www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Cardiotonic activity of aqueous extract of *Terminalia chebula* bark on isolated frog's heart

Ravindra Babu P*[1], Naveen Babu K[1], Satish Chandra[2], U. Bhaskar[2], G. Lakshman[2], J. Venkatesh[2]

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

ABSTRACT

The present study was undertaken to evaluate cardiotonic activity of aqueous extract of stem bark of *Terminalia chebula*. Different parts of this species are used in India for a wide range of medicinal purposes. Cardiotonic effect of aqueous extract of stem bark of *Terminalia chebula* was studied by using isolated frog heart perfusion technique (IFHP). Cardiotonic activity was studied on both normal and hypodynamic hearts. Calcium free Ringer solution was used as vehicle for administration of aqueous extract of *Terminalia chebula* as a test extract and digoxin as a standard. A significant increase in height of force of contraction (positive inotropic effect) no change in heart rate 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 was observed as the dose of test extract increased. The test extract produced cardiac arrest at 3 mg/ml, a higher concentration, as compared to standard, digoxin (15µg/ml). Compared to digoxin, a drug with narrow therapeutic window, *Terminalia chebula* showed wide therapeutic window.

Keywords: Cardiotonic; Hypodynamic heart; Positive inotropic effect; Terminalia chebula; Congestive heart failure

INTRODUCTION

Heart failure is a common and serious condition associated with high morbidity and mortality (Satoskar R S, 2005). Chronic heart disease ultimately leads to heart failure (HF), and the incidence of HF increases with age (Rosamond, 2007). Inotropic therapy to enhance cardiac contractile function for HF is still a significant component of the management of HF over decades (Katz et al, 2008). Current inotropic therapy has been associated with increased mortality to various degrees after long-term treatment via a variety of mechanisms, including arrhythmia and apoptosis (Stump, 2000). The toxicity of cardiotonic in chronic therapy has hampered the therapeutic value. Thus improvement of inotropic therapy remains one of main aims of the management of HF particularly to the patients who cannot benefit from hemodynamic therapies (Stevenson, 1998, Braunwald, 2008). It is generally accepted that the ionic environment of cell profoundly affects the cellular responses of the tissue. For example, the presence of sodium ions in the extracellular medium is necessary for the maintenance of the normal function in a variety of excitable tissues including heart. In several tissues it has been shown that sodium ions may compete with calcium ions, required for excitation-contraction coupling.

Cardiac glycosides are still the most important drugs in the treatment of congestive heart failure (CHF). Their exact mechanism of action is unknown, however it is accepted that they finally lead to an increase in the amount of intracellular Ca^{2+} to react with the contractile proteins. In cardiac tissue, the most important regulator of Ca^{2+} homeostasis is sarcoplasmic reticulum(SR), which serves as a sink for Ca^{2+} ions during relaxation and as a Ca^{2+} source during contraction (Lamb, 2009). Cardiac glycosides produce the positive inotropic action by inhibiting Na-K ATPase pump and hence facilitating the Calcium influx (Konschagg, 1973). It is widely known that a number of inotropic interventions share a common mechanism that governs the availability of Ca^{2+} ions at some sites critical for cardiac contraction.

Numbers of deaths in industrial world are increasing due to cardiac disease. Cardiac diseases are emerging as single largest contributors for morbidity in India. Cardiac glycosides and catecholamines are agents of choice in treatment of congestive cardiac failure (CCF) (Tripathi KD, 2004) but cardiac glycosides (e.g. digoxin) have narrow therapeutic index and hence cause many a times intoxication. Despite of the advancement of knowledge in understanding the basic pharmacology of

* Corresponding Author
Email: ravindrapingili@gmail.com
Contact: +91-9885589543
Received on: 06-10-2011
Revised on: 16-02-2012
Accepted on: 17-02-2012

cardioactive drugs glycosides still have its adverse effects in terms of toxication (Satoskar RS, 1999) hence, there is a need for new drug research with wide therapeutic index and good cardiac activity, and by this aim, we have chosen *Terminalia chebula* plant and evaluated its cardioactive potential.

The medicinal properties of *Terminalia chebula* Retz. (Combretaceae) have been known from ancient times and were described by Charaka in his text "Charaka Samhita" (Gandhi and Nair, 2005) and has traditionally been used as a popular folk medicine for homeostatic, antitussive, laxative, diuretic, and cardiotonic treatments (Singh C, 1990 & Barthakur N. N, 1991). *T. chebula* exhibits *in vitro* antioxidant and free radical-scavenging activities (Cheng H. Y, 2003). Its antimicrobial, antiviral (Yukawa T. A, 1996, Ahn M. J, 2002) antimutagenic (Kaue.S, 2002, Karur.S, 1998) and antidiabetic (Gandhipuram Periasamy Senthil Kumar, 2006) activities have been reported. The fruit powder of *Terminalia chebula* is used in India to treat several diseases ranging from digestive, coronary disorders to allergic and infectious diseases like cough and skin disorders (Barthakurand Arnold, 1991; Chattopadhyay and Bhattacharyya, 2007). The water or ethanolic extracts of the powder are used for treating diseases associated with oxidative stress as well as the cancerous diseases (Lee, 1995; Saleem, 2002). Its aqueous extract was reported to have free radical scavenging and radio-protector properties (Naik, 2004). Water soluble fraction of *Terminalia chebula* fruit was reported to have strong anti-anaphylactic actions, anti-inflammatory and analgesic properties (Shin, 2001; Chattopadhyay and Bhattacharyya, 2007). Although, fourteen hydrolysable tannins and related compounds were isolated from methanolic extract of the fruits of *Terminalia chebula* (Han, 2006), the nature of molecules involved and the biochemical nature of action of the constituents of the fruit in treating these diseases have not been investigated.

MATERIALS AND METHODS

Standard Drug: Digoxin

Test drug: Aqueous extract of *Terminalia chebula* bark

Physiological solutions: Ringer Solution and hypodynamic ringer solution

Animal: Frog (*Rana tigrina)*

Instruments: Sherrington Rotating Drum, Sterling's heart lever

Preparation of extract

The bark of *Terminalia chebula* was collected from Kothagudem forest, Khammam district, A.P, India. It was authenticated by B. Satyanaranaya, Lecturer in Govt. Degree College, kothagudem, Khammam district. One specimen was preserved in Department of Pharmacognosy of our institute for the reference. The bark was washed thoroughly to remove adhered material and fine powder was made by using hand grinder. 1gm of powder was mixed with 100ml 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:

A1-Undiluted filtrate

A2-1:1 (filtrate: distilled water)

A3-1:2 (filtrate: distilled water)

A4-1:4 (filtrate: distilled water)

All the preparations were evaluated for their cardiotonic 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 - 25µg/ml

S2 - 50µg/ml

Preparation of hypodynamic ringer solution

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

Evaluation of cardiotonic 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 (Kulkarni SK, 1993, Kale SR, 2003). The normal heart rate was noted. All test samples that is A1, A2, A3, A4, S1 and S2 were administered in different doses viz. 0.1ml, 0.2ml, 0.3ml respectively. The rate and force of heart contraction were noted as given in the following figures and tables.

RESULTS AND 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 Terminalia chebula bark which has been found to have excellent cardiotonic activity with the wide therapeutic index as compared to digoxin. Hence, test extract can be a safe alternative to digoxin in congestive cardiac failure. Free radicals play a main role in the prognosis of cardiovascular diseases, e.g. Free radicals cause endothelial dysfunction and activation of macrophages leading to atherosclerosis. In myocardial infraction, free radicals cause ischemic reperfusion injury and myocyte necrosis. *Terminalia chebula* bark aqueous extract was reported to have free radical scavenging activity (Naik, 2004) and hence, the plant poses itself as a substance for the prevention of cardiovascular diseases.

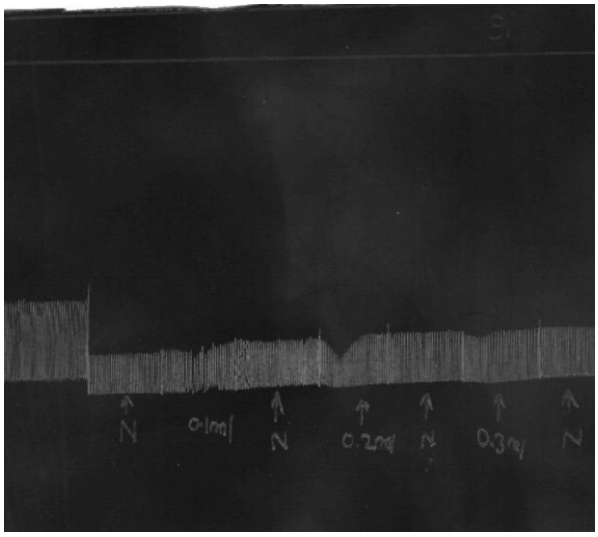

Figure 1: Effect of standard (S1) on hypodynamic heart

Table 1: Effect of S1on heart rate and change in force of contraction of hypodynamic heart

S. NO	Drug	Dose (ml)	Beats/min	Change in force
1	Control	0.0	32	Normal
2	B1	0.1	32	Normal
3	B1	0.2	31	Slight increase
4	B1	0.3	31	Slight increase

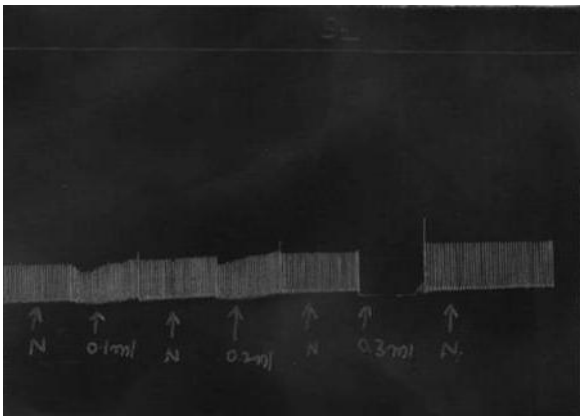

Figure 2: Effect of standard (S2) on hypodynamic heart

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

S. NO	Drug	Dose (ml)	Beats/min	Change in force
1	Control	0.0	32	Normal
2	B2	0.1	31	Slight increase
3	B2	0.2	31	Moderately increase
4	B2	0.3	10	Cardiac arrest

Figure 3: Effect of test (A4) on hypodynamic heart

Table 3: Effect of A4 on heart rate and change in force of contraction of hypodynamic heart

S. NO	Drug	Dose (ml)	Beats/min	Change in force
1	Control	0.0	32	Normal
2	A1	0.1	32	Normal
3	A1	0.2	31	Slight increase
4	A1	0.3	31	Slight increase

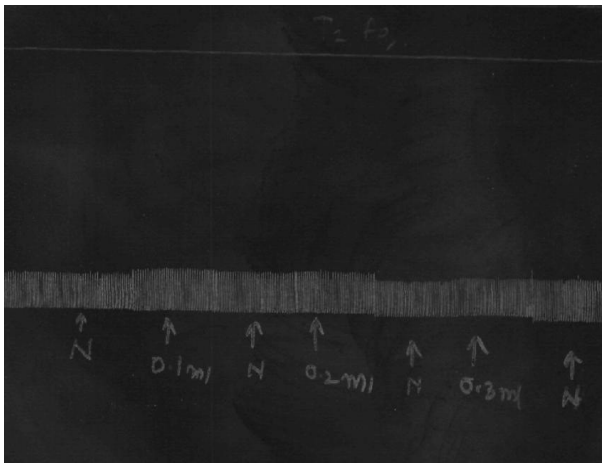

Figure 4: Effect of test (A3) on hypodynamic heart

Table 4: Effect of A3 on heart rate and change in force of contraction of hypodynamic heart

S. NO	Drug	Dose (ml)	Beats/min	Change in force
1	Control	0.0	32	Normal
2	A2	0.1	31	Normal
3	A2	0.2	31	Slight increase
4	A2	0.3	31	Slight increase

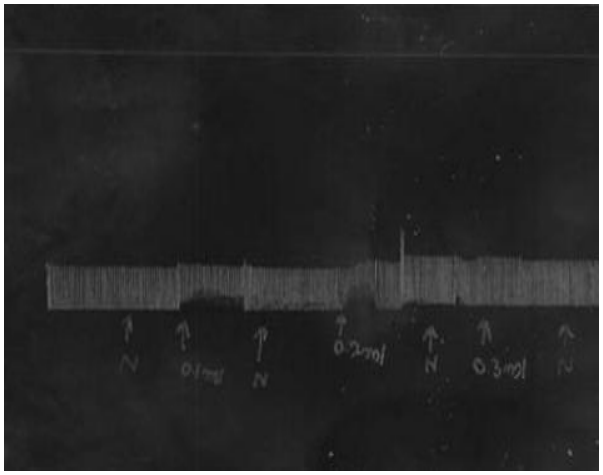

Figure 5: Effect of test (A2) on hypodynamic heart

Table 5: Effect of A2 on heart rate and change in force of contraction of hypodynamic heart

S. NO	Drug	Dose (ml)	Beats/min	Change in force
1	Control	0.0	32	Normal
2	A3	0.1	31	Slight increase
3	A3	0.2	31	Moderately increase
4	A3	0.3	31	Moderately increase

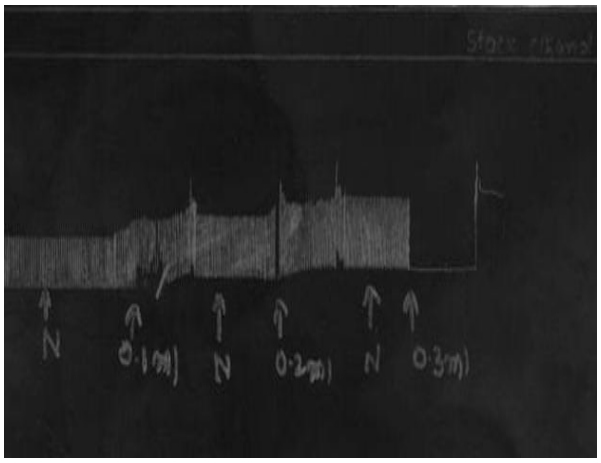

Figure 6: Effect of test (A1) on hypodynamic heart

Table 6: Effect of A1 on heart rate and change in force of contraction of hypodynamic heart

S. NO	Drug	Dose (ml)	Beats/min	Change in force
1	-	Normal	32	Normal
2	A4	0.1	31	Slight increase
3	A4	0.2	31	Moderately increase
4	A4	0.3	05	Cardiac arrest

CONCLUSION

In conclusion, we showed that *Terminalia chebula* dose dependently augments myocyte contraction by may be enhancing SR function. The cardiotonic effect of *Terminalia chebula* is consistent with the therapeutic property of *Terminalia chebula* bark used in ayurvedic medicine. *Terminalia chebula* is a promising and relatively safe cardiotonic 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 bark powder could be crucial to the efficacy and safety of *Terminalia chebula* bark in cardiac therapy. Further investigation is necessary for evaluation of traditional uses and phytochemical nature of the constituents that are responsible for cardiotonic 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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