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The subject of the present study was a pharmacological investigation on four indigenous herbs which were claimed to be diuretics, the four herbs being - Boerhaavia repens and rependa, Tribulus terrestris and Pedalium murex. Available literature about the chemistry, pharmacognosy and pharmacology of the herbs was reviewed and an attempt was made to study them, in a comparative manner and draw conclusions and inferences derived from experimental investigations.

The study of pharmacognosy was done on the fresh plants both macroscopically and microscopically. The points noted during such a study are mentioned below:

The plants grow wildly all over the country, Boerhaavia possessing small pinkish flowers and Tribulus and Pedalium having small yellowish flowers.

The study of the internal structure of Boerhaavia revealed the anomalous secondary growth which is a peculiar feature of this herb. (Hill - 1951). In the context the cork tissue cells consist of many layers of cells. The cambium is very thin. The vascular bundles are very irregularly distributed. In the outer layers of the cells throughout the section bundles of long needle-like crystalline raphides are seen. Air spaces are few and seen
mostly in leaves. Stomata are found in both the upper and lower epidermis. Plenty of starch grains are observed in the cells except in those of cork tissue. Pith is not distinguishable properly. (Fig. -I-)

Internal anatomy of tribulus terrestris and Pedaliun mmbex show more or less similar structure. In these starch grains are less, external cork tissue layers are smaller in number. Vascular bundles are arranged regularly around the pith which forms a central portion of the herbs. Rosette type of crystals are seen in the cells of leaf. They are yellowish and crystalline in form. (Fig.-W-)

The dry powders of herbs emanate a peculiar characteristic odour. When they are boiled with water, the watery extracts of tribulus and pedalium especially in the beginning yield aromatic musk like odour which disappears at a later stage. This is supported by the early workers like Ghoshal et al (1910), who have said that the odoriferous constituent is volatile in nature. It is also confirmed by the later workers like Agarwal and Dutt (1934), Nadakarni (1954) and Chopra (1958) who obtained this volatile aromatic constituent from the alcoholic extract.

The herbs Boerhaavia repens and rependa are bitter in taste. The aqueous as well as the alcoholic extracts are also bitter in taste. The active principle is isolated
in a crystalline form.

Preliminary chemical tests were conducted with the aqueous infusions and decoctions of the herbs mentioned previously. Later on Soxhlet extractions were done with the solvents like water, alcohol, chloroform and solvent ether (B.P.C. 1935). In addition the glycoside-like compound was extracted from the aqueous extract by lead method (Rosenthaler - 1925). The active principle was isolated from the herb Boerhaavia and identified according to the method of Agarwal and Dutt (1935), who named the alkaloid as "Punarnavine". The yield of this substance was very small (0.001%) and it was sufficient only for a limited number of experiments. The active principle was tested with Mayer's reagent and Dragendorff's reagent. There was change of colour and precipitate formation. This is supported by the paper chromatographic studies which showed the presence of an alkaloid. This was indicated by spraying Dragendorff's reagent on the paper and the area containing this substance changes colour to pink or purple. The ash was prepared by ignition of the crude herbs. The water soluble portion of the ash was used in the experiments. (Table - T-1).

The pH of all the aqueous as well as alcoholic extracts was ranging from 6 to 7 (as tested by B.D.H. pH paper).
Previous workers like Chopra (1958), Karandikar (1960), Guptha (1962), used mostly the aqueous extracts of Boerhaavia for their experimental work. In this study all the possible extracts were prepared and experiments were conducted using them.

No active principle could be isolated from herbs - Tribulus terrestris and Pedalium murex.

The effects on the cardiovascular system have been studied by performing perfusion experiments on the heart and blood vessels of frogs, by recording the effect of extracts on blood pressure on anaesthetised dogs and by observing the effects on the heart taking electrocardiogram on dogs.

Hearts of frogs were perfused according to the technique of Howell and Cooke (1893). The various effects of extracts were observed as follows:

- Latent period, which was the period between the time when the drug reached the heart and the time when the effects were visible, varied with the extracts, the quantity injected and the dilution. It was slightly greater in case of Boerhaavia than the other herbs.

- The increase in the amplitude of contraction varied with the quantity of the extract injected to which it was
directly proportional. Then the aqueous extracts were
injected in quantities like 0.5 ml., 1.0 ml., 1.5 ml., and
2 ml., of 1 in 100 dilution, it resulted in the increase in
the height of contraction to about 1½ to 1½ times and 2
times the normal. There was no diminution in the height
of contraction even after the addition of larger quantities
like 5 to 10 ml., or more of extracts, indicating the absence
of tachyphylaxis and damage to the heart. The extent of
increase in the amplitude of contraction was more in the
case of the aqueous extract of Tribulus than with those of
Boerhaavia and Pedalium. Further the effect of increase in
amplitude of contraction of frog's heart by the aqueous
extracts of Tribulus and Pedalium was not antagonised by
alcohol (0.2%), acetylcholine (10.0 mcg.), or quinine
hydrochloride (0.002 mg.%), (Fig.-4 ). Alcoholic
extract and the active principle of Boerhaavia showed
increase in the height of contraction whereas the alcoholic
extracts of Tribulus and Pedalium did not show significant
effect on amplitude of contraction. The glycoside-like
compound obtained from all the herbs showed increase in
the amplitude of contraction, which was of the same extent
as that of the aqueous extracts of the herbs. But the
glycoside-like compound from Boerhaavia showed slightly
greater stimulation than those of Tribulus and Pedalium.
The water soluble portion of the ash of all the herbs did
not show effect on the height of contraction of the heart. Atropinisation or pilocarpinisation of the heart did not alter the increase in the amplitude of contraction of the heart after perfusing it with various extracts.

Regularity in the rhythm of the heart was not altered by the extracts of the herbs except in toxic doses.

Rate of the heart was not much affected by any of the extracts in doses ranging from 0.5 ml. to 5 ml., except the active principle of Boerhaavia which showed slowing in the rate of contractions of the heart of frog when directly added into the perfusing cannula in doses ranging from 25 to 100 mcg. This was confirmed by the studies on electrocardiogram of dogs, in which the rate was reduced from 166/mt. to 125/mt. (Fig. 14-15).

The tone of the cardiac muscle was not much affected by the aqueous extracts of all the herbs in doses ranging from 0.5 ml. to 2.0 ml. In the doses of 3 ml. the tone increased for a short period of time (36 seconds) in case of Tribulus and Pedalium, but not in the case of Boerhaavia. Alcoholic extracts of all the herbs showed no significant alteration in the tone of the heart muscle. The glycoside-like compound, on the other hand, showed shifting of the base line upwards. This increase of tone was of short duration, when the glycoside-like compound of Boerhaavia was
perfused (in 4 mg.%), whereas it persisted as long as the glycoside-like compound obtained from Tribulus and Pedalium was passing through the heart in the same concentration. Later the tone became normal in every case after the normal Ringer was perfused. (Fig. - 13).

Duration of action was more and more with increased quantities of the extracts, that is, the duration of action was directly proportional to the amount of extract which was added, other factors remaining the same.

The outstanding points observed during the experiments were as follows:-

1. An increased amplitude of contraction of the heart was observed and it persisted for some time even after washing the extracts with normal frog Ringer solution, indicating the prolonged action of the extracts.

2. No change in the rhythm of the heart.

3. No change in the rate of the heart except by the active principle of Boerhavia which showed slowing.

4. Atropinisation or pilocarpinisation did not affect the stimulant effects of the extracts on the heart, indicating the probable direct muscular action.
5. Return of the heart movements to normal after the action of the extracts was over, indicating occurrence of no permanent damage to the heart.

6. In higher doses ranging from 11 to 12 ml., which acted like a toxic dose, the height of contraction decreased gradually but later on came to normal after washing the extract with normal frog Ringer, indicating that no permanent damage to the heart was caused and the extracts were not very toxic agents.

7. Though the results vary somewhat with the nature of the extract, the intensity of the stimulation produced appeared to be directly proportional to the dose and the concentration of the extract.

The stimulant action of the calcium on the frog's heart is well known and has been the subject of recent studies (Moulin et al 1955 and Niedergerke & Luttgau 1957). Several drugs have in common the properties of being fixed by the heart tissue and forming a calcium-drug complex on the heart surface (Loewi 1955). The stimulant action seems to be dependent in some way on the presence of calcium in the perfusion fluid. This was demonstrated by perfusing the hearts by continuous perfusion, with Ringer solution from which calcium was lessened in amount or omitted, and by adding the various extracts of herbs to the low-calcium or calcium-
free Ringer solution. The heart which was depressed with low calcium or calcium-free Ringer was stimulated on the addition of the extracts, showing thereby that the extracts possessed calcium-like effect on the heart. This stimulant effect was more in the aqueous extracts of Tribulus and Pedalium than with Boerhaavia; whereas the extent of stimulant effects were equal in case of glycoside-like compound of all herbs. The alcoholic extracts of Boerhaavia showed similar stimulant effect as that of the glycoside-like compound of Boerhaavia, whereas those of Tribulus and Pedalium did not show any stimulant effect on the depressed heart by the calcium-free Ringer. (Fig. -4-6).

From these experiments it was concluded that the extracts antagonised potassium effect in the same way as that of calcium. This is in accordance with the observations made by Burridge (1916) and Weizsacker (1917) that the cardiac glycosides act by sensitising the heart to calcium and these extracts behave in a similar manner and resemble digitalis in action.

Electrocardiogram in six dogs under intramuscular paraldehyde anaesthesia were studied before and after the injection of various extracts. Changes that appeared and the inference drawn are as follows:-
The mean value of the heart rate was 168/mt., and the variations might be at least partly due to the varying degree of excitement experienced by each dog. (Goldberger 1954). (Table -11).

Injection of most of the extracts of herbs showed no marked alterations either in the amplitude or width of the waves except in a few extracts in which there appeared mild changes in the width and amplitude of P, QRS, and T waves.

Normally the width of the P wave indicates the time taken for the wave of stimulus to spread through the auricles. The increased time duration after the injection of the active principle of Boerhaavia, Glycoside-like compound of Boerhaavia and aqueous extract of Pedalium indicates delay in conduction of impulses through the auricles. Similarly the width of QRS complex denotes normally, the time taken for the stimulus to pass through the ventricles. Only the active principle of Boerhaavia shows increase in the time duration of QRS complex, indicating the delay for the impulse to spread through the ventricles. It can be concluded that the delay in conduction both in auricles and in ventricles by the active principle of Boerhaavia indicates slowing of the heart rate or bradycardia which corresponds to the decreased rate observed when the isolated frog's heart was perfused by the same drug. (Fig. 7).
The mild peaking observed in T wave after the aqueous extract of Pedalium and potassium nitrate may be interpreted as due to hyperpotassasemia, because both correspond with each other. (Fig. 14-15).

The chief points to be noted are as follows:

1. Delay in the spread of impulse in auricles following the injection of active principle of Boerhaavia, the glycoside-like compound of Boerhaavia and the aqueous extract of Pedalium - as shown in the increase in the width of P wave as compared to the normal.

2. The increased time taken by QRS complex after the injection of the active principle of Boerhaavia, indicating the delay in conduction through ventricles.

3. Decrease in the rate of the heart in case of active principle of Boerhaavia as compared to the normal.

4. Mild peaking of T wave after the injections of potassium nitrate and aqueous extract of Pedalium, indicating the presence of more amount of potassium in the extract because T wave in comparable with the reference drug potassium nitrate.

The effect of the extracts on blood vessels was determined in frogs by perfusion through the abdominal aorta.
and recording the rate of flow of perfusion fluid by counting the number of drops falling out per minute. It was observed that the extracts of Boerhaavia produced slight vaso-constriction as was evident by the increased in resistance to the flow of perfusion fluid and subsequent diminution in the rate of flow of drops. This rise of pressure in perfusion fluid which would correspond to a rise in blood pressure in the dog, was apparent though it differs with each of the two herbs - Boerhaavia repens and Boerhaavia repanda - in intensity and duration. The pressor activity was observed in both varieties of Boerhaavia. (Table - A-9)

In the case of Tribulus and Pedalium no significant effect was observed as it was evident from the results which showed very mild vasodilator effect as compared with the reference drugs - potassium nitrate (1%) solution of which exhibited greater vasodilator effect.

The behaviour of the blood vessels in intact mammals was studied by recording the blood pressure from carotid artery in paraldehyde anaesthetised dogs. It was seen that the extracts of Boerhaavia produced a prompt but transient rise in blood pressure which came back to normal soon. This rise of pressure remained sustained for some more time when higher doses were injected. (Fig. 6-11).
In anaesthetised dogs the increase of blood pressure with the experimental extracts could be either due to the neuromuscular or a vasoconstrictor effect on the arterioles, or increased blood viscosity or blood volume or increased cardiac output.

The neuromuscular effect may not be the cause for the pressor activity since administration of Ergotoxine before the experimental extract did not abolish the hypertensive effect. But the administration of adrenaline along with the extracts showed additive effect, indicating thereby that the experimental extracts possess direct muscular effect on the blood vessels. Carotid occlusion did not modify or enhance the pressor activity of the extracts. The exact role which was played in the rise of blood pressure is difficult to assess. Probably the hypertensive response may be due to the stimulant effect on the heart as well as a mild vasoconstriction. (Fig. 16-2)

The effect of the extracts of Tribulus terrestris and Pedaliium murex were studied on anaesthetised dogs. It was shown that there was a transient fall of blood pressure in higher doses only. The extent of fall was greater in Tribulus than in Pedaliium. Duration of time to reahh normality was also greater in case of Tribulus than Pedaliium. (Fig. 16-13). This fall of blood pressure may be due to the vagal stimulation or vasodilatation by direct action. Vagal
stimulation does not seem to be the cause because atropinisation of the dog before the administration of the extracts do not modify the hypotensive response of Tribulus or Pedalium. So the second effect, that is, the mild direct action might be a probable cause for the slight fall of blood pressure.

In conclusion the experiments on blood pressure in dogs and perfused blood vessels of frogs show identical results, namely vasoconstriction in Boerhaavia and slight vasodilatation with Tribulus and Pedalium. It is difficult to assess the exact role played by these extracts on the vascular system. It is, however, probable the site of action may be directly on the blood vessels as well as on the heart.

The effects on respiration were observed on anaesthetised dogs. The observations made in dogs indicate that the respiration were not affected by the extracts. But the glycoside-like compound showed stimulation of both rate and depth of respiration coinciding with the fall of blood pressure. Atropinisation of the dog did not influence the respirations. The effect of the stimulation of respiration may be compensatory to the fall of blood pressure.

Diuretic activity of the four herbs was tested on saline loaded albino rats (Lipschitz et al - 1943), as well as on female dogs (Gaddum - 1961).
In the experiments on rats only Boerhaavia repens and repanda gave favourable results. Larger doses of these drugs were also tried. It was observed that no animal died even with large doses of the extracts, indicating thereby that the extracts were not toxic. Urea, aminophylline, acetazolamide and aprinox were taken as reference drugs for comparison during the present investigation. Potassium chloride and potassium nitrate were used as reference drugs also on the basis of observations of previous investigators (Chopra - 1958, and Karandikar - 1960), that the diuretic activity of the herbs was chiefly due to the content of potassium salts in the extracts.

The diuretic activity of all the drugs was tested for a period of five hours, as well as for a period of twenty-four hours to know whether the extracts were showing more diuretic effect after the duration of five hours.

Diuretic activity of the extracts was determined with preference to urea and is expressed in terms of after Van Arman (1954).

The chloride content of the extracts, as well as that of the samples of urine were estimated according to the method of Volhard (Hawk - 1954). The sodium content and the phosphate content of the samples of urine were estimated as per the methods of King (1956).
Bhide et al (1958) attributed the diuretic activity of Boerhaavia and Tribulus to their high potassium content. Gujral et al (1955) reported on the diuretic activity of the decoctions of both Boerhaavia and Tribulus using the method of Lipschitz et al (1943). However, they gave saline to their control group while urea was dissolved in distilled water and the decoctions of the herbs were prepared in distilled water. It is emphasized by Lipschitz et al in their original paper that saline and not water should be used as the hydrating substance for assaying the diuretic activity of drugs in rats. This has the advantage of keeping basal urinary excretion at a minimum. This was the reason why Jaretzky and Neuwald (1938) and Vollmer (1939), who used the method of Burn for the diuretic assay of drugs got controversial results. By the method of Burn the normal urinary excretion in rats is high and thus the method is meant essentially for the study of anti-diuretic substances.

Aqueous extracts of Boerhaavia, Tribulus and Pedalium possess diuretic effect. The diuretic activity of the extracts - both aqueous and alcoholic and active principle of Boerhaavia was slightly less than urea and other reference drugs like acetazolamide and aprinox but more than that of potassium salts like potassium chloride and potassium nitrate, used in experiments conducted on albino rats, but it was almost equal to urea in experiments on dogs. (Table -).
The diuretic effect of aqueous and alcoholic extracts of *Tribulus* and *Pedalium* was less than the reference drugs but comparable to potassium salts in experiments on rats. The extracts of *Tribulus* and *Pedalium* were not tried on dogs as they did not show any diuretic activity equivalent to standard drugs. The glycoside-like compound obtained from *Boerhaavia* of both varieties showed diuretic property intermediate between the aqueous and alcoholic extract. In the dose of 8 Gm./Kgm. the percentage of urine volume excreted was only 58.6% more or less equal to that of control group. The glycoside-like compound obtained from *Tribulus* and *Pedalium* showed diuretic effect equivalent to potassium salts. The percentage increased in volume of urine with extract of *Boerhaavia rependa* was 70% whereas with *Boerhaavia repens*, the percentage of increase in the volume of urine was 62.5%. Thus *Boerhaavia repens* showed comparatively lesser diuretic property than *Boerhaavia rependa*. (Table — )

The diuretic effect which was observed in anaesthetised dogs using the different extracts of *Boerhaavia* showed results which corresponded with those observed in case of experiments in rats. Even in dogs the active principle in the dose of 1 mgm./Kgm. produced a mean volume of urine — 32 ml., which was more or less equivalent to that produced by urea — 34 ml. (Table — ).
Aminophylline had a prompt and powerful but short lived action, whereas aprinax had a slower but more prolonged diuretic action. Acetazolamide produced only a moderate diuresis.

Aminophylline caused a pronounced and equal increase in the excretion of sodium and chloride. Aprinax produced a slightly less marked increase in chloride. Urea produced excretion of sodium and chloride equivalent to aprinax. Alcoholic extract and active principle of Boerhaavia showed greater increase in the excretion of both sodium as well as chloride in the urine samples collected for a period of 24 hours. In addition to the increased excretion of sodium and chloride, phosphate also increased in amount by the alcoholic extract and active principle of Boerhaavia whereas extracts of tribulus and pedalium resemble the control saline loaded groups in the excretion of sodium, chloride and phosphate. The mean chloride and sodium excretion in the samples of urine collected from the dogs for a period of five hours after the administration of the extracts of Boerhaavia also showed similar results and comparable to the experiments conducted on rats. (Table -XIV).

It was difficult to estimate the relative potencies of the four diuretics on the basis of rats experiments only. By attaching more importance to the sodium and chloride excretion and less to the volume it may be argued out that
aminophylline and aprinox are equally potent diuretics whereas urea is to a lesser extent. Boerhaavia is weaker to aprinox and aminophylline in producing diuresis but is equivalent to urea. It is a better diuretic than tribulus and pedalium.

The diuretic activity may be interpreted as follows: The extracts of Boerhaavia may act directly on the kidney of the action may be on the cardiovascular system or the action may be a combined one, to produce the diuretic effect. The reason for the greater diuretic activity of Boerhaavia than Tribulus and Pedalium can probably be explained as due to the combined effect on both cardiovascular and Renal systems - namely increase in the force of contraction of the heart as well as rise of blood pressure which are not observed with other extracts. Further a few experiments carried out on the active principle of Boerhaavia show greater effect on the cardiovascular system as well as on the Renal system, as compared to the other extracts. In addition the alcoholic extracts of Boerhaavia in which the active principle may be present show comparable results with that of active principle itself. The extracts of Boerhaavia also show effect on the Renal system as is evidenced by the greater increase in the volume of urine excreted by the extracts and phenol red treated rats than by the phenol red itself treated or by the control group.
These facts may lead to the conclusion that the diuretic activity of Boerhaavia at least in part is due to an active principle present in it and not entirely due to the potassium salts as observed by the previous investigators. (Fig. -1). Experiments on the action of the herbs on smooth muscles were conducted on the isolated loop of intestine obtained from freshly killed rabbits and guinea pigs, and on the intact intestines in dogs, and isolated uterus got from freshly killed albino rats and guinea pigs.

The aqueous extracts of Boerhaavia produced a diminution in the amplitude of contractions both in the isolated and intact intestines (Fig.2: 34), the extent depending upon the dose and concentration of the extracts.

The alcoholic extracts and active principle of Boerhaavia, on the other hand, showed transient stimulation of contractions in the isolated loops of intestine, but no effect was observed on the intact intestines in the doses administered in this experiment. (Curve -34).

The other extracts of Tribulus and Pedalium showed no marked effects on the intestines.

On the isolated uterus also the aqueous extract of Boerhaavia showed temporary inhibitory effect.
Inhibitory effect of the aqueous extract of Boerhaavia on the intestines and uterus may be a direct depressant effect on the smooth muscles and similarly the stimulant action produced by the alcoholic extract and active principle of Boerhaavia may be a direct stimulant action. Difference in the two cases may probably be due to the fact that in the case of aqueous extract the action may be due to several substances like potassium salts, whereas in the case of alcoholic extract, the active principle which is alkaloidal in nature may be acting. This is confirmed by the similar stimulation shown by the isolated active principle itself. (EI) - 30.31)

Experiments carried out by injecting albino rats with extracts and comparing them on normal rats showed no particular change in the general behaviour of the drugged animals as compared to the control ones. The several extracts showed no definite alteration in the size of the pupil of the rabbits.

Acute, subacute and chronic toxicity studies were conducted on albino rats. The various extracts obtained from Boerhaavia, Tribulus and Pedalium were tried in varying doses according the weights of the animals. As far as possible a uniformity in the administration of extracts was maintained. The extracts were administered orally. The purpose of this study was to observe whether any of these
extracts have any toxic effects on the Renal cells structure as evidenced by histopathological alteration.

In acute toxicity study although quite a heavy dose 500 mg./Kgm. was administered, the histopathological changes observed were very slight, and they were mild congestion and cloudy swelling of the cells. (Table \ref{tab:acute-toxicity})

The subacute toxicity and chronic toxicity studies were done for 15 days and 60 days respectively, utilising the same pattern of the dosage administration. The extracts were administered twice a day with an interval of six hours. Even these prolonged periods of treatment of albino rats with these extracts did not show any severe histopathological changes except mild congestion, cloudy swelling and cell proliferation, which were the only changes observed in histological sections of the kidney. (Fig.\ref{fig:subacute-toxicity} and Table \ref{tab:subacute-toxicity}).

From these observations it may be inferred that irrespective of the dosage of the extracts or the duration of administration of these extracts, the renal histological alterations were minimal and these point to the fact that no permanent damage or impairment of function was produced in the renal tissue. It might be concluded that the extracts are not toxic and do not produce any permanent damage of the renal tissue.