$\bar{x}$ = 10.0	$\bar{y}$ = 40.0
s.d.	7.91	42.43
s.e.m.	± 3.54	± 18.98

Slope = 5.2 intercept = - 12.0

R = 0.9690 $0.01 > p > 0.001$

LD50 = 11.0 mg/kg iv

s.d. = standard deviation
s.e.m. = standard error of mean.
R = correlation coefficient
p = propability
LD50 = 50% lethal dose

TABLE 4.15

RELATIONSHIP BETWEEN DOSE AND OBSERVED % MORTALITY AFTER INJECTING IV THE ETHANOL EXTRACT OF THE ROOTS OF ABRUS PRECATORIUS INTO CHICKS.

	x Dose (mg/kg)	y Observed % mortality
	10.0	100.0
	7.5	80.0
	5.0	60.0
	2.5	0.0
	0.0	0.0
Mean	$\bar{x}$ = 5.0	$\bar{y}$ = 48.0
s.d.	3.95	46.04
s.e.m.	± 1.77	± 20.59

Slope = 11.2 intercept = - 8.0

R = 0.9615 $0.01 > p > 0.001$

LD50 = 4.6 mg/kg iv

s.d. = standard deviation
s.e.m. = standard error of mean.
R = correlation coefficient
p = propability
LD50 = 50% lethal dose

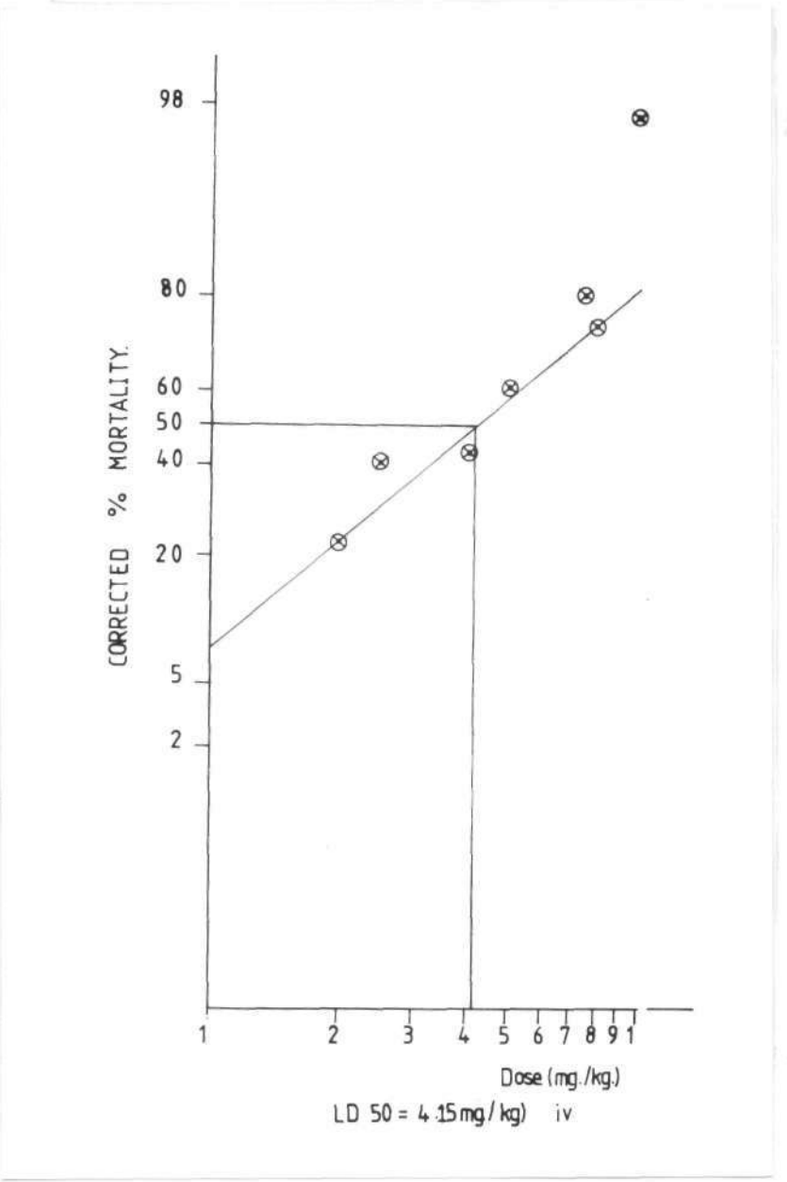


Figure 4.41: 50% lethal dose (LD50) of the ethanol extract of the seeds of Abrus precatorius.

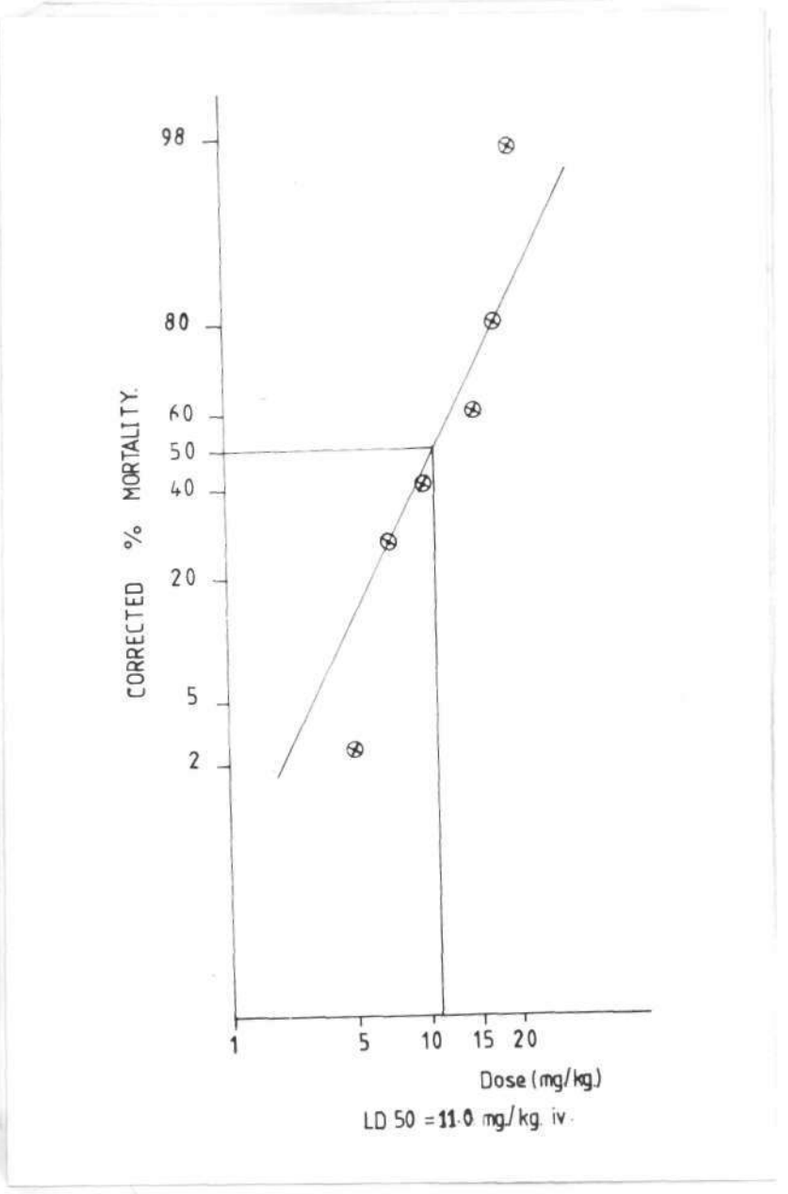
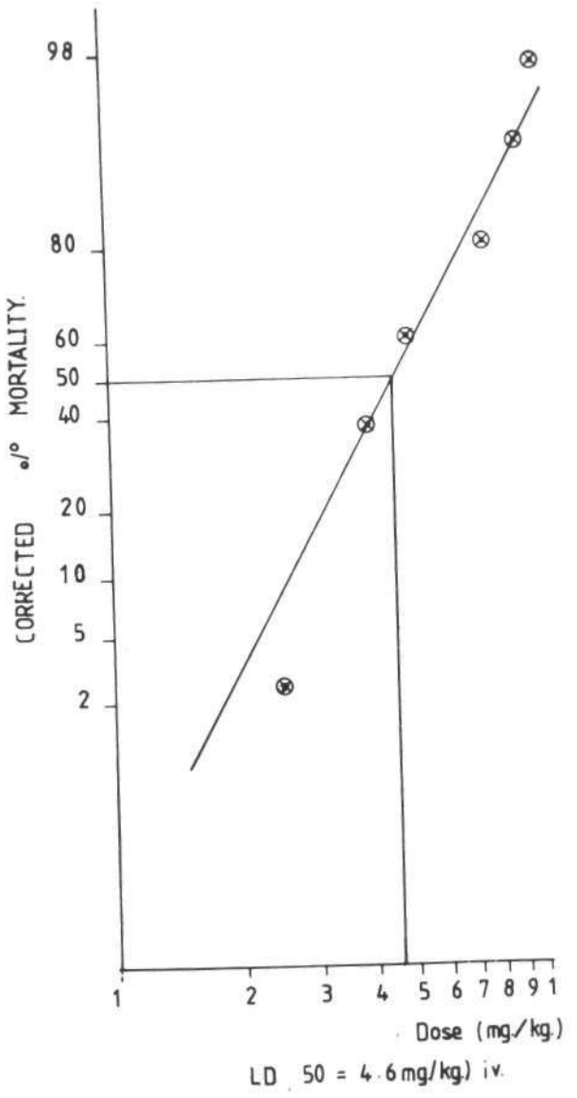


Figure 4.42: 50% lethal dose (LD50) of the ethanol extract of the leaves of Abrus precatorius.



4.43

Figure 4.43: 50% lethal dose (LD50) of the ethanol extract of the roots of Abrus precatorius.

CHAPTER 5. 0

DISCUSSION

Both the water and petroleum ether extracts of the seeds, leaves and roots of ABRUS PRECATORIUS LINN. had no noticeable effect on the isolated skeletal muscle preparations used in this project. On the other hand, the ethanol extracts of the seeds, leaves and roots induced significant antagonistic effects on both toad rectus abdominis muscle and rat phrenic nerve-diaphragm muscle preparations. These observations suggest that the active principle(s) in the seeds, leaves and roots, responsible for neuromuscular paralysis are extractable in ethanol.

In addition, ethanol extracts inhibited acetylcholine-induced contractions of the rectus abdominis muscle and nerve induced contraction of the diaphragm muscle in a concentration-dependent manner. The pattern of the neuromuscular blockade indicates that the effects were specific and might be attributed to some particular components in the ethanol extracts.

The inhibition induced by the ethanol extracts were pontentiated by d-tubocurare and reversed by esarine. This suggests that the ethanol extracts of Abrus precatorius acted on the postsynaptic membrane and induced a competitive type of neuromuscular blockade. Also, the ethanol extracts had no effect on direct electrical stimulation of the rat's diaphragm muscle. These neuromuscular effects of the ethanol extracts resemble those of d-tubocurare.

In 1937, Dalziel indicated that the seed of Abrus precatorius might be poisonous to nerve cells.

In this project, the ethanol extracts of the seeds, leaves and roots interfered with neuromuscular transmission. It is therefore possible that the neuromuscular paralysis reported in horses resulting from ingestion of the seeds of Abrus precatorius (Nwude, 1979) might be attributable to some neuronal toxic components in the seeds, leaves and roots. Thus, reservations by Akinloye and Adalumo (1980) about the use of the leaves of Abrus precatorius as a liquorice substitute may be justified.

When injected intraperitoneally into chicks, the hot water extracts of the seeds, leaves and roots of Abrus precatorius caused spastic paralysis, whereas the cold water extracts did not. This suggests that more of the toxic components were extracted in hot water whereas the same toxic components were either not extractable or extracted in smaller quantities in cold water. The petroleum ether extract of the seeds, when injected intraperitoneally into chicks, also resulted in spastic paralysis. The ethanol extracts of the seeds, leaves and roots of Abrus precatorius, when injected intravenously into chicks, resulted in flaccid paralysis. The difference in pattern of paralysis obtained with hot water extracts of the seeds, leaves and roots (spastic), petroleum ether extract of the seeds (spastic), and ethanol extracts of the seeds, leaves and roots (flaccid), might be attributable to different chemical components extracted by these solvents. The ethanol extract of the seeds is the most toxic (4.15 mg/kg iv), followed by that of the roots (4.6 mg/kg/ iv).

The ethanol extract of the leaves was the least toxic (11.0 mg/kg iv).

Although the results obtained with ethanol extracts of seeds, leaves and roots of Abrus precatorius on isolated toad's rectus abdominis showed a quick onset of action, this was not the case when rat's phrenic nerve-diaphragm was used. The results indicated that there was a time lag in each case within addition of extract and manifestation of neuromuscular blockade. Such an observation does not conform to the pattern of neuromuscular blockade by d-tubocurare. However, it is possible that the extracts contained chemical components which may have differential effect on neuromuscular transmission (i.e. competitive and non-competitive blockade). This suggestion is supported by results obtained when the extracts were injected into chicks, resulting in both flaccid (competitive blockade) and spastic (non-competitive blockade) paralysis. The difference in time course of action between the ethanol extracts and d-tubocurare might also be attributed to the crude nature of the extract. Thus a relatively longer time period might be required for the active components of the extracts to have access to the motor end plates.

The inhibition of nerve-induced contractions of the rat's diaphragm by the ethanol extract of the seeds was potentiated in the presence of reduced calcium ions while the inhibition was greatly reduced in the presence of increased calcium ions. On the other hand, magnesium ions induced opposite effects to those observed with calcium ions vis-a-vis inhibition of nerve-induced contraction of rat's diaphragm. The mutually antagonistic effects of calcium and magnesium ions were also repeated for the ethanol extracts of the leaves and roots. These results agree with the report that calcium ions are involved in the complex processes of neurotransmitter (acetylcholine) release at the peripheral nerve endings while magnesium ions inhibit transmitter release (Mayer, 1980). In addition, reduced potassium ions potentiated the inhibition of nerve-induced contraction of rat's diaphragm by the ethanol extracts of the seeds, leaves and roots. This observation is similar to the influence of potassium ions on neuromuscular blockade induced by d-tubocurare (Zaimis, 1976). Thus, an additional similarity between the neuromuscular effect of the ethanol extracts of the seeds, leaves and roots of Abrus precatorius and d-tubocurare has been identified.

In conclusion, Ghani (1981) indicated that despite the high potentiality of Nigerian indigenous medicinal plants, these plants can only be acceptable as pharmaceutical raw-materials after much research work has been carried out to eliminate the possible poisonous components of the plants. The antifertility activities of the steroidal oil faction from the petroleum ether extract of the seeds (Desai & Rupawala, 1967), and also of the petroleum ether and ethanol extracts of the roots (Agarwal et al, 1970) have been reported in rats and mice. Desai and Sirsi (1966) have also reported on the anti-microbial activity of the ethanol extract of the seeds. These are some of the basis of the medicinal uses of parts of Abrus precatorius.

In spite of the apparent scientific justification for the medicinal use of Abrus precatorius, the present data indicate that the neuronal poisonous component of the seeds, leaves and roots reside mainly in the ethanol extracts. In addition, the results show that the pattern of neuromuscular blockade induced by the ethanol extracts resemble that of d-tubocurare.

CHAPTER 6.0

SUMMARY AND CONCLUSIONS

6.1 Summary

Neuromuscular effects of the crude extracts of the seeds, leaves and roots of Abrus precatorius (Linn.) were investigated using the isolated toad's rectus abdominis and the rat's phrenic nerve-diaphragm muscle preparations, as well as young chicks. The cold and hot water extracts of the seeds, leaves and roots showed no significant neuromuscular activity of their own, nor on acetylcholine-induced contractions of the rectus abdominis, nor on phrenic nerve-diaphragm muscle preparations. Also the extracts had no effect on direct muscle stimulation of the diaphragm muscle. Similarly, the petroleum ether extracts of the seeds, leaves and roots did not show any significant neuromuscular activity on the isolated rectus abdominis of the toad and the phrenic nerve-diaphragm of the rat. The extracts did not exhibit any inhibitory or stimulatory effect on their own. The hot water extracts of the seeds, leaves and roots, and the petroleum ether extract of the seed induced spastic paralysis in chicks.

The ethanol extracts of the seeds, leaves and roots inhibited acetylcholine-induced contractions of both toad's rectus abdominis and rat's phrenic nerve-diaphragm muscle preparations. The effects were concentration-dependent and reversible. The extracts also caused flaccid paralysis when injected intravenously into young chicks. The extracts however had no effect on direct electrical stimulation of the rat's diaphragm. The inhibitory effect of the ethanol extract on the nerve-induced contraction of the rat's diaphragm was potentiated in the presence of reduced calcium ions, elevated magnesium ions or reduced potassium ions. Thus the ethanol extracts showed a similarity to d-tubocurare vis-a-vis the pattern of neuromuscular blockade. Apparently, the neuronal poisonous component of the seeds, leaves and roots of Abrus precatorius (Linn.) reside mainly in the ethanol fraction.

6.2 Conclusions

It is apparent that Abrus precatorius Linn. contributes to the paralysis that may develop after its use for medicinal purposes.

The ethanol extracts of the seeds, leaves and root of Abrus precatorius Linn. have neuromuscular blocking effects.

The ethanol extracts showed a similarity to d-tubocurare vis-a-vis the pattern of neuromuscular blockade.

Whatever the scientific justification for its medicinal use, Abrus precatorius Linn. may not be acceptable as pharmaceutical raw-materials until the neuronal poisonous component(s) has been isolated, purified and its characteristics studied.

REFERENCES

1. AGARWAL, S. S., GHATAK., ARORA, R. B. & BHARDWAJ, M. M. (1970), Antifertility activity of the roots of Abrus precatorius. Pharmacological Research Communication, 2 (2), 159 - 63.

2. AKINLOYE, B. A. (1981), Methodology and approach to chemical studies of medicinal plants. Seminar at Pharmacognosy Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria, in May 1981.

3. AKINLOYE, B. A. & ADALUMO, L. A. (1980), Abrus precatorius leaves - a source of glycyrrhizin. Presented at the 2nd International Conference on Medicinal Plants, held at the University of Ife, Ile-Ife, Nigeria.

4. AMBACHE, N. (1949), The peripheral action of Clostridium botulinum toxin. Journal of Physiology, 108, 127 - 41.

5. ARENA, J. M. (1974), Poisoning. Toxicology, Symptoms, Treatments. 3rd edition. Charles C. Thomas Publishers, Illinois, p492.

6. ARMITAGE, P. (1971), Statistical Methods in Medical Research. Blackwell Scientific Publications, Oxford. pp 105, 150 - 156, 239 & 462.

7. BAKER, P. F. (1966), The Nerve Axon. Scientific America, 214 (3), 74 - 82.

8. BOWMAN, W. C. & MARSHALL, I. G. (1972), Inhibitors of Ach synthesis. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford. Section 14, Volume 1, 357 - 90.

9. BULBRING, E. (1946), Observation on the isolated phrenic nerve-diaphragm preparation of the rat. British Journal of Pharmacology and Chemotherapy, 1, 38 - 61.

10. BURGEN, A. S. V. (1972), The action of botulinum toxin on neuromuscular transmission. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume 1, 169 - 74.

11. BURN, J. H. (1952), Practical Pharmacology, Blackwell Scientific Publications, Oxford.

12. CARBONESCHI, C. L. (1947), Chemistry and Toxicology of Abrus precatorius or seed of jequirity. Semana Med. (Buenos Aires), 2, 275 - 81.

13. CHANG, C. & LEE, C. Y. (1966), Models of action of purified toxins from elapid venoms on neuromuscular transmission. Mem. Inst. Butantan (Sao Paulo), 33 (2), 558 - 72.

14. CHEYMOL, J. & BOURILLET, F. (1972), Points of impact of modifiers of neuromuscular transmission. In: International Encyclopaedia of Pharmacology and Therapeutics (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume I, 135 - 40.

15. CHEYMOL, J. & BOURILLET, F. (1972), Tetradotoxin and Saxitoxin. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume 1, 175 - 86.

16. CHEYMOL, J. & BOURILLET, F. (1972), Inhibitors of postsynaptic receptors. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume 1, 297 - 356.

17. COUTEAUX, R. (1972), Structure and cytochemical characteristics of neuromuscular junction. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume 1, 7 - 56.

18. DALE, H. H. (1936), Release of acetylcholine at voluntary motor nerve endings. Journal of Physiology. 86, 353 - 80.

19. DALZIEL, J. M. (1937). The Useful Plants of West Tropical Africa. Crown Agents, London. 612.

20. DEBECKER, J. (1972), Activation of neuromuscular transmission - calcium, potassium, veratine, guanidine. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume II, 503 - 7.

21. DESAI, V. B. & SIRSI, M. (1966), Antimicrobial activity of Abrus precatorius. Indian Journal of Pharmaceutical Sciences. 28 (6); 164 - 5.

22. DESAI, R. V. & RUPAWALA, E. N. (1967), Antifertility activity of the steroidal oil of the seed of Abrus precatorius. Indian Journal of Pharmaceutical Sciences. 29 (8), 235 - 7.

23. DETTBARN, W. D. (1971), Mechanism of action of tetradotoxin (TTX) & Saxitoxin (STX). In: Neuropoisons - their pathophysiological actions. (ed. Lance L. Simpson), Plenum Press, New York, Volume 1, 169.

24. ENGBAEK, L. (1972), Inhibitor ion - Magnesium. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume 1, 391 - 405.

25. FISHER, R. A. & YATES, F. (1974), Statistical Tables for biological, agricultural and medical research. 6th edition. Reprinted by Longman Group Ltd. Edinburgh, 63.

26. GHANI, A. (1981), Potentiality of Nigerian Indigenous Medicinal Plants as Pharmaceutical Raw Materials. The Nigerian Journal of Pharmacy, 12 (4), 27 - 29.

27. GHOSAL, S. & DUTTA, S. K. (1971), Alkaloids of Abrus precatorius. Phytochemistry, 10, 195 - 8.

28. HUBBARD, J. I. (1972), Physiological and Biochemical aspects of the neuromuscular junction. In: International Encyclopaedia of Pharmacology and Therapeutics. (ed. C. Radouco-Thomas), Pergamon Press, Oxford, Section 14, Volume I, 57 - 105.

29. HUBBARD, J. I., JONES, S. F. & LANDAU, E. M. (1968a), Mechanism by which calcium and magnesium affect the spontaneous release of transmitter from mammalian motor nerve terminal. Journal of Physiology, 194, 355 - 80.

30. HUBBARD, J. I., JONES, S. F. & LANDAU, E. M. (1968b) Mechanism by which calcium and magnesium affect the release of transmitter by nerve impulse. Journal of Physiology, 196 (1), 75 - 86.

31. KATZ, B. (1952). The nerve impulse. Reprinted from Scientific America. November.

32. KATZ, B. & MILEDI, R. (1965), Acetylcholine release from motor nerve endings. Mech. Release Biogenic Amines, Proc. Int. Wenner-Guen Cent. Symp., Stockholm. (Published 1966), 417 - 24.

33. KATZ, B. & MILEDI, R. (1967a). Tetradotoxin and neuromuscular transmission. Proc. Roy. Soc. (London). 167 (10006), 8 - 22.

34. KATZ, B. & MILEDI, R. (1967 b). Ionic requirements of synaptic transmitter release. Nature. 215 (5101), 651.

35. KATZ, B. & MILEDI, R. (1968). The role of calcium in neuromuscular facilitation. Journal of Physiology (London), 195, 481 - 92.

36. KATZ, B. & MILEDI, R. (1969). Effect of divalent cations on transmission in the squid giant synaps. Pubbl. Sta. Zool. Napoli, 37 (2), 303 - 10.

37. KINGSBURY, J. M. (1964), Poisonous plants of the United States and Canada. 3rd edition. Prentice-Hall Inc. New Jersey. 303.

38. KOELLE, G. B. (1980), Neuromuscular Blocking Agents. In: Pharmacological Basis of Therapeutics. (eds. Gilman, A. G. and Goodman, L. S.). Macmillan Publishing Company, New York, 6th edition. 575 - 9.

39. MAYER, S. E. (1980), Neurohumoral transmission and the autonomic nervous system. In: Pharmacological Basis of Therapeutics. (eds. Gilman, A. G. and Goodman, L. S.). Macmillan Publishing Company, New York, 6th edition. 70.

40. MEYERS, J. A. (1980). New Plagues for Old? - Medicine. TIME, November 24, 47, 50.

41. MILLER, L. C. & TAINTER, M. L. (1944), Estimation of ED50 and its Error by means of Logarithmic - Probit Graph Paper. Pro. Soc. Exptl. Biology, 57, 261 - 264.

42. MORRIS, J. A. (1976), Sweetening Agents from Natural Sources. Lloydia, January - February, 39 (1), 25.

43. NWUDE, N. (1979), Poisonous Plants in Nigeria. Ahmadu Bello University Press. In print.

44. OLSNES, S. & PIHL, A. (1973). Isolation and properties of Abrin - A toxic protein inhibiting protein synthesis. European Journal of Biochemistry, 35, 179 - 85.

45. SELLIN, L. C. (1981). The action of botulinum toxin at the neuromuscular junction. Medical Biology, 59, 11 - 20.

46. SELLIN, L. C. & THESLEFF, S. (1981). Pre - and post-synaptic actions of botulinum toxin at the rat neuromuscular junction. Journal of Physiology, 317, 487 - 98.

47. SIMPSON, L. L. (1971). Ionic requirements for the neuromuscular action of botulinum toxin - Implications with regard to synaptic transmission. Neuropharmacology, 10 (6), 673 - 84.

48. SIMPSON, L. L. (1973), Interaction between divalent cations and botulinum toxin type A in the paralysis of the rat phrenic nerve - hemidiaphragm preparation. Neuropharmacology, 12(2), 165 - 76.

49. SU, C., CHANG, C. C & LEE, C. Y. (1966). Pharmacological properties of the neurotoxin of cobra venom. Animal Toxins, Collect. Pap. Int. Symp., 1st, Atlantic City, 259 - 67.

50. SUBRAMANIAN, E. H., VISWANATHAN, E. H. & KRISHNAMURTHY, G. (1973). An improved thin-layer chromatographic method for the detection of Abrus precatorius seeds. Current Science, 43 (14), 499.

51. THESLEFF, S. (1958. A study of the interaction between neuromuscular blocking agents and acetylcholine at the mammalian motor end-plate. Acts anesth. Scand., 2, 69 - 79.

52. WATANABE, S. & NARAHASHI, T. (1979), Cation selectivity of Ach-activated ionic channel of frog end plate. Journal of General Physiology, 74 (5), 615 - 28.

53. WATT, J. M. & BREYER-BRANDWIJK, M. G. (1962), Medicinal and Poisonous Plants of Southern and Eastern Africa. 4th edition, E & S Livingstone, Edinburgh, 535 - 6.

54. ZAIMIS, E. (1976). The neuromuscular junction-area of uncertainty. In: Neuromuscular junction. (ed. E. Zaimis). Springer-Verlag, Berlin, 365 - 420.

APPENDIX 1

CALCULATION OF THE SLOPE (b), INTERCEPT (a), AND CORRELATION COEFFICIENT (R) FOR THE ETHANOL EXTRACT OF THE SEEDS OF ABRUS PRECATORIUS

$x - \bar{x}$	$(x - \bar{x})^2$	$y - \bar{y}$	$(y - \bar{y})^2$	$(x - \bar{x})(y - \bar{y})$
10.0 - 4.33 = 5.67	32.15	100.0 - 46.67 = 53.33	2844.09	302.38
7.5 - 4.33 = 3.17	10.05	80.0 - 46.67 = 33.33	1110.89	105.66
5.0 - 4.33 = 0.67	0.45	60.0 - 46.67 = 13.33	177.69	8.93
2.5 - 4.33 = 1.83	3.35	40.0 - 46.67 = 6.67	44.49	12.21
1.0 - 4.33 = 3.33	11.09	0.0 - 46.67 = 46.67	2178.09	155.41
0.0 - 4.33 = 4.33	18.75	0.0 - 46.67 = 46.67	2178.09	202.08

$$b = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2} = \frac{786.67}{75.84} = 10.37$$

$$a = \bar{y} - b\bar{x} = 46.67 - (10.37 \times 4.33) = 46.67 - 44.90 = 1.77$$

$$R = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\{\sum (x - \bar{x})^2 \sum (y - \bar{y})^2\}}} = \frac{786.67}{\sqrt{75.84 \times 8533.34}} = 0.9779$$

APPENDIX 2

CALCULATION OF THE SLOPE (b), INTERCEPT (a), AND CORRELATION COEFFICIENT (R) FOR THE ETHANOL EXTRACT OF THE LEAVES OF ABRUS PRECATORIUS.

$x - \bar{x}$	$(x - \bar{x})^2$	$y - \bar{y}$	$(y - \bar{y})^2$	$(x - \bar{x})(y - \bar{y})$
20.0 - 10.0 = 10.0	100.0	100.0 - 40.0 = 60.0	3600.0	600.0
15.0 - 10.0 = 5.0	25.0	60.0 - 40.0 = 20.0	400.0	100.0
10.0 - 10.0 = 0.0	0.0	40.0 - 40.0 = 0.0	0.0	0.0
5.0 - 10.0 = -5.0	25.0	0.0 - 40.0 = -40.0	1600.0	200.0
0.0 - 10.0 = -10.0	100.0	0.0 - 40.0 = - 40	1600	400.0

$$b = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2} = \frac{1300.0}{250.0} = 5.2$$

$$a = \bar{y} - b\bar{x} = 40.0 - (5.2 \times 10.0) = 40.0 - 52.0 = -12.0$$

$$R = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\left\{\sum (x - \bar{x})^2 \quad (y - \bar{y})^2\right\}}} = \frac{1300.0}{\sqrt{250 \times 7200.0}} = 0.9690$$

APPENDIX 3

CALCULATION OF THE SLOPE (b), INTERCEPT (a), AND CORRELATION COEFFICIENT (R) FOR THE ETHANOL EXTRACT OF THE ROOTS OF ABRUS PRECATORIUS.

$x - \bar{x}$	$(x - x)^2$	$y - \bar{y}$	$(y - \bar{y})^2$	$(x - \bar{x})(y - \bar{y})$
10.0 - 5.0 = 5.0	25.0	100.0 - 48.0 = 52.0	2704.0	260.0
7.5 - 5.0 = 2.5	6.25	80.0 - 48.0 = 32.0	1024.0	80.0
5.0 - 5.0 = 0.0	0.0	60.0 - 48.0 = 12.0	144.0	0.0
2.5 - 5.0 = - 2.5	6.25	0.0 - 48.0 = - 48.0	2304.0	120.0
0.0 - 5.0 = - 50	25.0	0.0 - 48.0 = - 48.0	2304.0	240.0

$$b = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2} = \frac{700.00}{62.5} = 11.2$$

$$a = \bar{y} - b\bar{x} = 48.0 - (11.2 \times 5) = 48.0 - 56.0 = - 8.0$$

$$R = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \sum (y - y)^2}} = \frac{700.0}{\sqrt{62.5 \times 8480.0}} = 0.9615$$