PHARMACEUTICAL INVESTIGATIONS ON SALACIA MACROSPERMA-1

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ABSTRACT:

The chloroform, ethanol (95%) and aqueous extracts of roots of Salacia macrosperma (Hippocrataceae) were prepared by double maceration followed by vacuum evaporation. All the extracts were subjects to qualitative chemical tests to find out phytoconstituents present in them. The ethanolic extract showed significant hypoglycemic activity in fasted rabbits. The activity of ethanolic extract was also evaluated in alloxan induced hyperglycemic albino rats. It showed a mean blood sugar level reduction of 89.22 mg/100 ml which was significant when compared with mean variation in blood sugar levels of control group. Alcoholic and aqueous extracts were screened for their effect on both normal and hypodynamic isolated frog heart. Alcoholic extract showed considerable positive ionotropic activity and increased cardiac output without affecting heart rate both in normal and hypodynamic isolated frog heart.

Introduction

The roots and leaves of Salacia macrosperma (Hippocrataceae), a creeping shrub are used in the treatment of diabetes(1), as a tonic and blood purifier (2) and as a remedy for enlargement with congestion of liver and piles(3). Preliminary pharmacological screening of extract on C.N.S. has been reported(4). Reddy et al (5) isolated quinine methide, prestimerin, compounds related to prestimerin, tingenone and hydroxytingenone. In our present investigations, an attempt as been made to carry out systemic analysis of roots of carry out systemic analysis of roots of salacia macrosperma for their pharmaceutically useful phytoconstituents. Present report covers the preliminary screening of extracts of roots of D. macro sperma for hypoglycaemic activity, activity on isolated frog heart and isolation and purification of a film forming material.

Materials and Methods

Roots of S. macrosperma were collected at Belgaum, Karnataka state, India in the month of April 1987, after proper identification. The specimens are preserved in Pharmacy museum at Kakatiya University.
Warangal. The roots were powdered, passed through sieve no 10 and used for extraction.

(i) **Preparation of extracts:** the chloroform, ethanol (95%) and chloroform water extracts of powdered roots were prepared by double maceration at room temperature with drug-solvent ratio of 1:3. The solvents were distilled in vacuo and extracts were stored in well closed containers and preserved at refrigerated temperature

(ii) **Qualitative chemical tests:** the extracts were subjected to various qualitative chemical tests to detect phytoconstituents present in them (6,7 & 8).

(iii) **Hypoglycaemic activity of extracts in fasted rabbits:** For the studies with an individual extract, two groups containing of five male rabbits each weighing between 1.8 to 2.0 kg were fasted for 24 hours prior to experimentation. The extract of crude drug was suspended in 3% acacia mucilage. The chloroform extract, a solid matrix insoluble in water, was first dissolved in little amount of chloroform and was grinded with acacia powder. The chloroform was evaporated and made up to the required volume with distilled water to give a uniform suspension. A dose of 200 mg/kg of body weight was administered. The blood (0.5 ml) was withdrawn from marginal ear vein of rabbit before administration of extract, blood samples were collected at an interval of one hour for six hours and the blood sugar was estimated immediately.

(iv) **Hypoglycaemic activity of extracts in hyperglycaemic rats:** Hyperglycaemia in rats was induced by administering a single intraperitoneal injection of alloxan at a dose of 150mg/kg of body weight and the rats were maintained on standard diet (Hindustan Lever diet soaked with coconut oil). The studies were carried 72 hours after intraperitoneal injection of alloxan. Only alcoholic extract was evaluated for hypoglycaemic activity, which showed significant hypoglycaemic activity in fasted rabbits.

Two groups consisting of six male albino rats were fasted for 24 hours by withdrawing food. However, they were allowed to take water at libidum. Blood sample (0.2ml) was collected from tail vein of rat and initial blood sugar level was estimated colorimetrically (9). A dose of 200 mg/kg of body weight of extract in the form of suspension was administered orally with a syringe fitted with a bent needle and plastic cover over it. The control group received only 3% acacia mucilage. The blood samples were collected at 1, 2 and 4 hours after administration of alcoholic extract of crude drug and blood sugar levels in these samples were estimated immediately.

(v) **Effect of extracts on isolated frog heart:** The experiments were conducted both on normal and hypodynamic heart (10). The heart was made hypodynamic by perfusing frog-Ringer physiological solution containing half-calcium from another reservoir through Cyme’s canula.
The chloroform extract was not tried since it was a water insoluble solid mateix. The calcium content of alcoholic and chloroform-water extracts was determined by flame photometry (Systronics flame photometer: Mediflame 127) was found to be 0.23mg and 1.3 mg per gramme respectively. The weighed quantity of finely powdered alcoholic extract was powdered in frog-Ringer solution and frog-Ringer solution with half-calcium. The insoluble components of alcoholic extract were removed by filtering through Whatmann no. 1 filter paper. The experiments were carried out on 8 isolated frog hearts. The heart rate, force of contraction and cardiac out put were recorded. Various doses of extracts were tried to find out the
doseactivity relationship both on normal and hypodynamic hearts.

Results and Discussion

The alcoholic and chloroform-water extracts of crude drug were reddish-brown solids with 3.75 and 5.00% w/w yields respectively. The chloroform extract (1.90% w/w) was a solid matrix, which on repeated extraction with pyridine at 20°C temperature yielded an inert film forming material (1.02% w/w). Its elasticity was found to be good. It was insoluble in water but soluble in chloroform, carbon tetra chloride, benzene, solvent ether, toluene and xylene. Its utility in pharmaceutical industry as a film coating material could be exploited.

| TABLE -1 |
| Effect of alcoholic extract of S.macrosperma on blood sugar levels of mg/100ml) fasted rabbits |

| Treatment       | Initial blood sugar level | Blood sugar levels after |
|-----------------|---------------------------|--------------------------|
|                 |                           | 1hr | 2hr | 3hr | 4hr | 5hr | 6hr |
| Extract         | 110.7(5.8)*               | 99.2(5.5) | 91.9(5.3) | 93.2(4.0) | 96.6(4.0) | 100.4(7) | 104.6(8) |
| Control         | 109.4(4)                  | 108.5(3.5) | 108.1(3.4) | 107.4(3.5) | 107.3(3.3) | 107.3(3.4) | 106.6(3)  |

*The values in parenthesis indicate standard deviation.

The alcoholic extract showed positive tests for carbohydrates, resins, phenolic compounds, saponins and flavonoids, while water extract indicated presence of phenolic compounds and saponins and the chloroform extract showed the presence of phytosterols and inert polymeric compound.

Out of three extracts tested, alcoholic extract, 2 hours after administration of 200 mg/kg dose, showed considerable hypoglycaemic activity in fasted rabbits. The extent of reduction in blood sugar levels was 18.8mg/100 ml, which was significant when compared with control group values (Table: 1).

The activity of alcoholic extract on alloxan treated rats was studied since the extract showed significant hypoglycaemic activity in fasted rabbits. The blood sugar levels of drug treated and untreated alloxan induced
hyperglycaemic rats are shown a mean variation of -89.22 mg/100 ml of blood. 2 hours after oral administration of 200 mg/kg of body weight, whereas untreated group showed a mean variation of -9.0 mg/100 ml of blood, 2 hours after oral administration of 3% acacia mucilage. The alcoholic extract caused 41.45% reduction in initial blood sugar levels. The results indicate that his extract may become a potential source for new antidiabetic drug. The work is in progress to isolate active principle responsible for hypoglycaemic activity and to study mechanism of action.

The water extract did not show significant effect on isolated normal and hypodynamic frog heart. The alcoholic extract increased the force of contraction and cardiac out put significantly with a dose.

### TABLE-2
**Effect of alcoholic extract of *S. macrosperma* on blood levels (mg/100ml) of alloxan treated rats after a single dose (200mg/kg).**

| Treatment | Initial blood sugar level | Blood sugar levels after |
|-----------|--------------------------|--------------------------|
|           |                          | 1hr                      | 2hr                      | 3hr                      |
| Extract   | 213.68(28.6)*            | 126.25(16.5)             | 124.5(4.5)               | 127.65(16.83)            |
| Control   | 218.20(37.2)             | 210.07(22.5)             | 209.2(21.7)              | 208.73(22.48)            |

*The values in parenthesis indicate standard deviation.
Of 500 ug on normal and hypodynamic frog heart. However, the heart rate was unaffected with the extract. The force of contraction and cardiac output were increased with increased doses of extract up to 7mg, beyond which there was no appreciable change in activity (Fig:1). The force of contraction and cardiac output were brought to normal with a dose of 1mg of extract in hypodynamic heart (Fig:1). This clearly indicates the presence of cardiotonic principle in alcoholic extract of roots of S. macrosperma.

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ERRATA: Messrs. A. R. Somanathan, K. Sadanandan and N. P. Damodaran (1989) 'Ancient Science of Life' Vol. IX, No. 2, Pages 84–86. The authors wish to insert the following chart as supplementary to their article entitled Standardisation of Ayurvedic Medicines — Dasamulam Kasayam.

To read the following as 6th reference to the text:

6. Mooss: Single Drug remedies, Vaidyasarathy Press, Kottayam, p. 90–91 (1976)