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Vasodilatation Effect of Latex from *Calotropis gigantea* in Green Frog *Rana hexadactyla*

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Abstract : The latex of *Calotropis gigantea* is a rich source of useful components that has medicinal properties and one of the main applications is in controlling the heart muscle. The crude latex extract contained many proteins, which are highly basic in nature and exhibited strong dilatation activity. Blood vessel experiment of the latex from *Calotropis gigantea* was studied in the green frog (*Rana hexadactyla*). The diluted crude extract with distilled water to 1:10 and 1: 100 concentrations produces percentage increase in the cardiac output. Higher dilution factor increase the cardiac output 1:100 produces 66% output where as 1:10 produces 50% cardiac output. On treating with high dose, the latex damage the contractility of the cardiac muscle. Thus the present study reveals that the latex produces vasodilatation effect at fixed dose concentration.

Key words: Blood vessel, *Calotropis gigantea*, frog ringer, latex and *Rana hexadactyla*

INTRODUCTION

The presence of latex in plants is one of the characteristic features belonging to the families Euphorbiaceae, Asclepiadaceae, Moraceae and Apocyanaceae. The genus *Calotropis* (Asclepiadaceae) is comprised of about six species of shrubs distributed throughout tropical and subtropical Africa and Asia. Two of them, *Calotropis gigantea* L. and *Calotropis procera* L. occur in China, and are two sister species. *C. gigantea* is a high biomass, fast growing perennial shrub growing as a weed in the Hainan province of China. Plant latex is a complex mixture of several hydrolytic enzymes (Yagami *et al*., 1998) and also a rich source of wax, resins and lipid like substances. The physiological role of this latex in plants is not clearly understood. The plant resistant to variety of infectious diseases and to the extreme harsh conditions are partly attributed to the presence of hydrolytic enzymes of the latex especially proteases (Boller, 1986). These latex exhibited several pharmacological properties and are exploited in folk medicine (Ervatamia, 1952). Latex from several plant species has been shown to involve in hemostatis (Bolay, 1979; Gunter *et al*., 2002), wound healing and pain killing effects (Thankamma, 2003).

The people of the Li nationality, who are anonymous to Hainan Island in China, used it as a traditional folk medicine for the treatment of anthelmintic, carminative, cough, leprosy, and asthma. The chemical constituents of *C. gigantea* have been extensively investigated, leading to the isolation of many cardenolides (Mueen Ahmed, *et al.,* 2005; Singh and Rastogi, 1972; Lhinhatrakool and Sutthivaiyakit, 2006; Kiuchi, *et al*., 1998), flavonoids (Sen *et al*., 1992), terpenes (Anjaneyulu and Row, 1968; Thakur *et al*., 1984; Bhutani *et al*., 1992; Ali and Gupta, 1999), pregnanes (Kitagawa *et al*., 1992; Shibuya *et al*., 1992) and a nonprotein amino acid (Pari *et al.,* 1998). The latex is used as bitter, heating, oleaginous, purgative, cures, leucoderma, tumours, ascites. The latex is also used as caustic, acrid; expectorant, depilatory, anthelmintic; useful in leprosy scabies ring worm of the scalp, piles, eruptions on the body, asthma, enlargement of spleen and liver, dropsy applied to painful joint swellings (Kirtikar and Basu, 1999). The latex *C. gigantea* shows digitalis-like action on the heart. The latex also used to induce abortion, infanticide (The Wealth of India, 2004). Thus the present investigation was carried out in latex from *Calotropis gigantea* in green frog *Rana hexadactyla* for the dilatation effect for the treatment of cardiac diseases. The cardiac output was calculated by using the latex of *Calotropis gigantea.* Hence the study on further progressing may lead to the drug discovery from the terrestrial *Calotropis gigantea*.

MATERIALS AND METHODS

Collection and Preparation of samples: The latex oozing out from the plant was collected in sterile glass tube through giving 'V' shaped incision on the branches of the plant, from Portonovo and the green frog *Rana hexadactyla* weighed 25 – 30 g were collected from the Department of Pharmacy, Annamalai University, Tamil Nadu, South India, between 25 and 26 March 2006. The collected latex was diluted with distilled water to 1:10 and 1: 100 concentrations.

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Procedure: The frog was pithed and pinned up in the frog board. Incision was made in the midline of the abdomen. Pectoral gridle was removed and heart was exposed. Pericardium layer was removed and a few drops of frog ringer were sprayed over the heart, the inferior venacava was traced and a thread was inserted at the bottom of the venacava. A small cut was made inorder to inject the venous cannula, which was inturn connected to a perfusion bottle containing frog ringer. The cannula was inserted in the vein and the thread was tied, the venous pressure was adjusted by altering the height of the perfusion bottle, the effective venous pressure is the height in cm from level of the venous cannula and ringer level in the perfusion bottle. The use of Maniott's bottle helps to attain the constant pressure, start the perfusion by opening the screw clamp which was attached to the tube. The drop left from the ringer solution was collected in the beaker at every 5 minute after six readings 0.2ml and 0.4ml of latex from *Calotropis gigantea* at 1:100 concentrations into the perfusion tube was added very close to the nervous cannula. Same procedure was repeated at 1:10 concentration of the test extract.

RESULTS AND DISCUSSION

The effect of the latex from *Calotropis gigantea* in the green frog *Rana hexadactyla* on blood vessel preparation reveals a significant increase in cardiac output were given in Fig.1 flow rate i.e. 66% at 1:100 and 50% at 1:10 concentration. But there was some similarity found at 1:100 and 1:10 i.e. 0.4ml of crude extract at 1:100 and 0.2ml of crude extract at 1:10 showed same cardiac output. This denotes that the crude extract of lesser concentration showed a prominent cardiac output. The crude extract at 1:10 gave a significant cardiac output when compared with 1:100. The present study predicts that at concentration dependent manner the latex produces remarkable vasodilatation effect. The result similar to that the study of Endean *et al.*(1974) on the venom of *Conus magus*. Whether the latex produces a dilation effect upon the vascular smooth muscle also pose a further challenge. Sharma (1934) and Watt and Breyer-Brandwijk (1962) suggests an effect on the heart which showed that cardioactive glycosides were present in the latex and other parts of *C. procera*. The available evidence indicates that the prime action of latex upon cardiovascular system involves changes in the cation (Ca^{2+}/Na^{+}) permeability of the respective membrane. Therefore, the latex of *C. gigantea* is suspected to act in a similar way to *C. magus* by causing excitability of Ca^{2+} channels in heart muscle and also increase in coronary flow. Freeman and Turner (1972) stated that the increase in isotonic contractility of the isolated guinea pig heart could result from Ca^{2+} release from membrane binding site into mesoplasm. Paes de carvalho *et al.* (1969) stated that the importance of Ca^{2+} in the genesis of pacemaker potentials. The sinoatrial potentiation by

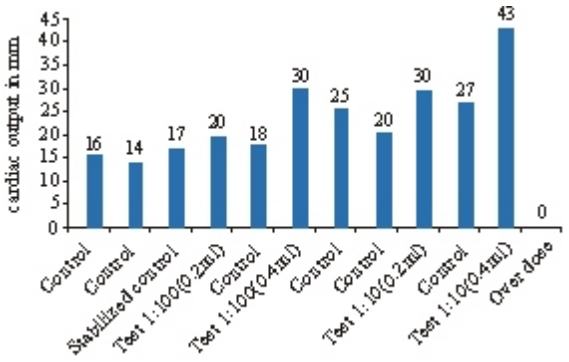

Fig. 1: Effect of latex from *Calotropis gigantea* showing the cardiac output of the frog

latex from *C. gigantea* would, therefore, consists with an increase in Ca^{2+} permeability. Thus, dilatation property is probably responsible for the observed pharmacological actions of the latex.

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