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HISTAMINE RELEASING ACTIVITY OF *CARISSA CARANDAS* ROOTS (APOCYANECEAE)

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The roots of *Carissa carandas L.* known as Karaunda in Hindi are used in the treatment of helminthiasis (1). They are also used to assess the intensity of snake poisoning (2). Joshi and Boyce (3) have investigated the roots of another species *Carissa congesta* and have reported the presence of glucoside. Since the roots of *Carissa carandas* are known to cause poisoning as a result of inadvertent contamination with other root material, it was decided to investigate the nature of the poisonous substance.

In this report we have an evidence for the histamine liberating activity in the ethanol extract obtained from the roots.

METHODS AND MATERIAL

The roots of *Carissa carandas* were collected from the Western Ghats (India). They were sun-dried and powdered. An ethanol extract was prepared. This extract was used for pharmacological investigation. Solid ethanol extract in weight of 23.09 g was obtained from 1 kg of powdered crude material.

The ethanol extract was insoluble in water. One hundred and fifty mg of extract were dissolved in 0.5 to 1.0 ml of 1% NaOH and the pH was adjusted to 7.6 and the final volume was made up with distilled water. The concentration of the solution used was 10 mg per ml. This was used as a stock solution. Working solution was prepared out of the stock solution by diluting with distilled water. The stock solution was preserved at 5°C.

1. Toxicity studies

Toxicity studies were carried out in mice and conscious cats. The drug was administered to albino mice in various doses, orally, and intraperitoneally to determine LD_{50}. Mortality was observed at the end of 24 hours.

The ethanol extract was administered, orally, intraperitoneally or intravenously in unanesthetized cats. The effect on the behavioural pattern was observed and the mortality at the end of 24 and 48 hours was noted.

2. Effect of ethanol extract on the blood pressure and plasma histamine content of cat

Cats were anesthetized with pentobarbitone sodium 40 mg/kg intraperitoneally. The carotid blood pressure was recorded by mercury manometer and the drug was ad-

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ministered through a polythene tube with attached metal connector, introduced in the femoral veins. The volume of the injection did not exceed 1 ml and was followed by a wash of 0.5 ml of normal saline. In all 10 experiments were done. Blood was collected from femoral artery at various intervals after intravenous administration of ethanol extract or 48/80. Plasma was separated and this was assayed for histamine like activity, on atropinized guinea-pig ileum.

3. Incubation of guinea-pig's lung tissue

Histamine releasing activity using minced guinea-pig lung was done by the method described by Monger and Schild (4). Lung tissue obtained from a guinea-pig killed by stunning, was divided into three portions. Each one was washed with Tyrode solution dried with a filter paper and weighed. Each portion was then cut into small pieces and then was ground with acidulated sand in a mortar. The volume was made up to 20 ml with Tyrode solution and incubated at 37°C in a metabolic shaker for 20 minutes. Ethanol extract or 48/80 was added to the tissue and the amount of free histamine liberated at the end of 20 minutes in each of the three portions was estimated. The lung tissue from each sample was separated by filtration, washed three times with Tyrode, dried on a filter paper and the residual histamine content was extracted (4) and estimated on atropinized guinea-pig ileum. The histamine release was expressed as percentage of initial histamine content (Fig. 1).

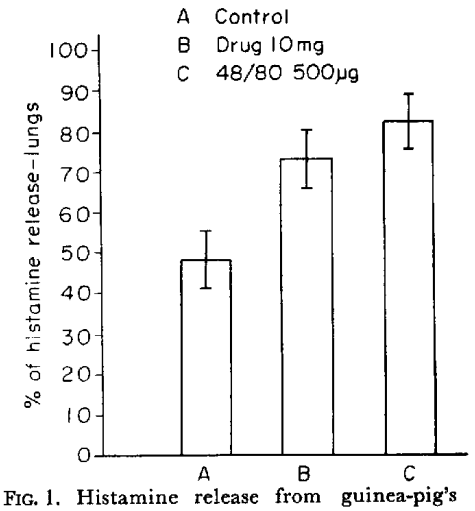

FIG. 1. Histamine release from guinea-pig's lung tissue in
A. Control.
B. Drug (E. Ex.) 10 mg.
C. 48/80 500 µg.

4. Rat hind limb perfusion

Rat hind limb perfusion was done by the method described by Feldberg and Monger (5). The perfusion cannula was introduced in the aorta, while the perfusion was collected through inferior vena cava after ligating all the branches except iliac vein. The perfusate was collected every 10 minutes and assayed for the presence of histamine before and after administration of the ethanol extract or 48/80. In all, six experiments were done. Histamine was assayed on atropinized guinea-pig ileum mounted in Tyrode. The presence of histamine was confirmed by the block produced by mepyramine maleate.

The following drugs were used: Histamine acid phosphate (doses expressed as base), atropine sulphate, mepyramine maleate and 48/80.

RESULTS

1. Effect of ethanol extract in conscious cats

Three cats were used for each dose and route. Ethanol extract administered in the

doses of 5 mg/kg to 150 mg/kg by various routes produced the effects like increase in respiratory rate, running of nose, drowsiness, vomiting, salivation, defecation with slimy stools, panting and death. Vomiting occurred after 3 to 5 minutes of intravenous administration of the drug; 10–20 minutes after intraperitoneal administration and 40–60 minutes after oral administration. All cats which received the extract intravenously died within a period of 1–2 hours. On post-mortem examination, liver was found to be congested, there were petechial hemorrhages in lungs and in the mucosa of the intestines.

The LD_{50} of ethanol extract in mice was found to be 175 mg/kg intraperitoneally.

2. Effect of ethanol extract on blood pressure in anesthetized cats

Intravenous administration of various doses of ethanol extract was found to produce a biphasic hypotensive effect. There was initial evanescent fall observed within 5 seconds followed by second fall, gradual in onset, but more prolonged and appeared not earlier than 30 seconds. The smallest dose that produced a definite effect was 250 μg/kg while 10 mg/kg i.v. resulted in the death of animals.

Fig. 2 shows a typical response after administration of ethanol extract (1 mg/kg i.v.). It was noted that the delayed fall in blood pressure after administration of 1 mg/kg of ethanol extract lasted for 4.5 hours. The same dose administered after 4.5 hours showed tachyphylaxis. However, when the dose was increased 10 times the initial dose, prolonged fall in blood pressure resulted in death of the animal.

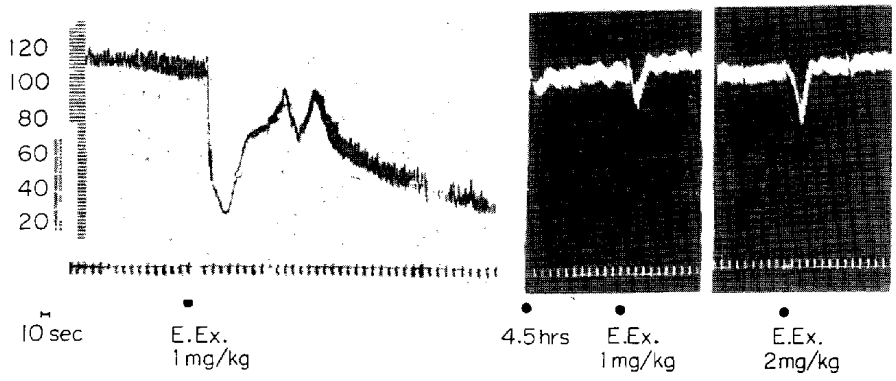

FIG. 2. Typical action of ethanol extract on blood pressure of cat and tachyphylaxis exhibited by the ethanol extract.

The initial transient fall in blood pressure was always present under these conditions of tachyphylaxis, although it was reduced. In three experiments it was observed that previous atropinization eliminated the initial evanescent fall in blood pressure. However, the second phase of hypotension was unimpaired.

Fig. 3 shows the effect of previous administration of 48/80 on the delayed hypotensive effect. It is quite evident that 48/80 administration eliminated delayed hypotensive phase of ethanol extract. This was consistently seen in three experiments. On the other hand, when tachyphylaxis to ethanol extract was established after repeated administration, the depressor effect of 48/80 (100 μg) was unaffected.

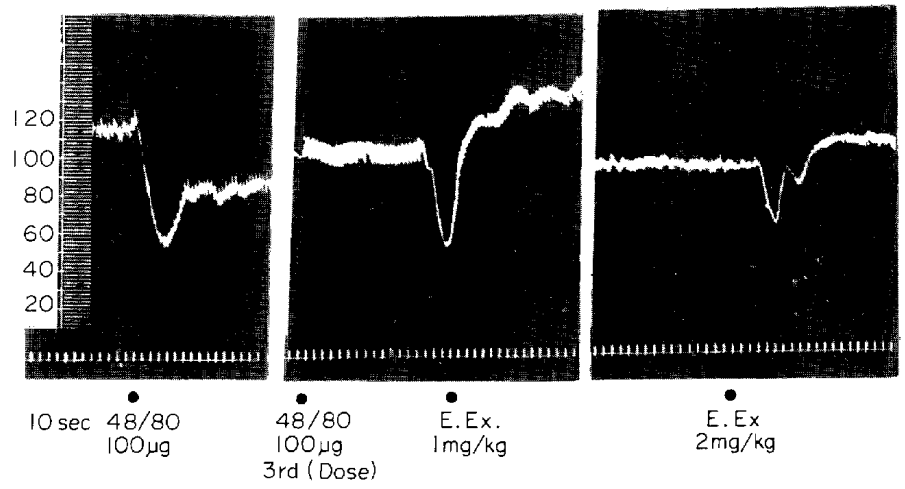

FIG. 3. Depletion of histamine by repeated administration of 48/80, followed by administration of ethanol extract on blood pressure of cat.

Previous administration of mepyramine maleate 2 mg/kg reduced the intensity of delayed hypotensive responses after administration of the ethanol extract.

3. Plasma histamine estimation

Fig. 4 shows the effect of ethanol extract (1 mg/kg i.v.) in comparison with 48/80 (100 µg, i.v.) on plasma histamine at various intervals after administration. It can be seen that the drug produced an immediate rise in plasma histamine to 10 times the control level (0.013–0.15 µg/ml). Whereas 48/80 produced a rise to 50 times the control levels (0.015–0.53 µg/ml). The peak rise in both cases occurred at about the same time, i.e., after 10 minutes.

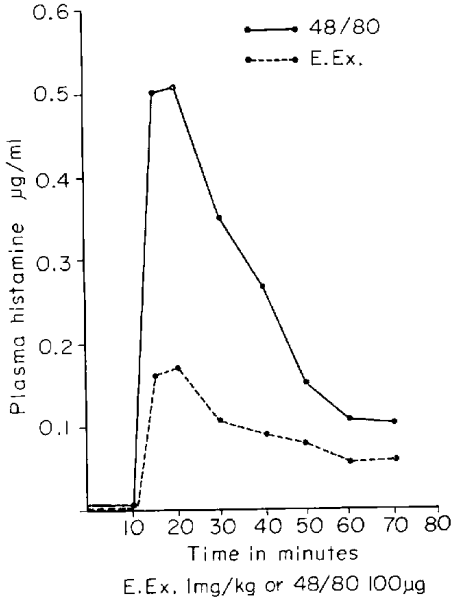

FIG. 4. Plasma histamine content after administration of 48/80 or ethanol extract.

4. Rat hind limb perfusion

Fig. 5 shows the results of average histamine content of the perfusate from rat hind quarters. It can be seen that administration of 10 mg ethanol extract increases the histamine content from 0.008 µg/ml to 0.09 µg/ml than that produced by 48/80 (10 µg) alone (Fig. 6). However, the rise in histamine content produced by 10 µg of 48/80 was seen to be reduced when 48/80 followed 10 mg of ethanol extract (Fig. 5).

5. Incubation of guinea-pig's lung tissue

Fig. 1 shows the histamine releasing activity of the extract in vitro when incubated

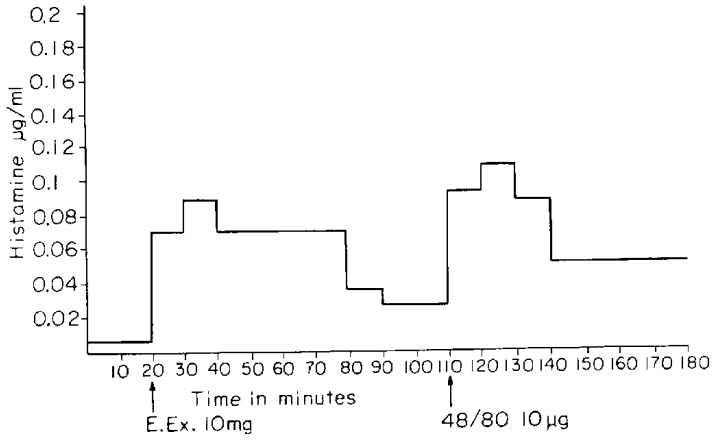

FIG. 5. Histamine content from the perfusate from rat hind-quarters after administration of ethanol extract and 48/80.

with lung tissue homogenate. It can be seen from the figure 48/80 produced a considerable rise in the free histamine over the control. Thus 500 µg/ml of 48/80 produces 32% greater release, while 10 mg/ml of drug produced 26% greater rise over the control.

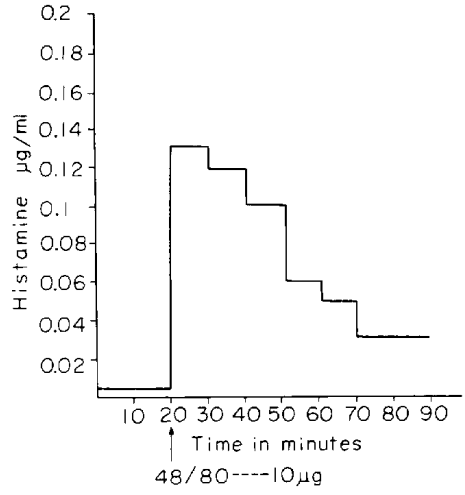

FIG. 6. Histamine content from the perfusate from rat hind-quarters after administration of 48/80.

DISCUSSION

Carissa carandas roots are reputed to possess poisonous properties. Its ethanol extract produced vomiting, rhinorrhoea, diarrhoea and increase in respiratory rate and panting, and finally convulsion and death in concious cats. Post-mortem examination revealed congestion of internal organs and petechial hemorrhages. These findings are suggestive of cardiovascular collapse. There is an evidence to suggest that the cardiovascular collapse was due to histamine release.

The drug has produced a biphasic depressor effect in anesthetized cats. Since the earlier evanescent component could be blocked by atropine it appears to be a cholinergic effect. The prolonged and delayed hypotensive effect is due to the release of histamine by the drug. The evidence in support of "histamine releasing" action of the drug can be summarized as follows:

1) The hypotensive effect is delayed in onset the effect is manifested after 30 seconds.

2) Tachyphylaxis occurs after second dose.

3) Cats pretreated with 48/80 do not show the delayed hypotensive effect of the drug.

4) After administration of the ethanol extract the plasma histamine of cat increased

from 0.008 μg/ml to 0.116 μg/ml.

5) The drug caused a substantial increase in free histamine in the guinea-pig lung homogenates.

6) Ethanol extract released histamine from the hind legs of rats.

The crude extract failed to produce hypotensive effect after second dose. However, 48/80 was found to be effective in releasing histamine in animals which had developed tachyphylaxis to ethanol extract. Similar phenomenon was seen in hind leg perfusion. From these results it appears that 48/80 produced more complete release of histamine as compared to the crude extract. The hypotensive effect produced by the drug is of much longer duration in comparison to the effect of 48/80. This suggests that the drug effect though weak, is of longer duration possibly due to irreversible drug receptor interaction.

SUMMARY

1. In conscious cats ethanol extract of *Carrissa carandas* produced vomiting, rhinorrhoea, diarrhoea, tachypnea, exhaustion and death.

2. In an anesthetized cat, it produced a biphasic hypotensive effect. The initial evanescent effect was abolished by atropine whereas the second prolonged hypotensive effect was reduced by mepyramine maleate.

3. The histamine releasing effect was observed by estimating plasma histamine in cats, histamine content in rat hind-limb perfusate and histamine content of the lung tissue.

4. The histamine releasing effect of the ethanol extract is compared with that of 48/80.

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