The cardiotonic effect of the crude ethanolic extract of *Nerium oleander* in the isolated guinea pig hearts

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ABSTRACT
Cardiovascular diseases are increasingly becoming one of the leading diseases causing morbidity and mortality in Uganda. Ethnographic evidence suggests that these diseases are often first managed by indigenous and related herbs before patients are referred for allopathic forms of management. One such herb of interest is *Nerium oleander*. Therefore the crude ethanolic extracts of the dried leaves of this herb were tested against the following parameters in the isolated guinea pig hearts: ***force of contraction***, ***heart rate*** and ***cardiac flow***. The extracts brought about dose-dependent increases in all these parameters from their baseline readings. Compared with graded doses of digoxin the effects closely mirrored the activities in a dose dependent manner. At the mechanism of action level, it would appear the extract works in the same as digoxin since their dose-contraction-reponse curves are parallel. This finding would tend to provide a strong rationale for the herb's traditional use in cardiovascular illness.

Key words: *contraction, cardiac flow, heart rate, Nerium oleander, extract, and digoxin*
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Introduction

The use of herbal preparations for therapeutic remedies is as old as man himself. In many parts of Uganda this tradition is still being practiced, mainly as a first choice form of medication before the patient is referred for management by the "western type" of medicine. In addition, there is a strong global trend for the revival of interest in the traditional system of medicines. In line with this trend, anecdotal evidence suggests that a number of plant products have been used traditionally for the management of cardiovascular diseases. Such herbs include the latex-producing plants used in African arrow poisons first described by Sir Thomas Fraser in 1890 [1]. *Neruim oleander* is one such herb, which although not a native to East and central Africa, has been introduced to the region as an ornamental plant[2].

Nerium oleander L (Apocynacee family) is a beautiful free flowering shrub bearing white or crimson flowers especially suited to dry and sunny localities. At least a dozen genera of the Apocynaceae are known. *Nerium oleander* is generally found in a wet habitat on sites well exposed to the sun. It occurs especially along the banks of streams and in the beds of streams.

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Although this plant is not indigenous to east Africa and was only introduced to the region where it has been cultivated freely for ornamental purposes[3], its traditional use has been observed. In Tanzania the hot water extract of its fresh leaves have been used for its anti bacterial activity [4]; in South Africa it has been used as an abortifacient[3]. In Iran the dried leaf extract has been used as a cardiotonic and diuretic in edema[5]. In Cuba it is a folklore medicine[6]. In Malaysia the plant has been used for its tumor promoting activity [7]. In India and Bangladesh it has also been used for its antibacterial activity [8].

On the other hand this plant has been reported to be quite toxic and can be dangerous to man and animals[9, 10, 11, 12].

The symptoms identified in the human include depression, dizziness, fever, nausea, bloody diarrhea, irregularity of heartbeat, weakened pulse, paralysis, loss of consciousness, and death through heart failure. In pharmacy, the fresh or dried leaves, an infusion, decoction, plaster or salve made from the leaves; the powdered bark or a decoction of the bark; a paste made from the roots, and the dried flowers are used [13]. It has been used to provoke menstruation, as an abortivum, and as an antispasmodic in the treatment of angina pectoris. As an external medicine it is used against all kinds of skin diseases[14]: rash, scabies, ringworm, lice, leprosy and boils, to treat skin eruptions or irritations in herpes and to destroy maggots in wounds.

Nerium oleander has also been used in the treatment of cancer[15]: the flowers, leaves, leaf juice or

latex, bark and roots have been used against corns, warts, cancerous ulcers, carcinoma, ulcerating or hard tumors.

The purpose of the present investigation was to test its ethanolic extract on three parameters of cardiac activity: force of contraction, heart rate and flow. The ethanolic extract was chosen because on polarity basis, it is the nearest to the preparation used traditionally.

Materials and Methods

Apparatus used

- Gallen Kemp hot box oven with fan size 1
- Waring commercial blender
- Stainless steel Impact Test Laboratory Sieve (500µm) BS410
- Stuart Scientific colony counter (UK)
- Metter PM16-N Electrical balance
- Isolated organ bath apparatus
- Oxygen cylinder
- Rotary evaporator
- Thermometer
- Kymograph (Isolated organ bath perfusion apparatus)

Chemicals

All chemicals were purchased from *Aldrich Chemicals* Ltd of England. These included: Ethanol AR, Adrenaline, Atopine, Acetylcholine- Sigma chemical company (USA), Adrenaline, Digoxin, Sodium chloride (NaCl), Potassium chloride (KCl) Calcium chloride ($CaCl_2$), Sodium bicarbonate ($NaHCO_3$), Sodium biphosphate ($NaHPO_4$), Magnesium chloride ($MgCl_2$), Glucose. Dimethyl sulphoxide, from BDH.

Materials

- Fresh samples of Nerium oleander (leaves, stem, flowers etc) for identification and herbarium.
- 2 healthy and mature guinea pigs.

Collection and treatment of the *nerium oleander* samples

The leaves and flowers of *nerium oleander* were collected, identified and examined for general physical appearance. The fresh leaves were washed to remove residual dust and dried in an oven at a constant temperature of 30°C over 48 hours. The dried leaves were reduced in size using a Waring commercial blender. Each batch of leaves was blended thrice to brake down the fibrous and venous parts of the leaf. The crushed leaves were passed through a stainless steel Impact Test laboratory sieve to separate the finer powder from the larger fibrous tissue.

Extraction

An ethanolic extract was prepared using the cold extraction method. One hundred (100) grams of the sieved powder of crushed Nerium oleander leaf was placed in a flask containing 500mls of cold ethanol and left in this position with intermittent shaking of the flask for 24 hours. The contents were then filtered and the filtrate collected was stored in the fridge to enhance stability. A second measure of 500mls of ethanol was run through the residue and left to soak for another 24hours. Again the filtrate was collected and stored in the fridge between 0-5°C. This procedure of cold extraction was repeated a third time. The filtrates were mixed together and a rotary vacuum pump extractor was used to remove the ethanol from the extract in a 250ml flask. The flask containing the concentrated extract was stored in a cool dry place until the next stage of the experiment with an isolated guinea pig heart.

Drug preparations

Ethanolic extract

One (1) g portions of the extract were scraped off the bottom of the flask and placed in 2 vials. To each of the vials 0.2 ml of dimethyl sulphoxide (DMSO) was added to the extract and then the mixture was topped up with 0.8mls of tyrode solution. The aim was to make the proportion of DMSO to tyrode solution in the final mixture of 1:5. This process gave a stock solution of 1g/ ml from which the following serial dilutions were made: 100mg/ml, 20mg/ml, 2mg/ml, 1mg/ml, 0.2mg/ml, and 0.02mg/ml,. One (1) ml of each concentration was tested against the heart preparation.

Acetylcholine

Acetylcholine reduces both heart rate and contraction. In order to check whether these parameters could be restored by the extract ACH was given then followed by the extract. A stock solution of 10mg/ml was made with tyrode solution. The following working concentrations were prepared from the stock: 1mg/ml and 0.1mg/ml. One (1) ml of each of this concentration was added to the bath and used as a negative control.

Adrenaline

Adrenaline increases heart rate and force of contraction. To see whether the extract would restore this parameter adrenaline was given then followed by the extract. From

the adrenaline vial contained 1mg per ml as the stock solution, the final working concentration of 100µg/ml was made with tyrode solution. One (1) ml of this concentration was used.

Digoxin
From the digoxin vial containing 0.25mg in 2ml, the following working dilutions were made with tyrode solution: 100µg/ml from which 0.2ml and 0.1ml were finally used in the bath; 10µg/ml from which 0.2 and 0.1ml were used; and 1µg/ml from which 0.2 and 0.1ml were used.

Animal preparation
Seven (7) guinea pigs of either sex, weighing between 600-800gm were injected with 1000 units of heparin in the ear vein to avoid irreparable damage by clots forming inside the heart before giving a sharp to the head. The throat was cut, the chest was opened and the heart was carefully removed. It was placed as quickly as possible in a dish containing Tyrode solution at room temperature. The preparation was gently squeezed several times in order to remove as much blood as possible. The aorta was located and dissected free and all other fascia tissue connected to the heart was trimmed away.

Langendorff method
To screen for the cardiotonic effect of *nerium oleander* Langendorff preparation[16] was used. The aorta was cut just below the point where it divides and the heart was transferred to the perfusion apparatus containing tyrode solution, constantly oxygenated and maintained at 37°C, where the aorta was tied onto the glass cannular. Care was taken to ensure that air bubbles did not enter the aorta, and any bubbles that did were immediately removed.

A funnel was placed beneath the suspended heart in order to allow for the collection of fluid flow from the heart to determine the flow rate with a graduate measuring cylinder and stopwatch.

A fine nylon thread was attached to the ventricle by a hook and to the auricle by a small spring clip. The thread was connected to spring levers to record the heart contractions. The heart was allowed to stabilize for a period of about 20 minutes. Readings of the rate of beating and of the coronary flow were most conveniently taken over a period of 30 seconds. Drugs: digoxin Acetylcholine, Adrenaline and the extract were added to the preparation by injection through the rubber tubing into the perfusion fluid. Any noted heart block was reversed by the administration of 0.1µg atropine.

Results
The results presented here are drawn from the readings of 5 guinea pig hearts. Baseline readings were noted to change with extract, digoxin, acetylchole and adrenaline. In typical case presented here the baseline amplitude was 22mm, the heart rate was 28 beats/min and a flow volume of 0.4ml/min. After the stabilization of the heart, 0.1ml of the diluted adrenaline was injected. This brought about a rise in amplitude, so did the heart rate and fluid flow from their baseline readings. This was followed by an administration of acetylcholine, which reduced all the parameters of measurement.

Figure 1: Increase of contraction by adrenaline and then reduction by acetylcholine then sub maximal doses of extract.

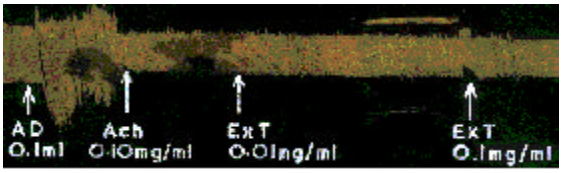
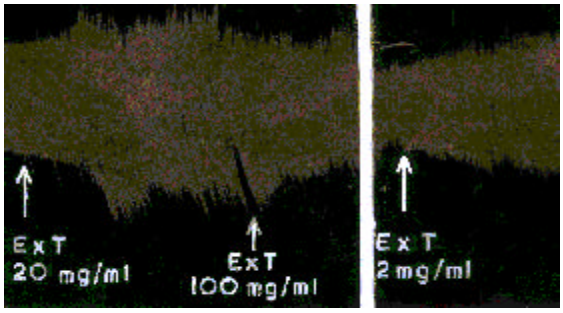


Fig. 2: Increase of myocardial contraction by different concentrations of the extract



A testing dose of 20µg of extract was administered followed by the administration of different concentrations of the extract. Graded responses of the parameters were obtained (table1, figs 1 and 2).

Fig. 3: Contraction of the heart produced by different doses of digoxin

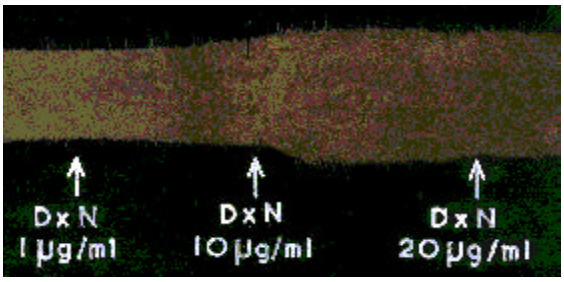


Fig. 4: Dose-contraction-response curve of the extract

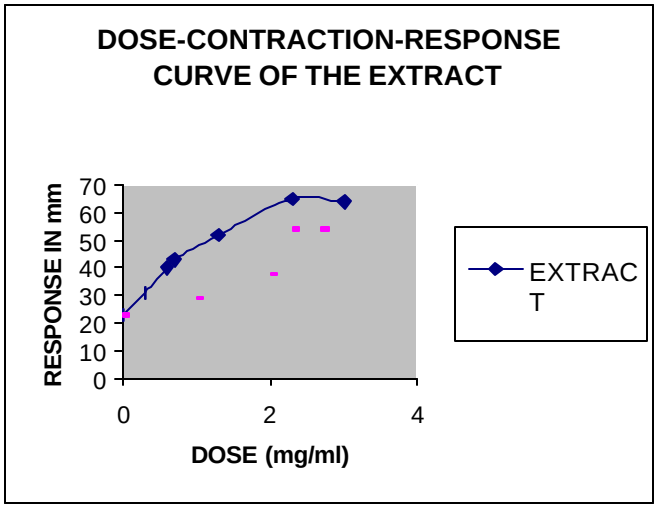


Table 1: Parameter responses to different concentrations of extract, digoxin, adrenaline and acetylcholine

Drugs & Extract	Dose	Heart Rate Beats/Min	Flow Volume ml/Min	Amplitude (mm)
Baseline		28	0.40	22
Adrenaline	0.1mg/ml	33	0.60	47
Acetylcholine	0.1mg/ml	24	0.3	20
Extract	0.01mg/ml	27	0.9	21
Extract	0.02mg/ml	29	1.0	22
Extract	0.1mg/ml	31	1.2	23
Extract	0.2mg/ml	35	1.5	41
Extract	2mg/ml	36	2.0	48
Extract	20mg/ml	38	2.1	48
Extract	100mg/ml	41	1.9	49
Digoxin	0.1µg/ml	24	0.7	12
Digoxin	0.2µg/ml	28	1.4	31
Digoxin	1µg/ml	30	1.6	24
Digoxin	2µg/ml	32	1.7	34
Digoxin	10µg/ml	35	1.7	54
Digoxin	20µg/ml	35	1.7	54

A dose of 0.1µg of digoxin was then administered followed by the administration of other concentrations as shown in Fig 3. There were also graded responses with the different doses with a tailing off of the response at a maximal dose where there was no more increased

Discussion

In this work ethanolic extract of *Nerium oleander* elicited a dose-dependent increase in amplitude of contraction that is in harmony with an increase of the force of contraction (Figs 1-3). This is a typical cardiac glycoside activity consistent with previous observations that its leaves contain cardenolides[17, 18]. A dose-contraction-response curve is shown in figure 4. It can be noted that doses below 2mg gave only small increases of contraction from the baseline, but above that significant increases were observed, peaking at a dose of 100mg/ml when further rise of contraction were not discernable. An attempt was made to compare *Nerium oleander* extract with standard digoxin. Even though Langdendorff's preparation is not considered suitable for

comparative purposes[19] in this work, table 1 shows the increase of contraction and heart rate with the dose of digoxin in the same fashion as the extract.

Acetylcholine has for long been known as a transmitter that has the property of lowering the blood pressure and also bringing about bradycardia through its muscarinic receptor [20]. It was used here as a negative control to test the possibility of the extract to restore the lowered contraction and heart rate. Higher doses however bring about extreme bradycardia with sino-arterial conduction failure. On the other hand adrenaline is a known autonomic nervous system stimulator working through both β and α adrenergic receptors to stimulate the contraction of the heart and also to increase the heart rate [21]. On the heart, it causes tachycardia. In high doses it can lead to arrhythmia. It was used here as a positive control. The Langdendorf experiment used in this work presents a good indicator of cardiac circulation because the pressure of the perfusion fluid closes the aortic valve, so the fluid flows only through the cardiac vessels to escape from the inferior vena cava.

The other parameter of investigation was heart rate. In the present study we noted a sustained increase in heart rate with the doses of the extract (table 1). Likewise we observed that the extract brought about dose-dependent increases in the cardiac flow.

Thus all the three parameters of study in this work seem to be positive and can be used to explain the possible benefit of the herb in cardiovascular diseases. The major control of the cardiovascular system is the autonomic regulatory activity. The increased force of contraction of the heart causes the heart to expel more blood into the arterial system with each beat. This is beneficial for especially a failing heart. With every heart beat the normally functioning heart expels as much blood as it has received in the proceeding diastole, as the heart muscle is known to stretch according to the load placed upon it, until a certain limit. This is a basic Starling law of the heart. The myocardium recovers as a result of the increased cardiac output and circulation. Ordinarily this increase of force on the myocardium brings about increased cardiac output, decreased heart rate, and decreased venous pressure volume, diuresis and relief of edema[22] If the amount of blood returning to the heart is increased, the stroke volume will increase correspondingly because of the increased stretch of the cardiac fibers. This is not so with the failing heart where the power of contraction is reduced and the heart can no longer eject all the blood which is returned to it. Blood accumulates in the heart, which becomes stretched beyond the point at which the Starling's law operates.

If an agent is able to produce a force of contraction, the power of cardiac contraction is improved and the failure is partially relieved, as well as reducing the size heart rate thus bringing it within the operation of the Starling law where the heart is again subject to the autonomic regulatory action of the returning blood [23].
In our present work the heart rate was increased. In experimental studies in intact animals the cardiac rate usually decreases with a cardiac glycoside in response to a reflex vagal effect and also to the rate of change and the extent of increase in arterial pressure[24]. In the isolated heart experiment like the present one, we see a sustained increase. The explanation may be based on the circumvention of the reflex mechanism with the isolated preparation. In the failing heart, cardiac glycosides slow the heart as a reflex response, consequent on the enhanced cardiac output. However in the isolated heart this reflex mechanism may not be operating.

In conclusion, although this work can only be considered as exploratory, it suggests that the plant contains a substance that has a cardiotonic activity in the heart. Although the Langdendorff preparation is not suitable for comparing and quantifying a new substance this work seems to strongly suggest that *Nerium oleander* has a similar activity with digoxin. In this work we used same hearts as their own experimental controls. This however would induce fatigue and hence low responses to digoxin and the extracts. It will be necessary to use different hearts for digoxin and extracts. That the tissue response varies with a very preparation our future work will consider only the degree of changes in the parameters.

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