STUDIES ON INDIGENOUS DRUGS USED IN UTERINE DISORDERS

PHARMACOLOGICAL ACTIONS OF THE EXTRACT OF THE FRUITS OF PIPER AURANTIACUM AND AN ATTEMPT FOR THE IDENTIFICATION OF ITS OXYTOCIC PRINCIPLES—I.

By

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Piper aurantiacum—wall. of family Piperaceae (Sanskrit : “Renuka”; Hindi : “Shambhaha-buji”; Bengali : “Renuk”) is a rather stout climber of a yellowish colour. It is a bitter, acid, refrigerant and is said to be beneficial in wind, thirst and poison. The fruits of the plant are extensively used by midwives as uterine stimulant. Arora, Dandiya and Sharma (1) carried out the preliminary investigation of the fruits of Piper aurantiacum and have reported that out of the different solvents employed for the extraction of the drug, alcohol extract showed maximum uterine activity. The extract was reported to have produced a marked contraction of the isolated uterus of the rat and lowered the blood pressure of the dog. In the present communication an attempt has been made to identify the oxytocic principle from these fruits and to study the smooth, skeletal and cardiac muscle pharmacology of the purified alcoholic extract of Piper aurantiacum.

CHEMICAL INVESTIGATION

Preparation of the Extract

Solvent extraction of the 250 gm. of the coarsely powdered fruits was carried out by employing Soxhlet apparatus. The following solvents were used successively and their extractive material obtained is noted against each solvent:

(i) Petroleum ether 60°-80°C 5.3%
(ii) Absolute alcohol 12.4%

The defatted ethanolic extract obtained by continuous extraction was concentrated, to remove most of the alcohol and was further purified by the removal of tannin by using strong solution of lead subacetate, excess of which was precipitated by passing hydrogen sulphide gas. After filtration the filtrate was concentrated under reduced pressure to a semi-solid mass. The residue was shaken with ethanol which resulted in the exclusion of a yellow substance which was separated by filtration. Thus the following two fractions were obtained:

(i) Defatted, tannin free, alcohol soluble alcoholic extract.
(ii) Defatted, tannin free, alcohol insoluble alcoholic extract.
Ethanol was removed from the (i) defatted, tannin-free, alcohol-soluble alcoholic extract by evaporation at room temperature and the semi-solid mass was weighed and dissolved in hot alcohol and diluted whenever required with sufficient warm water so that a 1% solution of the semi-solid mass did not contain more than 10% alcohol. Henceforth this will be referred as 'extract' throughout this paper.

The (ii) defatted, tannin-free, alcohol-insoluble alcoholic extract was dissolved in 15% of glacial acetic acid (being insoluble in most of the organic solvent and water) and the pH was adjusted between 7 and 8 by dropwise addition of a dilute solution of sodium hydroxide. The volume was finally made up with warm water to give 1% solution. This will now be referred as the 'residue'.

Identification of the uterine stimulant

With an object to identify the oxytocic principle, the activity of the different fractions of the ethanolic extract of the *Piper aurantiacum* were determined on the isolated rat uterus using Dejalon's solution as described elsewhere in this paper.

Both the 'residue' and the 'extract' were tested on the uterine muscle. The 'residue' was unable to elicit any effect on the isolated rat uterus even in concentrations as high as 5 mg/ml. The 'extract' however caused in 1.25 mg/ml concentration, a contraction which was nearly equal to that of 0.75 µg/ml of acetylcholine. The following experiments were therefore performed to find out if this activity of the 'extract' was due to some inorganic ion or some organic compound:

(i) Estimation of Potassium, Calcium and Magnesium—Estimations of the inorganic ions were carried out by well-known chemical methods and they were found to be present in traces and hence of little biological significance.

(ii) Thin layer chromatography—In order to determine whether the extract contained organic components, Thin Layer Chromatography was run. Various solvent systems e.g. benzene, mixture of benzene and alcohol (1:1 v/v) and mixture of benzene and chloroform (1:1 v/v) were used. Several reagents e.g. concentrated sulphuric acid, 80% sulphuric acid, iodine vapours were employed for the development of the coloured spots on the plate. During this study at least five spots could be observed indicating the presence of organic compounds. Element detection of the 'extract' was carried out by usual methods and it was found to contain nitrogen only. The activity exhibited by the 'extract' could therefore be due to some organic compounds which could possibly be separated out by paper chromatographic technique.

Paper Chromatography—The 'extract' was also fractionated by paper chromatography. As a result of trials with different solvent systems, it was found that butanol-acetic acid was
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system brought about the best separations. After running the chromatography the paper strips were sprayed with different colouring reagents. Many reagents (5) showed the presence of different carbohydrates (Rf between 0.74 to 0.83). Identification of carbohydrates was not undertaken. Alkaloidal reagents (5) also produced coloured spot on the paper (Rf 0.48).

In order to determine as to which of these fractions was responsible for the oxytocic activity, 15 mg of the ‘extract’ was spread on a line as the starting point on a chromatography paper sheet (Whatman No. 1) and after running, the paper chromatography strips were cut out of the undeveloped portion along the following:

(a) Spot that gave positive test with the alkaloidal reagents (Rf 0.48).

(b) Spot that gave positive test for the carbohydrates (Rf 0.74 to 0.83).

(c) Strip cut in between the spots mentioned above (Rf 0.5 to 0.73).

These strips were eluted with alcohol. Each elute after evaporation was dissolved in 1 ml of 10% alcohol and tested on isolated rat uterus. It was found that only (a) produced contraction of the uterus. Thus the oxytocic principle of the fruits of *Piper aurantiacum* was found to be resting in the fraction which gave positive test for the presence of the alkaloids.

**METHODS**

The following experiments were performed with the ‘extract’, each set was repeated thrice and results shown are the average of three experiments:

**Smooth Muscle**

The effect of ‘extract’ was studied on the isolated uterus of albino rat and isolated ileum of rabbit and rat by usual methods (8), (7) The horn of the uterus was suspended in an organ bath filled with oxygenated DeJalon’s solution or Ringer Dale between 29° to 32° C; the rabbit ileum was placed in an organ bath containing oxygenated Ringer solution at 37° C and the rat intestine was suspended in an organ bath filled with modified Kreb’s Ringer maintained at 29° C with constant slow stream of oxygen bubbling into it. The capacity of the bath used for these tissue experiments was 20 ml.

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*Composition* NaCl 9 g.; KCl 0.42 g.; CaCl₂ 0.06 g.; NaHCO₃ 0.5 g.; Glucose 0.5 g.; Distilled water to one litre.

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****Composition**, NaCl 6.8 g.; KCl 0.45 g.; CaCl₂ 0.278 g.; NaHCO₃ 2.1 g.; Glucose 1 g.; Acid Sodium Phosphate 0.143 g.; Distilled water to one liter.
The influence of different blocking agents like pentolinium 100 \( \mu g/ml \), pheniramine 1 \( \mu g/ml \), and atropine 10 \( \mu g/ml \) on the activity of the ‘extract’ upon various smooth muscle preparations were studied by first obtaining control responses of the drug. Then the blocking agent was permitted to act on the tissue for 2 minutes, at the end of this period, control amount of the ‘extract’ was added to the bath and the response so obtained was recorded.

**Skeletal Muscle:**

Frog rectus muscle preparation was set up according to the method described by Bulbring (2). Both the activity of the ‘extract’ on the muscle and its influence upon the acetylcholine response on the preparation was studied. Frog rectus muscle was mounted in a 10 cm bath containing aerated frog Ringer’s solution at room temperature. The preparation was left as such for one and a half to two hours in order to completely relax it.

To examine the effect of the ‘extract’ upon the acetylcholine response, 2 to 3 \( \mu g \) of acetylcholine was added to the bath and left in contact with the tissue for exactly 90 seconds. The muscle piece was then thoroughly washed and left to relax to the previous baseline, with the drum was stationary. An interval of at least 5 minutes was allowed between successive addition of acetylcholine. Two similar consecutive responses of acetylcholine served as control. The ‘extract’ was allowed to act for a period of two minutes before adding the control amount of acetylcholine and response recorded for a period of 90 seconds.

**Cardiac Muscle:**

Frog heart perfusion was carried out by Bulbring’s method as described by Burn (2). For the Straub heart preparation, the excised frog heart was prepared according to the Straub method as quoted by Gaddum (4). Perfusion of the isolated rabbit heart was performed by the modified Langendorff’s method as described by Burn (2). To elucidate parasympathetic mimetic like activity of the ‘extract’ all three experiments were repeated with 10 \( \mu g/ml \) of atropine in the perfusion fluid.

**RESULTS**

**Smooth Muscle:**

(i) Isolated rat uterus—0.1 mg/ml and 0.2 mg/ml of the ‘extract’ produced a sustained increase in the uterine tone and decrease in amplitude of contraction. (Fig. 1). A dose of 1.2 mg/ml was found equivalent to 0.35 \( \mu g/ml \) of acetylcholine on this preparation. Repeated dosage produced a gradual diminution in response and thus demonstrated the phenomenon of tachyphylaxis. Generally up to the third dose the response was not less than 90% of the initial dose but fifth or the sixth dose produced less than 60% of the contraction exhibited by the first dose. An attempt was made to find out the drug which blocked the action of the ‘extract’ on the isolated rat uterus. Both pentolinium and pheniramine failed to inhibit the response produced by the extract on the uterus. However, in presence of 5 to 10 \( \mu g/ml \) of atropine, th
The 'extract' did not elicit any response i.e. a 100% inhibition was observed. Since the 'extract' exhibited tachyphylaxis care was taken to use only one control response of the drug prior to the atropinization of the tissue which was subsequently followed with a higher dose of the 'extract' i.e. double the initial dose.

(ii) Isolated rabbit intestine—The 'extract' exhibited increased tone and diminished amplitude of contraction of the rabbit intestine. With successive doses of the 'extract' there was a steady decrease in the magnitude of its response indicating tachyphylaxis. It was observed that pentolinium and pheniramine were unable to block the response of the 'extract'. Atropine, however, completely prevented the contractile response of the 'extract' at 5 to 10 μg/ml concentrations.

(iii) Rat intestine—It exhibited a spasmogenic property on the rat intestine. 0.15 mg/ml of the 'extract' showed activity which was equivalent to 1 μg/ml of acetylcholine on this preparation.

Skeletal Muscles:

The 'extract' failed to contract the frog rectus muscle even when 0.5 mg/ml dose was employed. The acetylcholine response was not significantly altered.

Cardiac Muscle:

(i) Frog heart perfusion—The 'extract' in doses of 0.25 mg diminished the force of contraction of the ventricle to 50% and reduced the heart rate to a smaller extent. The 'extract' exhibited parasympathomimetic like activity on smooth muscles. The usual response of the 'extract' was inhibited in this preparation also when 10 μg/ml of atropine was added to the perfusion fluid.

(ii) Straub heart preparation—The 'extract' in 1:1000 concentration produced about 80% inhibition of the amplitude of contraction without markedly changing the rate. In presence of atropine 10 μg/ml, the 'extract' elicited a diminished response indicating that atropine blocked its action (Table I).

### Table I

<table>
<thead>
<tr>
<th>Dose</th>
<th>% decrease in amplitude</th>
<th>% decrease in heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piper extract 1:50,000</td>
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<td>0</td>
</tr>
<tr>
<td>Piper extract 1:10,000</td>
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<td>0</td>
</tr>
<tr>
<td>Piper extract 1:5000</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Piper extract 1:1000</td>
<td>79</td>
<td>13</td>
</tr>
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</table>
(iii) Isolated rabbit heart—The 'extract' exhibited a negative chronotropic and negative inotropic effect. The coronary flow was also decreased. When atropine was added to the perfusion fluid at a concentration of 1 to 10 \( \mu g/ml \) it partially counteracted the effects produced by the drug (Table II).

**TABLE II**

Summarises the effects of extract on the isolated rabbit heart
(Number of experiments performed were three in each case)

<table>
<thead>
<tr>
<th>Piper extract</th>
<th>Dose in mg</th>
<th>% decrease in amplitude</th>
<th>% decrease in coronary flow</th>
<th>% decrease in heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>19</td>
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<tr>
<td></td>
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<td>8</td>
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</tr>
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**DISCUSSION**

Preliminary pharmacological investigations were carried out of the tannin free alcoholic extract of the defatted fruits of *P. aurantiacum*. On smooth muscle preparations i.e. uterus and rabbit intestine it demonstrated an increase in tone and diminished the amplitude of contraction. On rat intestine where the rhythmic movements were prevented by the use of suitable physiological solution, the 'extract' produced a definite contraction. This action of the 'extract' on the smooth muscle could be blocked by atropine but not by pentolinium or pheniramine. These results are indicative of a parasympathomimetic like activity of the 'extract'.

In spite of the presence of parasympathomimetic like activity of the 'extract', it was found to be devoid of nicotinic effects as it failed to contract the frog rectus muscle even in concentration of 0.5 \( mg/ml \) of the bath fluid.

In frog heart perfusion, Straub heart preparation and isolated rabbit heart, the 'extract' acted like a parasympathomimetic drug by exhibiting a negative chronotropic and a negative inotropic effect. In the isolated rabbit heart, it decreased the coronary flow. The 'extract' activity was diminished in these preparations in the presence of atropine. This indicated a muscarinic activity of the 'extract' on cardiac muscle.

To identify the oxytocic principle the alcoholic extract when fractionated by various techniques revealed that the fraction giving positive tests for alkaloids, possessed the oxytocic activity. In the present study no attempts have been made to find out to which class of compound these alkaloids belong. This requires further investigation.
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To determine the nature of the oxytocic principle pharmacological activity of the 'extract' was studied. It produced a contraction of the isolated uterus and intestine which could be blocked by atropine. The 'extract' did not produce nicotinic effect of acetylcholine as seen in frog rectus muscle. Negative chronotropic and negative inotropic activity was observed in the 'extract' both in amphibian and mammalian cardiac muscle. The coronary flow was also reduced. These actions of the 'extract' on the heart muscles could be counteracted when the perfusion fluid contained atropine. It appears therefore that the oxytocic principle is muscarinelike in nature.

### Summary

The alcoholic extract of the fruits of *Piper aurantiacum* was found to possess oxytocic activity and was fractionated by chemical techniques. Application of paper chromatographical procedures to the alcoholic extract revealed the presence of a spot which gave positive tests with the alkaloidal reagents and exhibited oxytocic activity.

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### References

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