Sri Lanka Medical Association Oration 1999

YELLOW OLEANDER POISONING - IN SEARCH OF AN ANTIDOTE

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Introduction

The WHO definition of health had included physical, mental and social wellbeing in addition to lack of disease or infirmity. Many countries have concentrated on physical health as is happening in Sri Lanka and we have to a good extent focussed on social health supported by successive political will but sadly there has been much less interest and action on promoting mental health. There is now a growing trend to list mental health in the forefront of international public health.

This may be an important reason why a large number of suicides occur in the developing world particularly in Asian countries like Sri Lanka where "mental well being" is not carefully addressed. The work of Murray and Lopez highlights this when they estimated that nearly 600,000 people died by suicide in the developing world in the year 1990 making it the 12th most important cause of death. The larger issue is the many who attempt but do not intend suicide. This group now distinguished as the "deliberate self harm" group is receiving much publicity in the developing world.

Sri Lankan Situation

The deliberate self harm group are attempting to communicate distress, get back at people, achieve a selfish objective or deal with an overwhelming situation as a temporary method of coping with stress. Unfortunately the potential of these toxins to kill is grossly misjudged and results in unintentional death i.e. the person who takes the toxin does not fully understand its toxicity. Poisoning due to pesticides alone is listed as the 6th most important cause of hospitalized patients dying in Sri Lanka from 1993 to 1996. Organophosphates and Carbamates account for nearly 72% of all such deaths.

In this Oration I am addressing an important Plant Toxin which is killing large numbers of very young people in their second and third decades of life and appears to reflect a high number without true suicidal intent but simply an act of deliberate self harm. Other forms of poisoning by Yellow Oleander have been reported in India and the USA.

Sri Lanka has a very high suicide rate as depicted in the table given below

Sri Lanka	56.40/100.000
Hungary	36.00
Finland	30.00
Sweden	18.06
Japan	16.01
Singapore	13.01
United Kingdom	8.00

We have seen an alarming trend of increasing admissions in the north of this country of Yellow Oleander (Kaneru) Poisoning by seeds of the plant *Thevetia peruviana* (English:Yellow Oleander, Sinhala:Kaneru, Tamil:Manchal Alari).

As a part of a collaboration between University of Oxford, UK and University of Colombo we set up an OXCOL research study group primarily to study the feasibility to improving on the available anti venoms for snake bite in Sri Lanka. Our study base was Anuradhapura General Hospital, a secondary referral centre for 900,000 people living in the North Central Province. We observed at first hand another human tragedy and the tragic consequences of this emerging epidemic of Kaneru seed poisoning and its devastating effects on the families of affected persons. We also witnessed the issues the community had to face as it consumed the meagre ICU resources, the strain on its small fleet of ambulances and indeed deaths during transport to Colombo in addition to the usage of pacemaker and coronary care facilities at the tertiary cardiac referral centre in the country.

Series of Studies

Following a thorough literature search, we planned and set up the following studies and steps to assess the effects of this problem and address the possibility of developing an effective antidote to kaneru poisoning which can be utilized in the provinces and give relief to this tragedy and reduce mortality and morbidity.

1. Observational studies on patients admitted with Kaneru Poisoning in Anuradhapura
2. Studies on extracts of Kaneru seeds from Anuradhapura district in order to determine crossreactivity with anti-digoxin antibodies. Crossreactivity studies were done in Tab Laboratories in London at St Barthomeusz Hospital and Medical School and the Fab anti-digoxin Antibody production was done in a sheep farm and antiserum production facility in Wales,U.K.
3. Ehical Clearance was obtained and permission to import and use this drug as a clinical trial drug sought from the drug authority in Sri Lanka.
4. Dose Finding Studies conducted at the Institute of Cardiology, Colombo.
5. Double Blind Randomised Controlled Trial of Kanerutab (Anti-Digoxin Fab Antibody Fragments) in Yellow Oleander induced Cardiotoxicity

The Cardiotoxin

Prior to considering the clinical effects and studies it is important to briefly understand the botanical aspects and cardiotoxic effects of the plant and related plant toxins

Two Oleander species (Family Apocyanaceae) of plants are of importance viz. Common Oleander (*Nerium oleander*) and the common species in Sri Lanka used as a fence plant, the Yellow Oleander (*Thevetia peruviana*., Sinhala :Kaneru, Tamil: Manchal Alari)

These two plants contain a variety of cardiac glycosides of which at least 8 are dangerous

*Thevetin	*Oleandrin	*Oleandroside	*Digitoxigennin
*Peruvoside	*Nerioside	*Folinerin	*Rosagenin

The yellow oleander plant is a small tree which grows to a height of 15-20 feet and commonly encountered in India and Sri Lanka and some parts of United States. It has a beautiful yellow flower. The cardiac glycosides are found in all parts of the plant but highest in the seed

PART OF PLANT	% GLYCOSIDE
Leaf	0.070
Fruit	0.045
Seed(Kernal)	4.800
Milk	0.036

Oleander has been known to be poisonous to animals and human since the time of Hippocrates (460B.C). Oleander has been used as a medicinal plant as a folk medicine in many countries as an abortifacient, appetizer, and in the treatment of leprosy, ringworm, malaria and venereal disease. In Sri Lanka it is used in ayurvedic medicine as an ointment for the treatment of certain inflammatory skin conditions.

It has been reported that the most serious toxic effects of Oleander are seen in children taking yellow oleander (Kaneru) seeds and those taking common oleander leaves (as Tea as the cardiotoxins are not inactivated by boiling)[1]. Accidental poisoning in humans is recorded from chewing the flowers, leaves, eating meat cooked over oleander branches, stirring porridge with oleander stems. The ingestion of one oleander leaf may be lethal to human. Accidental poisoning has been reported in cattle, goats, sheep, horses and bears.

The cardiac glycoside causing toxicity in yellow oleander is Thevetin (oxydigitoxigenin- thevetose-gentiobios). The molecular structure of Thevetin is closely related to digitoxigenin. All cardiac glycosides have a steroid nucleus with an unsaturated lactose ring attached to the c-17 position and an attached sugar. They are well absorbed from the gastrointestinal tract.

The pharmacological actions of all cardiac glycosides are fundamentally similar. Cardiac glycosides reversibly bind to and inhibit the membrane bound Na-K ATPase (the so called digoxin receptor.) This results in an increase in intracellular sodium which in turn increases intracellular Ca through a Na/Ca exchange carrier mechanism. Cells in various parts of the heart show differing sensitivity to cardiac glycosides. Both direct and neurally mediated effects are involved in the pathogenesis of rhythm disturbances. The locus for neural augmentation of glycoside induced arrhythmias lies in the medulla. Vagal effects predominate in subjects without intrinsic disease of the cardiac conducting system. In "therapeutic" doses the increase in intracellular calcium in the myocardial cells invokes an inotropic response In severe digitalis toxicity however, marked excess of intracellular calcium leads to myocardial depression and asystole.

The increase in intracellular calcium together with increase in the intracellular sodium and decrease in intracellular potassium causes a decreased slope of upstroke of the action potential, shortening of the plateau phase, and spontaneous diastolic depolarization with progressive loss of resting potential. This results in increased automaticity and excitability with ectopic impulse activity. In the conducting tissue the effective refractory period is prolonged, and conduction velocity is reduced leading to varying degrees of heart block, sick sinus and sick nodal syndromes and the appearance of reentry circuits. A combination of vagal and direct effects on the sinus node contributes to sinus bradycardia as well as sick sinus syndrome, with sinuatrial arrests or sinuatrial exit block, which in turn leads to junctional escape rhythms. Increased vagal activity, and also high levels of potassium contribute to depression of atrioventricular nodal conduction.

The structural similarity to digoxin, as well as the considerable similarity in the toxic syndrome resulting from yellow oleander poisoning suggests strongly that the syndrome is similar to digitalis overdose. In the summer of 1995, the OXCOL research study group assayed digoxin like substances in 16 patients with yellow oleander poisoning admitted to GH Anuradhapura, Sri Lanka. The method used was a polarization fluoroimmunoassay. In these 16 patients,serum values in the range of 0.1 to 3.9ng/ml were seen. All patients who were clinically judged to require cardiac pacing had digoxin like substance levels of 2 ng/ml or more, while those who did not require pacing had levels lower than 2ng/ml. It should be borne in mind that the correlation between serum digoxin or digoxin like substances and toxicity is not entirely precise, especially as electrolyte disturbance also has an effect. The American Association of Poisons Control reported 2438 exposures to cardiac glycoside containing plants in 1988 and 633 of them from oleander.

Toxic Manifestations are virtually identical to that of digoxin toxicity. Initial nausea and vomiting is followed by abnormal rhythms and various types of sinus node dysfunction and atrioventricular blocks and premature ventricular beats. These can deteriorate to ventricular tachycardia, fibrillation and asystole. Potentially fatal hyperkalaemia characterises severe toxicity.

The use of digoxin specific antibody for treating oleander poisoning was suggested by Haynes et al 1985 in his case report of a single woman who died after oleander tea poisoning. Following this in 1987 Blum and Rieders failed to resuscitate a Haitian woman after 20 vials of a similar anti digoxin antibody drug called Digibind.

Shumaik et al 1988, saved one patient after one hour of ingestion by using this drug. In view of this controversy in the treatment of common oleander poisoning a canine model was developed by Clark et al 1991 and it was demonstrated that large doses of Digibind 60mg/kg was effective in dogs. They also showed a parallel drop in potassium level with response. The problem was that large doses (105 vials) had to be used to reverse toxicity in severe cases. The poison in the case of Yellow oleander is somewhat different. The molecular structure of the only active cardiac glycoside Thevetin is closely related to digitoxigenin. This will theoretically allow a better binding affinity and capacity with digoxin specific Fab. Our studies are the first human trials performed with Kanerutab produced by our research collaborates Therapeutic Antibodies (TAb) Inc., which is a highly purified high affinity ovine monospecific Fab anti digoxin antibody produced in a flock of sheep in Wales for this study.

In the case of Kaneru (Yellow Oleander) no reports are available of even successful anecdotal use

using Digibind. In both forms of Oleander poisoned patients no convincing demonstration is available by means of a double blind Randomised clinical trial. The studies done in Anuradhapura and Colombo is the first attempt at an organised study on this group of patients anywhere in the world as patients are hard to come by in such large numbers as occuring in our setting.

Coming back to our problem in Sri Lanka although this poison was known since Hippocrates, there were no incidences reported in Sri Lanka till 1983. The first reports in Jaffna University with deaths of 6 persons from yellow oleander and publicity in the newspapers only helped to spread the message quickly not only in Jaffna but it has now spread further south in the country and is now a major problem in Anuradhapura, Pollonaruwa and Kurunegala Districts. It is interesting to note that in the deep south of the country Kaneru poisoning is rare, although the plant is similarly distributed. There has been a general increase of poisoning in Anuradhapura district since 1984 not only using Kaneru but also Organophosphates. Since the mortality from kaneru is less than that of Organophosphates it is likely that the main reasons for kaneru poisoning is for deliberate self harm than well intended suicidal poisoning.

In 1984, 18% of the 2999 poisoned patients in Jaffna General Hospital was due to Yellow Oleander.

In our preliminary Observational study phase between April 1995 to March 1996 we recorded 415 cases of yellow oleander poisoning. During the same period 323 cases of yellow oleander poisoning was seen in Pollonaruwa Hospital. There was no central record of Oleander Poisoning in Sri Lanka until 1996 as it was included in the ICD-9 classification as "other poisoning and toxic effects (ICD-9 Section 980-989.1, 989.5-9) . The incidence of yellow oleander poisoning has doubled in the last 4 years. (Figure 1a & 1b) Of the patients admitted to Anuradapura Hospital during our period of observation, 40% needed transfer to Colombo. Each on of these transfers required an ambulance. On some nights 5 ambulances collected from other smaller hospitals from across the province had to make a four hour journey to the capital. Nurses & often doctors had to accompany these patients . In another study, 79 patients we followed prospectively, 5 (6%) died waiting for a ambulance, 1 died in transit to Colombo. 33 (42%) had to be transferred accompanied by a house officer. 41 patients had mild poisoning and got better with supportive therapy. (Gastric lavage, Cardiac monitoring, IV atropine etc) Facilities for cardiac pacing were not available in Anuradhapura and Polonnaruwa.

In another study done at the Institute of Cardiology from Dec 1995 to June 1996 yellow oleander poisoning accounted for 8.1 % of all intensive care admissions (118/1451) and for 9.8% of all CCU/ICCU deaths. All these cases were self poisoning and none accidental poisoning, and all were in the 2nd and 3rd decade of life.

These observations serve to highlight the enormous burden and strain placed on health facilities, and on the health staff of all levels, primary, secondary and tertiary as a result of the Yellow Oleander epidemic. It is prudent to recall that Pesticides poisonings occurring side by side place accounted for nearly 50% of the ICU beds at Anuradhapura. (figure 2).

Thus for the Health financier viz the Ministry of Health this epidemic of Yellow Oleander poisoning based on our observation in Anuradapura and Colombo Cardiology Institute is draining enormous finances and therefore expensive and proves dangerous to the poisoned patient being transferred. No cost analysis of the epidemic has yet being undertaken.

We then set out to get ethical clearance from the University of Colombo Ethical Committee which was obtained. We then obtained temporary registration to import the clinical trial drug KaneruTab which is a highly purified ovine polyclonal anti-digoxin Fab antibody.

We agreed on a very strict protocol for a randomized, placebo-controlled, double-blind clinical trial to be conducted and the Institute of Cardiology, Colombo. Cross reactivity of the KaneruTab antibody samples with Thevetin extracted from kernel obtained from Yellow Oleander Seeds sent from Anuradhapura was demonstrated in vitro. These data was acceptable to the ethical committee. (Figure 3 & 4)

An initial dose finding study was conducted on 16 patients. Doses of 400mg, 800mg, 1200mg and 1600mg of KaneruTab were used. The most satisfactory response was seen with doses of 1200mg and 1600mg. Based on the most effective and economically feasible dose, a randomized double blind placebo controlled clinical trial was designed. This compared 1200mg of antidigoxin antibodies against placebo, which was an equal volume of normal saline, given as an intravenous infusion over 20 minutes.

The setting was the Institute of Cardiology of the National Hospital of Sri Lanka, where most patients are transferred for temporary cardiac pacing. The study took place between April and October 1997. An overview of the study design and plan is shown in figure 5.

All patients admitted to the institute of cardiology with a history of yellow oleander seed poisoning were screened within one hour of admission. For this purpose two team members were available on a 24 hour basis. All patients with a history of yello oleander seed ingestion who were over the age of 11 years were eligible. Patients with concurrent poisoning due to other causes ,severe systemic illness, and pregnant women were excluded. Very severely ill patients were excluded from the randomized controlled trial and were considered for open labelled treatment.

Patients with the following ECG abnormalities on screening were included: severe sinus bradycardia with a heart rate below 40 beats per minute, sick sinus syndrome type one or type two, nodal rhythm, 2nd degree or complete heart block, atrial flutter or fibrillation with complete heart block; these arrhythmias being defined as "life threatening cardiac arrhythmias (L TCA)" needing pacing.

Sinus rhythm, 1st degree heart block, sinus bradycardia with a satisfactory heart rate (more than 40 beats per minute) were considered comparatively benign or non life threatening arrhythmias (NL TCA) not requiring cardiac pacing. This classification was based on the criteria used by the clinicians for temporary cardiac pacing.

Informed written consent was obtained in the patienfs own language. Patients were then randomized using a preset randomization order to receive either the intervention which was 1200mg of KaneruTab in 200ml or placebo 200ml of normal saline infused over one hour. Two members of the study team team were present during treatment. One member would perform the randomization while the other blinded member would observe the patient clinically and obtain ECG tracings and blood samples. Observations were made at preset intervals. 10,20,30,45,60, minutes, 2hours, 4 hours, 8 hours, 12 hours, 24 hours, 36 hours and 48 hours. Serum was separated and frozen within 2 hours of bleeding. In the event of adverse reactions, a standard protocol of management was followed. Prior to treatment, all patients were prophylactically temporarily paced. Pacemakers were kept at an on demand rate of 30 beats per minute and were tempotarily switched off while obtaining ECG tracings if so required.

Concurrent treatment with atropine or adrenoreceptor agonists like salbutamol was avoided whenever possible. Other drugs like antiemetics and antibiotics were permitted. Data was recorded on a standard form by the blinded investigator.

On discharge, follow up was arranged at 21 days. ECG tracings were interpreted by a cardiologist who was blind to the trial. This was particularly important in view of the highly variable nature of the ECG arrhythmias even in individual patients. Reversal of LTCA to NLTCA was taken as a positive response.

Results

203 transferred patients with a history of yellow oleander seeds were screened. Of them, 69 patients with LTCA were eligible for randomization. The others were in sinus rhythm or first degree heart block, with heart rates more than 40 beats per minute. 2 patients refused consent, and received conventional therapy. 1 patient could not be randomized since it was not possible to insert prophylactic pacemaker due to trade union action by nursing staff.

The patient characteristics at the start of the treatment in each group were comparable. (Table 1). Vomiting and diarrhoea were the most frequently seen symptoms. The mean number of seeds ingested were similar in the two groups (Table 2) . No correlation was seen between the number of seeds ingested and the degree of intoxication or the type of arrhythmia at presentation. Second degree and third degree heart block and sick sinus syndrome were the commonest LTCAs seen (Table 3). Atrial fibrillation/flutter with complete heart block, one of the classical arrhythmias) seen with digoxin toxicity was seen in one patient in each group.

The heart rate response at 2 hours and 8 hours is shown in Table 4 and Figure 6. In the patients who received KaneruTab a significant increase in the mean heart rate was seen at 2 hours and was maintained at 8 hours. The mean heart rate was maintained at over 60 beats per minute until the end of the study period of 48 hours. The placebo group showed no significant improvement of the mean heart rate during the first 8 hours, and showed a slow recovery over 24 to 48 hours.

An increase in the heart rate alone, however was is not the best index of reduction of toxicity. Particularly as in glycoside toxicity patients could maintain a reasonable heart rate while remaining in a LTCA. This is particularly true of arrhythmias like sick sinus syndrome Type II, where a stable heart rate could be accompanied by long and dangerous periods of sinus node arrest. Nodal rhythm and atrial flutter/fibrillation with block is another example. For these reasons a reversal of ECG rhythm from one of the above named LTCAs to a NLTCA was an important outcome measure.

Treatment with KaneruTab resulted in a significantly larger percentage of patients to revert from a life threatening arrhythmia to a non life threatening arrhythmia within 8 hours of treatment. (Figure 7)

Of the 34 patients treated with antidigoxin antibodies, 17 patients returned to a NLTCA within 2 hours of therapy whilst in the placebo group only 2 spontaneously reverted. (Figure 8). The results showed a significant benefit concurred by treatment with KaneruTab. (Odds ratio:0.07.95% CI 0.01 to 0.37; Odds reduction:93%; Relative Benefit increase 733%; Absolute Benefit Increase: 44%,95% CI 25 to 63%). The Number needed to Treat was 2 (95% CI 2 to 4), indicating that a positive result could be expected for every two persons treated. Reversal to sinus rhythm at 8hours was achieved satisfactorily in 26 of the 34 patients treated with the drug. Whist only 6 out of 32 responding in the placebo group. The results were again statistically highly significant (Odds ratio: 0.07,95% CI 0.01 to 0.37, Odds Reduction: 93%; Relative Benefit increase 320%; Absolute Benefit Increase: 58%, 95% CI 38 to78; Number Needed to Treat 2, 95% CI 1 to 3). Of the patients who returned to sinus rhythm, 2 patients returned to a life threatening arrhythmia 24 hours after treatment and 4 more did so at 48 hours. Their clinical condition however had improved by this time . It was postulated that the relapse was due to delayed absorption of the toxin. Follow up at day 21 did not show significant clinical /ECG complications due to poisoning or treatment.

KaneruTab also showed a significant reduction in serum potassium concentrations compared to the placebo group. In this study a dose of 1200mg of KaneruTab was sufficient in most cases, but this dose may need to be repeated in severe cases.

Clinical improvement especially reduction in restlessness was seen in the majority of patients receiving KaneruTab. Adverse reactions although monitored for very closely were very few and mostly mild. 4 patients developed mild urticarial reactions with itching and a single urticarial wheal. Prompt treatment was given in all cases. 1 patient developed moderate bronchospasm- she gave a past history of asthma. We did not have to withhold the trial drug in any of the cases. All adverse reactions occurred in the first 2 hours of treatment. There were no reactions in the placebo arm. Non of the patients developed haemodynamic disturbances. In the light of evidence obtained from the dose finding study, it was considered unethical to withhold treatment in situations where antibodies would be the ideal therapy - on this basis, patients with very severe intoxication, in cardiogenic shock and / or ventricular tachycardia who were exclusions in the RCT received antibodies on a open labeled basis. Decisions in regard to this compassionate therapy was made at the request of the two senior cardiologists in the team, in consultation with the trial managers.

6 severely intoxicated patients who did not fall within trial inclusion criteria were treated on humanitarian grounds. 3 of these patients died although transient improvement in their clinical and ECG status was noted. The other three responded satisfactorily and made a complete recovery. It was noted that the most severely affected and intoxicated patients had tachyarrhythmias rather than bradyarrhythmias.

Conclusions

The KaneruTab raised as an Ovine polyclonal purified drug was found to be an effective and safe medication in the treatment of life threatening cardiac arrhythmias due to yellow oleander poisoning seen in Sri Lanka. Both improvements in heart rate in bradycardic patients, and reversal of arrhythmias to sinus rhythm can be anticipated within 8 hours of therapy, and even as early as 2 hours after therapy. Consequent improvement in haemodynamic status together with overall clinical improvement reduced overall morbidity. Treatment if made available at the site of first contact will significantly reduce the need for cardiac pacing and the need or duration of intensive care, Whilst the effect on mortality cannot be evaluated in a study of this design excluding seriously ill patients the reversal of LTCAs to NLTCAs is taken as the closest endpoint to simulating reduction in mortality. The treatment in moribund patients on compassionate grounds adds weight to the excellent trial results.

Getting Results into practice is the next stage of our deliberations. The results of the series of studies were formally presented to the Ministry of Health by the study team and firm decisions were taken to import the drug and make it available to people in this country. The Drug Regulation Authority will go over the registration process in the usual way and the necessary documentation has been forwarded by the manufacturers and suppliers. The Medical Supplies Division has used the trial data and worked out the requirements and decisions to make it available at the larger hospitals in the relevant provinces in addition to the main Cardiac Centres. Our team will continue post marketing surveillance studies for a year or two and follow up on each vial used in this country.

It is presumed that we can by this treatment strategy bring down the deaths due to Yellow Oleander poisoning in Sri Lanka and perhaps the availability of the data to many other countries who do not have a proven effective therapy for this malady. The educational efforts to improve mental health would be the long term strategy to rid of Yellow Oleander poisoning. Until we rid ourselves of the social stresses which promote deliberate self harm or suicidal intent it will continue to occupy a high position in our mortality charts for many more years to come. It is said that when one finds an antidote to one toxin the people shift to a brand new substance and so work for the profession would not cease.

Kaneru Tab may also be effective in treating severe intoxication caused by other plant and animal glycosides found in common pink oleander, pheasant's eye, yew berry, foxglove, Chan Su and cane toad venom

I have presented data on focussing on a national problem and how we have planned and executed a series of observations and carefully planned and executed a randomized double blind clinical trial which has hitherto not been done anywhere in the world and it has shown good results and we have promoted the development and use of an agent not only in Sri Lanka but also in many other countries in the region and USA where Oleander poisoning is seen.

Table 1 Demographic Data

Values are the mean, ± SD and range in brackets

	Placebo Group (n = 32)	DigiT Ab Group (n = 34)	p-value	All Patients (n = 66)
Women/Men	19/13	13/21	0.09	32/34
Age, years	27 ± 14	25 ± 10	0.54	26 ± 12 [13-70]
Weight, kg	48 ±9	47 ± 8	0.52	47 ±8 [31-68]
Height, cm	159 ± 8	162 ± 9	0.29	161 ± 8 [143-180]

Table 2 Number of Seeds Ingested

Values are the mean, ± SD and range in brackets

Placeebo Group(n=32)	Digi Tab Group (n=34)	P-value	All Patients (n=66)
4.8± 3.1 [1-13]	4.4± 3.0 [1-10]	0.58	4.6 ± 3.0 [1-13]

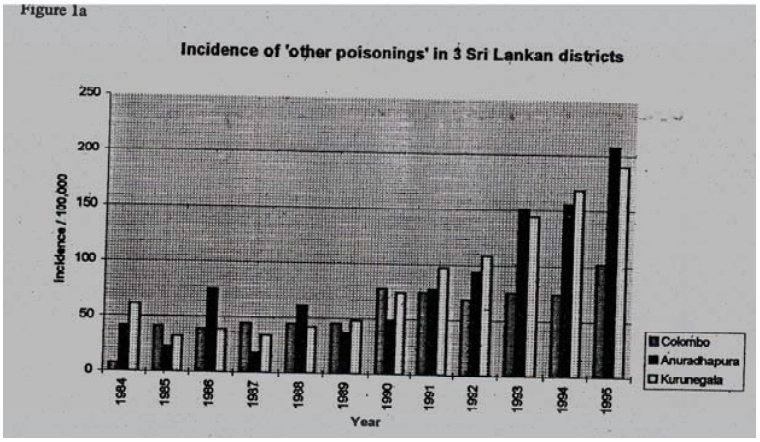
Table 3 Cardiac Arrhythmias on Admission

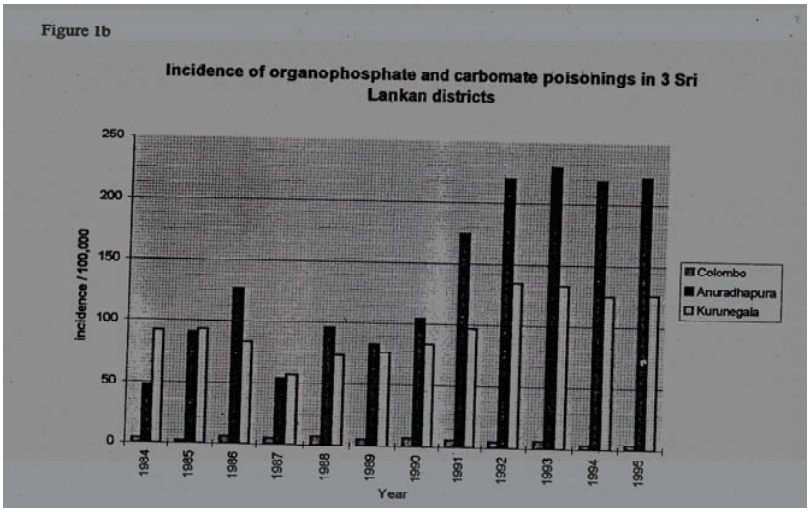
Arrhythmia	Placebo Group (n=32)	Drug Group (n=34)
Sick sinus syndrome	20(62%)	15(44%)
Sinus bradycardia (≤ 40bpm)	4(12%)	7(21%)
2^0 Heart block	11(34%)	9(26%)
3^0 Heart block	4 (12%)	8(24%)
Atrial flutter	0	1(3%)

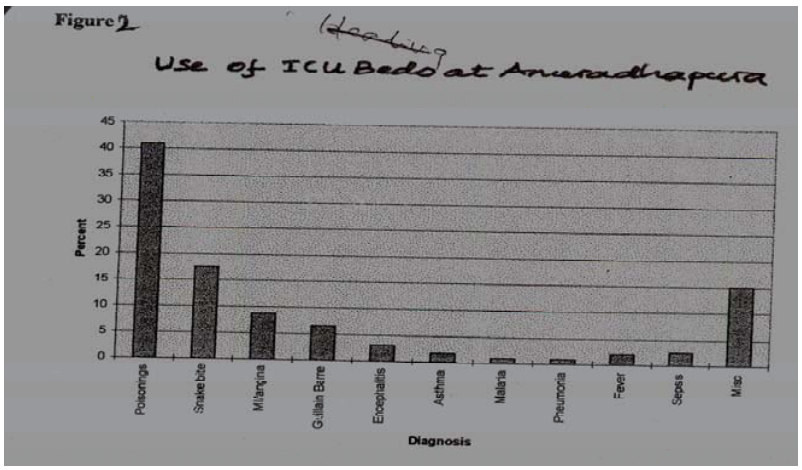
Table 4 Heart Rates at 2hr and 8hr after Treatment

	Baseline	2hr	p-value 2hr	8hr	p-value 8hr
Drug Group (n=34)	49.0 ± 9.8	66.6±18.7	<0.001	68.6±17.7	<0.001
Placebo Group (n=32)	50.6± 12.6	50.5±11.8		53.4± 14.4	

Figure 1a





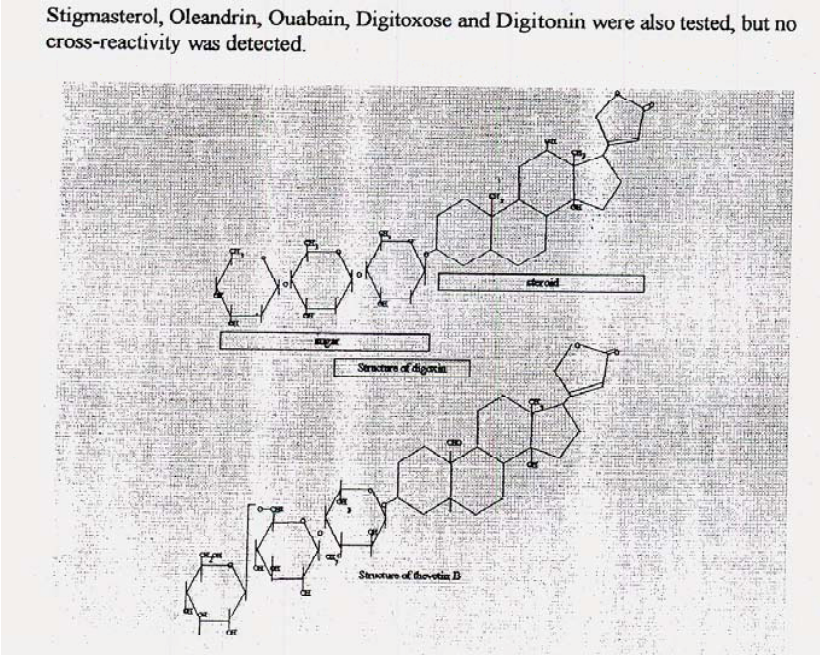


Results

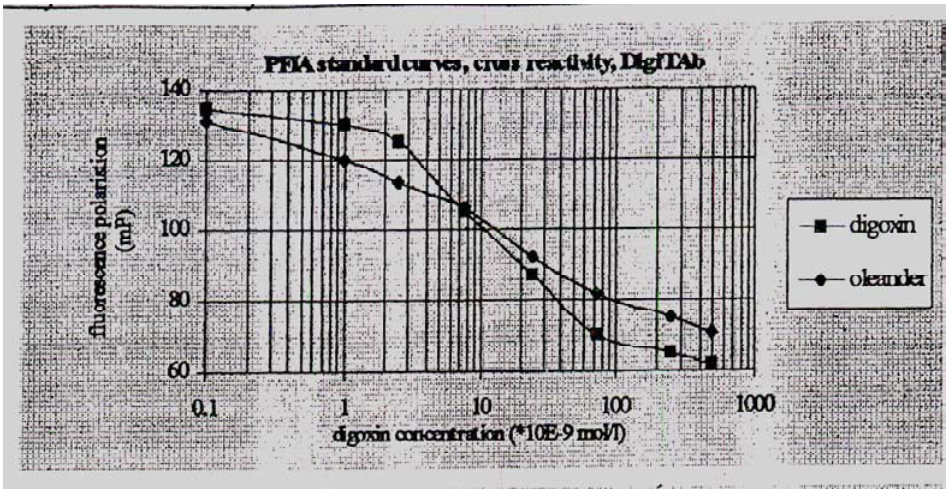
Cross reactivity study

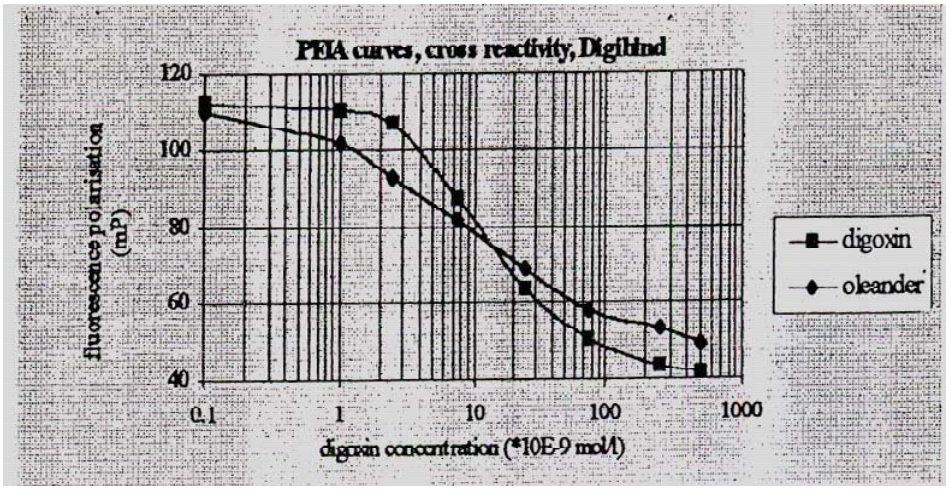
	% Cross-Reactivity			Structure
	Digi TAb	Digibind	Digitalis Antidot	
Digoxin	100	100	100	Steroid+rri-digitoxose
Lanatoside C	100	100	100	Third sugar molecule contains an acetyl group+ 1 extra glucose
Digitoxigenin	68	50	48	No susgar
Digitoxin	40	50	68	Missing one OH group on the steroid structure
Digitoxigenin	10	17	16	Missing one OH group on the steroid structure, no sugar
Proscillaridin A	4	10	21	Differences in the steroid structure, single sugar resi due with slightly different orientation
Thevetin B	17	21	33	Missing one OH group on the steroid structure, different sugar

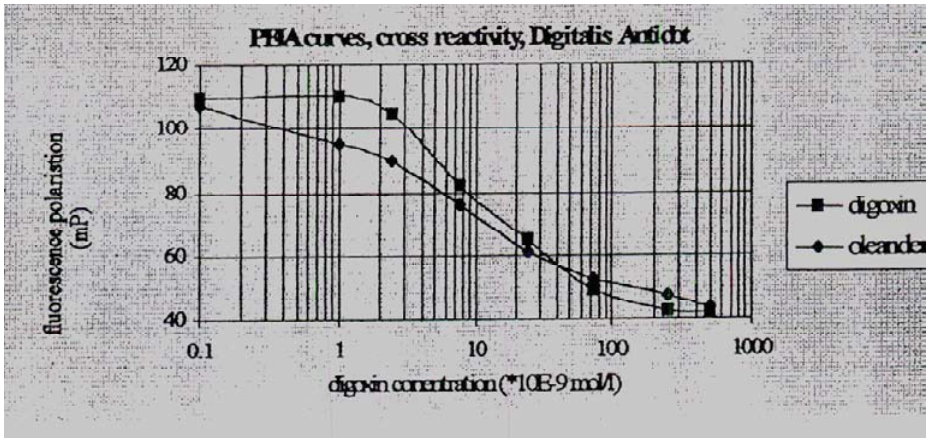
Stigmasterol, Oleandrin, Ouabain, Digitoxose and Digitonin were also tested, but no cross-reactivity was detected.



Cross-reactivity with a crude yellow oleander seed extract







Curves exhibited non-parallelism, therefore corss-reactivity was determined at 75% 50% and 25% binding

Fab	% cross reactivity		
	at 75% binding	at 50 % binding	at 25% binding
Digi Tab ™	219	95	40
Digibind ®	206	100	41
Digititalis antidote BM	350	200	100

12.1 OVERALL STUDY DESIGN AND PLAN:DESCRIPTION

Figure 1. Study Schematic

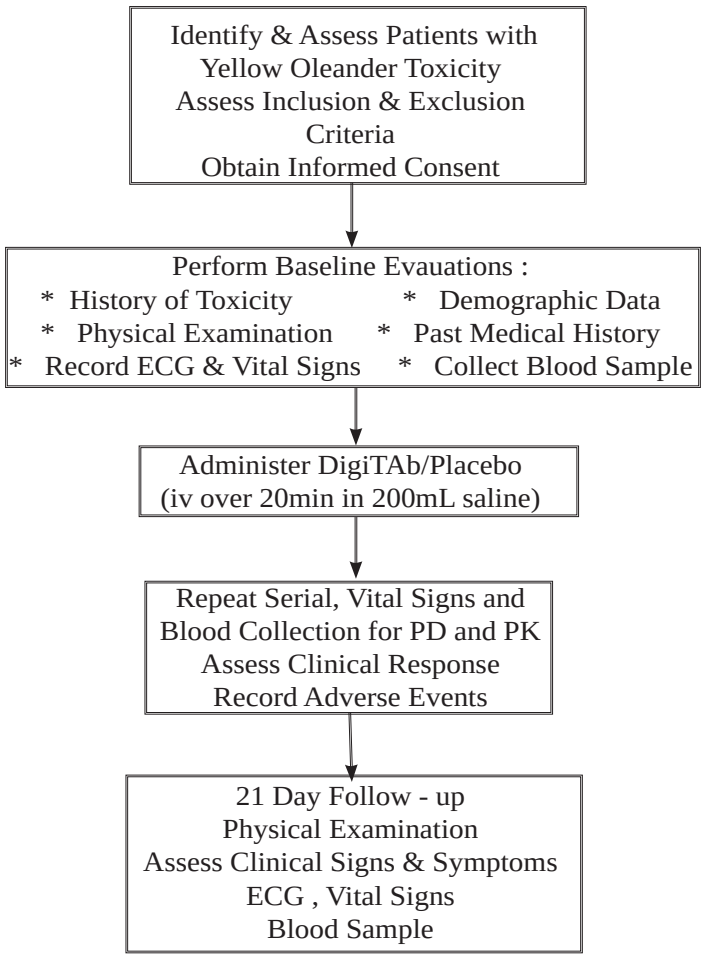
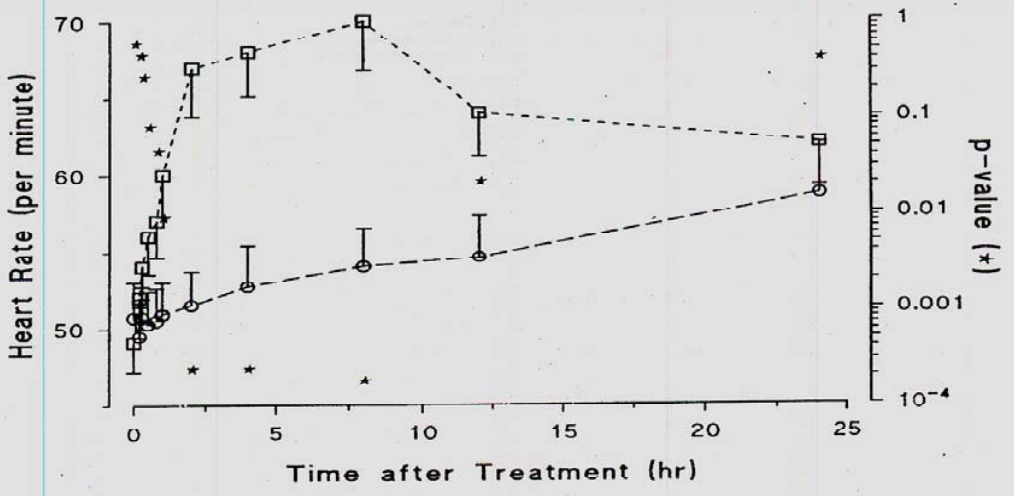


Figure 6. Mean Heart Rates in the Placebo Group (o) and Study Drug Group (□)(±SEM). Stars represents p-value.



CONFIDENTIAL

Therapeutic Antibodies Inc
Yellow Oleander Poisoning
Randomised Trial, TAb015-01

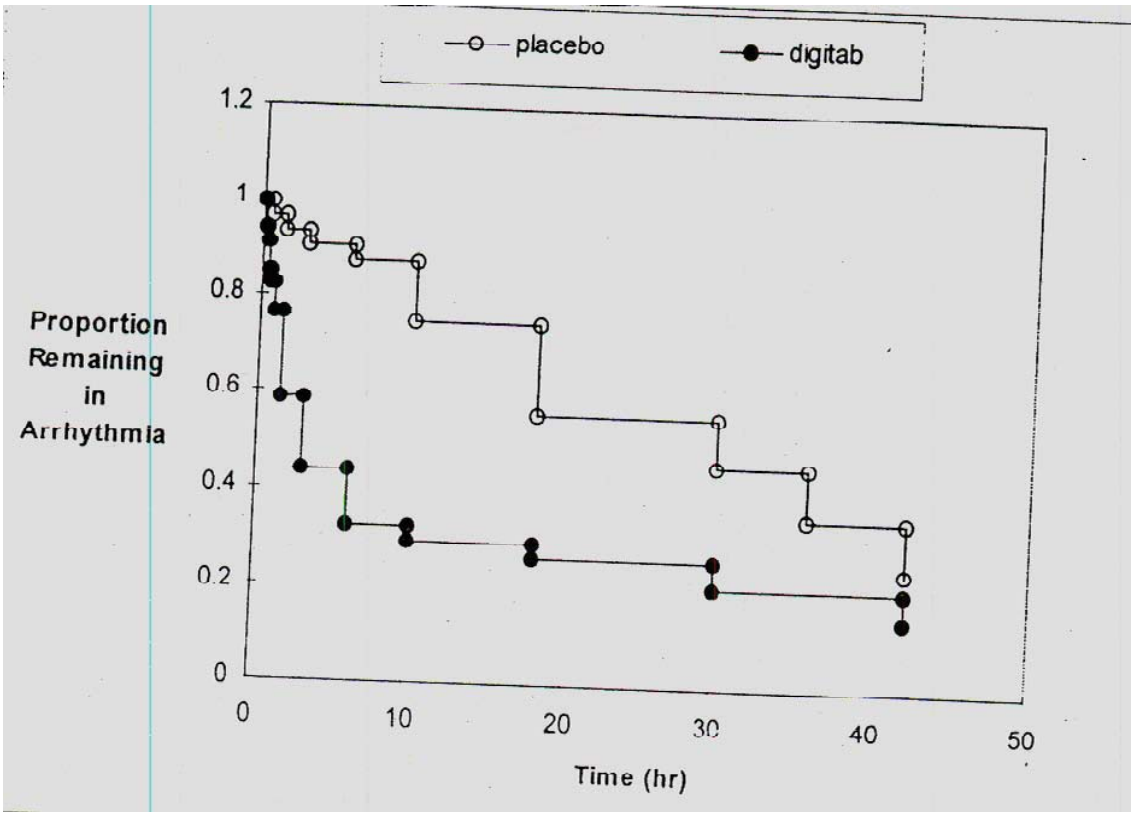


Figure 8. ECG on Admission and Eight Hours after Treatment with DigiTAb for Three Patients.