PHARMACOLOGY OF THE CRUDE EXTRACT OF ANONA SQUAMOSA, (SHARIFA)

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The pharmacology and toxicology of the water soluble portion of the alcoholic extract of the leaves of *Anona squamosa* (Sharifa) has been studied. The extract was found to stimulate the isolated rabbit heart, relax the isolated rabbit duodenum and raise the blood pressure of anaesthetised animals. It also caused a contraction of the nictitating membrane of the cat. The actions were very similar to those produced by adrenaline. Adrenergic blocking agents like dibenamine and ergotamine completely blocked and reversed the pressor response of the extract.

The results indicate that the active substance is very similar to adrenaline in it's pharmacological actions.

Introduction

Anona squamosa Linn., is an indigenous plant belonging to the family' Anonaceae and is oommonly known as "SHARIFA". Various preparations made from its leaves and seeds have been described to posses anthelminthic and insecticidal properties.[1] In indigenous medicine decoction of Sharifa leaves has also been used in cases of cardiovascular collapse. Santos et al[2-4] studied thc chemistry of thc extract of the leaves and seeds and found gum, resins, oils and an alkaloid "Anonaine", both in the leaves and the seeds. The pharmacological activity of the crude extract of the leaves as well as that of the alkaloid "Anonaine" has not so far been described. The paper deals with the pharmacology and toxicology of the water soluble portion of the alcoholic extract of Sharifa leaves.

Preparation of Extract

Fresh, undried leaves were weighed, cut into small pieces and percolated with alcohol for seven days. Percolation was repeated three times, and the alcohol was removed *in vacu* at 40°C. The extract was treated with petroleum ether and the semisolid mass was taken up in *distilled* water. This pale strawcoloured solution with a pH of 5-6 was found to be active.

Pharmacology

The pharmacology of the extract was studied on the following preparations:—

1. *Isolated Rabbit Heart.*—Langendorff isolated heart preparation was set up according to the method of Burn.[5] Mc Ewan's solution was used for perfusion. Contractions of the heart were recorded on the smoked drum.

2. *Isolated Rabbit Duodenum.*—A piece of rabbit duodenum was perfused in oxygenated Tyrode's solution at 37°C. Contractions were recorded on smoked drum by a frontal writing lever.

3. *Blood Pressure of Dog.*—Dogs were anaesthetised with a dose of Pentothal 15 mg./kg. combined with Gardenal 25 mg./kg. given i-v, followed by an injection of Sodium Gardenal 75 mg./kg. given intraperitoneally.

4. *Blood Pressure of Cat.*—Normal cats were anaesthetised with Gardenal 1 mg./kg. intraperitoneally. Blood pressure was recorded from the carotid artery and the drugs were injected into the femoral vein. In certain experiments the contractions of the nictitating membrane were recorded by a frontal writing lever on smoked drum.

The above studies were repeated on three "reserpinised" cats. Reserpine was injected intraperitoneally in a dose of 1 mg./kg. b.w. on the first day, 2 mg./kg. b.w. on the second day and 3 mg./kg. b.w. on the third day. Experiments were done on the fourth day. Contractions of the nictitating membrane were also recorded.

5. *Rat Blood Pressure.*—Adult male rats were anaesthetised with chloralose 15 mg./kg. body weight given intraperitoneally. Blood pressure was recorded with a mercury manometer from the carotid artery. Injections were given via cannulated jugular vein.

Results

1. *Isolated Rabbit Heart.*—The extract was injected through a polythene cannula opening near the coronary artery into the perfusion fluid. A dose of 0.1 ml. produced a sharp rise in the amplitude and the rate of the heart. The contractions of the heart returned to normal after about 2 minutes. These responses were very closely matched by a dose of 0.5μg of adrenaline given in a similar way (Fig. 1). The response

was repeatable and no tachyphylaxis was observed. The subsequent responses to adrenaline were not at all affected.

2. *Isolated Rabbit Duodenum.*—The extract when added to the organ bath relaxed the rabbit duodenum. The normal response to acetylcholine was depressed. The response returned

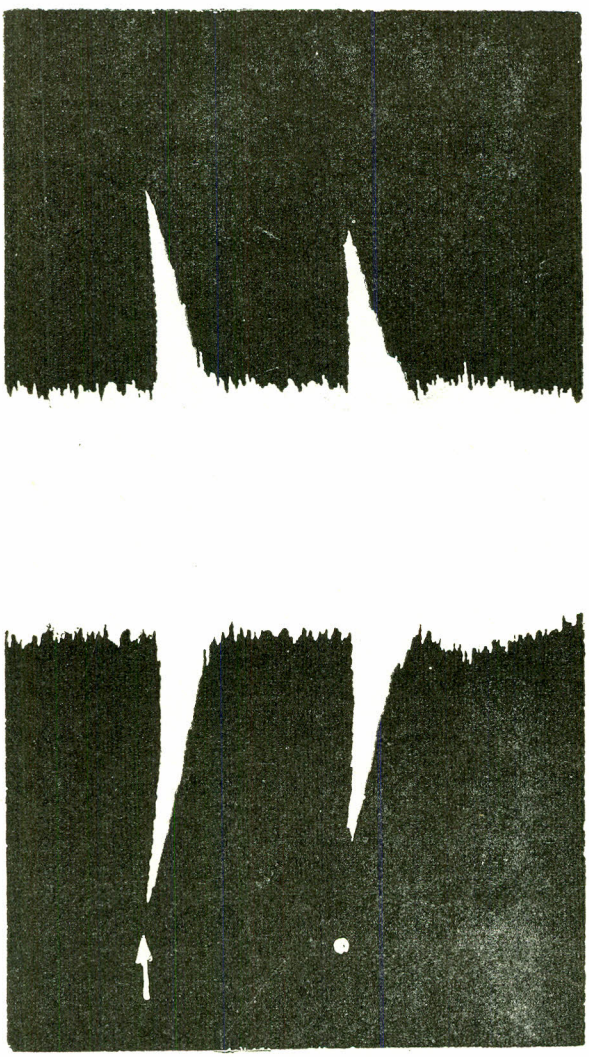

Fig. 1.—Contractions of the isolated perfused rabbit heart. At ●, 2 μg. ardenaline was injected. 0.5 ml. Sharifa leave extract was injected at ↑.

to normal after three minutes. This antiacetylcholine action of the extract was matched by the similar action of adrenaline. Fig. 2. shows this action.

3. *Blood Pressure of Dog.*—A sharp rise in the blood pressure was noticed when 2 to 3 cc. of extract was injected intravenously into the femoral

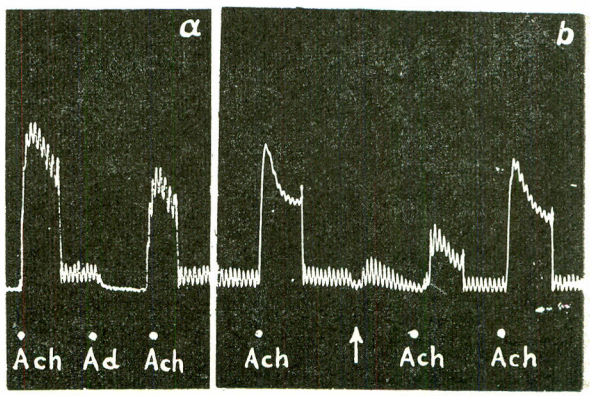

Fig. 2.—Isolated rabbit duodenum. Acetylcholine, 2μg. added at 'Ach'. At Ad, 0.5μg./ml. adrenaline was added to the bath. Acetylcholine was repeated without washing. Sharifa leave extract (0.5 mg.), was added in similar way at ↑.

vein. Blood pressure returned to normal in about 3 to 5 minutes depending on the dose. This response was very similar to that obtained after intravenous injection of 20 μg adrenaline (Fig. 3). It could not on the other hand be matched with the response obtained after intravenous injection of 20 μg of noradrenaline or 2 mg. of ephedrine (Fig. 4). These pressor responses were repeatable and no tachyphylaxis was observed.

In another series of experiments on dogs, the injections of adrenaline and Sharifa leaves extract were given before and after intravenous injections of ergotamine 1.0 mg./kg. body weight. As is shown in Fig. 5, the extract, like adrenaline, produced a fall in the blood pressure of the dog when given after ergotamine.

The fall in blood pressure produced by the extract was much more pronounced than that of adrenaline.

4. *Cat Blood Pressure.*—The extract produced a rise in the blood pressure of the cat. Like the responses in the dog, these responses were repeatable and did not show any tachyphylaxis. The responses could be matched with those of adrenaline.

In the nictitating membrane preparation of the cat, a contraction was observed together with a rise in the blood pressure. These contractions of the nictitating membrane and the pressor responses, were closely matched with adrenaline

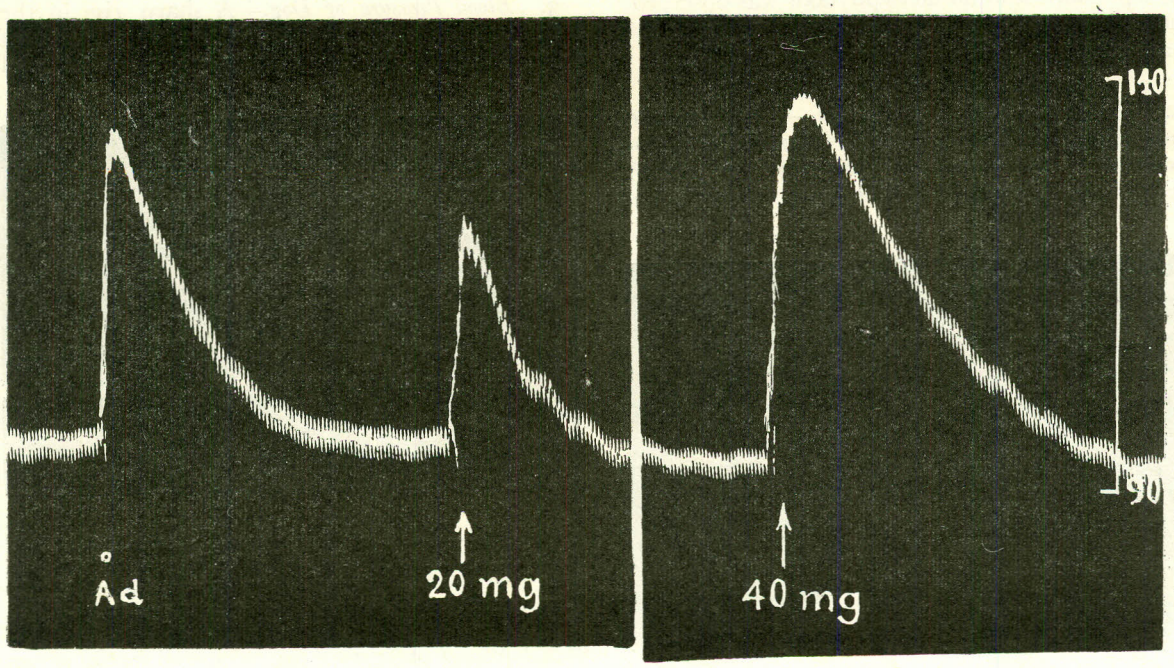

Fig. 3.—Dog blood pressure. The effect of intravenous injections of 20 mg. and 40 mg. Sharifa leaves extract could be matched with adrenaline 20 μg. given in similar way.

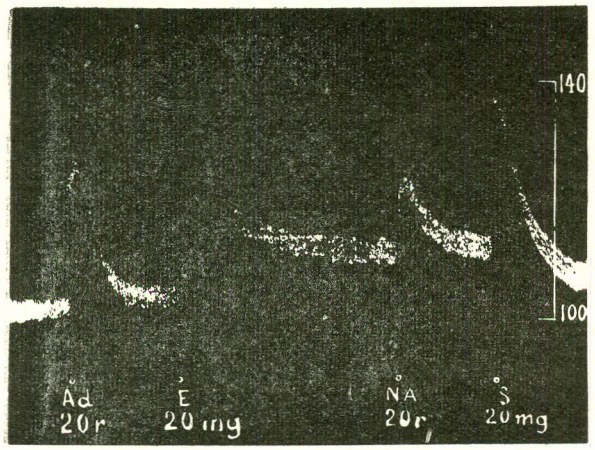

Fig. 4.—Dog blood pressure. Pressor responses obtained by intravenous injections of adrenaline (Ad) 20 μg., ephedrine (E) 20 mg., noradrenaline (NA) 20 μg, and Sharifa leaves extract (S) 20 mg. The response of the extract matches well with that of adrenaline.

injected intravenously. (Fig. 6) The pressor response as well as the contractions of the nictitating membrane showed a clear dose response relationship.

5. *'Reserpinised' Cat.*—In cats who were treated with reserpine for three days, the extract produced rise in the blood pressure and a contraction of the nictitating membrane in a way similar to that seen in untreated cats. The dose-response relationship was seen best in these preparations. Fig. 7 illustrates one such experiment.

6. *Blood Pressure of Rat.*—Injection of the extract produced a rise in the blood pressure of anaesthetised rats. There was however an initial fall of the blood pressure although the pressor action was predominant. Six repeated intravenous injections of 100 μg. dibenamine per 100 g. bodyweight were given at 5 minutes intervals. After a period of 30 minutes the injections of the extract were repeated. These injections produced a marked fall of blood pressure as against a rise seen in the untreated animals. One such experiment is illustrated in Fig. 8.

7. *Toxicity.*—Adult, male albino rats weighing 150-200 g. were used. Increasing doses of the extract were injected intraperitoneally. No deaths were, however, noted even when the dose of the dessicated extract was 1 g./kg. of the body weight. The animals were observed for eight days.

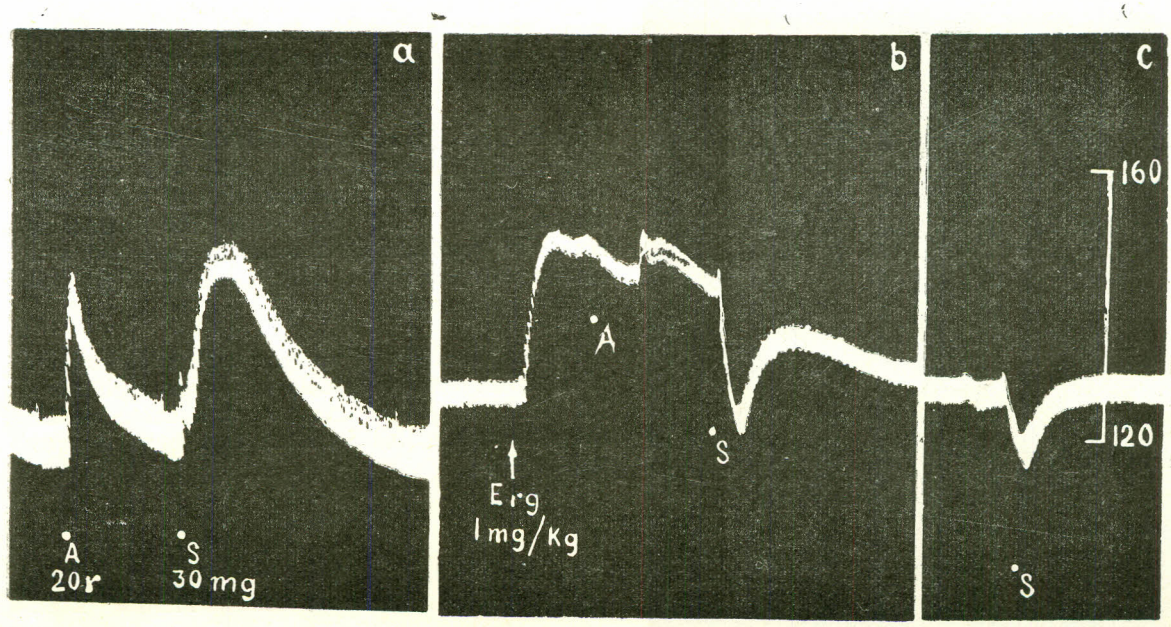

Fig. 5.—Dog blood pressure: 'a' shows the pressor response obtained by intravenous injection of adrenaline (A) 20 μg. and Sharifa leave extract (S) 30 mg. At 'b' ergotamine tartrate (Erg) was injected intravenously 1 mg./kg. When injections of adrenaline (A) and the extract (S) were repeated now, they produced a fall in the blood pressure. Time interval between and 'c' 30 min.

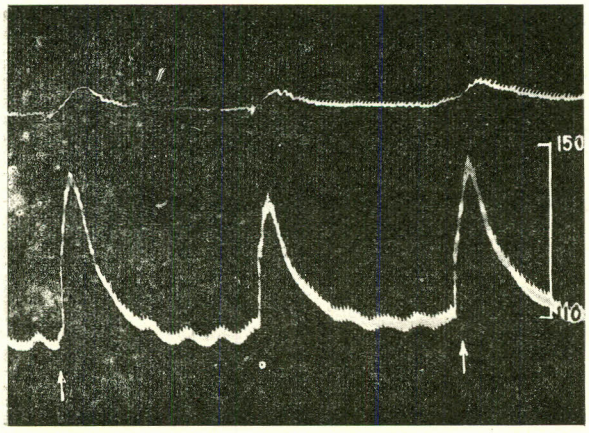

Fig. 6.—Cat blood pressure, nictitating membrane preparation. At ↑ , 30 mg. dried Sharifa leaves extract injected intravenously. This pressor response and the contraction of the nictitating membrane was matched with the response of 20 μg adrenaline given at ● .

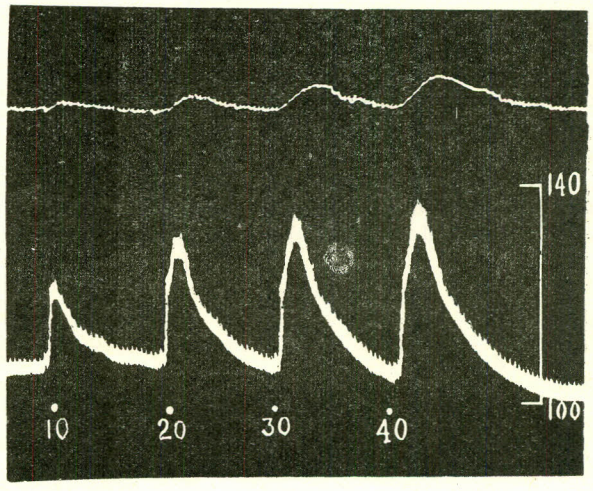

Fig. 7.—"Reserpinised" Cat. Blood pressure, nictitating membrame preparation. Uniform graded responses obtained after intravenous injection of increasing doses of the extract. Figures de note dose in mg.

Discussion

It is clear from the experiments described above, that the water soluble fraction of the alcoholic extract of the Sharifa leaves has a very marked activity on the cardiovascular system, as well as on the smooth muscle of the intestine. The actions on both the systems resemble very closely those of adrenaline. The extract stimulates the isolated

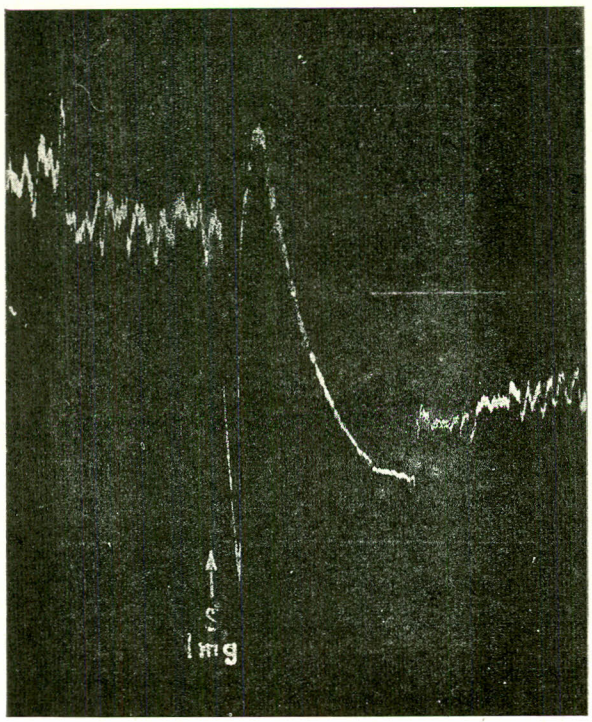

Fig. 8.—Dibenaminised rat blood pressure. Sharifa leaves extract(S) produces a fall in the blood pressure.

rabbit heart in a way very similar to adrenaline. Similarly its action on the dog and the cat blood pressure is like that of adrenaline and does not resemble noradrenaline or ephedrine. The action is abrupt and short lived. This pressor response is also blocked and reversed by adrenergic blocking agents like ergotamine and dibenamine. The extract contracts the nictitating membrane of the cat and relaxes the rabbit duodenum as well as antagonises the action of acetylcholine on the same tissue. It could therefore be concluded that the active principle of the extract resemble adrenaline very closely.

It is well known that reserpine depletes the tissues of their stores of the catecholamines including adrenaline, noradrenaline and 5-hydroxytryptamine.[6-8] The fact that the extract is active in 'reserpinised' animals as well, shows it clearly that the action is independent of the endogenous adrenaline.

It could therefore be summarised that the extract of the fresh undried Sharifa leaves has a pharmacologically active substance or substances, which resemble adrenaline in its action both *in vitro* and *in vivo*.

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