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Cardiotonic Activity of Alcoholic Bark Extract of *Xylocarpus Granatum* with Emphasis on Its Mechanism of Action

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Abstract:*The aim of the present study was to scan and evaluate cardiotonic activity of alcoholic bark extract of Xylocarpus granatum and to establish its mechanism of action. The alcoholic extract of the bark of Xylocarpus granatum was prepared by the process of continuous extraction (soxhlation). The cardiotonic activity of the extract was evaluated on isolated normal and hypodynamic frog heart using Syme's technique. Potassium loss was estimated in the effluents collected from the isolated perfused hypodynamic heart (control and inhibitory activity of the extract was estimated using the same frog heart muscle. The extract showed dose dependent positive ionotropic effect on normal and hypodynamic frog heart, loss of potassium ions and the percentage inhibition of Na^+, K^+ ATPase activity. The percentage inhibition of Na^+, K^+ ATPase activity with digoxin at a dose of 300 µg was found to be 93.80 ± 1.98%. Similarly, the extract also showed dose dependent (2, 20, 200 mg) percentage inhibition of Na^+, K^+ ATPase activity and was found to be 35.20 ± 4.96%, 40.10 ± 1.27%, 56.74 ± 3.01% respectively. Results clearly indicated that the bark extract of Xylocarpus granatum possesses significant cardiotonic activity and follows the similar mechanism of digoxin (inhibition of Na^+, K^+ ATPase enzyme activity).*

Keywords: *Cardiotonic activity, Frog heart, Na^+, K^+ ATPase, Xylocarpus granatum.*

I. Introduction

Nature is an abundant reservoir of medicinal plants gifted by the god which has been the most productive source of leads for the development of drugs[1]. This is widely accepted to be true because most of the drugs discovered, starting from miracle drug Aspirin to Digoxin, the only life saving drug molecule available till date to treat congestive heart failure (CHF) were discovered from natural resource[2]. Congestive heart failure is a syndrome characterized by persistent inability of the ventricle to eject blood volume associated with deterioration in hemodynamic status and exercise intolerance. The drugs available to treat congestive heart failure are cardiotonic agents like digoxin and digitoxin which pose a great value in treating congestive heart failure but their efficacy remains controversial as their therapeutic margin is narrow (0.5 ng to 2.5 ng/ml for digoxin and 10 ng to 35 ng/ml for digitoxin)[3]. If the therapeutic window is low, there is a possibility for showing adverse effects and sometimes, it may be lethal. Other allopathic drugs available for congestive heart failure are diuretics, cardiac stimulants (dopamine, dobutamine), Angiotensin Converting Enzyme Inhibitors (Captopril), Angiotensin receptor blockers (Losartan), Vasodilators and Phosphodiesterase inhibitors but, they are not specific in action and produce adverse effects like dysrhythmias[4]. Further, cardiac glycosides toxicity continues to be a main problem due to their narrow therapeutic window, there by leading to deterioration in quality of life and increased incidence of mortality. Thus, there is indispensability for further research in this discipline, to discover potential leads with less toxicity and greater efficacy.*Xylocarpus granatum* belongs to the family Meliaceae, and commonly known as Cannonball mangrove. Species of *Xylocarpus granatum* are rich in tannins and flavonoid type[5] of compounds which are known for their medicinal properties and have been traditionally used by people of tropical places like East Asia, Madagascar, Tropical Australia and Polynesia. The astringent bark is used for treating ailments like dysentery, diarrhoea, some abdominal troubles and as a febrifuge[6,7]. The oil extracted from the seeds has been used as illuminant and hair oil. The Indians use the fruit to treat swellings of breast and also elephantiasis. Preliminary screening reports on various parts of the plant (leaves, bark, stem and root) were found to possess activities like antimicrobial, antimalarial, antifilarial, antiulcer, antidiarrhoeal, fungicidal, cancer and wound healing[8-15]. Keeping in view the limitations of available cardiotonics and folkloric claims on the plant, the present study has been undertaken to scan and evaluate the cardiotonic activity of *Xylocarpus granatum.*

II. Materials And Methods

2.1. Plant material

The *Xylocarpus granatum* bark was collected from Santhasramam region of Bay of Bengal, Visakhapatnam, Andhra Pradesh, India.

2.2. Preparation of alcoholic extract

Freshly collected bark of *Xylocarpus granatum* was dried under shade and dried material was milled to obtain coarse powder. The alcoholic extract of the powder was prepared by the process of continous extraction (Soxhlation), such that 1 g of alcoholic extract is equivalent to 2.35 g of crude drug.

2.3. Animals

Adult healthy frogs (Rana tigrina) of either sex weighing 200 to 250 g were selected. All studies were performed in accordance with Institutional Animal Ethics Committee (IAECNO: 439/PO/01/a/CPCSEA)

2.4. Experimental protocol

2.4.1. Effect of the extract on isolated frog heart

The frogs were sacrificed by pithing. The heart was isolated briskly and washed with saline, mounted on Syme's cannula using Syme's technique. Normal heart was run with Ringer's solution and hypodynamic heart was developed with half calcium Ringer. The alcoholic extract was made into a solution using 10% Dimethyl sulfoxide (DMSO) and the final volume was adjusted with saline to obtain various concentrations of the extracts. The extract was administered as spot doses of gradient concentrations into the Syme's cannula. The responses were recorded on Sherrington Kymograph at a speed of 0.12 mm/sec[16].

2.4.2. Estimation of Potassium loss from the isolated frog heart using flame Photometry

Effluents were collected from the isolated perfused hypodynamic heart (control and treatment groups) for the estimation of potassium ions with the aid of flame photometer (ELICO CL 361)[17].

2.4.3. Estimation of Na^+, K^+ ATP-ase activity on frog heart muscle

The frog was sacrificed and the heart was isolated rapidly and washed in ice-cold normal saline to remove any clots present. The ventricular portion was excised, minced, homogenized in 20 mM Tris-HCl buffer at 0ºC for 5 minutes, filtered and the filtrate was adjusted to contain about 10% protein by the addition of Tris-HCl buffer (pH 7.4) and 0.2 ml aliquots of the whole tissue homogenate was used as source of ATP-ase. ATP-ase activity was estimated in a reaction mixture of 1 ml containing 140 mM NaCl, 14 mM KCl, 3 mM EDTA, 20 mM Tris-HCl buffer and 1 mM EGTA. The solutions of the extracts of *Xylocarpus granatum* and digoxin were prepared using double distilled water. The enzyme (0.2 ml aliquots) was pre-incubated in the reaction mixture with 300 µg of Digoxin and gradient concentrations of *Xylocarpus granatum* respectively for 10 minutes at 31 ± 1ºC. The reaction was initiated by the addition of 0.05 ml of 30 mM Na-ATP solution and maintained at same temperature for 30 minutes with occasional shaking. The reaction was terminated by addition of 1 ml of 10% Trichloroaceticacid (TCA). The above solution was centrifuged for 15 minutes at 3500 rpm. To 1 ml of supernatant liquid, 5 ml of 10% TCA, 1 ml of ammonium molybdate solution, 0.4 ml of 1, 2, 5 tri amino nitro sulfonic acid (121 ANSA) reagents were added and the final volume was made up to 10 ml with distilled water. The difference in the specific activities in the presence and absence of Digoxin and extract was considered due to (Na^+, K^+) ATPase[18,19]. The specific activities were expressed as n Moles Pi liberated/mg protein/minute. The inorganic phosphates liberated in the reaction were determined by the method of Gomori[20].

2.5. Statistical analysis

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison tests using Graph pad Prism 5 software. A probability level of 0.001 or less was accepted as significant.

III. Results

In normal and hypodynamic hearts the alcoholic bark extract of *Xylocarpus granatum* and digoxin produced a dose dependent positive ionotropic effect and an increase in cardiac output (Table1; Fig. 1, 2, 3). The positive ionotropic effect of the extract was not blocked by Timolol. Potassium ions level in freshly prepared half calcium ringer was found to be 20.45 ± 0.29 ppm. In control group (hypodynamic heart), the K^+ levels were found to be 15.50 ± 0.31 ppm. The alcoholic bark extract and digoxin showed a dose dependent increase in K^+ ion levels in the effluents (Table 2). The percentage inhibition of Na^+, K^+ ATPase in digoxin (300 µg) and extract (200 mg) treated groups was found to be 93.80 ± 1.98% and 56.74 ± 3.01% respectively (Table 3; Fig. 4).

IV. Discussion

Cardiac activity is regulated by many mechanisms to meet the need of the circulation rate from time to time through Autonomic nervous system (ANS), renin-angiotensin system (RAS) and baroreceptors. In congestive heart failure, the defect lies in contraction coupling element due to Ca^{2+} insufficiency or improper utilization of Ca^{2+} by the cell. At this juncture, drugs which increase the intracellular Ca^{2+} will show beneficial effects. The positive ionotropic action of various drugs may be through direct stimulation of adrenergic receptors or indirect sympathomimetic action, inhibiting the phosphodiesterase III or direct action on the cardiac muscle[21] through Na^+, K^+ ATPase inhibition. In the present study, the alcoholic extract of *Xylocarpus granatum* produced a dose dependent positive ionotropic effect on isolated frog heart in normal and hypodynamic conditions. The positive ionotropic effect produced by the extract may be through cardiac stimulation or by increasing the availability of intracellular Ca^{2+} in myocytes or by varied mechanisms. The results with Timolol, a β-receptor antagonist, ruled out the involvement of β receptors by showing inability in inhibiting the positive ionotropic effect produced by the alcoholic extract of *Xylocarpus granatum*. It is reported that Digoxin increases K^+ levels in the effluent due to the inhibition of Na^+, K^+ ATPase pump[22]. It is observed that the bark extract of *Xylocarpus granatum* also increased the K^+ levels in the effluent in a dose dependent manner. This supports that the possible mechanism of the extract may be through inhibition of Na^+, K^+ ATPase pump. Further studies on Na^+, K^+ ATPase inhibitory activity revealed that the positive ionotropic effect produced by the extract is through the inhibiton of Na^+, K^+ ATPase pump as it is evidenced by the reduction in the release of inorganic phosphates from ATP. Overall, the present study clearly indicates that the positive ionotropic effects produced by the extract may be due to inhibition of Na^+, K^+ ATPase pump which simulates the action of digoxin.

V. Conclusions

The current study reveals that the alcoholic bark extract of *Xylocarpus granatum* possesses good cardiotonic activity and its possible mechanism may be through inhibition of Na^+, K^+ ATPase pump. Further studies on bioassay guided fractionation and purification may yield the bio-active molecules responsible.

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Table 1: Effect of *Xylocarpus granatum* & Digoxin on isolated normal and hypodynamic frog heart.

Sample	Force of Contraction (height in cm)		Change in response (height in cm)		% increase in response (height)		% increase in cardiac output	
	Normal Ringer	½ Ca^{2+} Ringer	Normal Ringer	½ Ca^{2+} Ringer	Normal Ringer	½ Ca^{2+} Ringer	Normal Ringer	½ Ca^{2+} Ringer
Control	1.3	0.8	-	-	-	-	-	-
X. g E 1 mg	1.4	1.1	0.1	0.3	-	30%	-	-
X. g E 2 mg	1.4	1.2*	0.1	0.4	10%	40%	10%	5%
X. g E 3 mg	1.7*	1.2*	0.4	0.4	40%	40%	15%	5%
X. g E 10 mg	2.4*	1.3*	1.1	0.5	110%	50%	20%	10%
X. g E 20 mg	2.5*	2.0*	1.2	1.2	120%	120%	25%	15%
Digoxin 50µg	-	1.0	-	0.2	-	20%	-	-
Digoxin 150 µg	-	1.1	-	0.3	-	30%	-	10%
Digoxin 250 µg	-	1.6*	-	0.6	-	60%	-	15%

Values are mean ± SEM of 3 Experiments, *p<0.001 compared with control.

X. g E: *Xylocarpus granatum* extract

Table 2: Estimation of Potassium ions (ppm) in effluent fluids of normal and hypodynamic frog heart treated with alcoholic extract and digoxin.

Sample	Normal Ringer (ppm)	Half calcium Ringer (ppm)
Ringer	20.65 ± 0.18	20.45 ± 0.29
Control (normal)	17.02 ± 0.31	15.50 ± 0.31
Alcoholic extract of *X. granatum* 1mg	17.05* ± 0.35	17.08* ± 0.33
Alcoholic extract of *X. granatum* 2 mg	18.33* ± 0.53	22.70* ± 0.24
Alcoholic extract of *X. granatum* 3 mg	20.13* ± 0.19	25.55* ± 0.46
Alcoholic extract of *X. granatum* 10 mg	25.28* ± 0.69	30.48* ± 0.35
Alcoholic extract of *X. granatum* 20 mg	31.43* ± 0.34	35.85* ± 0.68
Digoxin 50 µg	-	21.35* ± 0.41
Digoxin 150 µg	-	32.70* ± 0.87
Digoxin 250 µg	-	40.15* ± 0.62

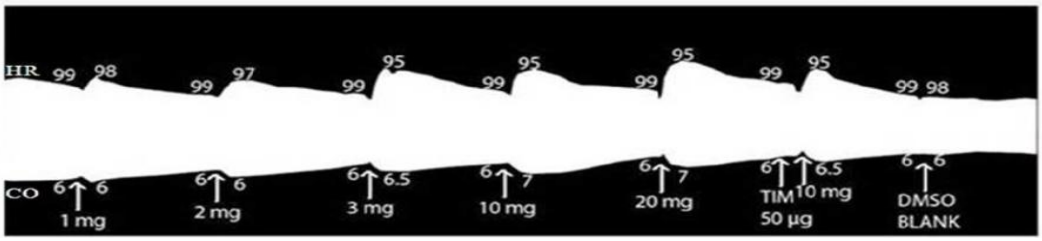
Values are mean ± SEM of 3 Experiments, *p<0.001 compared with control.

Table 3: Na^+, K^+ ATPase inhibitory activity of alcoholic bark extract of *Xylocarpus granatum* using frog heart muscle

Treatment/dose	Inorganic phosphates (n moles/ mg of tissue/minute)	% inhibition of Na^+, K^+ ATP ase
Control	12.0 ± 0.15	-----
Digoxin 300 µg	0.74* ± 023	93.80 ± 1.98
Alcoholic extract of *X. granatum* 2 mg	7.76* ± 0.50	35.20 ± 4.96
Alcoholic extract of *X. granatum* 20 mg	7.18* ± 0.06	40.10 ± 1.27
Alcoholic extract of *X. granatum* 200 mg	5.18* ± 0.32	56.74 ± 3.01

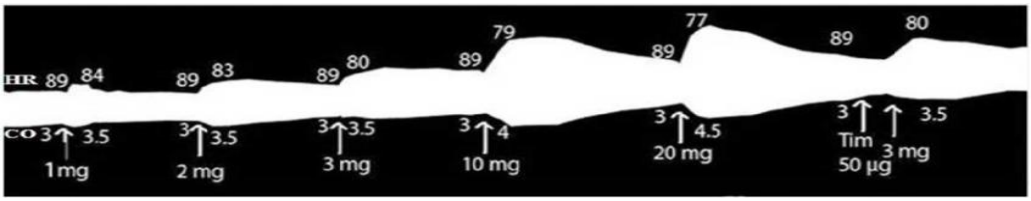
Values are mean ± SEM of 3 Experiments, *p<0.001 compared with control.

Fig. 1: Effect of alcoholic bark extract of *Xylocarpus granatum* on isolated normal frog heart



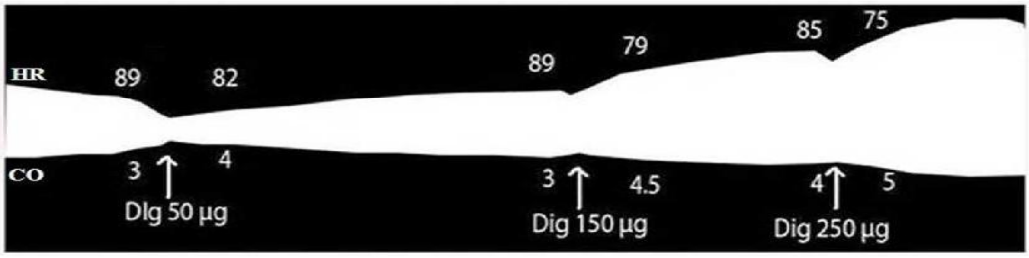
HR: Heart rate; CO: Cardiac output; Tim: Timolol

Fig. 2: Effect of alcoholic bark extract of *Xylocarpus granatum* on isolated hypodynamic frog heart



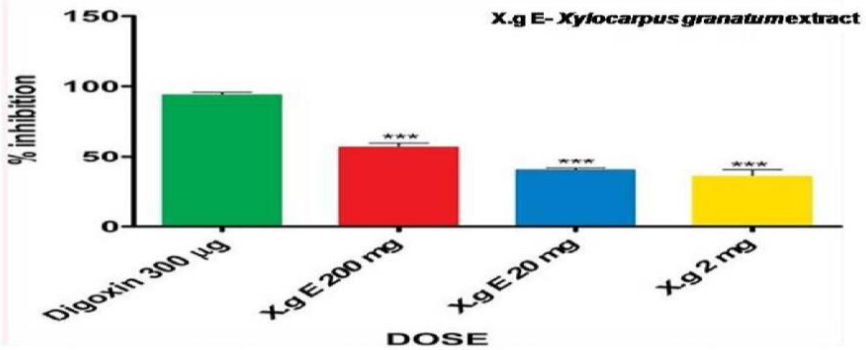
HR: Heart rate; CO: Cardiac output

Fig. 3: Effect of Digoxin on isolated hypodynamic frog heart



HR: Heart rate; CO: Cardiac output

Fig. 4: % Inhibition of Na^+, K^+ ATPase inhibitory activity of alcoholic extract of *Xylocarpus granatum* using frog heart muscle.



Results were expressed as mean ± SEM of 3 experiments.

***p<0.001, compared with Digoxin.