PART XIV
PHARMACOLOGICAL INVESTIGATIONS OF
i WATER INSOLUBLE ALKALOID
ii 1-STACHYDRINE, ITS AMIDES AND ESTERS
PHARMACOLOGICAL INVESTIGATIONS OF THE ALKALOID ISOLATED FROM ROOT BARK

Root bark in powder form or its infusion is being used in Ayurvedic system of medicines for rheumatism, gout, cough, dropsy and palsy etc. It is also used as analgesic, diaphoretic, alexeteric and anthelmintic. During the chemical investigations, an alkaloid has been isolated from root and stem bark which was found to be absent in flowers and fruit. This alkaloid has anthelmintic activity when tested against earthworms. Thus it seemed interesting to carry out pharmacological investigations of the alkaloid.

Pharmacological investigations have been carried out on frog's heart, rabbit's intestine, dog's blood pressure and respiration, and guinea pig's uterus.

Action of alkaloid hydrochloride on rabbit's intestine.

The method used for studying the effect of alkaloid hydrochloride on rabbit's intestine was same as of Magnus. Experimental. Rabbit weighing 1.5 kg. was fasted for 24 hours before starting the experiment. The animal was killed by stunning. The abdomen was opened and small pieces of about 3 cm. were dissected out of the proximal portion of the small intestine and were transferred immediately to a container containing Tyrode solution. The strips were then suspended in an organ bath containing 50 ml. oxygenated Tyrode solution maintained at 37°C. Oxygen was bubbled through the solution at a rate of about 40 bubbles per minute. A period of 10 minutes was allowed for the movements to become regular and uniform. The normal tracings of the intestine were first obtained. The effect of alkaloid
Plate showing the action of alk. HCl on isolated pieces of rabbit's intestine.
Upper tracings: action of alk. HCl (2 ml, 1% sol.)
Lower tracings: action of alk. HCl (1 ml, 1% sol.)

Plate showing the action of BaCl₂ and acetylcholine in presence of alk. HCl on isolated pieces of rabbit's intestine.
Upper tracings: action of BaCl₂ (1 ml, 10% sol.) in presence of alk. HCl (1 ml, 1% sol.)
Lower tracings: action of acetylcholine (0.1 ml and 0.2 ml, 0.01% sol.) in presence of alk. HCl (1 ml, 1% sol.)
hydrochloride was studied in the following order:-

1. Effect of alkaloid hydrochloride in presence of acetyl-choline.

1 ml. aqueous solution of alkaloid hydrochloride (1% w/v) was added to organ bath, and then followed by acetylcholine solution (0.01 % w/v ) 1 ml. and then 2 ml.

2. Effect of alkaloid hydrochloride in presence of barium-chloride.

1.5 ml. solution of alkaloid hydrochloride (1% w/v) was added to organ bath and followed by 2 ml. barium chloride solution (10% w/v).

3. Effect of alkaloid hydrochloride only.

2.0 ml. solution of alkaloid hydrochloride (1% w/v) was added to organ bath. (Every time the previous Tyrode solution was removed, the intestinal strip was washed and fresh oxygenated Tyrode solution added to mark of 50 ml.)

Results:

When 1 ml. of alkaloid hydrochloride solution was added, there was slight inhibition in the intestinal movements which was not effected by adding 1 ml. of acetylcholine solution, but however, the movements became normal when 2 ml. of acetylcholine solution was added. Addition of 1.5 ml. solution of alkaloid hydrochloride showed more inhibition of intestinal movements, and when 2 ml. of barium-chloride solution was added, there was stimulation of intestinal movements. Addition of solution of 2 ml. alkaloid hydrochloride completely arrested the movements of the intestinal strip.
Plate showing the action of oxytocin and alk. HCl in presence of atropine sulphate (1 ml, 0.1% sol.) on isolated piece of guinea pig's uterus.
Action of alkaloid hydrochloride on guinea pig's uterus.

The method used for studying the action of alkaloid hydrochloride was same as for rabbit's intestine as described above.

Guinea pig was injected 1 ml. of solution of stilboestrol dipropionate (containing 1 mg.) in three divided doses, once daily for three continuous days before starting the experiment. On fourth day the animal was killed by stunning. The abdomen was opened and piece of uterus was taken out. The strip of uterus was suspended in an organ bath containing 50 ml. oxygenated Dale solution maintained at 37±1°C. Rest of the procedure was same as described earlier. The normal tracings were first obtained. The effect of alkaloid hydrochloride was studied in the following order:

1. **Action of alkaloid hydrochloride only.**
   
   1 ml. (1% w/v) aqueous solution of alkaloid hydrochloride was added to organ bath.

2. **Action of alkaloid hydrochloride in presence of atropine**
   
   1 ml. (0.1% w/v) atropine sulphate solution was added to organ bath and then followed by 1 ml. (1%) aqueous solution of alkaloid hydrochloride.

   Every time the previous Dale solution was removed, the uterus strip was washed and fresh oxygenated Dale solution added to mark of 50 ml.

**Results:**

When 1 ml. solution of alkaloid hydrochloride was added, there was stimulation of uterus, but the addition of alkaloid hydrochloride in presence of atropine had no
Plate showing the action of alkaloid hydrochloride on frog's heart.
stimulating effect on uterus. Posterior pituitary extract stimulated uterus in presence of atropine.

**Action of alkaloid hydrochloride on Frog's heart.**

The method used for finding the effect of alkaloid hydrochloride on frog's heart was same as of Bulbring.  

**Experimental**

Frog was pithed and the heart was exposed by removing the skin and the sternum. The connected tissues were removed from the vessels as much as possible. A cut was made in the inferior vena cavae and cannula was inserted in it, the other end of the cannula was attached to the bottle containing frog's Ringer solution through rubber tubing. A nick was made in the right aorta. The height of the bottle containing the frog's Ringer solution was so adjusted as to maintain slow and uniform rate of perfusion throughout the experiment. A pin hook was prepared and passed through the apex of the heart, and the other end of the pin hook was attached to the heart lever with the help of thread. The normal heart tracings were recorded, and the drug was injected in the following order:-

First 0.5 ml. aqueous solution of alkaloid hydrochloride (1% w/v) was injected through the rubber tubing near the cannula, and then followed by 0.75 ml. solution of alkaloid hydrochloride. Each dose was injected after the effect of previous dose was over and the tracings obtained were normal.

**Results**

Injection of 0.5 ml. solution of
Plate showing the action of alkaloid hydrochloride on dog's blood pressure and respiration.

i). Upper tracings of respiration.

ii). Lower tracings of blood pressure.
alkaloid hydrochloride produced slight blocking manifestation of heart. There was little more temporarily inhibition of the movements of heart when 0.75 ml. of the solution was injected, but injection of 1 ml. alkaloid hydrochloride solution completely blocked the heart.

Action of alkaloid hydrochloride on dog's blood pressure and respiration.

Male dog weighing 13 kg. was selected for the experiment. The animal was anaesthetised with an intravenous injection of pentobarbitone in dose of 35 mg. per kg. The trachea was exposed and cannulated. The tracheal cannula was connected with a tambour. One of the carotid artery on one side of the trachea was exposed. A ligature was applied distally and a bull dog forceps was placed proximally on a carotid artery, a snip was given between the two, and carotid cannula inserted and tied in position. The cannula was previously connected to a mercury manometer through a rubber tubing having a by pass attached to pressure bottle containing 10% sodium citrate solution. The citrate solution was run into the cannula by releasing the screw to remove the air. When all the air had passed out, the screw was closed and the bull dog forceps removed from the carotid artery, and the recording lever of the manometer was adjusted. The right femoral vein was exposed for intravenous injection of the drug solutions. The kymographic recordings of the respiration and blood pressure were taken on a smoked drum. After recording normal movements, the doses of 0.1 ml. and 1 ml. of the solution of alkaloid hydrochloride were injected into the exposed femoral vein.
Results

The dose of 0.1 ml. showed no effect on blood pressure and respiration, but when 1 ml. was injected there was a fall of blood pressure, but no effect on respiration.

DISCUSSION

From the experimental data, it may be inferred that the alkaloid hydrochloride is depressant to the smooth muscles of rabbit's intestine and can block the effect of small doses of acetylcholine but cannot block the effect of larger doses of acetylcholine. The effect of barium chloride is however not blocked by alkaloid hydrochloride. The alkaloid has stimulating effect on smooth muscles of guinea pig's uterus, but the effect seems to be non-specific one, as it is blocked by atropine. The alkaloid hydrochloride depresses both the rate and the force of the heart and thus produces the blocking manifestation of heart. Alkaloid hydrochloride lowers blood pressure in larger doses, but the dose which lowers blood pressure does not effect the respiration.
Plate showing the action of 1-stachydrine, its amides and esters on isolated pieces of rabbit's intestine.
PHARMACOLOGICAL INVESTIGATIONS OF STACHYDRINE, ITS AMIDES AND ESTERS ON RABBIT'S INTESTINE AND FROG'S HEART

1. Action on rabbit's intestine

The method followed was same as of Magnus, as described earlier.

It is evident from the tracings, neither 1-stachydrine nor its amides of methylamine, ethylamine and ethylene diamine have any effect on rabbit's intestine in doses of 1 and 2 ml. (1% solution). Similarly it was found that methyl, ethyl, propyl and butyl esters of 1-stachydrine had no effect in doses of 1 and 2 ml. (1% solution) on intestinal movements.

2. Action on frog's heart

The method followed was same as of Bulbring, as described earlier.

The tracings indicate that amide of 1-stachydrine with ethylene diamine (1,2-bis(N-methylamido)ethane dimethochloride) has slight depressant effect on the force of palpitation of heart in doses of 1 and 2 ml. (1% solution), but has no effect on heart rate. Methyl and ethylamide of 1-stachydrine has no effect in doses of 1 and 2 ml. (1% solution) on heart. Similarly it was found that 1-stachydrine and its methyl, ethyl, propyl and butyl esters had no effect on heart.

Thus the esters and amides of 1-stachydrine unlike esters and amides of betaine are pharmacologically inactive.
SOLUTIONS USED IN PHARMACOLOGICAL INVESTIGATIONS

1. Tyrode Solution

- Sodium chloride 8.00 gm.
- Potassium chloride 0.20 gm.
- Calcium chloride 0.20 gm.
- Sodium bicarbonate 1.00 gm.
- Magnesium chloride 0.10 gm.
- Glucose 1.00 gm.
- Sodium dihydrogen phosphate 0.05 gm.

Distilled water in sufficient quantity to make 1000 ml.

2. Frog's Ringer Solution

- Sodium chloride 6.50 gm.
- Potassium chloride 0.14 gm.
- Calcium chloride 0.12 gm.
- Sodium bicarbonate 0.20 gm.
- Sodium dihydrogen phosphate 0.01 gm.

Distilled water in sufficient quantity to make 1000 ml.

3. Dale's Solution for Uterus

- Sodium chloride 9.00 gm.
- Potassium chloride 0.42 gm.
- Calcium chloride 0.24 gm.
- Magnesium chloride 0.005 gm.
- Sodium bicarbonate 0.50 gm.
- Glucose 0.50 gm.

Distilled water in sufficient quantity to make 1000 ml.
REFERENCES

