In vitro testing of some West African and Indian plants used to treat snakebites

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This paper is presented in honour of Professor Norman Bisset, a member of the European society of Ethnopharmacology and of the Organising Committee of the Symposium, whose untimely death occurred on 12th February 1993.

RÉSUMÉ

Plus de 700 espèces de plantes à fleur ont été recensées pour le traitement des morsures de serpent, mais seulement quelques-unes ont été testées scientifiquement. L'activité de l'extrait de plante est testée sur des animaux. La mortalité du groupe à qui l'on injecte une dose létale de venin est comparée avec celle du groupe à qui l'on donne la même dose de venin, ainsi que des doses d'extraits de plantes. Cette méthode n'est pas recommandée pour un fractionnement « bioassay-guided » ; de ce fait, des tests *in vitro* ont été mis au point. La plante doit être spécifique de l'activité du venin. Les espèces de cobra (*Naja* spp.) fabriquent un venin qui affecte les membranes des cellules musculaires et nerveuses. Des tests ont été mis au point, qui mesurent les effets du venin sur les cellules nerveuses du poussin (*Chick biventer cervicis*), ainsi qu'une réduction de ces effets par l'administration d'extraits de plantes. Un autre test utilisé met en valeur les effets de l'extrait de plante sur une hémolyse déclenchée par le venin. Le venin de *Echis carinatus* provoque une coagulation, et la possible action inhibitrice de l'extrait de plante peut être mise en évidence par l'augmentation du temps de coagulation (induit par une dose standard de venin). Ces méthodes ont été appliquées pour quelques plantes d'Afrique de l'Ouest et de l'Inde, où elles étaient traditionnellement utilisées pour traiter les morsures de serpent, et ces effets inhibiteurs ont été retenus pour toutes les plantes testées.

1. INTRODUCTION

1.1. SNAKEBITE AS A PUBLIC HEALTH PROBLEM

Although death by snakebite is not common in temperate parts of the world it is estimated that every year it accounts for as many as 40 000 fatalities throughout the world, particularly in tropical countries where access to hospital stores of antivenin is very difficult because of distance (HABERMEHL, 1981). It is usually not possible to accurately identify the species of snake involved in cases of envenomation and, since different families of snake produce venoms with different pharmacological activities, clinical treatment often involves use of a polyvalent antivenin. These have disadvantages such as the need to be kept at low temperatures and the allergic reactions which occur in some patients.

1.2. PLANTS USED AGAINST SNAKEBITE

In contrast to the difficulty of availability of this modern treatment in large areas of the developing world many societies who live in such places use plants to treat snakebite. Such plants are recorded in texts dealing with the ethnopharmacology of geographical areas but in most instances there is no information on the method of use of the plant, the part of the plant used or the type of snake whose venom it is supposed to counteract. In recent months the subject of plants used to treat snakebite has attracted the attention of several reviewers (MORS, 1991; MARTZ, 1992; HOUGHTON and OSIBOGUN, 1993). This attention has served to highlight the lack of scientific information regarding the efficacy, chemistry and basis of activity of the plants in question.

1.3. OVERVIEW OF PLANTS REPUTEDLY USEFUL IN TREATMENT OF SNAKEBITE

The most recent review contains the results of a search through the literature comprising a catalogue of over 700 species of flowering plant used to treat snakebite (HOUGHTON and OSIBOGUN, 1993). This list revealed some interesting points such as the widespread geographical use of members of particular families or genera (see Table 1). Thus many species of *Aristolochia* are used. An indication of possible effectiveness might be the occurrence of aristolochic acid in many members of this genus. This compound has been shown to have a general immunostimulant effect and also to inhibit the phospholipases which frequently form part of the venom and which are associated with the inflammatory response (WAGNER and PROKSCH, 1985; VISHNAWATH *et al.*, 1987*a,b,c*).

Table 1

Families and genera containing plants with a widespread geographical distribution and reputed use against snakebite

Family	Genera
DICOTYLEDONÆ	
Acanthaceæ	
Amaranthaceæ	*Amaranthus*
Apocynaceæ	*Rauwolfia*
Compositæ	*Eupatorium*
	Vernonia
Convolvulaceæ	*Ipomœa*
Cucurbitaceæ	
Euphorbiaceæ	*Acalypha*
	Croton
	Euphorbia
	Phyllanthus
Labiatæ	*Leucas*
	Ocimum
Leguminosæ	*Acacia*
	Cassia
	Uraria
Menispermacea	
Moraceæ	
Piperaceæ	*Piper*
Polygalaceæ	*Polygala*
Rubiaceæ	
Rutaceæ	*Zanthoxylu*
Solanaceæ	
Verbenaceæ	*Clerodendron*
	Lantana
MONOCOTYLEDONÆ	
Araceæ	
Commelinaceæ	

Plants are included in the list which might not inhibit the action of the venom directly but may produce some symptomatic relief. The inclusion of *Rauwolfia* spp. might be accounted for by the tranquilising effect of the alkaloids present *e.g.* reserpine. Victims of snakebite frequently panic even if a non-venomous species is involved and a tranquilliser would minimise this response. *Papaver somniferum* is included which contains the well-known alkaloid morphine and its powerful analgesic activity would reduce the pain often associated with envenomation. Other plants mentioned have anti-inflammatory and immunostimulant effects which could also alleviate the effects of envenomation (Tables 2, 3). The practice of some peoples of growing or tying plants to the exterior of buildings or compounds to deter snakes might imply that snakes may be repelled by some compounds produced from plants although no work has been carried out to test this intriguing hypothesis.

It should be noted that the recorded connection of a plant with snakes may be based more on an approach related to the "doctrine of signature" than any proven efficacy. Thus several roots in commerce have the appellation "Snakeroot"' because of their tortuosity *e.g. Rauwolfia serpentina* and *Aristolochia reticulata.*

In spite of such considerations it is not unreasonable to believe that a plant might have a direct effect on a venom. This is likely to occur by two mechanisms viz. chemical interaction with one or more of the venom constituents so that they cannot bind to the receptors in the body or pharmacological inhibition of the venom at the receptor site itself.

Comparatively few of the plants in the list have been tested for activity against snake venoms and such assessment is still in its infancy. The development of such tests is vital if bioassay-guided fractionation is to be carried out to determine the nature of any active compounds present in the plant.

Table 2

Plants used to treat snakebite shown to have anti-inflammatory effects (LEWIS, 1989)

Species	Family
Anacardium occidentale	*Anacardiaceæ*
Argemone mexicana	*Papaveraceæ*
Boswellia serrata	*Burseraceæ*
Brunfelsia uniflora	*Solanaceæ*
Capparis spp.	*Capparidaceæ*
Casearia sylvestris	*Flacourtiaceæ*
Cyperus rotundus	*Cyperaceæ*
Dolichos lablab	*Leguminosæ*
Ficus carica	*Moraceæ*
Morus alba	*Moraceæ*
Prosopis spicigera	*Leguminosæ*
Santolina chamæcyparissus	*Compositæ*
Securidaca longepedunculata	*Polygalaceæ*
Stachytarpheta dichotoma	*Verbenaceæ*
Terminalia spp.	*Combretaceæ*
Withania somnifera	*Solanaceæ*
Zanthoxylum spp.	*Rutaceae*

Table 3

Plants and constituents with immunostimulant effects (WAGNER and PROKSCH, 1985)

Species	(Family)	Immunostimulant constituent(s)
Aristolochia spp.	*Aristolochiaceæ*	Aristolochic acid I (1)
Stephania tetrandra	*Menispermaceæ*	Cepharanthine
Tylophora ovata	*Asclepiadaceæ*	Tylophorine
Many species	*Compositæ*	Sesquiterpene lactones
Echinacea angustifolia	*Compositæ*	Polysaccharides

2. TESTS FOR ANTIVENOM ACTIVITY

2.1. *INTRODUCTION*

The testing of plant extracts for anti-venom activity illustrates the dilemma facing investigators who are seeking to validate the traditional use of plants in treatment of snakebite. Tests should reproduce the conditions and effects of envenomization but also be as economic as possible, suitable for a high throughput of samples and be socially acceptable. The tests which have been used reflect the predicament experienced in balancing these considerations.

2.2. IN VIVO *WHOLE ANIMAL TESTING*

The protection of whole animals against a dose of venom afforded by extracts or compounds is the method that approximates most closely the field situation. This method has been used by many workers, beginning with the Herculean work in India in 1931, reported by CHOPRA (1958), when dogs were used to test any protective activity of extracts from 250 species of plants against cobra and Russell's viper venoms.

Most recent work has been carried out with mice for the testing of total crude extracts and is summarised in Table 4. In most cases a previously determined lethal dose of the venom was mixed with varying doses of the plant extract and incubated prior to injection. The survival rate with and without the extract was determined and any significant decrease in mortality was taken to imply protection due to deactivation of the venom by compounds present in the plant. A few studies have been carried out where the extract was given prior to injection of the venom or where the plant extract was given after administration of the venom; the latter procedure is most analogous to cases of snakebite occurring amongst the general population.

Some work has also been carried out using preparations used in traditional Chinese medicine for the treatment of snakebite (MARTZ, 1992). These preparations are often a mixture of

Table 4

Plant extracts showing activity against snake venom when tested *in vivo* in mice

Plant	Part	Extract	Snake species	Reference
Andrographis paniculata (Acanthaceæ)	PL	90% ethanol	*Naja naja*	Nazimudeen *et al.* (1978)
Alocasia cucullata (Araceæ)	RT	80% ethanol	*Naja naja*	
			Naja hannah	Wang *et al.*(1986)
Apuleia leiocarpa (Leguminosæ)		Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Arlstolochia sp. *(Arlstolochiaceæ)*	RT	Ether	*Naja naja atra*	Tsai *et al.* (1980)
Bredemeyera florlbunda (Polygalaceæ)	RT	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Brunfelsla uniflora (Solanacea)	LV	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Casearla sylvestris (Flacourtiaceæ)	SB	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Chlococca brachiata (Rubiaceæ)	RT	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Cynara scolymus (Compositæ)	LV	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Diospyros kaki (Ebenaceæ)	F	Tannin	*Laticauda semifasciata*	
			Trimeresurus flavoviridis	Okonogi *et al.* (1979)
Dorstenia brasiliensis (Moraceæ)	RT	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Eclipta prostrata (Compositæ)	PL	40% ethanol	*Crotalus durissus*	Mors *et al.* (1989)
Elephantopus scaber (Compositæ)	LV	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Geranium sp. *(Geraniaceæ)*	PL	Water	*Vipera* sp.	Luzhinskii *et al.* (1968)
Marsyplanthes hyptoides (Labiatæ)	PL	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Mlkanla glomerata (Compositæ)	LV	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Periandra mediterranea (Leguminosea)	RT	Water	*Bothrops jararaca*	Pereira *et al.* (1991)
Perlandra pujalu (Leguminosæ)	RT	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Phyllanthus klotzschianus (Euphorbiaceæ)	LV	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Picrasma quassioides (Simaroubaceæ)	LV	Water	*Bungarus multicinctus*	
			Naja naja	
			Agkistrodon acutus	Liang (1987)
Schumanniophyton magnificum (Rubiaceæ)	RB	Water	*Naja melanoleuca*	
			Naja kaouthia	Akunyili *et al.* (1986)
				Houghton *et al.* (1991)
Stachytarpheta dichotoma (Verbenacea)	PL	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Vernonia condensata (Compositæ)	LV	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)
Wilbrandia ebracteata (Compositæ)	RT	Water	*Bothrops jaracaca*	Pereira *et al.* (1991)

extracts of several plants, therefore, it is impossible to determine which one contains ingredients which neutralise the effects of venom and which one(s) exert other beneficial effects, such as a reduction in inflammation.

Whole animal work gives the greatest indication of the efficacy of an extract in real life but its use in bioassay-guided fractionation is impossible for financial and ethical reasons. It is these constraints which have led to the development of *in vitro* tests for antivenom activity.

2.3. IN VITRO *TESTS USING ISOLATED ORGAN PREPARATIONS*

2.3.1. Introduction

The use of isolated tissue for testing for biological activity does not require such large doses or long periods as experiments using whole animals and, if the effects produced can be removed by washing out, the preparation can be re-used for a series of experiments. In addition, a more quantitative approach can be used to measure the effects. The tests which have been developed correspond to the types of venoms and consist of measurements on nerve-muscle preparations, isolated muscles and studies on blood-clotting procedures.

The work described below has been used to investigate the possible antivenom effects of eight plants against two types of venom. Four of the plants chosen have a reputation for use against snakebite in west Africa, *i.e. Schumanniophyton magnificum* Harms. stem bark (AKUNYILI and AKUBUE, 1987), *Strophanthus gratus* Baill. and *Strophanthus hispidus* DC. leaves and the leaves of *Mucuna pruriens* var. *utilis* (Wall. *ex* Wight) Bak. *ex* Burck (ABBIW, 1990). The other four species are used in the Indian sub-continent and comprise the roots of *Rauwolfia serpentina* Benth., *Rauwolfia tetrandra* L. (CHOPRA, 1958), *Ophiorrhiza mungos* L. (GUNAWARDENA, 1975) and the bark of *Cassia tora* L. (CHOPRA, 1958).

2.3.2. *THE CHICK BIVENTER CERVICIS NERVE-MUSCLE PREPARATION*

The cobra venoms and their constituents which impair neuromuscular transmission have been extensively studied using the nerve-muscle preparations from the neck of the chick (*Biventer cervicis*) and abdomen of the rat (phrenic nerve-hemidiaphragm) since indirect stimulation of these preparations is inhibited by the venom components (HARVEY *et al.*, 1982). Consequently any plant extracts containing anti-venom activity might reduce or even reverse these inhibitory effects.

When an active plant extract is added to the chick biventer cervicis preparation and a dose of venom is introduced a reduced contracture and increased time to 100% twitch block and time of maximum contracture is observed compared with the response given by the same dose of venom alone (see Fig. 1).

Fig. 1
Test for anti-cobra venom activity using the *Chick biventer vervicis* preparation

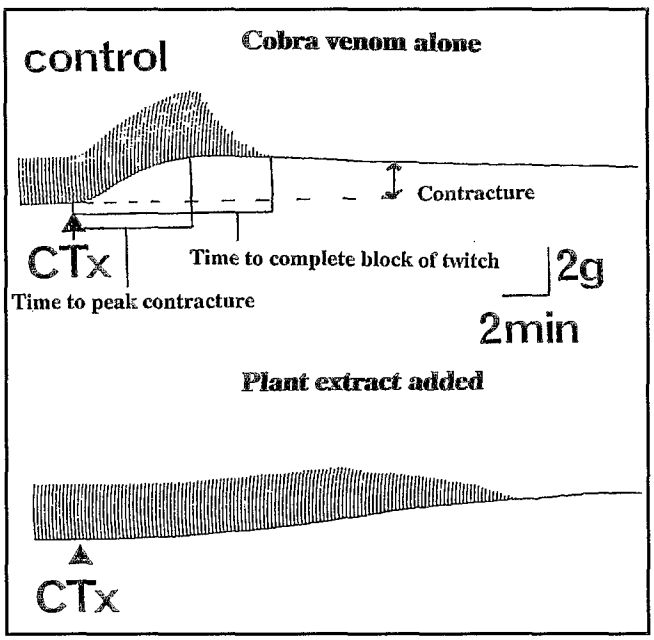
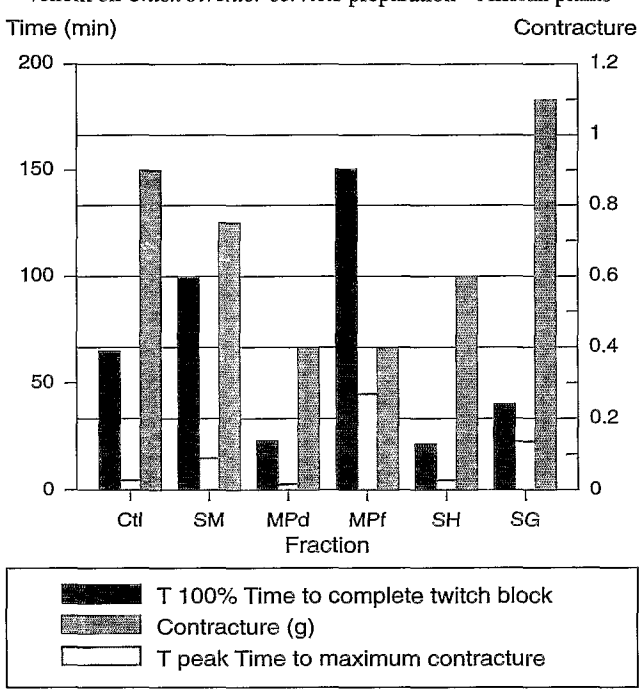


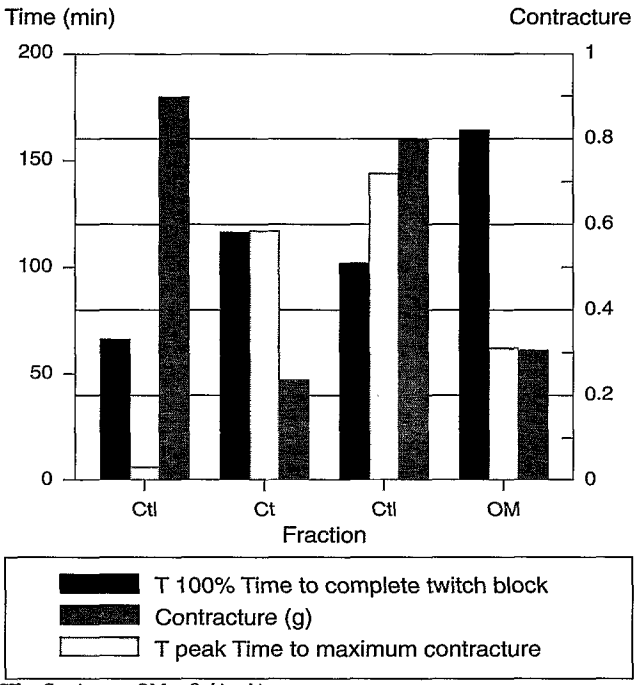
Fig. 2
Action of 2 mg/ml aqueous plant extracts and 25 µg *Naja nigricollis* venom on *Chick biventer cervicis* preparation – African plants



SM = *Schumanniophyton magnificum*; MC = *Mucuna pruriens* var. *utilis*; SH = *Strophanthus hispidus*; SG = *S. gratus*

Fig. 3

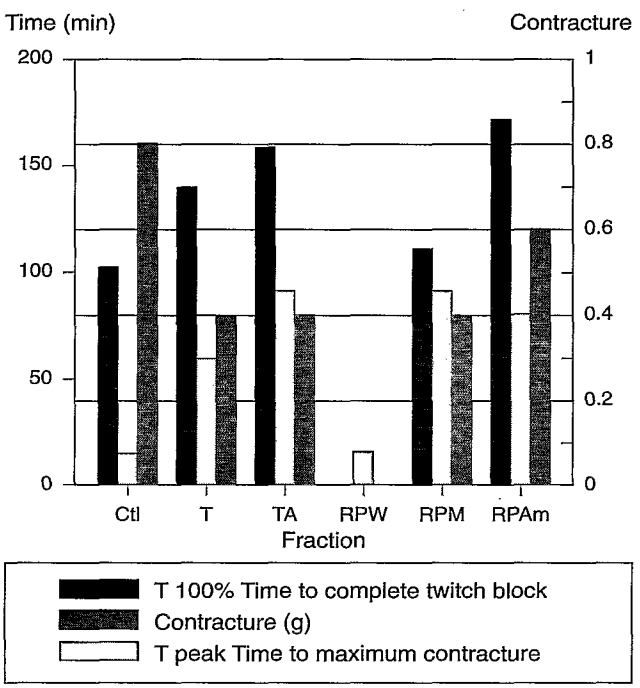
Action of 2 mg/ml aqueous plant extracts and 25 µg *Naja nigricollis* venom on *Chick biventer cervicis* preparation – Indian plants



CT = *Cassia tora*; OM = *Ophiorrhiza mungos*

Fig. 4

Action of O. mungos root eluates on 25 µg *Naja nigricollis* venom on *Chick biventer cervicis* preparation



T = total water; A = water EtOAc extd; RP = RP silica eluates; W = water; M = methanol; Am = ammonia

Such inhibitory effects have been noted in tests using extracts of *Curcuma* (CHERDCHU *et al.*, 1978; CHERDCHU and KARLSSON, 1983).

The chick biventer cervicis preparation has been used in our laboratories to monitor the reputed activity of the plant species under investigation. The results are shown in Fig. 2 and 3. Clear indication of inhibition of the effects of *Naja nigricollis* venom is shown by the extracts of *Schumanniophyton magnificum* bark, by fresh *Mucuna pruriens* leaves, by *Ophiorrhiza mungos* roots and by *Cassia tora* bark. The extracts of both species of *Rauwolfia* caused muscle contracture in the absence of venom so they could not be tested using this system.

Some work has been done to determine the nature of the compounds responsible for the effects noted in the species showing activity. The extract of *Schumanniophyton magnificum* has been subjected to bioassay-guided fractionation using the chick biventer preparation. The aqueous extract was passed through a reverse-phase silica column and the most active fraction subjected to gel filtration through Sephadex G-25. Preparative chromatography of the active eluate so obtained led to the isolation of a peptide with a molecular weight of about 6kD which showed a dose-related response (HOUGHTON *et al.*, 1992). Such tests are open to the criticism that the plant extract is added to the preparation some time before the venom which does not correspond to the situation in the field but is necessary both to check that the extract does not have an intrinsic effect on the preparation and also because the effect of the venom occurs very quickly. It has been shown however that RW12 confers some protection even if added to the preparation 1 minute after the venom (see Table 6).

Preliminary fractionation studies have been performed on the aqueous extract of *O. mungos* and the water eluate from a reverse-phase silica fractionation shows the greatest activity (see Fig. 4).

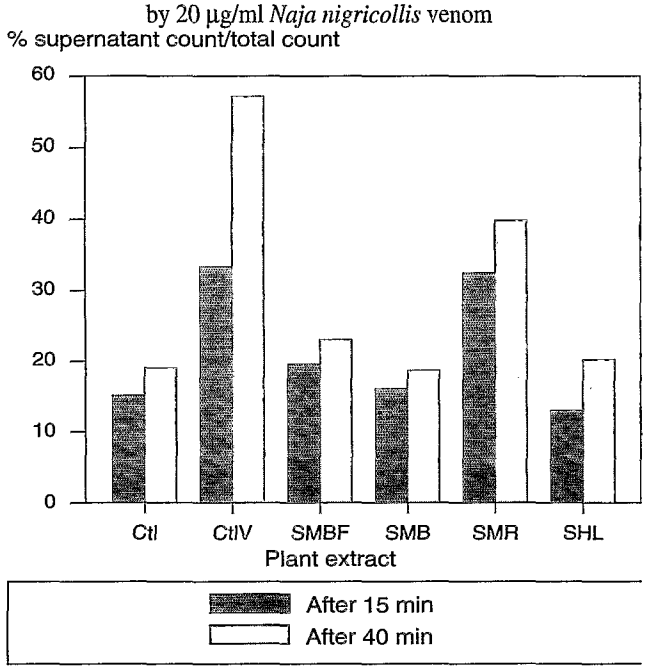
2.3.3. MEASUREMENT OF HAEMAGLOBIN RELEASE

The cardiotoxin components of cobra venoms cause an increase in permeability in the membranes of many cells although the exact mechanisms by which this occurs are not clearly understood.

Thus a reduction of the membrane permeability induced by a dose of cobra venom when an extract or compound is added to the system may indicate the presence of compounds which antagonise the action of the venom. If erythrocytes are used then the haemaglobin released into the surrounding medium can be measured as an indication of membrane permeability. When colourless compounds are tested the amount of haemaglobin released can be measured spectroscopically but if plant extracts are employed they are usually coloured and such colorimetric assay is precluded. Studies carried out by ourselves have overcome this problem by labelling the haemaglobin with Fe and

Fig. 5

Effect of 15 mg/ml plant extracts on blood haemolysis induced by 20 µg/ml *Naja nigricollis* venom



Ctl = Buffer; CtlV = Buffer + venom; SM = *S. magnificum*; SH = *Stroph. hispidus*; B = Stembark; R = Roots; L = Leaves; F = Fresh

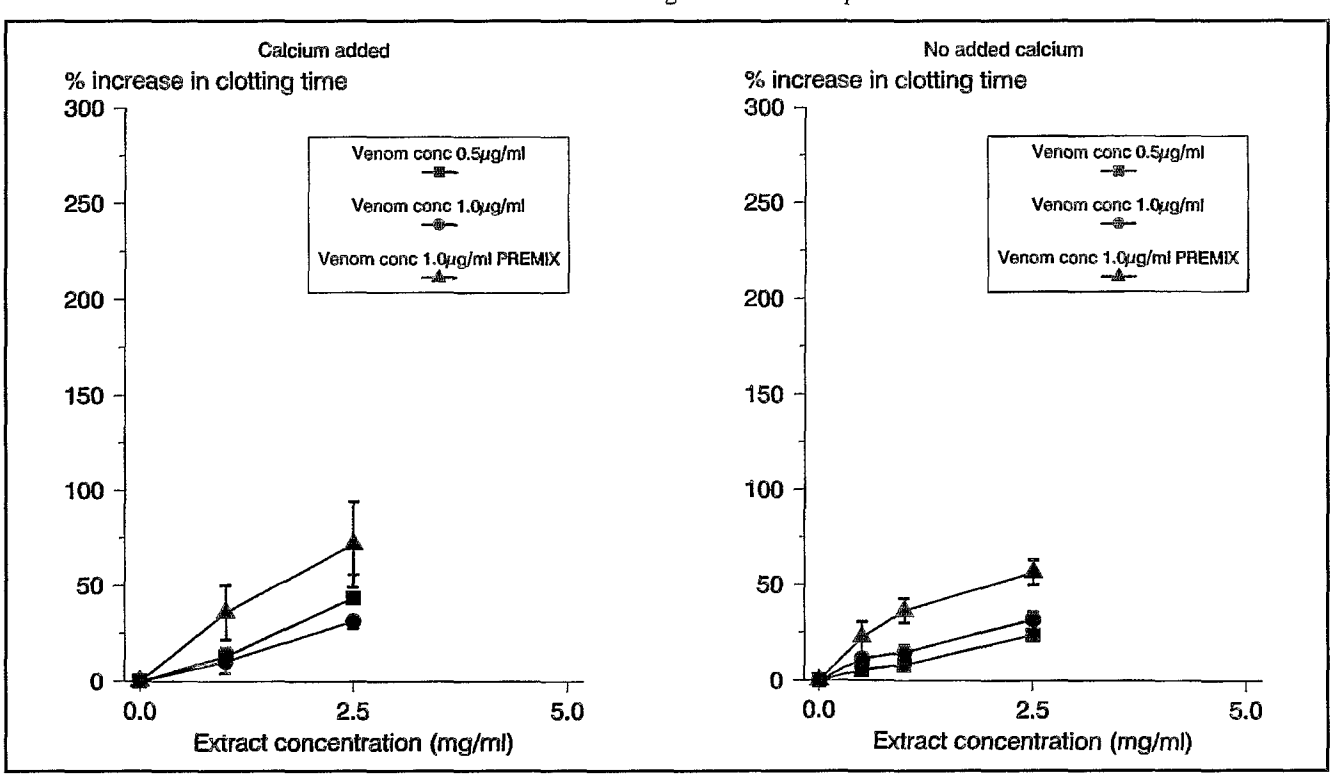
measuring its release by measurement of the radioactivity of the surrounding medium after the incubation time.

Erythrocytes suspended in buffer are incubated with Fe citrate. After washing to remove unincorporated iron the cells are suspended in buffer, incubated and silicone oil added before the mixture is centrifuged. The buffer is lense dense than the oil whilst the erythrocytes form a pellet at the base of the oil. The top layer of buffer is separated and its activity determined followed by the counting of the whole preparation. This is carried out after 15 and 40 minutes. The percentage of the total activity given by the top layer is calculated to give an indication of the haemaglobin loss. Controls are carried out using the buffer alone, a previously determined dose of venom alone and the dose of plant extract alone. A decrease in the percentage count of the top layer between the venom alone and venom plus extract is considered to indicate some inhibition of the venom by the plant extract. This test cannot be used if the plant extract itself causes haemaglobin release to any extent.

The results of preliminary studies on total extracts using this test are shown in Figure 5. The aqueous extracts of *Schumanniophyton magnificum* bark and *Strophanthus hispidus* leaves show some inhibition of the venom. *Mucuna pruriens* leaf extract was also used but it induced direct haemolysis.

Fig. 6

Increase in venom-induced clotting time — *Mucuna pruriens* leaves



2.3.4. INHIBITION OF BLOOD CLOTTING INDUCED BY ECHIS CARINATUS *VENOM*

The effect of plant extracts which produce changes in the clotting of blood has only recently been studied by measurement using *in vitro* systems. *Bothrops* species cause haemorrhage at the point of injection due to inhibition of the clotting mechanism and inhibition of this activity has been quantified by measuring the change in optical density of the skin at the site of injection in sacrificed mice (MELO, 1990) who detected some inhibition when *Eclipta prostrata* extracts were used.

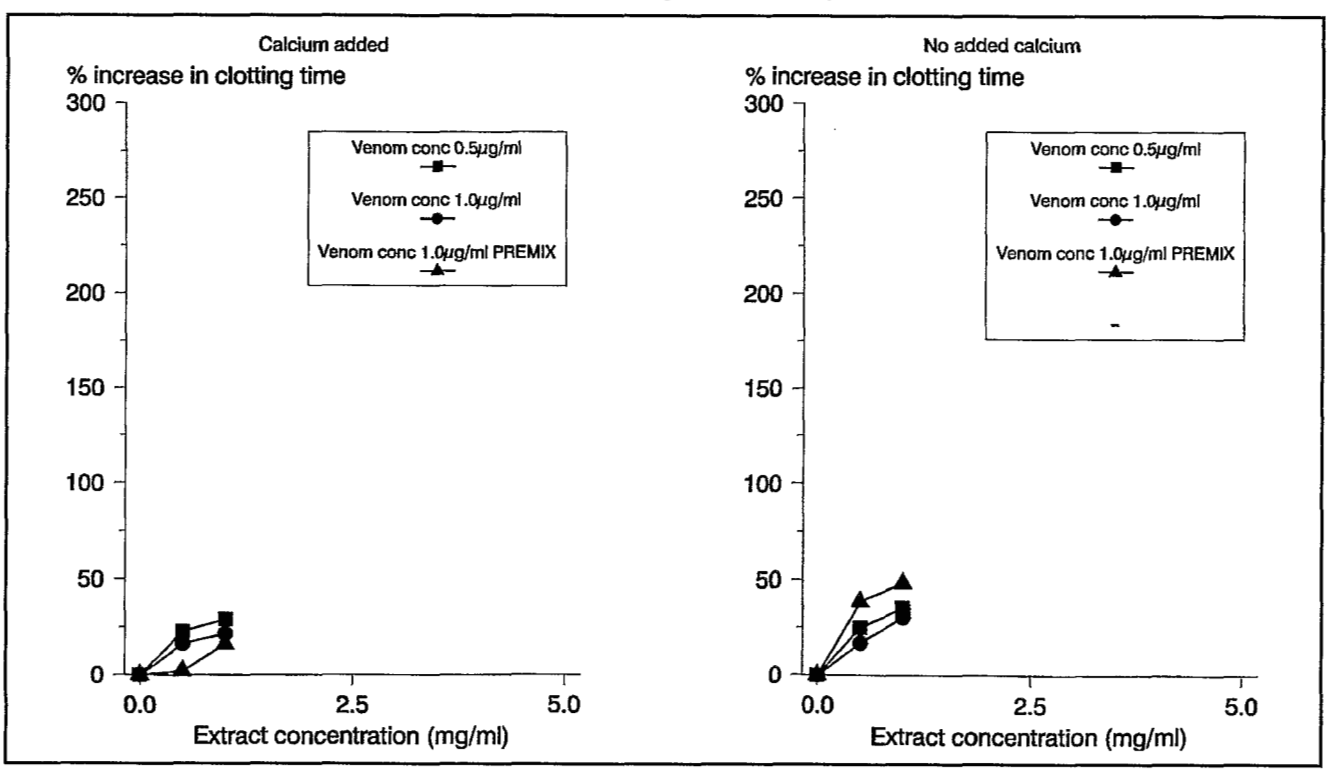
The venom of the carpet viper *Echis carinatus* causes rapid intra-arterial clotting of blood which leads to death in small animals. In larger animals and man death occurs due to internal haemorrhage resulting from the depletion of fibrinogen. Envenomation by this snake causes many hundreds of death each year in sub-Saharan Africa and in the Indian subcontinent. ONUAGULUCHI and OKEKE (1989) used the increase in venom-induced clotting time to test the effect of *Diodia scandens* (*Rubiaceæ*) and found significant activity.

The method pioneered by these workers has been developed in our laboratory to test other plant extracts using blood consisting of expired stock from hospital transfusion stocks. This contains citrate to prevent clotting so the tests are carried out with and without the presence of calcium ions. The calcium chelates the citrate and thus removes one potential inhibitor of clotting.

The test consists of the measurement of clotting time of a fixed volume of blood after the addition of a previously-determined dose of *Echis carinatus* venom. This time is then compared with that given when a dose of plant extract is added to the mixture or when the venom and plant extract are premixed before adding to the blood. This latter procedure gives some indication that an increase in clotting time may be due to complexation between the venom peptides and constituents of the extract which involves the sites on the venom which promote clotting.

These tests were carried out on a range of doses of aqueous extracts of all the eight species under investigation and all the plants showed some dose-related activity as shown in fig. 6-13. *Strophanthus hispidus* leaves gave the strongest inhibitory effect especially when it was premixed with the venom and no calcium was added (see Fig. 13). Fractionation of the *Strophanthus* extract on polyvinylpyrollidone (PVPP) showed that the majority of the activity resided in the fraction that was eluted with ammonia after water and alcohol elution (Fig. 14). This fraction is likely to contain polyphenolic substances which could complex with venom peptides and also with calcium ions

Fig. 7
Increase in venom-induced clotting time — *Rauwolfia tetrandra* root



and thus account for the lower activity shown when the extract was tested in the presence of Ca_2^+ (Fig. 13).

This possible venom-plant constituent complexation is also shown by the extracts of *Mucuna pruriens*, *Strophanthus gratus*, *Cassia tora* and *Ophiorrhiza mungos* (Fig. 6, 10-12). However in the case of the other three plants viz. *Schumanniophyton magnificum*, *Rauwolfia serpentina* and *Rauwolfia tetrandra* no significant difference was observed when premixing occurred and these plants may therefore inhibit the promotion of clotting by the venom directly (Fig. 7-9).

It should be emphasised that these results are of a preliminary nature and more investigation is needed to elucidate the nature of the compounds responsible and their mechanism of action.

2.4.1. TESTS USING ENZYMES

The development of enzyme-based assays in the last fifteen years has permitted automated testing for enzyme-inhibition or enzyme-activation of large numbers of plant extracts. This technique is applicable only where a particular enzyme can be linked to a disease state; since the action of snake venoms is diverse and several enzyme systems may be involved, it has not been used for any general screening procedures. Besides, enzyme assays have the disadvantage that any activity displayed *in vitro* in such a test may not bear very much relation to effects in the whole animal.

Compounds isolated from some plants used against snakebite, have, however been tested in enzyme assays, particularly those associated with the inflammatory response. The potassium salt of gymnemic acid isolated from *Gymnema sylvestre* has been shown to inhibit ATPase from the venoms of *Naja naja* and *Vipera russelli* (KINI and GOWDA, 1982*a, b*). The inhibition occurs due to competitive binding between the gymnemate and ATP. A set of studies has been published on the interaction of aristolochic acid with phospholipase A_2 from *Trimeresurus flavoviridis* and *Vipera russelli* venoms (VISHNAWATH and GOWDA, 1987; VISHNAWATH *et al.* , 1987*a, b*).

3. CONCLUSION

Tests are being developed which do not necessitate the use of whole animals but correlation between positive results given by these *in vitro* assessments and *in vivo* protection has not yet been established. It is interesting to note however that many plants with a traditional reputation for treating snakebite do in fact display some inhibitory effect against at least one of the venoms in the tests described above.

Fig. 8

Increase in venom-induced clotting time — *Rauwolfia serpentina* roots

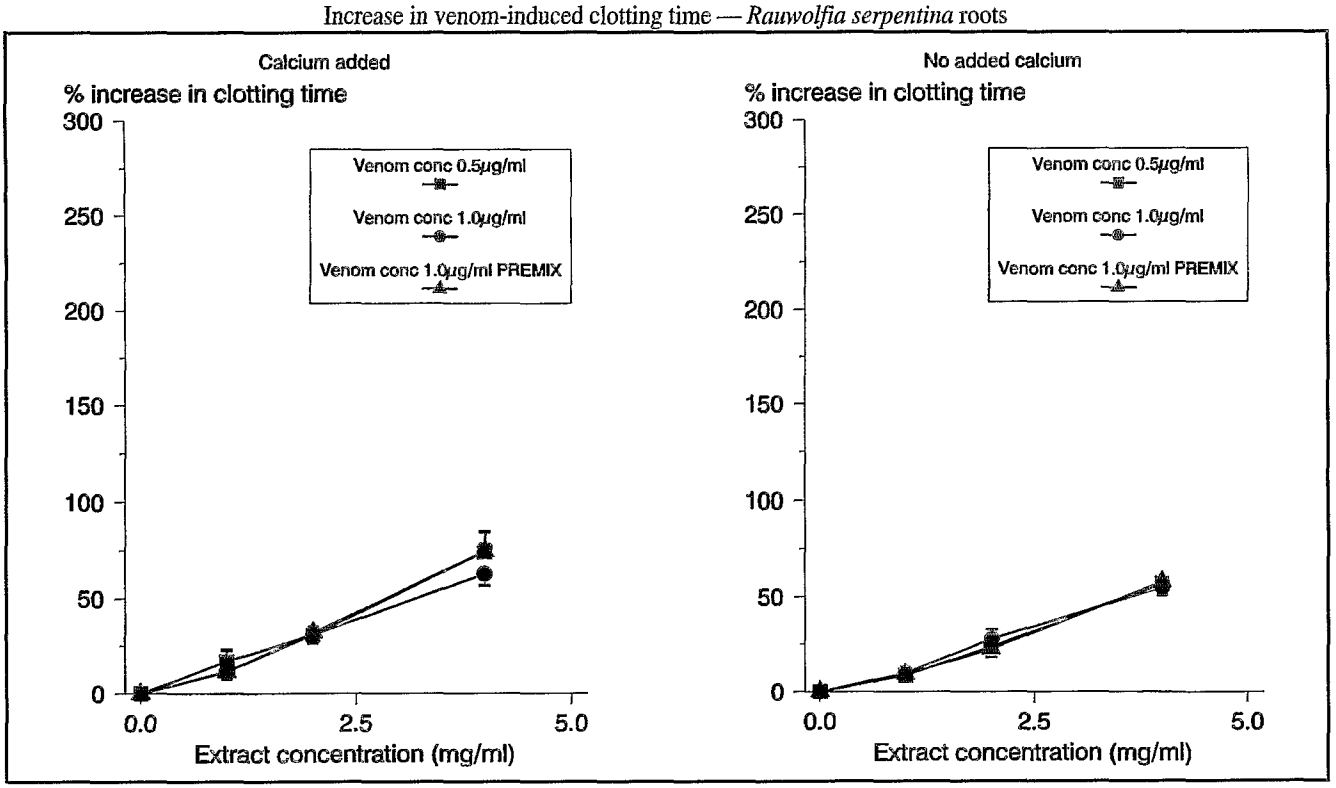


Fig. 9

Increase in venom-induced clotting time — *Schumanniophyton magnificum* stembark

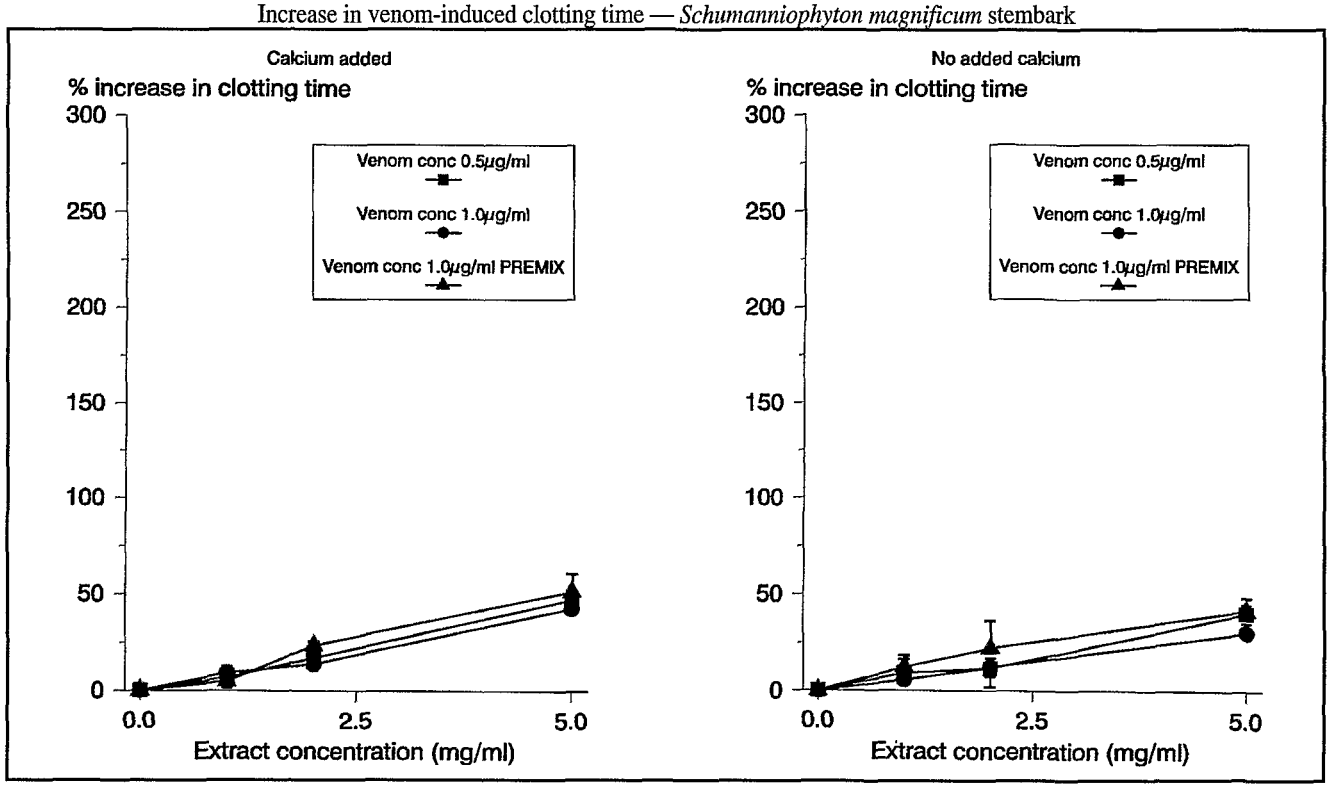


Fig.10

Increase in venom-induced clotting time — *Ophiorrhiza mungos* root

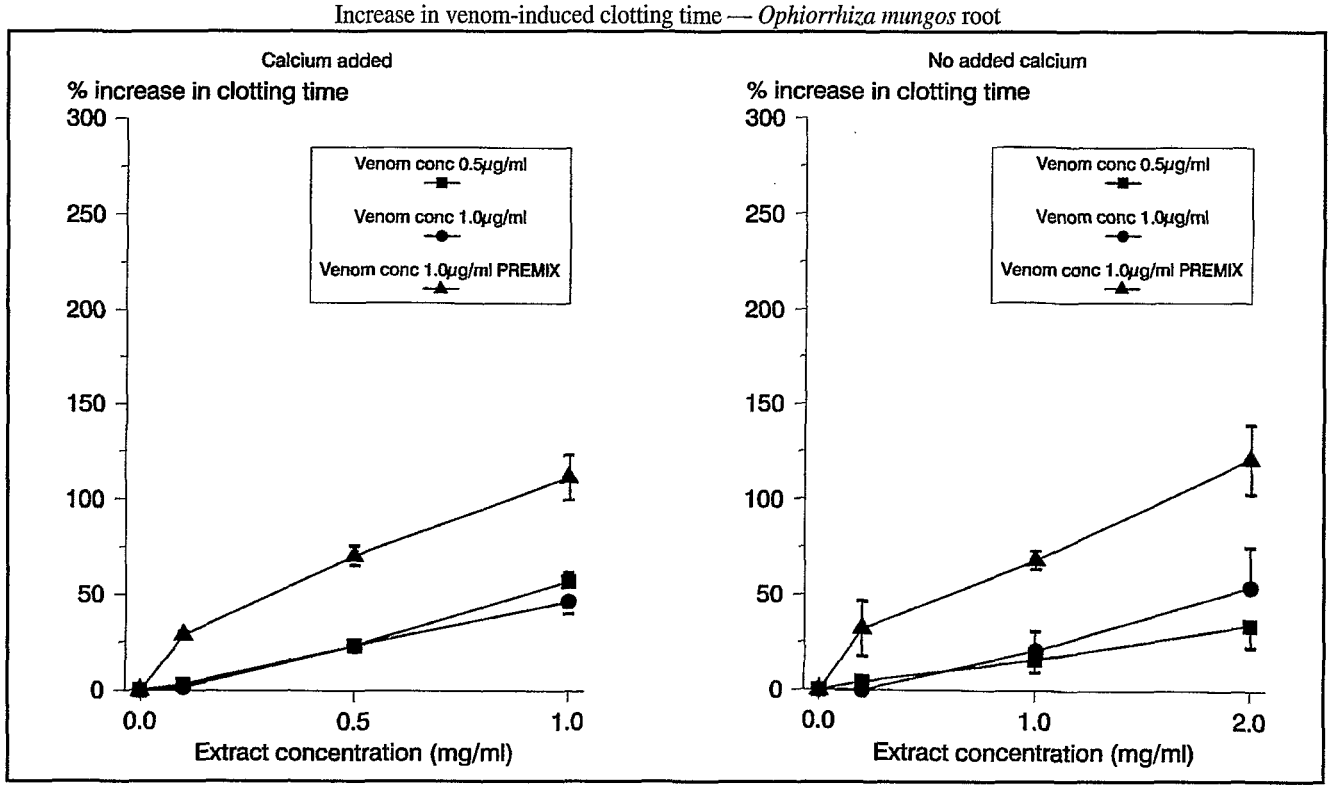


Fig. 11

Increase in venom-induced clotting time — *Cassia tora* stembark

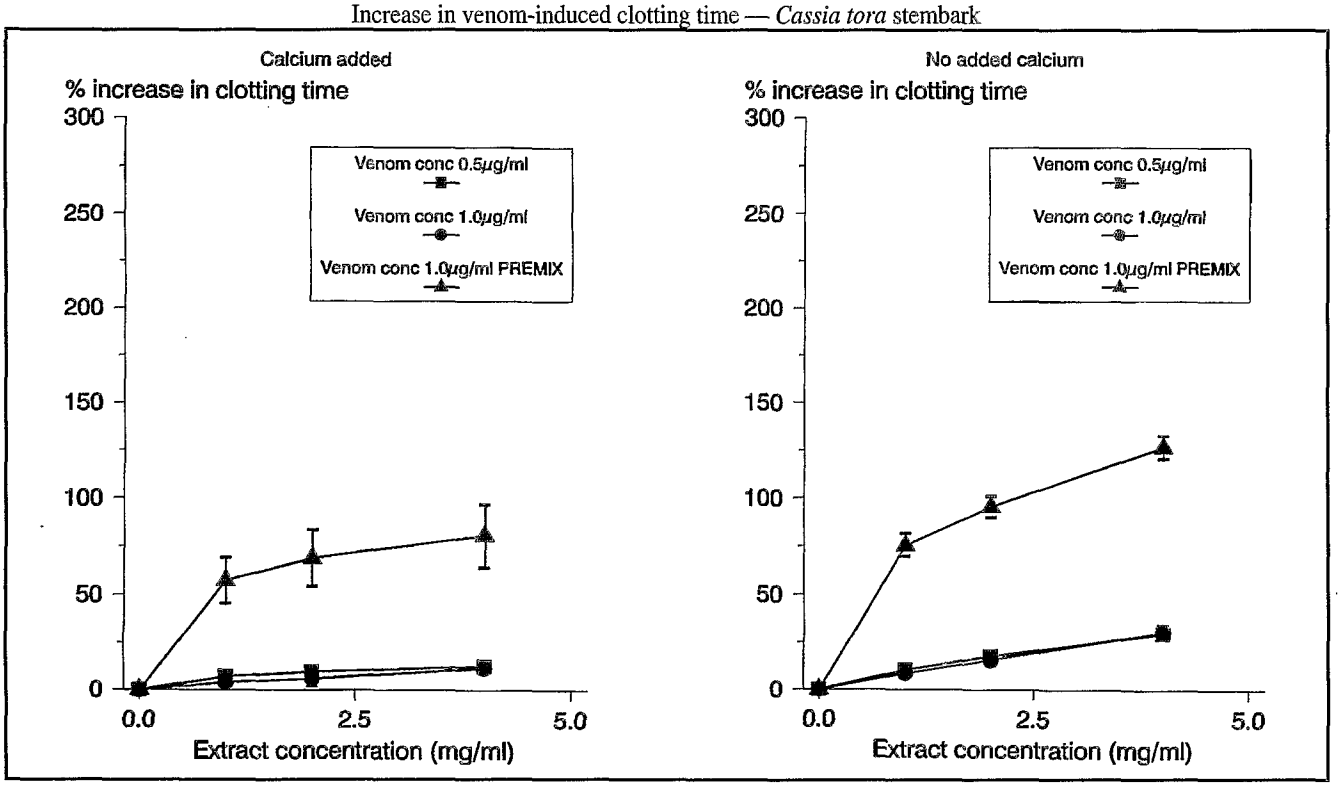


Fig.12

Increase in venom-induced clotting time — *Strophanthus gratus* leaves

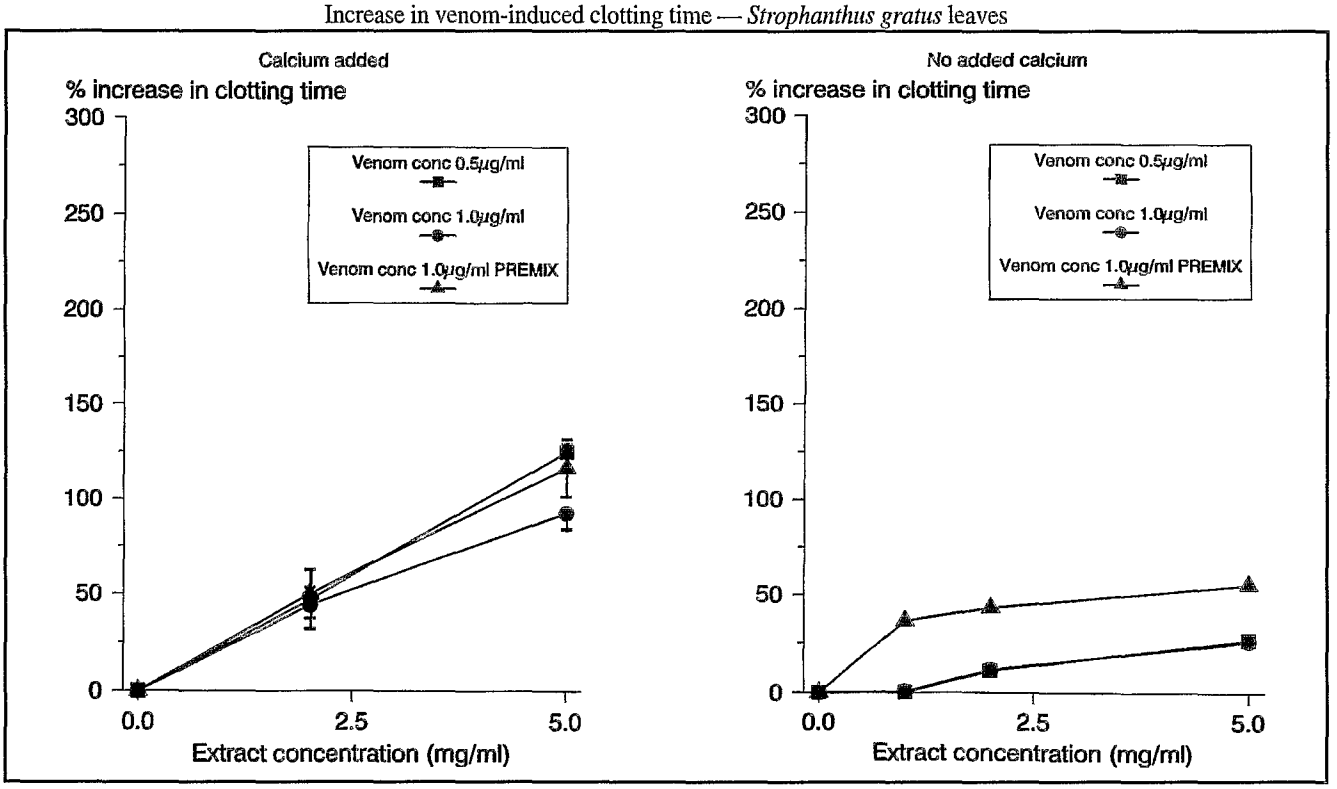
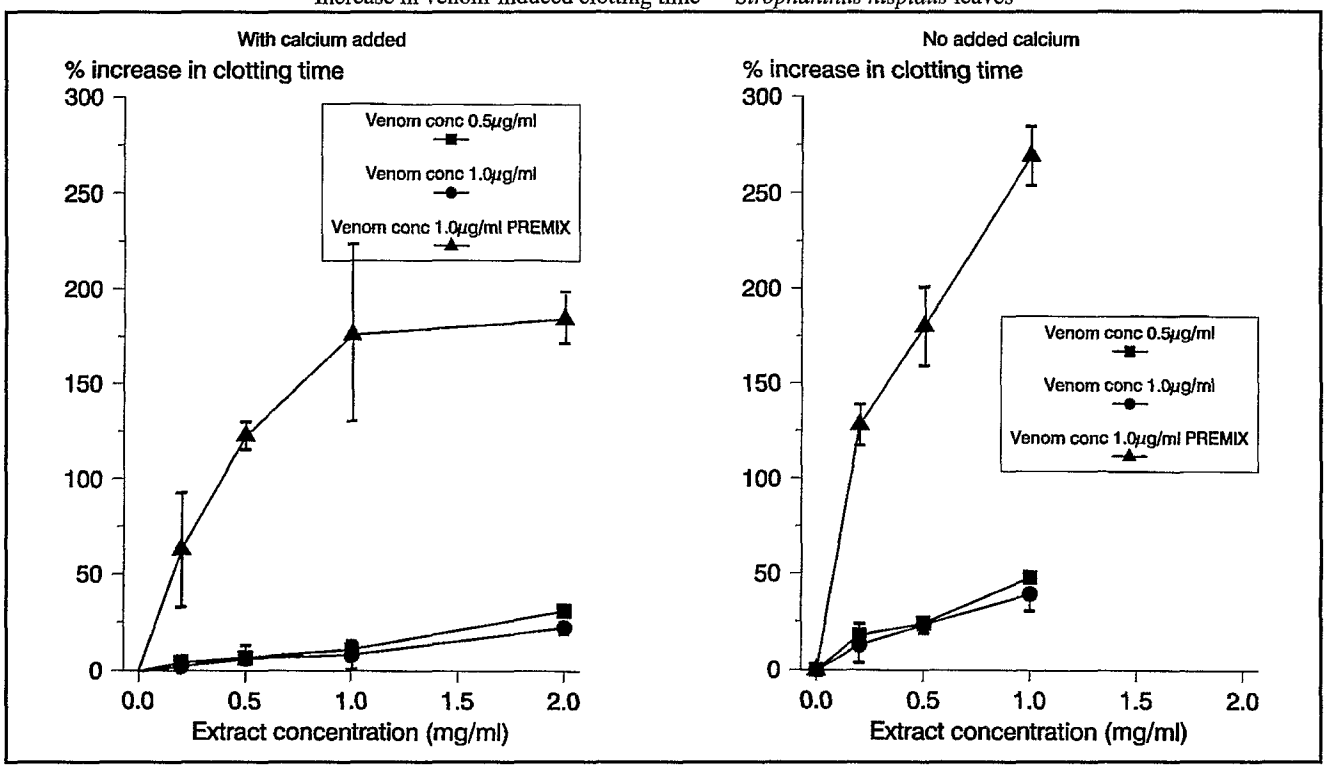


Fig.13

Increase in venom-induced clotting time — *Strophanthus hispidus* leaves



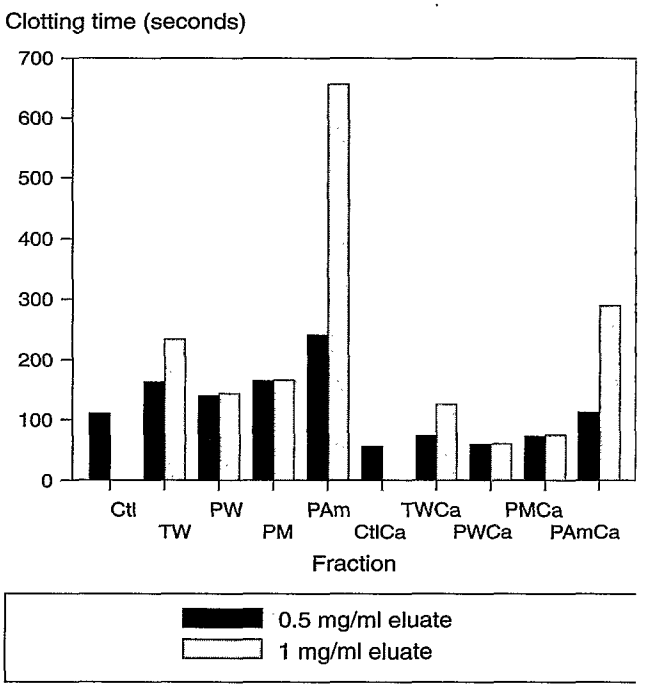
Some of the plants reported to have been used live in areas where the common venomous snakes belong to a group for which no test has been developed and there is a need to develop new assays for these categories of venom.

A standardised form of a compound or extract with proven *in vivo* antivenom activity would be a useful adjunct to antivenin therapy, particularly as a first aid measure.

Even though no direct link between positive results in *in vitro* tests and protection against envenomation might be found these tests might lead to the discovery of new pharmacological tools or other compounds with interesting biochemical characteristics. Although in some cases the activity of an extract seems to be due to complexation between polyphenolics, such as tannins, and the venom peptides in other instances inhibition at the receptor level may occur. The study of such mechanisms might cast new light on receptor properties as well as on the structure and function of the venoms themselves and the plant constituents.

Fig. 14

Clotting time for *Srophanthus hispidus* fractions – PVP eluates



1 µg/ml venom used

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ACKNOWLEDGEMENTS

The Wellcome Trust has provided financial support for some of the work described above. Dr Surinder Singh, Dr Ronke Osibogun, Dr Lawrence Woo and Mr Solomon Tafesse are to be thanked for assistance in the laboratory. Professor Alan Harvey (Strathclyde University), Professor Bob Hider (KCL) and Dr David Theakston (Liverpool School of Tropical Medicine) have provided useful ideas and discussions.