

POSITIVE INOTROPIC ACTIVITY OF AQUEOUS EXTRACT OF PERICARP OF PUNICA GRANATUM ON ISOLATED FROG'S HEART

P. RAVINDRA BABU^{1*}, HAROLED PETER P. L¹, M. ANKAIAH², P. HEMANTH SAIRAM², M. RAMESH²

¹KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, A. P, 520 010, ²Department of Pharmacology, Sree College of Pharmacy, Nayakula Gudem (vil), Kothagudem (Mandal), Khammam district, A. P, India, 507120.

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ABSTRACT

The present paper describes the cardiac effect of aqueous extract of *Punica granatum* pericarp using isolated frog heart. Different parts of this species are used in India for a wide range of medicinal purposes. Cardiotonic activity was studied on both normal and hypodynamic hearts. Calcium free Ringer solution was used as vehicle for administration of aqueous extract of *punica granatum* pericarp as a test extract and digoxin as a standard. A significant increase in height of force of contraction (positive inotropic effect) and decrease in heart rate (negative chronotropic effect) at a very low concentration was observed with test extract as compared to the same dose of a standard digoxin. The present results indicated that a significant increase in height of force of contraction with decrease in heart rate was observed as the dose of test extract increased. The test extract produced cardiac arrest at 4 mg/ml, a higher concentration, as compared to standard, digoxin (15µg/ml). Compared to digoxin, a drug with narrow therapeutic window, *punica granatum* pericarp showed wide therapeutic window.

Keywords: Chronotropic effect, Pericarp, CHF, Hypodynamic heart, Cardiotonic.

INTRODUCTION

Cardiovascular diseases are leading cause of death and lost of life expectancy in most developed countries and also one of the most important causes of morbidity and health care costs^{1, 2}. In the United States, congestive heart failure (CHF) is a common cardiovascular condition with approximately 5,700,000 individuals diagnosed in 2006. Complications associated with heart failure include increased emergency room visits, hospitalizations, and a risk for sudden cardiac death. In 2005, CHF was noted as the second most heart-related condition listed as the reason for admission to the hospital through the emergency department. Thus, the appropriate treatment of CHF is essential for those diagnosed with this disease³.

Congestive heart failure (CHF) is one of the leading causes of death in the Western world^{4, 5}. The prevalence of CHF in developed countries is 1%-2% of the adult population overall, and approximately 6%-10% in the elderly^{6, 7}. It is now the leading cause of hospitalization in the population older than 65 years. With an aging population, the prevalence of CHF is likely to rise in coming decades, and increasing costs will be attributable to both the expanding number of patients and the greater sophistication of therapy. Despite advances in the treatment of CHF, the prognosis from the time of diagnosis is still poor.

Recent therapeutic advances in the management of congestive heart failure (CHF) on the basis of systolic dysfunction are reflected in the large number of studies on CHF published or presented at meetings in the last two years. While some therapies previously thought to be promising have been shown to be efficacious, others are associated with adverse outcomes, and new therapeutic

Options have emerged which hold significant potential for the future.

Heart failure (HF) is a complex pathophysiological syndrome that arises from a primary defect in the ability of the heart to fill and/or eject blood sufficiently. The clinical manifestations of HF result from the primary myocardial insult (most commonly coronary artery disease, hypertension or genetic factors) and the attendant sequelae. In general, the primary insult brings about an increase in myocardial wall stress that induces an orchestrated cascade of remodeling stimuli within the heart, as well as neurohormonal, vascular, renal and skeletal muscle alterations. Within this conceptual framework, chronic HF is generally a progressive disorder that results from continued left-ventricular remodeling and a progressive loss of function. It should be noted

that abnormalities of systolic and/or diastolic function can result in similar symptoms and they might share some common underlying mechanisms. Within the clinical HF spectrum, the severity of symptoms often fluctuates considerably. Acute episodes of HF due to cardiac decompensation often in the setting of an acute inter current illness such as myocardial infarction, arrhythmia or sepsis are also well recognized and require distinct forms of clinical management.

Punica granatum L. (Punicaceae), commonly known as pomegranate, is a shrub or a small tree native to the Mediterranean region is widely used for therapeutic formulae, cosmetics, and food seasoning. Pomegranate, also easily acquired from traditional medicine markets, was usually used as an astringent agent⁸, for eliminating parasites⁹ and as an antipyretic. The pharmacological functions of pomegranate include antioxidation¹⁰ antitumour¹¹, antihepatotoxicity¹², anti diarrhoeal¹³ antilipoperoxidation¹⁴ and anti bacteria properties¹⁵. In hematology, pomegranate could reduce the common carotid intima medium thickness, thus lowering blood pressure and decreasing low-density lipoprotein (LDL) oxidation and the incidence of heart disease¹⁶. In our previous studies, we found that extract from the dried peel of the pomegranate could significantly inhibit NO production. The fruit juice of *punica granatum* is also reported for cardiotonic activity¹⁷. We studied cardiotonic activity of *punica granatum* pericarp; it was shown better effect than fruit juice.

MATERIALS AND METHODS

Standard Drug: Digoxin

Test drug: Aqueous extract of *Punica granatum* pericarp

Physiological solutions: Ringer Solution and hypodynamic ringer solution

Animal: Frog

Instruments: Sherrington Rotating Drum, Sterling's heart lever

Preparation of extract

The fresh fruits of *Punica granatum* were collected from local market and it was authenticated by B. Satyanarany, Lecturer in Govt. Degree College, kothagudem, Khammam district. One specimen was preserved in Department of Pharmacognosy of our institute for the reference. The fruits were washed thoroughly to remove adhered material. The pericarp of fruit was removed carefully and grind thoroughly in mixer. 1 gm of powder was mixed with 100 ml

distilled water with the help of magnetic stirrer for half an hour. The material was filtered through Whatman filter paper no.40 and filtrate was collected. The prepared infusion was diluted with the help of distilled water in varying proportion and labeled as follows:

- T1- Undiluted filtrate
- T2- 1:1 (filtrate: distilled water)
- T3- 1:2 (filtrate: distilled water)
- T4- 1:4 (filtrate: distilled water)

All the preparations were evaluated for their cardiotoxic activity by using isolated frog heart assembly. The rate and force of heart contraction was determined.

Preparation of digoxin solution

Digoxin ampoules (Sun Pharma Ltd.) were purchased from local pharmacy. Various different dilutions were made with distilled water and labeled as follows:

- S1- 25 µg/ml,
- S2- 50 µg/ml.

Above prepared samples were evaluated for their cardiotoxic activity and treated as standard.

Preparation of hypodynamic ringer solution

Hypodynamic ringer solution was prepared by using standard method (Kulkarni S K, 1993)¹⁸

Evaluation of cardiotoxic activity

The frog of species *Rana tigrina* was pithed and pinned it to the frog board. A midline incision was given on the abdomen, the pectoral girdle was removed and the heart was exposed. The pericardium was carefully removed and put a few drops of hypodynamic frog ringer over the heart. The inferior venacava was traced, put a thread around it and given a small cut in order to insert the venous cannula. The cannula was inserted in the vein and the thread was tied to assure the cannula in place which is in turn connected to a saline bottle containing hypodynamic frog ringer solution. A small cut in one of the aorta was given for the ringer to come out. Heart was isolated and attached to the stand with moderate flow of ringer. A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread attached to the hook; it was tied to the free limb of the Sterling's heart lever which was fixed to a stand. A proper tension was adjusted by altering the height of the lever^{18, 19}. The normal heart rate was noted. All test samples that is A1, A2, A3, A4, B1 and B2 were administered in different doses viz. 0.1 ml, 0.2 ml, 0.3 ml respectively. The rate and force of heart contraction were noted as given in the following figures and tables.

RESULTS



Fig. 1: Effect of Standard (S1) on Hypodynamic heart

Table 1: Heart rate and change in force of contraction with S1 on hypodynamic heart

S. No.	Drug	Dose(ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	S1	0.1	32	Normal
3	S1	0.2	31	Slight increase
4	S1	0.3	31	Slight increase

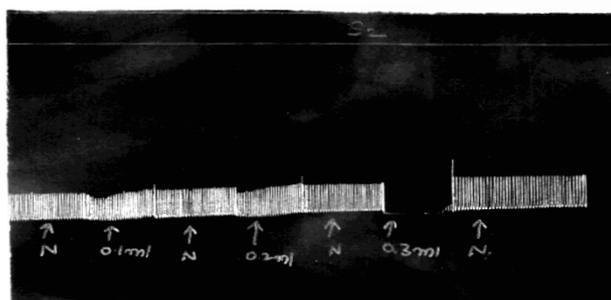


Fig. 2: Effect of Standard (S2) on Hypodynamic heart

Table 2: Heart rate and change in force of contraction with S2 on hypodynamic heart

S. No.	Drug	Dose(ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	S2	0.1	31	Slight increase
3	S2	0.2	31	moderately increase
4	S2	0.3	10	Cardiac arrest

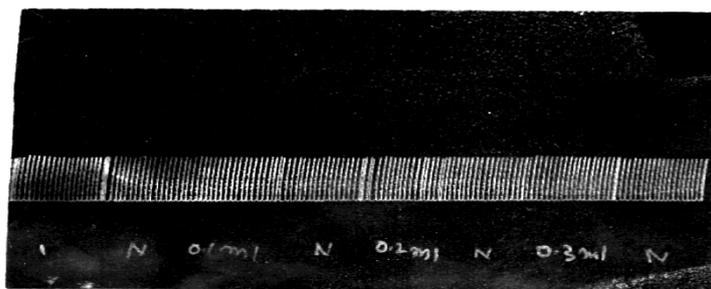


Fig. 3: Effect of Test (T4) on Hypodynamic heart

Table 3: Heart rate and change in force of contraction with T4 on hypodynamic heart

S. No.	Drug	Dose(ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	T4	0.1	32	Normal
3	T4	0.2	31	Slight increase
4	T4	0.3	31	Slight increase

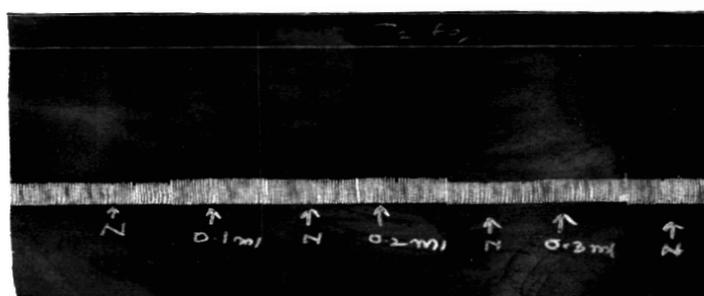


Fig. 4: Effect of Test (T3) on Hypodynamic heart

Table 4: Heart rate and change in force of contraction with T3 on hypodynamic heart

S. No.	Drug	Dose(ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	T3	0.1	31	Normal
3	T3	0.2	31	Slight increase
4	T3	0.3	31	Slight increase

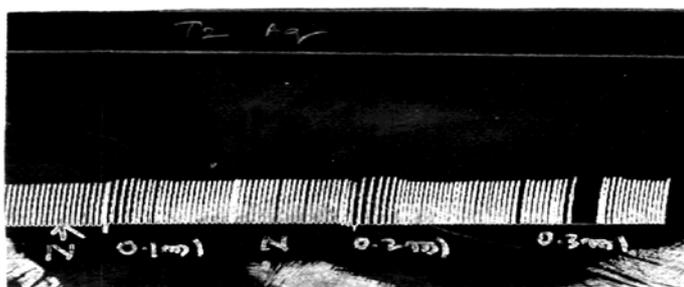


Fig. 5: Effect of Test (T2) on Hypodynamic heart

Table 5: Heart rate and change in force of contraction with T2 on hypodynamic heart

S. No.	Drug	Dose(ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	T2	0.1	31	Slight increase
3	T2	0.2	30	moderately increase
4	T2	0.3	30	moderately increase

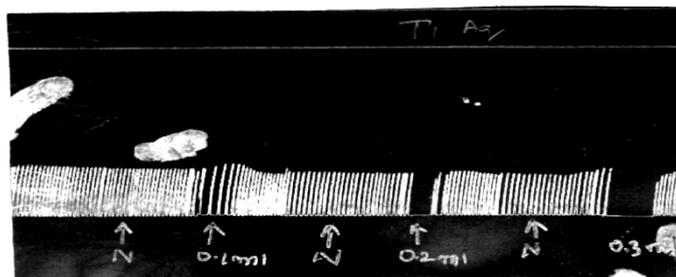


Fig. 6: Effect of Test (T1) on Hypodynamic heart

Table 6: Heart rate and change in force of contraction with T1 on hypodynamic heart

S. No.	Drug	Dose (ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	T1	0.1	31	Slight increase
3	T1	0.2	30	moderately increase
4	T1	0.3	5	Cardiac arrest

DISCUSSION

Kymograph obtained indicates that even lower doses of test extract give a significant increase in height of contraction. The dose at which digoxin showed cardiac arrest was 0.2 mg and test extract showed a therapeutic effect in the range of 0.25 - 2 mg without any cardiac arrest. Hence, as compared to digoxin, test extract showed wide therapeutic index. We all know the adverse effects shown by digoxin and difficulty in its dose adjustments. Also, in the market, there is still no safer alternative for digoxin and it is considered as a sole drug for the treatment of congestive cardiac failure. From the above-shown observations, the limitation of using digoxin can be overcome by using the aqueous extract of punica granatum pericarp which has been found to have excellent cardiotoxic activity with the wide therapeutic index as compared to digoxin. Hence, test extract can be a safe alternative to digoxin in congestive cardiac failure.

In conclusion, we showed that punica granatum pericarp concentration dependently augments myocyte contraction by may be enhancing SR function. The cardiotoxic effect of punica granatum pericarp is consistent with the therapeutic property of punica granatum pericarp used in ayurvedic medicine. Punica granatum pericarp is a promising and relatively safe cardiotoxic that can be beneficial to the healthy heart and the inotropic therapy for chronic heart failure. The method of administration and/or selective omission of hydrophobic components from pericarp powder could be crucial to the efficacy and safety of punica granatum pericarp in cardiac therapy. Further investigation is necessary for evaluation of traditional uses and phytochemical nature of the constituents that are responsible for cardiotoxic activity. This is the preliminary study and if proper constituents responsible for the effects are isolated, and in turn, if they can be synthesized, then the drug can add its value in the market.

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