

Cardiac stimulant activity of bark and wood of *Premna serratifolia*

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Abstract

Premna serratifolia Lin., (Verbenaceae) contains alkaloids and iridoid glycoside and is believed to prevent cardiovascular disease. The stem-bark and stem-wood were extracted with 95% ethanol and distilled water. These extracts were screened for their effects by "Isolated Frog Heart Perfusion Technique" and biochemical parameters in heart tissue and serum of albino rats after administering the extracts for 7 days. The ethanol extract produced significant positive inotropic and negative chronotropic actions similar to that of digoxin on frog heart and its effect was inhibited by nifedipine and not by propranolol. A significant decrease in membrane Na⁺K⁺ATPase and Mg²⁺ATPase and an increase in Ca²⁺ATPase further confirmed its cardiotonic activity. Aqueous extract produced positive inotropic and chronotropic effects similar to that of Adrenaline and its effect was antagonized by propranolol and nifedipine. The results suggest that the ethanol extract produced cardiotonic effect and the aqueous extract produced β -adrenergic effect.

Introduction

Cardiovascular disease incurs a greater economic constraint than any other illness especially in the developing countries. It is the most common cause of death by the year 2020. The risk factors for heart disease are family history, sex, increased lipid levels, diabetes mellitus, hypertension, obesity and cigarette smoking. There is overwhelming evidence indicating hypercholesterolemia and other lipid abnormalities as major risk factors in the development of atherosclerosis and coronary heart disease. Therefore, cardiovascular disease becomes a very common problem in the affluent societies related to their life style (Trivedi, 2004).

In developed countries, coronary vascular diseases now constitute the principal cause of human mortality. Not surprisingly, therefore, this is an intensive research, not entirely devoted to treatment, but also to the prevention of these diseases. Many factors affect the complex regulation of the heart and there is a large group of drugs which will affect the heart's performance, often in a manner not directly associated with the heart muscle itself. However, the therapeutic use of drugs for the treatment of the failing heart is confined to a small group of glycosides that act directly on the heart muscle (Evans, 1996). Cardiovascular disorder leads to variety of pathological changes including endothelial, pulmonary, he-



patic, renal, endocrine, skeletal muscle abnormalities and the state of multi-organ impairment (Poole-Wilson et al., 1997). Cardiac glycosides and catecholamine have been used as the main therapeutic drugs in the treatment of coronary heart disease (Kitada et al., 1987). There is no evidence that digitalis prolongs survival of coronary heart disease patients (Tripathi, 2001), major limitations in the use of cardiac glycosides are low margin of safety, inability to retard the process which caused the heart to fail and intoxication are well documented (Beller et al., 1971). Catecholamine use is limited by its, insufficient differentiation between positive inotropic and chronotropic actions, potential arrhythmogenic properties, tachyphylaxis due to receptor down-regulation and causes a severe oxidative stress in the myocardium through free radical formation (Kitada et al., 1987). There are so many popular herbs used in traditional practices to cure cardiovascular problems. *Premna serratifolia* Lin., is having an important place in such cardiovascular medicinal herbs (Yoganarasimhan, 2000) and its synonym is *Premna integrifolia* Lin. It is known as "Munney" in Tamil, "Agnimantha" in Ayurveda and used as cardiogenic, antibiotic, antihyperglycemic (Natkarni, 1976). It is widespread throughout Micronesia and tropical Asia. Root forms an ingredient in well known Ayurvedic formulation "Dasamula" for variety of affections (Anonymous, 1972). It has shown anticoagulant activity (Gopal and Purushothaman, 1984) and the decoction exhibited antiinflammatory and antiarthritic activity (Rathore et al., 1977). However, its cardiogenic activity has not been investigated still now. Hence it was considered to evaluate the cardioactive potential and its mechanism of action.

Materials and Methods

Preparation of extracts: Fresh stem-bark and stem-wood of *P. serratifolia* Lin., was collected from The Indian Medical Practitioners Co-operative Pharmacy and Stores garden, Chennai, Tamil Nadu. Plant material was identified and authenticated (PARC/2007/71) by a Botanist, Dr. P. Jayaraman, Plant Anatomical Research Centre, Chennai. Materials were cleaned with water and dried in the shade until a constant weight was obtained. It was extracted with 95% ethanol and double distilled water in a Soxhlet extractor. Extracts were

concentrated; the percentage yield for ethanol and aqueous extracts were 7.9% and 7.7%. For pharmacological studies, since the ethanol extract was not soluble in water, it is suspended in 5% gum acacia and aqueous extract was being water soluble, hence an aqueous solution was used.

Drugs and chemicals: Digoxin, adrenaline, propranolol and nifedipine were procured from Government General Hospital, Chennai. All other chemicals used were of higher quality analytical grade.

Animals: Frogs of *Rana hexadactyla* species (procured from animal house of Madras Medical College) and male Wistar albino rats (200 g) housed in cages at $27 \pm 2^\circ\text{C}$ on a 12 hour light/dark cycle was used. The animals were fed with food and water *ad libitum*. The animals were maintained as per the norms of CPCSEA (6/243/CPCSEA/2007) and cleared by CPCSEA and institutional ethics committee (Madras Medical College).

Phytochemical screening: Ethanol extract showed positive reaction for alkaloids, glycosides, flavonoids and steroids whereas the aqueous extract showed positive reaction for alkaloids, glycosides and phenolic compounds. HPTLC profile of ethanol extract showed 10 peaks in the solvent system of n-hexane: ethylacetate (3:1) at 260 nm and aqueous extract showed 7 peaks in the solvent system of chloroform: methanol: water (7:2.6:0.4) at 260 nm.

Isolated frog heart perfusion technique (Muralidharan and Dhananjayan, 2004): Frogs were pithed and heart was exposed. The inferior vena cava was traced and cannulated for perfusing the heart with the frog's Ringer solution. (Composition of frog Ringer solution in mmol: NaCl-110; KCl-1.9; CaCl₂-1.1; NaHCO₃-2.4; NaH₂PO₄-0.06; Glucose-11.1). The basal cardiac contraction was recorded on a smoked kymographic drum after the administration of frog Ringer's solution and 5% gum-acacia. Administration of gum acacia was done to see that it did not contribute to the effects of extracts. Drugs and extracts were administered through the cannula. The average heart rate and the contraction amplitude were recorded on a smoked kymographic drum and it was found to be 64 beats/min and 8 mm respectively. The effects obtained with the drugs and extracts were transposed to the respec-

tive percentage of the basal values. Graded dose-response was recorded for each extract and the dose which caused the maximum effect was chosen as the experimental dose. The frog heart was washed with the Ringer solution after every administration of extracts and drugs till it was brought back to the normal state. The frog heart was perfused with propranolol, at 30 μ M concentration in frog Ringer solution for 60 sec followed by the administration of extracts and the recording were noted. Nifedipine, at 28.8 μ M concentration in frog Ringer solution was administered for 60 sec followed by extracts and the recordings were noted.

Biochemical studies in Wistar albino rats: Animals were randomized into 3 groups of 6 rats each. Group I was treated with gum acacia suspension i. p. for 7 days which served as control. Group II and Group III animals were treated with ethanol and aqueous extracts at a dose of 200mg/kg (approximately 1/10 of the LD₅₀) body weight i. p. for 7 days. On 8th day, all the animals from 3 groups were sacrificed. Blood was collected by cardiac puncture and heart-tissue was collected and serum was separated from the blood. The heart was washed in ice-cold saline and 100 mg of tissue was weighed and homogenized in chilled 0.1 M Tris-HCl Buffer (pH 7) and the homogenate was used for the assay of Na⁺K⁺ATPase (Bonting, 1970), Ca²⁺ATPase (Hjerten and Pan, 1983) and Mg²⁺ATPase (Ohinishi et al., 1982). Serum and homogenized samples were assayed for the clinical marker enzymes like CPK (Okinaka et al., 1961), LDH (King, 1965a), AST and ALT (King, 1965b).

Histopathological studies: A portion of heart from each group was stored in 10% formalin, for processing and sectioning. By following routine histological techniques, these samples were put into paraffin and serial cross sections of 5 μ m, which were taken from tissue blocks stained with hematoxylin and eosin. The preparations were evaluated under a photomicroscope and were photographed.

Statistical analysis: Results were expressed as mean \pm standard error for 6 animals in each group. Differences between groups were assessed by one-way ANOVA using Bonferroni test. Post hoc testing was performed for inter-group comparisons using the least significance difference

test. P-values <0.05 have been considered as significant.

Results

Ethanol extract produced significant positive inotropic and negative chronotropic effects similar to that of digoxin on frog heart and it is indicated by an increase in the force of contraction (Table I) and a decrease in the heart rate (Table II). This cardiotoxic effect of the ethanol extract was not antagonized by propranolol, whereas nifedipine treatment significantly reduced the cardiotoxic effect. There was a significant decrease in membranous Na⁺K⁺ATPase and Mg²⁺ATPase (p<0.05) and an increase in Ca²⁺ATPase (Table III) when compared with that of the control and this further confirmed its cardiotoxic effect.

Aqueous extract produced a significant positive inotropic and positive chronotropic actions similar to that of Adrenaline on frog heart and it is indicated by an increase in the force of contraction and the heart rate. Propranolol and nifedipine antagonized the effect of the aqueous extract. No significant changes were observed in membrane bound phosphatase in rat.

Both the extracts do not produce any significant changes in the levels of CPK, LDH, AST and ALT in heart and in serum samples when compared to that of the control (Table IV). Therefore, it indicated that both the extracts do not alter the physiological conditions of the heart.

Histopathological studies of both the extracts do not produce any significant pathological changes in the heart when compared to that of the control (data not shown) and therefore it is indicated that both the extracts do not alter the physiological conditions of the heart.

Discussion

Ethanol extract produced cardiotoxic effect, which was characterized by positive inotropic and negative chronotropic actions. This effect was not significantly blocked by propranolol whereas nifedipine, antagonized the effect significantly. Cardiac enzyme profile indicates that the ethanol extract exhibited powerful cardiotoxic like activ-

Table I: Effect of *Premna serratifolia* extract on force of contraction using isolated frog heart perfusion technique

	Frog Ringer		Frog Ringer + propranolol (1 mg/ml)		Frog Ringer + nifedipine (1 mg/ml)	
	Force of contraction (mm)	Change (%)	Force of contraction (mm)	Change (%)	Force of contraction (mm)	Change (%)
Digoxin	14.1 ± 0.40 ^{a e}	76.25	-	-	10.3 ± 0.68 ^{a e}	28.75
Adrenaline	21.5 ± 0.56 ^{a b d}	168.75	13.6 ± 0.33 ^{a e}	70	-	-
Ethanol extract	14.83 ± .30 ^{a c e}	75.38	14.16 ± 0.40 ^{a e}	77	10.6 ± 0.49 ^{a e}	32.5
Aqueous extract	20.5 ± 0.32 ^{a b d}	156.25	16.1 ± 0.70 ^{a c d}	101.25	13.8 ± 0.66 ^{a b d}	72.5
F value	196.219		63.836		19.448	
P value	<0.001		<0.001		<0.001	

Control value of force of contraction: 8 ± 0.06 mm. n=6. Values are mean ± SE. Values with different superscripts in a column are significantly different from each other at p<0.05 significant, by one-way ANOVA using Bonferroni test. ^acomparison with control; ^bcomparison with Digoxin; ^ccomparison with adrenaline; ^dcomparison with ethanol extract; ^ecomparison with aqueous extract

ity which manifested as a result of general decrease in the activity of Na⁺K⁺ATPase and Mg²⁺ATPase and an increase in Ca²⁺ATPase.

Most important electrolytes like sodium, potassium, calcium, magnesium and bicarbonate provide inorganic chemicals for biochemical proc-

esses as well as act at the cell membrane to allow transmission of electrochemical impulse in nerve and muscle fibers (Kokko and Tannen, 1990). The intracellular cation plays a significant role in the regulation of normal physiology and biochemistry of cardiac and smooth muscles. Dysregulation of these processes is an important factor in the

Table II: Effect of *Premna serratifolia* extract on heart rate using isolated frog heart perfusion technique

	Heart rate (per min)		
	Frog Ringer	Frog Ringer + propranolol (1 mg/ml)	Frog Ringer + nifedipine (1 mg/ml)
Digoxin	58.6 ± 0.42 ^{c e}	-	55.0 ± 0.63 ^d
Adrenaline	136.3 ± 0.80 ^{a b d e}	107.0 ± 0.70 ^{a d}	-
Ethanol extract	57.8 ± 0.67 ^{c e}	59.5 ± 0.34 ^{c e}	37.6 ± 0.54 ^{a b e}
Aqueous extract	108.2 ± 0.47 ^{a b c d}	96.3 ± 0.82 ^{a d}	55.8 ± 1.05 ^d
F value	246.27	96.30	13.22
P value	<0.001	<0.001	<0.001

Control value of heart rate: 66.0 ± 1.25 / min; n=6. Values are mean ± SE. Values with different superscripts in a column are significantly different from each other at p<0.05 significant, by one-way ANOVA using Bonferroni test. ^acomparison with control; ^bcomparison with digoxin; ^ccomparison with adrenaline; ^dcomparison with ethanol extract; ^ecomparison with aqueous extract

Table III: Effect of the extracts of *Premna serratifolia* extract on membrane bound phosphatases in rats

Groups	Na ⁺ K ⁺ ATPase	Ca ²⁺ ATPase	Mg ²⁺ ATPase
Control	0.5333 ± 0.020	0.2483 ± 0.005	0.3850 ± 0.008
Ethanol extract	0.4133 ± 0.0110 ^{A*} B*	0.3983 ± 0.0137 ^{A*} B*	0.2998 ± 0.006 ^{A*} B*
Aqueous extract	0.5110 ± 0.007 ^{B*} C*	0.2508 ± 0.0129 ^{B*} C*	0.3661 ± 0.0041 ^{B*} C*
F value	25.537	56.017	19.531
p value	<0.001	<0.001	<0.001

n=6. Values are mean ± SE. ^aComparison with control; ^bComparison with ethanol extract; ^cComparison with aqueous extract; p<0.05 significant, by one-way ANOVA using Bonferroni test. Enzyme unit: micromoles of phosphorous liberated/min/mg of protein

Table IV: Effect of the extracts of *Premna serratifolia* n the clinical marker enzymes in rats

Marker enzymes	Control		Ethanol extract		Aqueous extract	
	Heart	Serum	Heart	Serum	Heart	Serum
Creatinine phosphokinase	0.55 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	8.96 ± 0.01	0.59 ± 0.01	8.97 ± 0.01
Lactate dehydrogenas	2.92 ± 0.03	2.92 ± 0.03	2.92 ± 0.03	5.63 ± 0.02	2.97 ± 0.05	5.22 ± 0.03
Alanine transaminase	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.59 ± 0.01	0.10 ± 0.01	0.60 ± 0.01
Aspartate transaminae	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.52 ± 0.01	0.20 ± 0.01	0.52 ± 0.01

n = 6; values are expressed as mean ± SE; Enzyme units- for heart creatinine phosphokinase: μmoles of phosphorus liberated/min/mg protein, For serum creatinine phosphokinase: μmoles × 10⁻³ of phosphorus liberated/min/mg protein; Lactate dehydrogenase: μmoles × 10⁻¹ of pyruvate liberated/min/mg protein. Aminotransferases: μmoles×10⁻² of pyruvate liberated/min/mg protein.

genesis of various serious arrhythmias (Bassett et al., 1997). Various studies have reported inhibition of Na⁺K⁺ATPase activity during cardiovascular problems (Less, 1991). This inhibition of Na⁺K⁺ATPase is similar to the action of cardiac glycosides (Akera and Brody, 1997). Cardiac glycosides are specific and unique inhibitors of Na⁺K⁺ATPase at normal concentrations (10⁻⁸ to 10⁻⁹M) (Goto et al., 1992). Na⁺K⁺ATPase inhibition by cardiac glycosides leads ultimately to increase intracellular Ca²⁺ concentrations through Na⁺/Ca²⁺ exchange and an associated increase in slow inward Ca²⁺ current (Wang et al., 2002) as well as in transient Ca²⁺ current (McGarry and Williams, 1993). Ca²⁺-induced Ca²⁺ release is a general mechanism that most cells use to amplify Ca²⁺

signals (Wang et al., 2002). In heart cells, this mechanism is operated between voltage-gated L-type calcium channels (LCCs) in the plasma membrane and calcium release channel in the sarcoplasmic reticulum (Fabiato, 1985). Nifedipine is a LCC antagonist (Wang et al., 2002). Since nifedipine, blocks the cardiotoxic action of the ethanol extract significantly, the extract might have produced its action by opening the voltage sensitive slow Ca²⁺ channel. In connection with the cardiotoxic effects observed one could see a relationship that exists between the inhibitory levels of the activities of Mg²⁺ATPase and Na⁺K⁺ATPase (Chen et al., 1992). The significant rise in the level of activity of Ca²⁺ATPase might be due to the rise of cytosolic Ca²⁺ (Kelly and

Smith, 1996). Therefore this cardiotoxic action of the ethanol extract might be attributed to the phytoconstituents present in it.

Aqueous extract produced positive chronotropic and positive inotropic effects which were antagonized by propranolol indicating that these might have been mediated through β -adrenergic receptors. Nifedipine also blocks the action of the aqueous extract.

CPK is found in high concentration in skeletal muscle, myocardium and brain but not found in liver and kidney, small amounts are found in lungs not found in RB cells and its level is not affected by hemolysis. It appears to be a sensitive measure of myocardial infarction. LDH has gained much clinical interest recently and measurement of its activity in blood is considered useful in the diagnosis of certain cardiovascular disease conditions. AST level increase markedly in conditions of extensive damage to muscle especially cardiac muscles. Estimation of this enzyme is widely sought for, to confirm diagnosis of myocardial infarction. In pathological conditions, the enzymes such as CPK, LDH, AST and ALT leak from the necrotic heart cells to the serum, which are important measures of cardiac injury. These enzymes are not specific for myocardial injury individually; however, evaluation of these enzymes together may be an indicator of myocardial injury (Al-Shabanah et al., 1998; Chopra et al., 1995). Levels of CPK, LDH, AST and ALT of ethanol and aqueous extracts do not change when compared to that of the control both in the serum and also in the heart. Therefore, it confirms that both the extracts do not alter the physiological conditions of the heart when given at the dose of 200 mg/kg body weight.

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