SOME NEUROMUSCULAR EFFECTS OF THE CRUDE EXTRACTS OF THE ROOTS OF ABRUS PRECATORIUS LINN

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SUMMARY

The effects of the crude water, petroleum ether and ethanol extracts of the root of Abrus precatorius Linn (family: Leguminosae) were investigated using the isolated toad's rectus abdominis and the rat's phrenic nerve-diaphragm preparations. The ethanol extract (0.5 – 4.0 mg/ml inhibited (20.1 – 85%) acetylcholine (1 – 4 ug/ml) – induced contractions of toad's rectus abdominis. Similarly, the ethanol extract (0.3 – 2.4 mg/ml) antagonised (4.5 – 86.5%) to contraction of the rat diaphragm muscle elicited via electrical stimulation of the phrenic nerve. In both cases, the antagonism was concentration-dependent, reversed by addition of eserine or washing and potentiated by d-tubocurare. The inhibitory effect of the ethanol extract on the rat's phrenic nerve-diaphragm preparation was potentiated in the presence of reduced Ca^{++} (1.25mM), elevated Mg^{++} (2.50mM) or reduced K^{+} (0.9 mM). The ethanol extract also induced flaccid paralysis when injected intravenously into young chicks. Both the petroleum ether and water extracts showed no observable effects on the skeletal muscle preparations used in this project. The present data indicates that the ethanol fraction of the roots of Abrus precatorius Linn possesses neuromuscular blocking effects similar to those of d-tubocurare.

INTRODUCTION

According to Watt and Breyer-Brandwijk (1962) the fresh roots of *Abrus precatorius* Linn were chewed to induce aphrodisiac effect while the dried pulverised roots were employed in East Africa for the treatment of snake bite. In addition, different parts of *Abrus precatorius* plant have been powdered and included in toxic preparations used as arrow poison in hunting wild animals (Watt and Breyer-Bandwijk, 1962). Recently, Wambebe and Amosun (1982) reported that the crude extracts of the leaves of *Abrus precatorius* Linn possessed neuromuscular blocking effect. In this paper, the effect of water, petroleum ether and ethanol extracts of the roots of *Abrus precatorius* Linn on neuromuscular transmission using young chicks, isolated rectus abdominis muscle of the toad and phrenic nerve-diaphragm muscle of the rat will be reported.

Materials and methods

Plant and animals.

The roots were obtained from Abrus precatorius plant which grows naturally in the Kubani Dam area, Ahmadu Bello University main campus in Zaira, Nigeria. The plant was identified by Mr. Ohaeri, a taxonomist in the Department of Biological Sciences, Facultry of Science, Ahmadu Bello University, Zaria.

Extractions

(a) Water.

20 g. dried, ground roots were added to 100.0 mls of cold de-ionised water and left for 24 hours. Another 20.0 g portion of the roots were added to 100.0 mls of boiling de-ionised

water for 1 hour. After filtration, the residue was discarded while the filtrate was used for the study after evaporation of water in a rotavapour.

(b) Ethanol.

25f. of the roots were added to 90% v/v ethanol at 60-70°C under reflux, in a soxhlet apparatus and left for 24 hours. After this procedure, ethanol was evaporated to dryness using a rotavapour.

(c) Petroleum ether.

50 g. of the roots were added to petroleum ether, under reflux, in a soxhlet apparatus and left for 24 hours. Petroleum ether was evaporated in a rotavapour leaving a yellowish oily liquid.

Drug solutions.

Stock solutions of the extracts were prepared by disolving a weighed amount of each extract in a known volume of normal saline (0.9% w/v NaCl). The solutions were stored in the refrigerator until ready for use. Other durg solutions used were acetylcholine (Sigma Chemical Co.), d-tubocurare (Sigma Chemical Co.) and eserine (Sigma Chemical Co.). Stock solutions of acetylcholine, d-tubocurare and eserine were made by dissolving, in each case, a known weight of the drug in a volume of normal saline (0.9% w/v NaCl).

Toad Rectus Abdominis Muscle Preparation (Burn, 1952, modified).

The toad was stunned, decapitated and then the spinal cord was destroyed with blunt needles. The toad was placed in a supine position afterwhich the skin of the abdomen was cut open to expose the whole of the abdomen. A peice was cut from one of the two rectus abdominis muscles and immediately transferred into a petri dish containing freshly prepared Frog Ringer's solution (concentrations of solutes (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, $CaCl_2$(M) = 0.12). The Frog Ringer's solution was aerated continuously with ordinary air.

After the tissue was freed of fatty materials, a thread was sewn through one end of the tissue, a longer thread was sewn for attachment to the transducer. The preparation was then transferred into the tissue bath (containing 20 mls of Ringer's solution) afterwhich the longer thread was tied to the transducer. The aeration of the tissue was maintained throughout the period of the experiment. The temperature of the tissue bath was maintained at a room temperature of about 28°C with the aid of a thermostatically-controlled water circulator. The preparation was left in the tissue bath to equilibrate for 30 minutes before studying the effects of drugs on it.

Rat Phrenic Nerve-diaphragm Preparation (Bulbring, 1946, modified)

The rat was killed by a blow on the head, and bled. The skin was removed from the middle of the chest. The muscles were freed from the chest wall, and the ribs were then 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. 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 intact. The nerve was then cut below the thymus and the whole preparation was transferred into a petri dish containing Kreb's solution (concentration of solutes (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, $CaCl_2$ = 0.28) which was continuously aerated with 95% v/v O_2 and 5% v/v CO_2. The base of the fan shaped muscle piece was attached to the electrode with a piece of thread. Similarly, the nerve was connected to the electrode. A longer thread was sewn through the apex of the tissue, which was then attached to the transducer. The whole preparation was then transferred into the organ bath (containing 100 mls of the physiological solution), while the longer thread was attached to the transducer. The temperature of the organ bath was maintained at 37°C using a thermostatically-controlled water circulator. The preparation was then left in the organ bath for 30 minutes to equilibrate before studying the effects of durgs on it.

Recording Apparatus.

The Ugo Basile recording microdynamometer (model No. 7050) was used to record the isometric contractions of the various isolated muscle preaparations. The speed of the recorder was set at 24 mm/min. The stimulating parameters for the phrenic nerve were 10 volts, a frequency of 1 pulse per second and duration of 10 milliseconds. The stimulating parameters for the diaphragm muscle were maintained as above except the voltage which was increased to 20 volts (i.e. supramaximal voltage). Acetylcholine, d-tubocurare and eserine were used as reference agents. Four concentrations (1.0 - 4.0, 2.1 - 16.8, 6.0 - 4.8.0 mg/ml of the ethanol, water and petroleum ether extracts respectively) of the extracts were then tested, and each concentration was repeated four times, using the Latin Square randomisation process. The solvent vehicle was also tested on the tissues. After each test, the tissues were repeatedly washed and allowed to rest for up to 30 minutes. Acute Toxicity Test Using Young chicks.

5–days old chicks were used to study the type of paralysis caused by the injected extracts and also to determine the acute toxicity of the extracts. Different doses of the hydrophylic extracts were injected intravenously into separate groups of chicks. The lethal dose was determine for extracts which showed toxic effects within 24 hours by using the Graphic Method of Miller & Tainter (1944). Signs of toxicity were observed and the number of dead and survivors were recorded.

The formula used to calculate the corrected percent mortality is shown below:

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

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

when n = number of animals in each group = 5

Variation in Ion Concentration.

The influence of K^+, Na^+, Ca^{++} and Mg^{++} on the effects of the extracts on neuromuscular transmission was also studied. Hypo- and hyper-concentrations of each ion were varied in the physiological solutions for each of the tissues. Control recordings were first obtained using the nomal physiological solution. The influence of physiological solutions containing hypo- or hyper-concentrations of either K^+, Na^+, Ca^{++} or Mg^{++} on nerve-induced contraction of rat diaphragm was then examined. Where a difinite effect was indicated, the extracts were then tested in the presence of the physiological fluid containing hypo- or hyper- concentration of a particular ion.

RESULTS.

Extraction.

Brown coloured solid extracts were obtained from the water (cold and hot) extraction of the roots. The percentage yields were 0.4 and 0.8 for cold and hot water fractions respectively.

An oily light brown liquid was obtained after extraction of the roots with petroleum ether. The percentage yield was 2.0.

Ethanol extraction of the roots produced a brown coloured solid material with a percentage yeild of 5.2.

Pharmacology.

Effect of the extracts on Toad's rectus abdominis.

The responses of the toad's abdominis muscle to acetylcholine (0.5 ug/ml) were neither inhibited nor enhanced by the cold water extract (0.43 – 3.44 mg/ml hot water extract (0.89 – 7.12 mg/ml and petroleum ether extract (1.25 – 10.0 mg/ml). For the phrenic nerve-diaphragm, both the cold (0.17 – 1.36 mg/ml) and hot water extracts (0.36 – 2.88 mg/ml) had no effect on the muscle contraction either via nerve stimulation or direct muscle stimulation.

The solvent vehicle (normal saline solution) also showed no effect on both tissues.

However, the rectus abdominis muscle response to acetylcholine (0.5 ug/ml) was antogonised (20.8 – 85.8%) by the ethanol extract (0.5 – 4.) mg/ml; Fig. 1). The antagonism

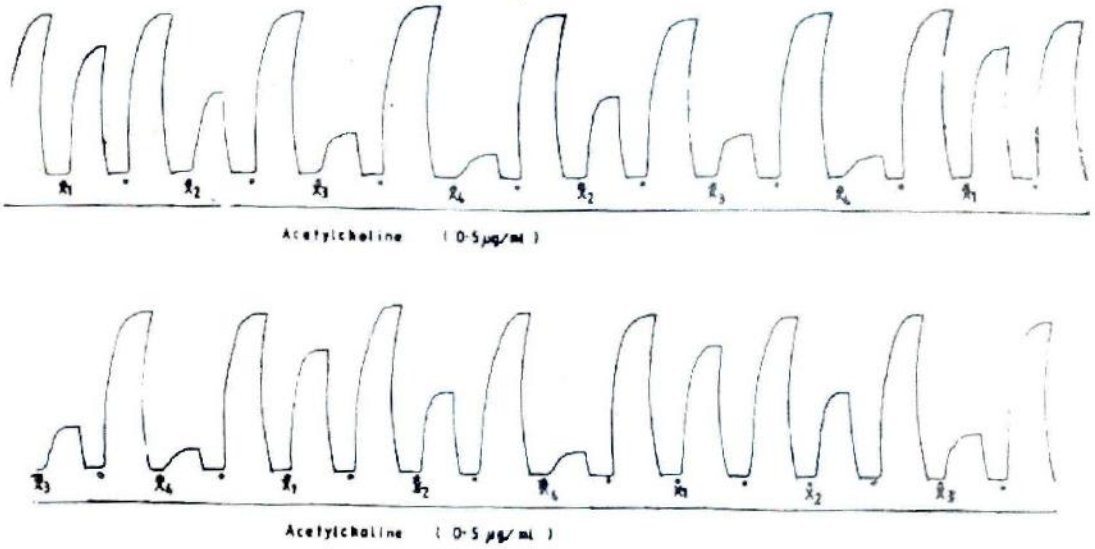


Fig. 1: Influence of the ethanol extract of the roots of *Abrus precatorius* on toad's rectus abdominis mucle. X_1, X_2, X_3 and X_4 represent 3.125, 6.25, 12.50 and 25.0 mg/ml of the tissue bath respectively.

was reversible after repeated washings of the tissue. Using the student's-t-test, the antagonism was higher significant (p 0.001).

Effect of the extracts on the Rat phrenic nerve-diaphragm preparation.

The ethanol extract (0.3 - 2.4 mg/ml) inhibited (4.9 - 86.5%) the responses of the diaphragm muscle to nerve stimulation (Fig. 2) the inhibition caused by the extract was statistically significant (P 0.001), using the student's -t - test. The inhibition was potentiated by d-tubocurare (0.5 ug/ml) but reversed by eserine (1.0 ug/ml; Fig. 3). In all cases, either the extract alone or the solvent vehicle had no observable effect on toad rectus abdominis muscle or rat phrenic nerve diaphragm preparations when they were not stimulated either by acetylcholine or electrically.

The $\log_{10}$ concentration – response graph for the inhibitory effects of the extract on both isolated tissues indicated that the inhibition was concentration-dependent (Fig. 4).

Influence of Ions on the Effects of the roots of *Abrus precatorius* Linn extracts.

In the Frog Ringer's solution, variation in concentration of Ca^{++} (0.54 mM and 2.16 mM), K^+ (0.9 mM and 3.6 mM);

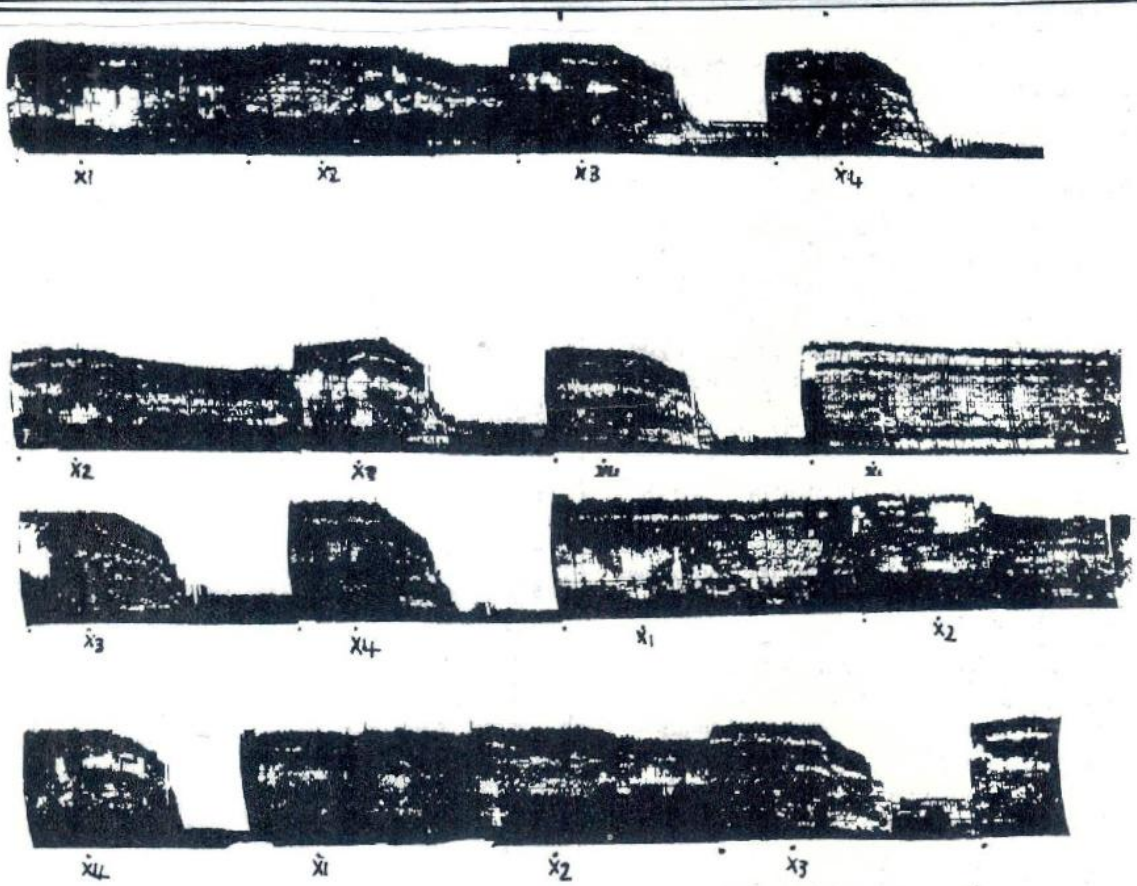


Fig. 2: (a & b). Influence of the ethanol extract of the roots of *Abrus precatorius* on nerve-induced contraction of the diaphragm muscle. X_1, X_2, X_3 and X_4 represent 0.5, 1.0, 2.0 and 4.0 mg/ml of the tissue bath respectively.

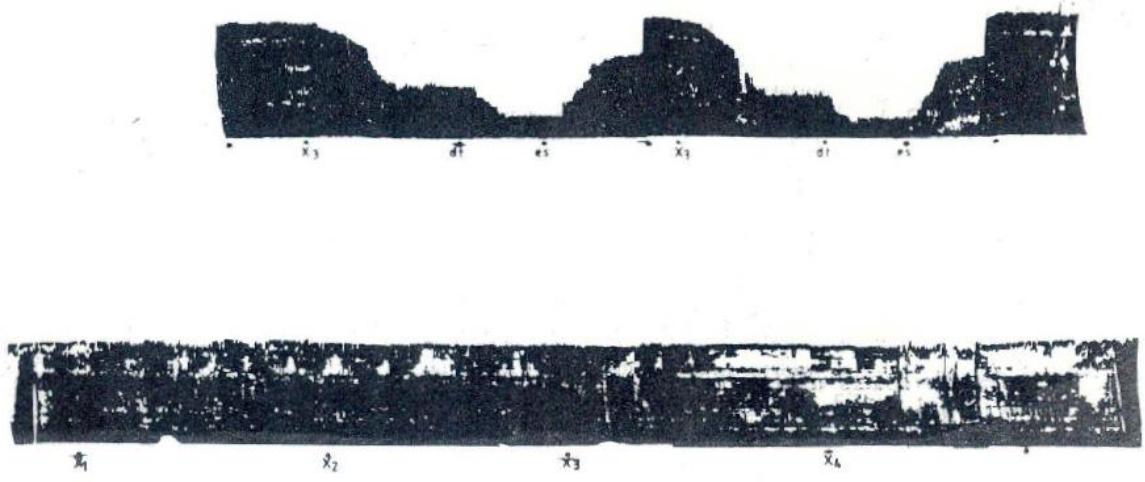


Fig. 3: Influence of d-tubocurare (dt) and eserine (es) on the effect of the ethanol extract of the roots of *Abrus precatorius* on phrenic nerver-induced contraction of rat's diaphragm (upper row) and on the direct electrical stimulation of the diaphragm (lower row). X_1, X_2, X_3 and X_4 represent 100, 200, 400 and 800 ug/ml of the tissue bath.

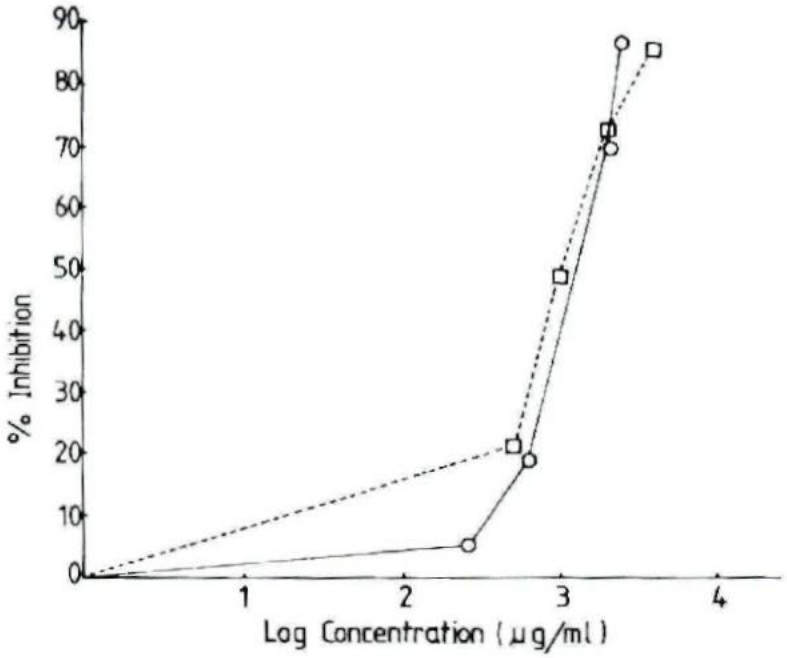
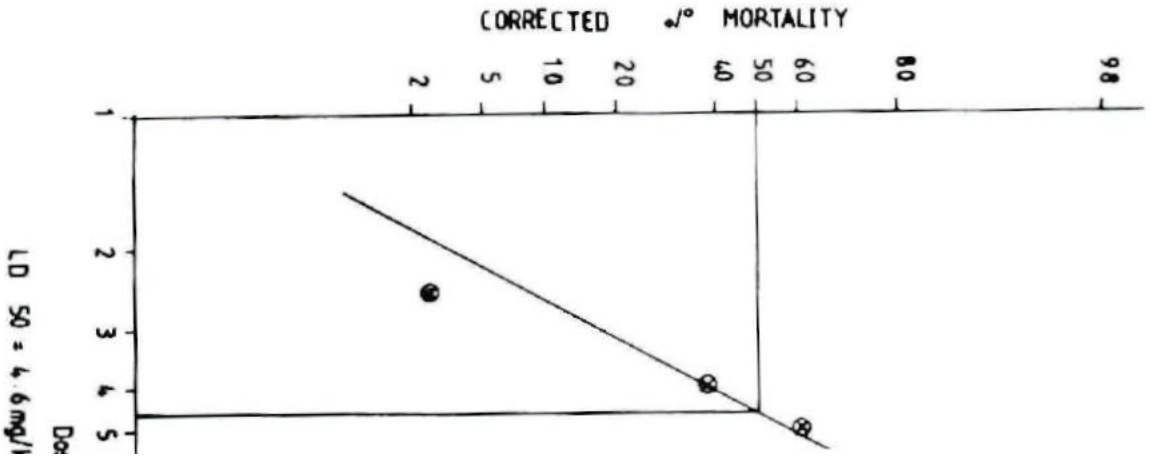


Fig. 4: Log_{10} concentration-response graph of the ethanol extract of the roots of *Abrus precatorius* on toad's rectus abdominis mucle and phrenic nerve-induced contraction of the rat's diaphragm. A represents road's rectus abdominis contraction, while B represents the results obtained with rat phrenic nerve-diaphragm muscle preparation.



and Na^+ (0,06 M and 0.24 M) had no significant effect on the contraction produced by applied ACh (0.5 ug/ml) on the isolated rectus abdominis muscle of the toad. Since Mg^{++} ions are normally absent in Frog Ringer's solution, it was not included in this study.

The influence of variation in Ca^{++} on the effect of the extract on nerve-induced contraction of rat's diaphragm is shown on Table 1. In the presence of reduced Ca^{++} concentration (1.24 mM), the extract (0.3 - 2 mg/ml inhibited (62.6 - 100%) the phrenic nerve-induced contraction of the diaphragm (Table 1). The extract (1.2 - 2.4 mg/ml) inhibited (30.7 - 62.8%) the contraction of the diaphragm in the presence of increased Ca(++ concentration (5.0 mM. Table 1).

The influence of variation in Mg^{++} concentration on the effect of the extract on nerve-induced contraction of the rat's diaphragm is shown on Table 1. The extract (0.3 -2.4 mg/ml) inhibited (53.4 – 100%) the contraction of the diaphragm in the presence of 2.5 mM Mg++ 1.2 and 2.4 mg/ml of the extract inhibited (20.1 – 49.6%) the same contraction in the presence of 0.63 mM Mg^{++} (Table 1).

		% Inhibition					
Conc. of drug (mg/ml)	Normal Physiological fluid	Ca^{++} 1.25 mM	5.0 mM	Mg^{++} 0.63 mM	2.5 mM	K^+ 0 9 mM	3.6mM
X_1 (0.3)	4.89	62.62	0.00	0.00	53.39	58.17	0.00
X_2 (0.6)	19.21	82.56	0.00	0.00	78.81	76.23	0.00
X_3 (1.2)	69.76	100.00	30.68	20.09	100.00	100.00	0.00
X_4 (2.4)	86.47	100.00	62.76	49.59	100.00	100.00	0.00

Table 1: Influence of alteration fo Mg^{++}, Ca^{++} and K^+ concentration on the effects of the ethanol extract of the roots of *Abrus precatorius* on nerve-induced contraction of the rat diaphragm.

The influence of variation in K^+ concentration on the effect of the extract on nerve-induced contraction of the rat's diaphragm is shown on Table 1. In the presence of reduced K+ concentration (0.9 mM), the extract (0.3 – 2.4 mg/ml) inhibited (58.2 – 100%) nerve-induced contraction of the diaphragm (Table 1). An increase in K+ concentration (3.6 mM) in the physiological solution did not influence the normal contraction of the diaphragm muscle (Table 1).

Acute toxicity test using young chicks

When injected intravenously into 5-days old chicks, a dose of 20 mg/kg of the hot water extract resulted in spastic paralysis and death (40%) within 32 hours. Similarly, 20 mg/kg of the ethanol extract injected intravenously into young chicks induced flaccid paralysis and death (100%) within 1½ hours. The 50% Lethal Dose (LD_{50}) was 4.6 mg/kg i.v. (Fig. 5).

DISCUSSION

Both the water and petroleum ether extracts had no effect on the isolated skeletal muscal preparations used in this project. On the other hand, the ethanol extract induced profound antogonistic effects on toad abdominis muscle and rat phrenic nerve-diaphragm muscle preparations. These observations suggest that the active ingredient in the roots responsible for neuromuscular paralysis reside mainly in the ethanol extract. These results are similar to those obtained with the extracts of the leaves of *Abrus precatorius* (Wambebe & Amosun, 1983).

Similarly, the ethanol extract of *Abrus precatorius* roots inhibited acetylcholine-induced contractions of both the rectus abdominis muscle and rat phrenic nerve-diaphragm muscle preparations in a concentration-dependent manner. The pattern of the neuro-muscular blockade induced by the extract indicated that specific chemical components might be responsible. The extract had no effect on electrical stimulation of the rat's diaphragm muscle. These neuromuscular effects of the extract resemble those of d-tubocurare. These observations also show that *Abrus precatorius* roots may contain the neuronal toxic component just like the seeds (Wambebe et al, 1981) and the leaves (Wambebe & Amosun, 1983). It is therefore possible that the neuromuscular paralysis accompanies by muscle weakness in man resulting from ingestion of parts of *Abrus precatorius* might be attributable to a chemical compound present in the seeds, leaves and roots. Similarly, the use of parts of *Abrus precatorius* plant as poison for hunting wild animals might be related to neuromuscular paralysis induced by the toxic components in the plant.

The inhibition of the nerve-induced contraction of rat's diaphragm by the ethanol extract 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 vis-a-vis inhibition of nerve-induced contraction of rat's diaphragm by the ethanol extract. These results agree with the report that calcium is in the complex processes of transmitter release at autonomic nerve endings while magnesium inhibited transmitter release (Mayer, 1980). In addition, reduced potassium ions potentiated the inhibition of nerve-induced contraction of rat's diaphragm by the ethanol extract. 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 extract of *Abrus precatorius* roots and d-tubocurare has been identified.

The present data indicate that the neuronal poisonous component of *Abrus precatorius* roots reside mainly in the ethanol fraction. It is therefore unadvisable to chew the roots of *Abrus precatorius* for aphrodisiac purposes. In addition, the results show that the patter of neuromuscular blockade induced by the extract resemble that of d-tubocurare. These conclusions agree with those reported using the extracts of the seeds and the leaves (Wambebe et at, 1981; Wambebe and Amosun, 1983).

Acknowledgements

The authors acknowledge with thanks the post-graduate scholarship granted to Amosun, S. L. by the Federal Government of Nigeria.

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