THE CARDIOTONIC AND OTHER PHARMACOLOGIC ACTIONS OF *THEVETIA NERIIFOLIA*

By

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The extract obtained from the plant of *Thevetia neriifolia*, Yellow Oleander, contains pharmacologically active substances such as glycosides. The typical digitalis like action of *thevetia* was demonstrated in isolated frog heart and in anaesthetized dog. Results indicated that the induced cardiac failure by increased venous pressure in frog heart may be used for the study of the therapeutic activity of cardiac glycosides with rapid action.

It was confirmed that the active substance is taken up by the heart muscle with slow but persistent fixation during its passage in the heart. It was not immediately destroyed or metabolized by the cardiac tissue. It has been suggested that *thevetia* glycosides may be included in the list of drugs active in restoring a failing myocardium.

Since its discovery by William Withering in 1775, Digitalis has remained the sheet-anchor for the treatment of congestive heart failure. During the past thirty years, the studies of Cloetta (1929), Windaus (1928), Jacobs (1933), Elderfield (1935), Stoll (1937) and of Mendez *et al.*, (1951) have clarified to a great extent the chemical structure of the cardiac glycosides. A digitalis like substance, thevetin, has been isolated from the following species of the genus thevetia, viz *Thevetia neriifolia* (De Vry, 1863 ; Toja, 1952) and *Thevetia yocottli* (Chen and Chen, 1938). Thevetoidin, which is a white crystalline moderately hygroscopic substance has been isolated by Mendez *et al* (1951) from the seeds of *Thevetia gaumeri* in the National Institute of Cardiology, Mexico, and has been found to contain a cardiac glycoside, acetyl-thevetin, and small amount of a muscarine derivative. Studies on the Indian species of *Thevetia neriifolia* have been conducted by Ghatak and Pendse (1932). These workers also claim to have obtained thevetin.

Toja (1952) claims that complete digitalization with thevetia can be obtained with one half of the toxic dose whereas the digitalizing dose with

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other digitalis preparations is two thirds to one third of the toxic dose. With these data as a background, I undertook a pharmacological study of thevetia extract believing that I was dealing with a substance with a very rapid transient action similar to that of thevetoidin studied by Mendez and his collaborators. In view of the fact that the safety margin with thevetia is wide, it was decided to study the pharmacological actions of this substance on various preparations. It was further decided to induce cardiac failure in perfused frog heart by high venous pressure and to compare the effect of injecting thevetia to that of digitalis and stropanthus glycosides.

METHODS

The plant of *Thevetia neriifolia* (Yellow Oleander, Piia Kaner), which is found through out India, was procured for the present study, locally (Fig. 1).

Fig. 1.—*Thevetia Neriifolia* (Leaves Long, Lanceolate and Smooth; Flowers Large, Yellow and Bell-Shaped).

The identification of this plant was done from an examination of the roots, leaves, flowers, fruits etc. and was verified from a description of the same as given by Chopra (1958). The extraction procedure of thevetia from the root, bark and stem of this plant was followed according to Mendez *et al.* (1951). It was thought essential to test chemically for the presence or absence of the various possible constituents in this extract. The chemical investigations were done according to the method described by Clark (1949) and the extract was found to contain the active substance glycoside (besides carbohydrate, protein etc.) since the following tests were positive.

Tests For Glycoside :—(a) Five ml of thevetia was first tested for reducing substances by boiling with qualitative Benedict's reagent—change of colour to green but no precipitation took place. Filtered. The filtrate was heated with 2 ml diluted hydrochloric acid for hydrolysing any glycoside in it and splitting off the sugar. It was cooled and sodium carbonate 1 per cent solution was added drop by drop till all free acid was neutralized. The solution obtained after filtration was boiled with five volumes of qualitative Benedict's reagent—an orange coloured precipitate was produced.

(b) Conc. Sulphuric acid (2 ml) was added to 2 ml of thevetia solution—black colour was produced.

Induction of Cardiac Failure in Perfused Frog Heart :—For the experiments on isolated frog heart and determining the effect of increasing venous pressure on cardiac output Bulbring's method (1930) of perfusion was followed. *Rana temporaria* were set up, the inferior vena cava was canulated and the venous pressure readings were taken from the height of the column of Ringer solution in the perfusion bottle and measured directly as cm of water. Records of the heart tracings were taken and cardiac output was measured at the corresponding venous pressure.

Dog Blood Pressure :—Dogs of the mongrel variety were selected and anaesthetized with 1 per cent chloralose solution given intravenously in the dose of 80 mg per kg of body weight. Artificial respiration was instituted through a tracheal canula and adequate ventilation was ensured. Drugs were given by injecting into a venous canula tied in the femoral vein. The blood pressure was recorded from the carotid artery by a mercury manometer.

Longitudinal Movements of Guineapig Ileum :—Guineapigs were starved overnight and killed with a blow on the head. A portion of the terminal ileum was removed and washed gently in Tyrode solution. This was next mounted

in an isolated organ bath and perfused with Tyrode solution at a constant temperature of 37°C.

Rhythmic Longitudinal Contractions Of Rabbit Ileum And Pregnant Rabbit Uterus. —The experimental procedure for this set-up was similar to that of guineapig ileum. A portion of the terminal ileum was removed from a freshly killed rabbit and washed and perfused with Tyrode solution at 37°C. Normal rhythmic movements of intestine were recorded for a few minutes on a smoked drum before administering any drug in the bath.

Strips of the horn of pregnant rabbit uterus were next mounted as usual in an isolated organ bath and perfused with de Jalon fluid at a constant temp of 31°C. Even in this solution, the spontaneous rhythmic contractions of the isolated uterus continued for long periods of time.

RESULTS

The Effect of Thevetia On Cardiac Failure Induced In Perfused Frog Heart And Comparison With Digitalis Glycosides.— Using the isolated frog heart it was seen that while increase in venous filling pressure raised the cardiac output, this relationship was held over a certain physiological range only. If the venous pressure was raised to a certain point at which the heart began to be over-

TABLE I

Effect of variation of venous pressure on cardiac output of frog. Line 5 (+) shows the effect of stropanthin (0.25 mgm) at high venous pressure

Serial No (frogs)	Venous Pressure cm water	Cardiac Output (ml per min)
I	(1) 20.0	1.20
	(2) 52.0	2.15
	(3) 70.0	2.25
	(4) 78.0	1.50
	+(5) 78.0	4.80
II	(1) 17.0	0.90
	(2) 38.0	1.50
	(3) 60.0	2.00
	(4) 65.0	0.95
	+(5) 65.0	3.60
III	(1) 18.0	1.00
	(2) 55.0	1.95
	(3) 68.0	2.30
	(4) 75.0	1.60
	+(5) 75.0	4.50

stretched, then no further rise in cardiac output took place, while still further increase in venous filling pressure was accompanied by a definite fall in cardiac output. Finally at this limiting pressure when the outflow became less, stropanthin injected in the dose of 0.25 mg acted very rapidly on the heart and rise in cardiac output was the first manifest response (Table I, Fig. 2). In another perfused frog, following such cardiac standstill, 0.5 ml of thevetia administration was followed by a further rise in cardiac output, positive inotropic effect and reversal of the cardiac failure (Table II, Fig. 3 and 4). At the end of ten minutes, a further 0.5 ml of thevetia administration was followed by a further rise in cardiac output.

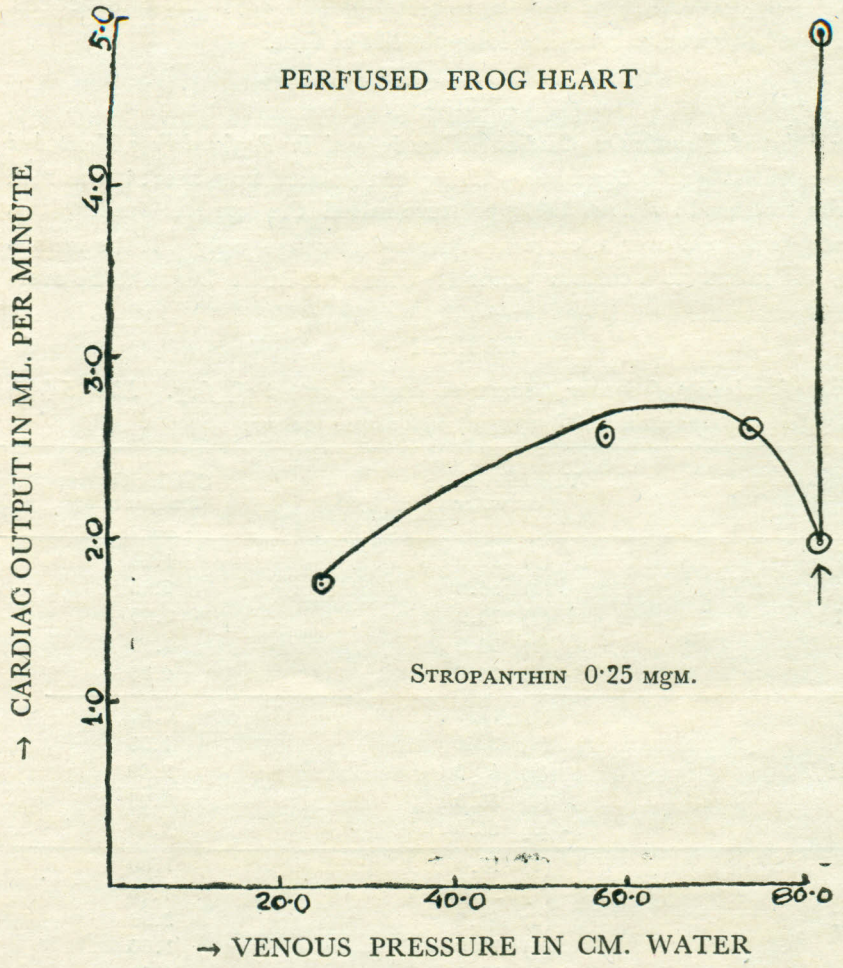


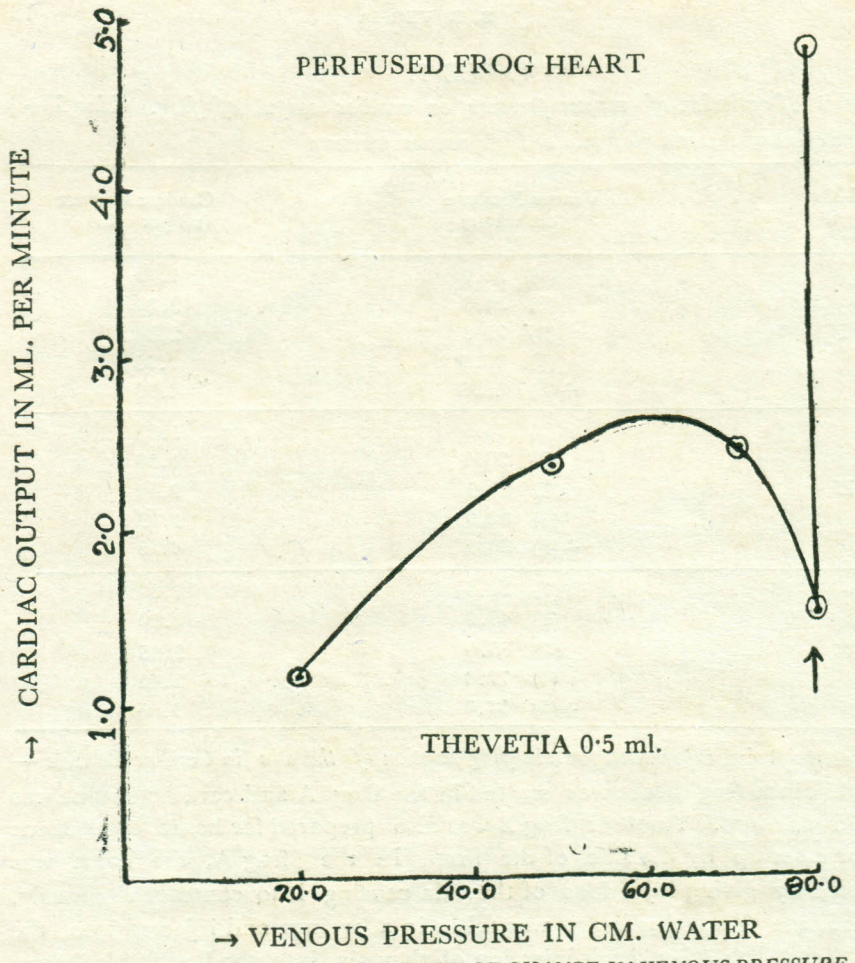
Fig. 2.—Shows the Results Obtained When Cardiac Failure is Induced in Perfused Frog's Heart and the Effect of Stropanthin 0·25 mgm At high Venous Pressure

TABLE II

Effect of variation of venous pressure on cardiac output of frog. Line 5(+) shows the effect of thevetia (0.5 ml) at high venous pressure

Serial No (frogs)	Venous Pressure (cm water)	Cardiac Output (ml per min)
I	(1) 22.0	1.70
	(2) 54.8	2.20
	(3) 75.8	2.30
	(4) 80.3	1.75
	+(5) 80.3	4.90
II	(1) 18.5	1.00
	(2) 36.0	1.50
	(3) 65.0	2.10
	(4) 80.0	1.10
	+(5) 80.0	4.25
III	(1) 20.5	1.30
	(2) 45.0	1.90
	(3) 65.0	2.25
	(4) 77.0	1.40
	+(5) 77.0	3.95

Cross perfusion experiment for studying fixation of thevetia in Cardiac Muscle.— Perfusion of the frog heart was started in the frog A and cardiac output was collected as usual. Another frog B was also prepared for heart experiment and it was set up by the side of the first. In the frog A, the heart was stimulated by giving injections of thevetia causing it to contract vigorously. The perfusate from this heart was taken in a syringe and injected into the second heart B. After each injection of thevetia into the first frog's heart, the perfusate was collected and injected into the frog B, but without any effect on the latter (Fig. 5). This could be either due to fixation or destruction of the drug during its passage in the heart. The first possibility was tested by recovering the active substance from the first heart A by cutting off the heart, grinding it and making a heart extract with water. The heart extract of A when injected into the frog B produced a similar stimulating effect. This result was checked in a control study by injecting a normal heart extract in the frog B which however did not elicit any response. Also a normal frog's heart was removed and after soaking it for five min with thevetia solution and washing it thoroughly in distilled water thevetia was found to pass and get fixed in the heart completely during its first contact with the heart muscle. The heart extract of this frog also elicited a stimulating effect on the heart of frog B. This experiment proved that the drug was fixed in the heart.



Fig, 3.—Shows the Results Obtained When Cardiac Failure is Induced in Perfused Frog's Heart and the Effect of Thevetia 0·5 ml. at High Venous Pressure,

PERFUSED FROG HEART EFFECT OF CHANGE IN VENOUS PRESSURE ON CARDIAC OUTPUT
(At arrow E_1 0·5 ml was injected)

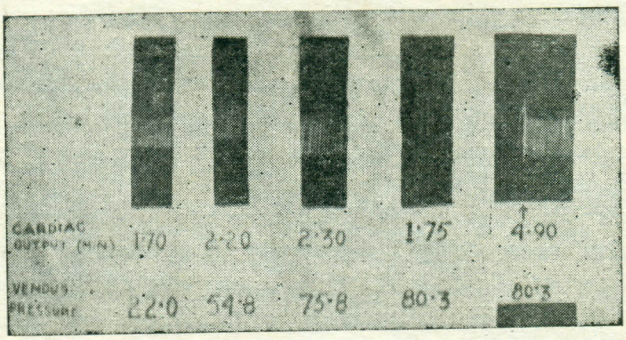
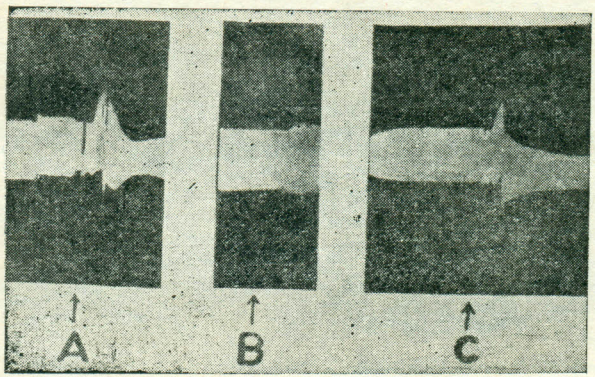


Fig. 4—Shows the tracings obtained when cardiac failure is induced on perfused frog's heart and the effect of Thevetia at high venous pressure on the contractility and cardiac output of frog

Experiments were next undertaken for elucidating the mechanism of the digitalis like action of thevetia on frog's heart. The effect on the ventricular muscle of the heart was also demonstrated by pre-treatment with the various blocking agents like atropine (10^{-6}), pentolinium (10^{-5}) and priscol (10^{-3}). No change in the action of thevetia was observed when it was administered along with them. As such thevetia was a direct acting drug.

Dog blood presssure —On administration of thevetia, a rise in blood pressure by 15 to 20 mm Hg was observed, which was maintained for nearly 10 min before descending slowly to the original level. This was not abolished by previous injection of atropine, pentolinium or priscol. An irregular blood pressure was seen due to repeated doses of thevetia given regularly for two hrs or so. The experiment was repeated six times. When the animal was killed finally, by injecting air intravenously through the venous canula, the ventricles were seen tonically contracted in each of the six dogs.

PERFUSED FROG HEART



A—O.5 ml of E_1 was injected in the frog A.
B—The perfusate of frog A was injected in frog B.
C—At C, the heart-extract of the frog A was injected in frog B.

Fig. 5. Shows the fixation of thevetia in the cardiac tissue of frog during the process of perfusion of heart.

Guineapig ileum.—Thevetia itself did not produce any visible action in moderate doses. However, when thevetia was administered in the bath, it increased spasm due to high doses of acetylcholine, histamine and barium, but was antispasmodic to low doses when given together in the bath (Fig. 6). This effect was not antagonised by a atropine (10^{-6}) pentolinium (10^{-5}) and mepyramine (10^{-7}).

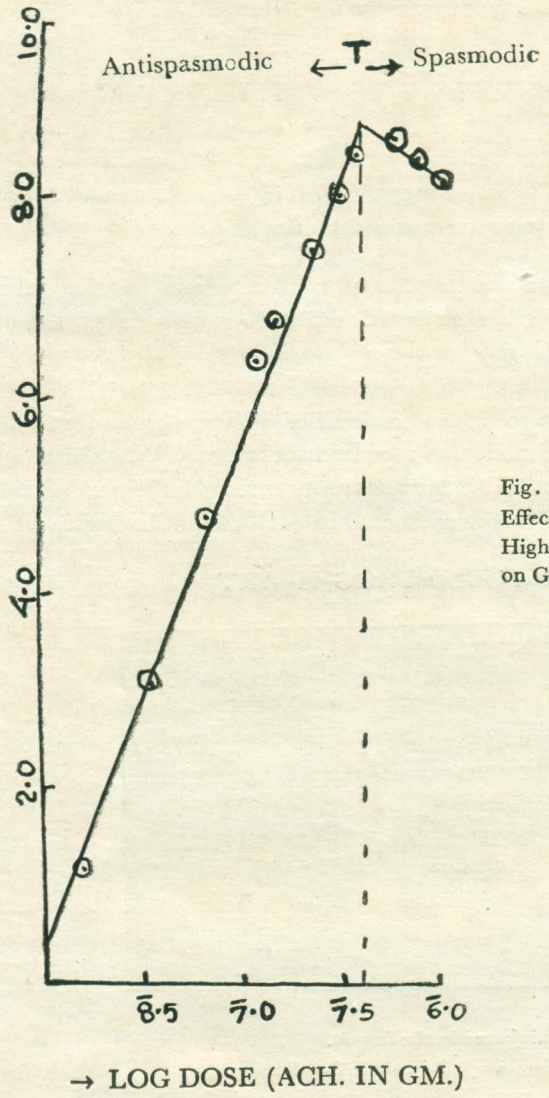


Fig. 6—Shows Log-Dose Response and Effect of Thevetia on Spasm Induced by High & Low Doses of Acetyl-Choline on Guinea-pig Ileum.

Rabbit ileum and pregnant rabbit uterus.—When *thevetia* was added into the bath, an increased tonicity and sustained contraction of the muscle was observed. The spontaneous contraction was not changed by *thevetia.* The Spasm so induced by the drug was not reduced by pre-treatment with the various blocking agents.

DISCUSSION

Disorders of the cardiovascular system are quite common and take a fairly heavy toll of human lives. Digitalis remains the most important drug in the therapy of failing myocardium and any research revealing drugs which might supplement or supplant digitalis is worth while. Thevetia glycoside seems to be one such drug and its claim for inclusion in the list of drugs active in restoring a failing myocardium is based on the following points :—

(1) A determination of the stimulating action of its glycosides in certain forms of failing myocardium (experimental).

(2) A demonstration of the clinical improvement in certain types of heart failure (Chavez *et al.*, 1952).

(3) Comparatively low toxicity (Celice *et al.*, 1953).

However, the drug shows certain amount of cumulative action and has got some pharmacologic actions elsewhere in the body.

A pre-requisite of introduction of a drug in therapy is its isolation and purification and, if possible, an economic method of synthesis. The glycosides (thevetin, thevetoidin etc.) have been isolated in fairly pure state and this has already been discussed before. During the course of present investigations, it has been shown that the activity of thevetia extract was present mostly in the fraction containing the glycosides. The next logical step in this research would be determination of the chemical structure, pharmacologic studies and a method of synthesis or manufacture of the drug in a larger scale in order to enable one to undertake clinical and toxicological study. The pharmacologic studies under taken may be classified as :—

(1) Action on the failing myocardium.

(2) Action on the blood pressure of experimental animals.

(3) Action on the smooth muscles.

There is an adequate support for a stimulating action of thevetia on the myocardium particularly when the heart has failed under certain experimental conditions. In an isolated perfused frog heart, the perfusion pressure is directly related to cardiac output over a small range. Raising the pressure beyond this limit ultimately leads to myocardial stretching and dilatation. The increase in perfusion pressure embarrasses the heart and leads to a decrease in cardiac output thereby producing a vicious circle. A type of cardiac failure is thus created which can be called "overload failure".

There are reasonable grounds to believe that many forms of cardiac failure in human beings are the result of a somewhat similar mechanism. The evidence that the failing human heart behaves as though it were overloaded is afforded by :—

(1) The dramatic relief of venesection in cardiac failure (McMichael, 1938, 1948).

(2) Transfusion which if given too rapidly to the anaemic heart may induce serious failure (Sharpey-Schafer, 1944).

(3) Exercise, which in normal persons is associated with a slight rise in venous pressure, is accompanied by a considerable rise in venous pressure in patients with heart failure, but the cardiac output usually shows a decline instead of an increase (Hickam and Cargill, 1948 ; McMichael, 1948).

There are several ways by which cardiac output may be improved after setting of failure in the experimental setup :—

(I) Lowering of venous pressure.

(2) Use of cardiac glycosides like stropanthin.

(3) Use of thevetia.

The increase in cardiac output i s most marked with the use of stropanthin, then with the use of thevetia and lastly wlth the lowering of venous pressure. Both stropanthin and thevetia intravenously raised the cardiac output considerably. An immediate consequence of these substances was a rise in cardiac output from 1.50 ml to 4.80 ml by stropanthin and from 1.75 ml to 4.90 by thevetia, During this phase the venous pressure remained high but inspite of this the cardiac output was definitely raised. On the basis of this result it may be postulated that thevetia glycosides may be a second choice in conditions of cardiac failure. Clinical studies undertaken so far have corroborated this statement(Chavez *et at.*, 1952; Celice *et al* , 1952; Toja, 1952; Lian, 1952; Ambrosio & Mangiesi 1954; Kramer, 1955). The mechanism of action of this drug is, however, obscure. Chemical studies to determine its fate and excretion in body have not so far been undertaken and the conclusions on these points are indirect. There is good evidence to suggest that the drug is fixed in the heart in as much as the perfusate when tested on a second heart contains little activity but if the heart itself, to which the drug was originally perfused, is re-extracted with water it shows, under controlled circumstances, presence of cardiotonic activity. Clinical evidence mostly supports this data (Chavez *et al* 1952 ; Toja 1952 ; Kramer, 1955;). The action of the drug on the heart and on other organs and tissues does not resemble anyone of the known auto-pharmacologic substances and as such it

is unlikely that the drug action is mediated through the release of local hormones. Both thevetia and adrenaline have quite similar effect on contractility and tone of frog's heart, but the mechanism of antispasmodic action of these two substances on guineapig ileum is different (thevetia produces sharp relaxation after acetylcholine, histamine or barium induced spasm in low dosage only when given separately on guineapig ileum, whereas adrenaline always decreases spasm). Moreover the effect of adrenaline on blood pressure of dog is always blocked by priscol, but it does not block that of thevetia The action of non-adrenaline also resembles thevetia, but the former is again differentiated in being blocked by priscol. Similarly, thevetia has an identical action on blood pressure as 5—HT, but the mechanism of the latter drug is different (excitatory) on guineapig ileum. Finally, thevetia is distinguished from acetylcholine, histamine and 5—HT due to lack of direct action on guineapig ileum.

In experimental animals, thevetia has got vasopressor action which lasts for 10 to 15 min. This is not abolished by previous administration of pentolinium and priscol and, therefore, the possibility of any neurogenic mechanism is ruled out. It appears to be a direct stimulating effect on the cardiovascular system. Similarly, on the isolated uterus of the rabbit, the effect of thevetia is a direct action on the smooth muscle.

Having excluded the possibility that the drug acts either by neurogenic or chemical mediation, the only possibility that remains is that the drug has got a direct action on its target organs. Such action is antispasmodic when the smooth muscle is firmly contracted whereas on the cardiac muscle such action is stimulant. On the analogy of the action on smooth muscle, it may be speculated that even in the heart the drug might facilitate diastole, thereby improving cardiac nutrition and contractility and increased cardiac reserve. Electrocardiographic studies (Mendez *et al.*, 1951 ; Chavez *et al.*, 1952) have shown that the P—R interval is increased and this may be taken to support the above view which is further strengthened by the observation that one of the earliest effect of the drug is a shortening of the Q—T interval which indicates that the duration of the ventricular systole is very much reduced. What causes the relaxation in smooth muscle and whether the above conjecture is correct in the heart muscle as well, cannot at the moment be asserted. It is possible that the antispasmodic action is due to changes in the cell membrane and ionic transfer across the same (Benson 1955).

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