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Cardiotonic Activity of Aqueous Extract of *Emblica officinalis* Fruits

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Emblica officinalis is known for its medicinal and therapeutic properties from the ancient time in India and considered as a wonder fruit for health conscious population. It is one of the important herbal drugs used in Unani (Graeco-Arab) and Ayurvedic systems of medicine. It is used both as a medicine and as a tonic to build up lost vitality and vigour. In Unani medicine, it is described as a cardiotonic. Although acclaimed traditionally as cardiotonic there are not many scientific studies regarding the cardiotonic activity of *Emblica officinalis*. The present study was conducted to evaluate the cardiotonic activity of aqueous extract of *Emblica officinalis* (EAE) fruits in graded doses using frog-heart *in situ* preparation (normal and hypodynamic condition) and to explore the possible mechanism of action by conducting interaction study of EAE with β-blocker (propranolol) and calcium-channel blocker (Diltiazem). Enzyme studies such as Na^+K^+ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase were done on the heart tissue aspartate transaminases (AST), alanine transaminases (ALT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were estimated in the heart tissue and serum of albino rats after administering the extract for 7 d. The aqueous extract of *Emblica officinalis* fruits exhibited a cardiotonic activity and its possible mechanism of action is through calcium channels.

Key Words: Ionotropic activity, Chronotropic activity, Cardiotonic.

INTRODUCTION

Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine were used commonly in India and China since ancient times. Such practice has now spread to developed countries, such as the USA, in the form of health supplements.

Emblica officinalis Geartn (Syn. *Phyllanthus Emblica* Linn.) (Family, Euphorbiaceae) is commonly known as 'Adiphala' in Sanskrit and 'usirikaya' in Telugu. Triphala is a traditional Ayurvedic herbal formulation consisting of the

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dried fruits of three medicinal plants, *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis*. In Unani medicine, it is described as a cardiotonic and many polyherbal formulations containing *Emblica officinalis* as one of the main ingredient are described as a tonic for heart and brain[1]. Its fruits have been reported to possess expectorant, purgative, spasmolytic, antibacterial, hypoglycemic[2,3], hepatoprotective[4], hypolipidemic[5] properties. The *Emblica officinalis* has been reported to produce cytoprotective effects against oxidative damage by augmenting endogenous antioxidants and protecting rat heart against oxidative stress associated with ischemic reperfusion injury[6-8]. The fruit contains tannins and vitamin-C like substances in abundance. Their constituents include gallic acid, ellagic acid, phyllantidine, phyllantine, punigliconin, pedunculagin and some flavanoids[1,9,10].

Although acclaimed traditionally as cardiotonic there are not many scientific studies regarding the cardiotonic activity of *Emblica officinalis* except ethanolic fruit extract of Amla has been reported to possess slight stimulation action on isolated frog heart[1]. This study intended to investigate the cardiotonic activity and possible mechanism of action of aqueous extract of *Emblica officinalis* fruits in frogs and rats.

EXPERIMENTAL

The aqueous extract of dried fruits of *Emblica officinalis* was obtained from M/s. Laila Impex, Vijayawada, Andhra Pradesh as a gift sample and was used in the graded concentrations of 10 to 300 µg/mL. Propranolol (β-adrenergic blocker) and diltiazem (calcium-channel blocker) were supplied by Neon Laboratories, Mumbai and Dr. Reddy's Laboratories, Hyderabad respectively. All the biochemicals and chemicals used were of analytical grade.

Frogs of Rana hexadactyla species and male wistar albino rats (150-200 g) were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 ºC and 50 ± 15 % relative humidity with a 12 h light/12 h dark cycle. Rats were fed with commercial pellet diet (Rayans Biotechnologies Pvt Ltd., Hyderabad) and water *ad libitum*. The study was performed in accordance with our institutional animal ethics committee.

Frog-heart *in situ* preparation: Frogs were pithed so as to destroy the CNS but without causing any injury to heart and associated blood vessels and the heart was exposed. Then the frog was placed on the myoboard. The inferior vena cava was cannulated for perfusing the heart with the frog's Ringer solution (The composition of the frog's ringer solution in millimoles is NaCl-110; KCl-1.9; $CaCl_2$-1.1; $NaHCO_3$ - 2.4; NaH_2PO_4 - 0.06; glucose - 11.1)[11]. The basal cardiac response was recorded on a smoked kymographic drum after the administration of frog Ringer solution using starling heart lever. The drugs and extract were administered through the straub's tube along with frog Ringer solution. The frog heart was washed with the frog Ringer solution after every administration of extract and drugs till it was brought back to the normal state. The cardiac responses were recorded for a constant time

(1 min) and it was maintained throughout the study. The graded dose-response of EAE was recorded (10, 20, 40, 80, 100, 200 and 300 µg). Cardiovascular parameters like force of contraction, heart rate, rhythm and tone were determined from the kymogram throughout the study.

Hypodynamic frog-heart *in situ* preparation: The frog Ringer solution containing 1/4th the normal calcium content was used to produce a hypodynamic state of the heart[12,13]. The hypodynamic heart response and graded dose-response of EAE were recorded (10, 20, 40, 80, 100 and 200 µg).

Interaction study: This interaction study was conducted on frog-heart *in situ* preparation. The dose at which the EAE showed significant activity was chosen for this interaction study (EAE-40 µg). The frog heart was perfused with propranolol, a β-adrenergic blocker at 10 µg concentration in frog Ringer solution for 1 min followed by administration of extract and recordings were noted. Diltiazem, a calcium-channel blocker at 10 µg concentration in frog Ringer solution was administered for 1 min followed by extract and the recordings were noted.

Biochemical studies: Wistar albino rats were divided into 2 groups of 6 animals each. Group I received with normal saline, which served as control. Group 2 treated with EAE at a dose of 40 mg/kg (*ca.* 1/10th of the LD^{50}) body weight i.p. for 7 d. On the 8th day the animals were sacrificed and the blood and heart tissue were collected and the serum was separated from the blood. The heart was washed in ice-cold saline and about 100 mg of tissue was weighed and homogenized in chilled 0.1 M *Tris*-HCl buffer in Patter-Elvejhem Teflon homogenizer. The serum and homogenized samples were assayed for clinical marker enzymes like CPK[14], LDH[15] and transaminases AST and ALT[16]. Heart homogenate samples were also assayed for Na^+ K^+ATPase[17], Ca^{2+} ATPase[18] and Mg^{2+} ATPase[19].

Statistical analysis: All the frog heart *in situ* preparation results were analyzed using SPSS version-6 soft ware. One-way ANOVA and post ANOVA t-test were performed to compare in between groups. The biochemical parameters obtained were subjected to one-way ANOVA followed by Turkey's multiple comparison test. p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Frog-heart *in situ* preparation: EAE showed a biphasic response on ionotropic activity with a significant and dose dependent positive ionotropic activity (10, 20 and 40 µg) followed by non-significant and dose dependent negative ionotropic activity (80, 100, 200 and 300 µg). However, at 300 µg there was cardiac arrest and negative ionotropic activity was significant. EAE produced marked and dose dependent negative chronotropic activity. EAE produced significant increase in tone at 10, 20 and 40 µg and it produced disturbance in the rhythm of the frog heart at 200 and 300 µg. The data was presented in Table-1.

Hypodynamic frog-heart *in situ* preparation: EAE produced significant and dose dependent positive ionotropic activity on the hypodynamic heart of the frog.

At the dose of 300 µg there was temporary cardiac arrest. EAE has no effect on the chronotropic activity and tone of the hypodynamic heart. However, at 200 µg there was statistically decrease in heart rate. EAE produced slight disturbance in the rhythm at the doses of 100 and 200 µg. The data was presented in Table-1.

TABLE-1
EFFECT OF EAE ON FROG-HEART *in situ* PREPARATION AND FROG-HYPODYNAMIC HEART *in situ* PREPARATION

Treatment	Force of contraction (amplitude in mm)	Heart rate (beats/min)	Rhythm	Tone
Frog-heart *in situ* preparation				
Normal	21.02 ± 0.72	38.80 ± 2.16	Normal	Normal
EAE-10 µg	22.26 ± 1.29	37.60 ± 2.07	Normal	Increase
EAE-20 µg	24.26 ± 1.35*	37.40 ± 2.40	Normal	Increase
EAE-40 µg	26.28 ± 1.36*	36.80 ± 1.92	Normal	Increase
EAE-80 µg	20.08 ± 1.53	36.20 ± 4.14	Normal	Normal
EAE-100 µg	19.64 ± 2.41	35.00 ± 2.50*	Slight disturbance	Normal
EAE-200 µg	19.62 ± 1.61	33.20 ± 1.30*	Marked disturbance	Normal
EAE-300 µg	18.00 ± 1.94*	31.60 ± 1.81*	Total disturbance	Normal
Frog-hypodynamic heart *in situ* preparation				
Hypo dynamic heart	7.20 ± 0.83	15.20 ± 0.83	Normal	Normal
EAE-10 µg	9.20 ± 1.30*	15.20 ± 0.83	Normal	Normal
EAE-20 µg	13.00 ± 1.58*	15.00 ± 0.72	Normal	Normal
EAE-40 µg	13.60 ± 1.39*	15.30 ± 0.84	Normal	Normal
EAE-80 µg	13.54 ± 1.44*	15.40 ± 0.72	Normal	Normal
EAE-100 µg	8.60 ± 1.14	14.80 ± 1.09	Slight disturbance	Normal
EAE-200 µg	7.70 ± 0.83	12.80 ± 0.83*	Slight disturbance	Normal

*Statistically significant as compared to control, $p < 0.05$; All values are expressed as mean ± SD; EAE = Aqueous extract of *Emblica officinalis* fruits.

Interaction study: The data was presented in Table-2. The positive ionotropic activity of EAE was not antagonized by propranolol. The negative chronotropic activity was not potentiated by propranolol. These results are statistically significant. The increase in tone of the frog heart with EAE comes to normal by propranolol treatment. Propranolol has no significant effect on rhythm of EAE treated frog heart.

Diltiazem pretreatment significantly antagonized the ionotropic activity of EAE and significantly potentiated the negative chronotropic effect of EAE. Diltiazem significantly decreased the increase in tone of the EAE treated frog heart and disturbed the rhythm of the extract treated frog heart.

Biochemical studies: There was a significant decrease in membranous Na^+ K^+ ATPase, Mg^{2+} ATPase and an increase in Ca^{2+} ATPase (Fig. 1). EAE did not produce any significant changes in the levels of ALT, AST, LDH and CPK in heart and in serum samples when compared with the control group. The data was presented in Table-3.

TABLE-2
INTERACTION OF EAE AND PROPRANOLOL/DILTIAZEM ON
FROG- HEART *in situ* PREPARATION

Treatment	Force of contraction (amplitude in mm)	Heart rate (beats/min)	Rhythm	Tone
Interaction of EAE and propranolol				
EAE-40 µg	26.40 ± 1.14	36.20 ± 1.43	Normal	Increase
Propranolol-10 µg	08.40 ± 0.54[a]	12.40 ± 2.07 [a]	Disturbance	Normal
Propranolol-10 µg + EAE-40 µg	22.20 ± 3.49[*]	32.80 ± 3.12 [*]	Slight disturbance	Normal
Interaction of EAE and diltiazem				
EAE-40 µg	26.62 ± 1.44	36.40 ± 1.67	Normal	Increase
Diltiazem-10 µg	05.60 ± 1.14[a]	10.80 ± 1.09 [a]	Disturbance	Decrease
Diltiazem-10 µg + EAE-40 µg	10.70 ± 0.83[a**]	06.80 ±0.83 [a**]	Disturbance	Decrease

[a]Statistically significant as compared to EAE-40 µg, $p < 0.05$; *Statistically significant as compared to Propranolol-10 µg, $p < 0.05$; **Statistically significant as compared to diltiazem-10 µg, $p < 0.05$; All values are expressed as mean ± SD; EAE: Aqueous extract of *Emblica officinalis* fruits.

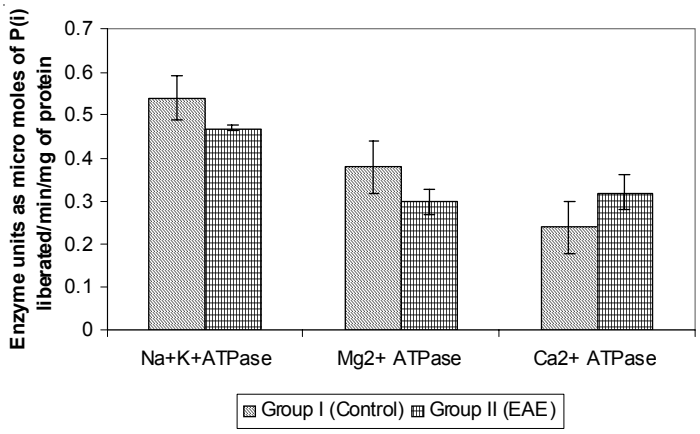

Fig. 1. Effect of EAE on heart tissue ATPases of wistar albino rats; Group II values were significant when compared to control ($p < 0.001$)

The frog-heart *in situ* preparation was found to be very convenient and exhibited reproducible results. The aqueous extract of *Emblica officinalis* (EAE) elicited powerful cardiotonic effect, which was characterized by positive ionotropic and negative chronotropic actions. This was supported by some earlier reports, which shown the ethanolic fruit extract has slight stimulant action on isolated frog heart[1]. Moreover, present statement was supported by the results obtained from hypodynamic heart *in situ* preparation and biochemical studies in rats. The mechanism behind transient cardiac arrest at 300 µg has to be studied further after purification of the *Emblica officinalis* active components.

TABLE-3
EFFECT OF EAE ON THE MARKER ENZYMES IN RATS

Marker enzymes	Groups I (control)		Group II (EAE)	
	Heart	Serum	Heart	Serum
AST	0.1940 ± 0.006	0.514 ± 0.024	0.1980 ± 0.007	0.518 ± 0.027
ALT	0.0954 ± 0.004	0.612 ± 0.032	0.0960 ± 0.006	0.616 ± 0.036
LDH	2.9400 ± 0.080	5.220 ± 0.180	3.0000 ± 0.080	5.226 ± 0.184
CPK	0.5800 ± 0.060	8.564 ± 0.322	0.6200 ± 0.040	8.580 ± 0.326

Values are expressed as mean ± SEM; NS = Non significant; EAE = Aqueous extract of *Emblica officinalis* fruits.
Enzyme Units; Aminotransferases (AST, ALT) = µmol × 10^{-2} of pyruvate liberated/min/mg protein; LDH = µmol × 10^{-1} of pyruvate liberated/min/mg protein; Heart CPK = µmol of phosphorus liberated/min/mg protein; Serum CPK = µmol × 10^{-3} of phosphorus liberated/min/mg protein.

In frogs, both α- and β-adrenoceptors are known to be present in the myocardium[20]. The cardiotonic activity of EAE was not antagonized by propranolol (β-adrenergic blocker). These results indicating that β-adrenergic receptors are not involved in cardiovascular effects of EAE. This statement was supported by earlier report that the hypotensive effect of *Emblica officinalis* was not mediating through β-adrenergic receptors in dogs[21].

The cardiac enzyme profile indicates that EAE exhibited cardiotonic activity which manifested as a result of general decrease in membranous Na^+ K^+ ATPase, Mg^{2+} ATPase and an increase in Ca^{2+} ATPase. This inhibition of Na^+ K^+ ATPase is similar to the action of cardiac glycosides[22]. Na^+ K^+ ATPase inhibition by cardiac glycosides leads ultimately to increase intracellular Ca^{2+} concentrations through Na^+ / Ca^{2+} exchange and an associated increase in slow inward Ca^{2+} current[23] as well as in transient Ca^{2+} current. Ca^{2+} induced Ca^{2+} release is a general mechanism that most cells use to amplify Ca^{2+} signals[23]. In heart cells, this mechanism is operated between voltage-gated L-type calcium channels (LCCS) in the plasma membrane and calcium release channel, commonly known as ryanodine receptors in the sarcoplasmic reticulum[24,25]. Diltiazem is LCC antagonist[26]. Since Diltiazem, blocks the cardiotonic action of EAE significantly, the extract might have produced its action by opening the voltage sensitive slow Ca^{2+} channel. In connection with the cardiotonic effects observed one could see a relationship that exists between the inhibitory levels of the activities of Mg^{2+} ATPase and Na^+ K^+ ATPase[27]. The significant rise in the level of activity of Ca^{2+} ATPase might be due to the rise of cytosolic calcium ions[28].

Conclusion

Based on the results, the aqueous extract of *Emblica officinalis* fruits produced significant cardiotonic activity. This cardiotonic activity was not mediated by β-receptors and the possible mechanism of action is through calcium channels. A limited scope of this study however needs further elaboration by conducting on other species and elucidating the effects of the active principles involved, in future studies.

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