Pak. J. Sci. Ind. Res. 2004 ***47*** *(5) 390-393*

EFFECT OF AQUEOUS EXTRACT OF *AVICINNIA MARINA* ON MYOCARDIAL CONTRACTION OF ISOLATED MAMMALIAN HEART

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(Received May 09, 2002; accepted September 02, 2004)

Aqueous extract of *Avicinnia marina* commonly known as mangrove showed a marked inhibitory action on isolated mammalian heart *in-vitro*. The degree of inhibition was found to be highly dose dependent. A dose of 100 mg/kg caused cardiac arrest for few seconds. The force and magnitude of cardiac contractions were regained to pre-injection level after 1.6-2 min. Pretreatment with atropine was found to have no effect on myocardial contractions. The amplitude of cardiac contractions was reduced slightly after the administration of adrenaline.

Key words : *Avicinnia marina*, Mangrove, Mammalian heart.

Introduction

Avicinnia marina Linn. (Verbenaceae) commonly known as mangrove is a coastal vegetation. It fringes shores and estuaries and also proliferate luxuriantly in swamp and marshy places. It is found abundantly all along 150 miles coast line of Sindh (Jafri 1966, 1973; Saifullah 1982).

Mangrove plant is not only a land builder and retainer but shows an interesting array of diverse uses i.e commercial and industrial uses in paper, plywood, adhesive, leather, dying and textile industries (Combs and Anderson 1949; Koeppen and Cahen 1956; Julia 1965; Vetter *et al* 1995; Sowunni *et al* 1996) and as a food and feed for livestock and marine organisms.(Borris *et al* 1949; Taedo 1962; Julia 1965). Also used in medicines locally as well as internally (Julia 1965; Shahnaz *et al* 1995; Itoigawa *et al* 2001).

An ample data available on the chemical composition of mangrove reveals the presence of a number of chemical constituents and bioactive compounds (Gosh and Patra 1979; Kokpol Udom *et al* 1990; Adrian *et al* 1998; Sharaf *et al* 2000; Ito *et al* 2000; Bandara-nayake 2002) beside minerals, amino acids, vitamins, fatty acids, lipids and waxes (Drude *et al* 1986; Rashid *et al* 1986; Misra *et al* 1987; Joshi and Kumar 1989; Mohan *et al* 1998; Datta *et al* 2003). While on the other hand very little data is available on its biological and pharmacotoxicological properties. Therefore, it was decided to study the pharmacotoxicological properties of *A. marina* with profound basis regarding the search for a new useful pharmaceutical product capable of benefitting both applied and basic research sides.

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Materials and Methods

Collection and identification. Mangrove plant was collected from the coastal areas of Karachi, identified by a taxonomist and a voucher specimen was kept in our laboratory under PCSIR Herbarium No. 585 for future reference.

Preparation of extract. The collected plant material was washed thoroughly first with tap water and then with distilled water. Dried in air at room temperature and then chopped into small bits. 1.0 kg of chopped material was soaked in 95% ethanol for a period of 5 days with continuous stirring for 6 h per day. Solvent was then decanted and concentrated under reduced pressure at room temperature. The resultant gel like alcoholic extract was partitioned with (V/V 2:1 ratio) water and pet ether with vigorous shaking in separating funnel. The resultant aqueous layer thus formed was separated and concentrated to maximum dryness in rotary evaporator. The mass thus obtained as aqueous extract was used for the study.

Standard reference solutions. Adrenaline, acetylcholine and atropine were used as standard references. These solutions were made in sterile distilled water.

Test solution. Mac. Ewen's solution (Robert 1971) having the following formula was used to perfuse the isolated heart.

NaCl	7.60 g	$NaHCO_3$	2.10 g
KCl	0.24 g	Dextrose	2.00 g
$CaCl_2$ (anhydrous)	0.19 g	Sucrose	4.50 g

Double glass distilled water ———Vol. made to 1 liter.

Animal used. Healthy adult rabbits (2.5 - 3.0 kg) of either sex were used and kept under observation for a period of 7 days before using for the experiment.

Experimental procedure (Lagendorff 1895). Rabbit was killed by dislocating the neck and the beating heart was immediately taken out of the body with at least 1.0 cm aorta attached by cutting the chest wall with sharp scissors. The heart was immediately immersed in a dish containing 200 ml of Heparinized (100 i.u. of Heparin) Mac Ewen solution maintained at 37°C. The heart was gently squeezed to remove blood or any blood clot present in it. All the rudiment and tissues attached to the aorta were removed with care and precaution. The aorta was then cut from the point where it divides and the heart was tied to the perfusion apparatus on glass cannula. Perfusion of the isolated mammalian heart averages 3.24cc per gram of the organ, in conditions similar to those taking place in the body at rest (Aldo 1954). Care was taken to avoid air bubbles to enter the aorta. The oxygenated perfusion solution maintained at a constant temperature of 37°C. The perfusion pressure was also kept constant. One end of the thread was attached to the apex of the heart by a hook and other end to a spring lever through a system of pullies to record the amplitude of cardiac contraction on the smoked kymograph.

The drug was inserted in the heart through a cannula attached to the perfusion apparatus near the aorta. The height of tracings were taken as a force of cardiac contractions. The only modification made in this method was that instead of using ringer solution, McEwen's solution was used, which also resembles much to, plasma minus protein like Ringer's solution (Aldo 1954).

Mangrove extract in a dose of 25 mg/kg, 50 mg/kg and 100 mg/kg was used to assess the affect on myocardial contractions. Before the administration of extract normal responses of an isolated heart against the standard known drugs i.e. adrenaline and acetylcholine were checked.

Results and Discussion

Isolated perfused rabbit heart showed a normal behaviour against the standard known drugs i.e. adrenaline and acetylcholine. Adrenaline showed a positive inotropic effect as shown in Fig 1.

Aqueous mangrove extract showed a significant action on an isolated heart. It significantly reduced the force of cardiac contraction. Decrease in cardiac contractions were found to be highly dose dependent (Fig 2). This effect becomes progressively more intense and marked as the concentration of drug is increased. A dose of 100 mg/kg stopped the heart in partial diastolic condition for few seconds (4 -7 sec) which gradually regained the pre-injection level after 1.6-2 min (Fig 2).

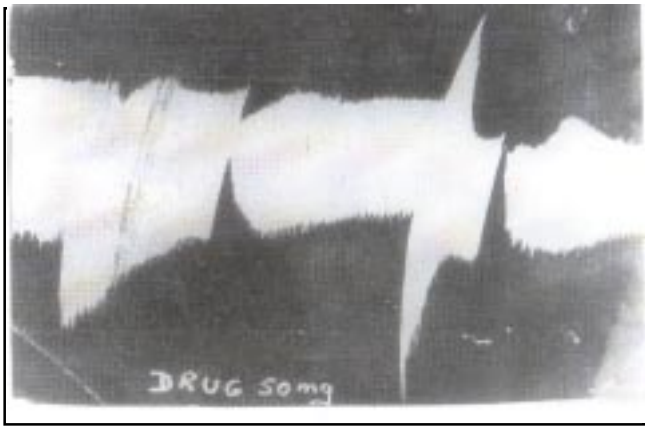

Fig 1. Contractions of isolated rabbit heart on a smoked drum. Note: Positive ionotropic action of adrenaline & a transient reduction in the force of cardiac contraction at a dose of 50 mg of aqueous mangrove extract

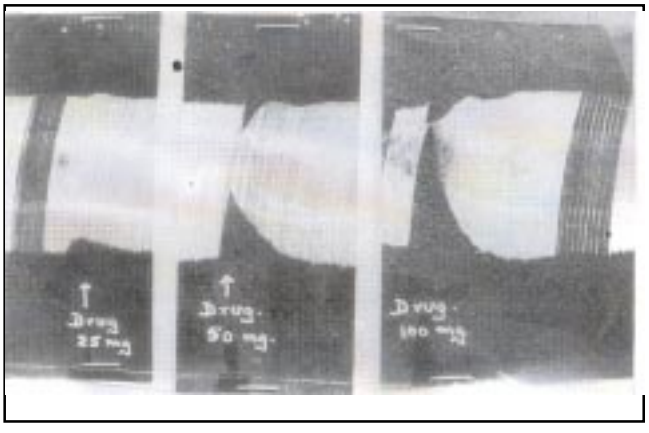

Fig 2. Dose proportionate cardiac inhibition. Extract causes the transient cardiac arrest in partial diastolic condition

Effect of atropinisation. Atropine which is standard known anticholinergic drug when injected in a dose of 2.0 mg/kg abolished bradycardia induced by cholinergic agent (i.e acetylcholine) and resulted in partial heart block. The aqueous mangrove extract in a dose of 100 mg/kg when administered after atropinization produced same inhibitory effects on myocardial contractions as before the administration of atropine i.e heart stopped in partial diastole for 4 - 7 sec and then gradually regained the pre-injection level, (Fig 3). It was further noticed that the amplitude of cardiac contractions were reduced when extract was administered after standard drug i.e adrenaline.

From the study it was evident that the aqueous mangrove extract decreases the contractility of myocardium in isolated

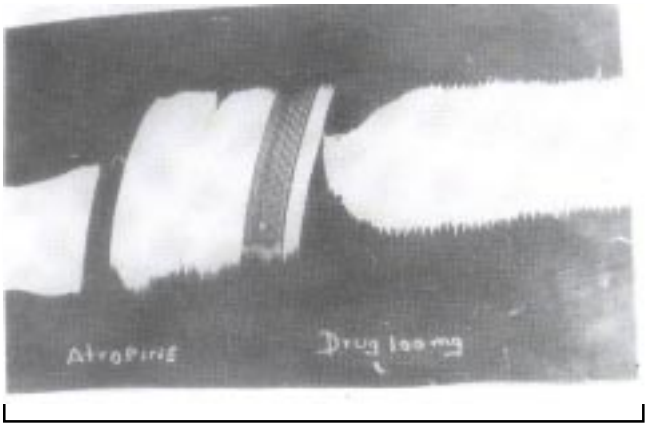

Fig 3. Effect of atropinization and 100mg/kg dose of aqueous mangrove extract on cardiac contraction

rabbit heart preparation in a dose dependent manner (Fig 2). It resulted in cardiac arrest / inhibition for few seconds in higher doses. As soon as the action of the extract was washed, the heart again regained the force and magnitude of contractions to pre injection level.

The amplitude of cardiac contraction was reduced slightly after the administration of adrenaline. Atropinization fails to inhibit the action of extract. This indicates that the extract does not act either through the cholinomimetic or adrenergic mechanism. It can be assumed probably that the mechanism of action of the extract seems to be through sodium channel blockers such as in Quinidine. Literature citation depicts that Quinidine in higher concentration decreases myocardial contraction and produces transient asystole (Andres 1976; Laurence and Bennet 1992) a negative ionotropic effect, as produced by aqueous mangrove extract. Furthermore, the chemical composition of mangrove reveals the presence of polyphenols, tannic acid and napthaquinones (Gosh *et al* 1979; Kokpoludom *et al* 1990; Ito *et al* 2000; Itoigawa *et al* 2001) which also strengthens its mode of action like Quinidine. However, crude extract needs to be further processed to have pure compound(s) which can then be tested for the claimed pharmacological activity.

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