INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

CARDIOTONIC ACTIVITY OF ISOLATED CARDIAC GLYCOSIDE FROM THE FRUITS OF *CORCHORUS AESTUANS* LINN

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Article Received on: 10/04/12 Revised on: 23/05/12 Approved for publication: 06/06/12

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ABSTRACT
Preliminary phytochemical investigation of various extracts of fruits of *Corchorus aestuans* Linn showed the presence of cardiac glycosides, triterpenes, carbohydrates, saponins, flavonoids, tannins and fatty acids. An attempt has been made to isolate cardiac glycoside and perform cardiotonic activity of the same. The cardiotonic effect was studied by using isolated frog heart perfusion technique (IFHP). Ringer solution without calcium was used as a vehicle for administration of isolated cardiac glycoside as test and digoxin as standard. A significant increase in the height of force of contraction (positive inotropic effect) and decrease in heart rate (negative chronotropic effect) was observed at smaller doses (0.4 mg). The effect increased as dose was increased. The test compound had not produced cardiac arrest even at a dose of 2 mg, a higher concentration as compared to standard, digoxin that showed cardiac arrest at dose of 0.2 mg. Hence, as compared to standard, test drug showed wide therapeutic index.
KEY WORDS: Cardiotonic, Isolated frog heart perfusion technique, Digoxin, Isolated cardiac glycoside

INTRODUCTION

Numbers of deaths in industrial world are increasing due to cardiac disease. Cardiac glycosides and catecholamine are agents of choice in treatment of congestive cardiac failure (CCF) [1]. But cardiac glycosides (e.g. digoxin) have narrow therapeutic index and hence cause many a times intoxication. Despite continuing advances in understanding the basic pharmacology of cardiac glycosides, digitalis intoxication remains a common clinical problem.[2] It necessitates research for new nature based drugs which increase cardiac muscle contractility with a broad therapeutic index and good cardiac activity, and by this aim, we have chosen *Corchorus aestuans* Linn plant and evaluated its cardioactive potential.

Corchorus aestuans Linn (Syn. *Corchorus acutangulus* Lam.), family-Tiliaceae, is an annual herb occurring through out the hotter parts of the Subcontinent, Indo-china, Australia, Tropical Africa, West Indies, and Central America.[3,4] It is popularly known as Jute. The roots and leaves are said to cure gonorrhea and used in making an injection for urethral discharge. The seeds are stomachic and used in pneumonia.[5,6]

The plant is said to possess anticancer, antipyretic, anticonvulsant, stomachic and digitalis glycosides like action.[7] Many cardiac glycosides have been isolated from the seeds of the above said plant. Hence in the present study an attempt has been made to isolate new cardiac glycoside from the fruits of *Corchorus aestuans* Linn and perform the cardiotonic potential of the isolated compound.

MATERIALS AND METHODS

Collection and identification of the plant material

The plant *Corchorus aestuans* Linn was collected from the local area of Kheda district, Gujarat, India, in the month of August 2009 and its authentication was confirmed by Dr. M. S. Jangid, Botany Department, Sir P. T. Science College, Modasa, Gujarat, India. Herbarium of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Dist. Panchmahal, Gujarat, India for future reference.

Preparation of the extract

1kg of 40# powdered drug was extracted by heating for 4 hour under reflux with 3 litre of methanol, with the addition of 500ml of 10% lead-(II)-acetate solution. After cooling and filtration the solution was extracted by shaking with three 1.5 litre quantities of dichloromethane (3:2); shaking must be gentle to avoid emulsion formation. The combined lower phases were filtered over anhydrous sodium sulphate and evaporated to dryness (5.2g yield). The white colored amorphous residue was dissolved in 20ml dichloromethane (3:2). The extract was subjected to qualitative chemical tests to identify phytoconstituents. Thin layer chromatographic studies were carried out for the above extract to confirm the presence of phytoconstituents detected in qualitative chemical tests.[8]

Isolation of cardiac glycosides

The alcoholic extract prepared by above procedure was subjected to TLC study to confirm the presence of cardiac glycosides. Ethyl acetate: methanol: water (100:13.5:10) was determined as the solvent system for the TLC of cardiac glycosides. After spraying the plate with Antimony-III-chloride reagent, followed by heating at 110˚C for 5-6 minutes was observed in visible light and UV light (365 nm). The plate showed five spots. The R_f values and color of the spots were recorded which are shown in [Table 1]. The extract was then subjected to column chromatographic study by using ethyl acetate as elution solvent for further purification. Each fraction of 25ml at the rate of 20 drops/minute was collected and tested on TLC plate using ethyl acetate: methanol: water (100:13.5:10) as mobile phase. The fractions (17-93) showing single spot with same R_f value and color were mixed, evaporated and again tested on TLC to confirm their purity. This way all the five compounds of the extract which had resolved on TLC plate were separated. All the five compounds were tested for presence of cardiac glycosides by Keller-Killiani's test and Libermann Burchard's test and their $UV_{\lambda max}$ were also taken. The results are shown in [Table 2].[9]

Cardiotonic activity

Animals

Frogs of *Rana tagrina* species were obtained from the animal house of Zydus Research Centre, Ahmedabad, Gujarat, India. Animals were feed with food and water *ad libitum*. They were maintained as per the norms of CPCSEA, and the experiment was conducted as per CPCSEA norms.

Test compound

The isolated compound identified as cardiac glycoside by chemical tests and TLC studies from the methanolic extract of fruits of *Corchorus aestuans* Linn was used for the study.

Experimental methodology

'Isolated Frog Heart Perfusion Technique' was used for the study of cardiotonic activity [10, 11]. Here, the activity of test extract was compared with digoxin. The methodology was divided into four sections.

1) Effect of different concentrations of calcium

Here the effect of different concentrations of calcium (as in ringer solution) was tested on frog heart. And ringer solution without calcium was used for testing the activity as it represented failing heart very well.

2) Recording cardiotonic activity

Basal cardiac contraction was recorded on a kymograph with calcium-free ringer solution that was 42beats/min and contraction amplitude (HFC) was 9 mm. Then responses showed by digoxin and test extract were recorded at different concentrations. Effects were converted to respective percentages. In between the results, the heart was washed with calcium-free ringer solution.

3) Effect of drug on blocked heart

The heart when blocked with 0.2 mg of digoxin, it was not washed with ringer, rather was perfuse with drug extract. The effect of isolated compound is shown in [Table 3].

4) Effect of drug on failed heart

Here the effect of drug on failed heart was analyzed. The washing with ringer in between different concentrations of drug extract was not provided and the effect of isolated compound was recorded which is shown in [Table 4].

RESULTS

The preliminary phytochemical study of the fruits of *Corchorus aestuans* Linn revealed the presence of cardiac glycosides, triterpenes, carbohydrates, saponins, flavonoids and sterol in its alcoholic extract. Chromatographic studies on this extract substantiated the presence of chemical constituents detected in the qualitative chemical tests. Five spots of different R_f values were observed in the TLC of cardiac glycosides [Table 1]. All the five compounds were tested for presence of cardiac glycosides by Keller-Killiani's test and Libermann Burchard's test and their UVλ_{max} (MeOH) were also taken. The results are shown in [Table 2]. Isolated compounds 1, 2 and 5 (R_f – 0.89, 0.78 and 0.53) showed negative chemical tests for cardiac glycosides while compounds 3 and 4 (R_f – 0.72 and 0.67) showed positive chemical tests for cardiac glycosides. Compound 3 showed UVλ_{max} (220 nm) and compound 4 showed UV λ_{max} (253 nm).

Cardiac glycosides showed UVλ_{max} at 218-222 nm and hence compound 3 was kept in dilute alcohol for crystallization. The M.P. of white crystals was taken, it was 162-168°C, and Molisch's test was performed for the detection of glycosidal nature. It was positive which confirmed presence of glycone moiety in the isolated compound.

Effect of isolated test compound on blocked heart and failed heart is shown in [Table 3] and [Table 4] respectively. With decreasing concentrations of calcium there is decrease in height of force of contraction while increase in heart rate i.e. positive chronotropic and negative inotropic effect [Table 5]. The incremental dosage of isolated test compound (*C. aestuans* Linn.) produced positive inotropic and negative chronotropic effects. The cardiotonic effect shown by isolated test compound at various concentrations is given in [Table 6]. The effect of digoxin is shown in [Table 6]. Similarly, the blocked heart started its normal rhythm when perfuse with isolated test compound extract.

The test compound also shows its promising effect on failed heart with a successive increase in the height of force of contraction without any ringer washings.

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 [Figure 1]. Also, test compound extract showed its promising effect on the blocked and failed heart without ringer washings.

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 isolated cardiac glycoside from the fruits of *C. aestuans* Linn which has been found to have excellent cardiotonic activity with the wide therapeutic index as compared to digoxin. Hence, isolated test compound can be a safe alternative to digoxin in congestive cardiac failure.

CONCLUSION

The incremental dosage of isolated cardiac glycoside from the methanol extract of fruits of *Corchorus aestuans* L. produced positive inotropic and negative chronotropic effects. 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. Also, test compound extract showed its promising effect on the blocked and failed heart without ringer washings. Further, study is needed to characterize the isolated cardiac glycoside in accordance.

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Table 1: TLC studies of fruits of *Corchorus aestuans* Linn.

Spot no.	Fraction no.	Color of the spot	R_f value
First	17-22	Yellow	0.89
Second	33-38	Purple	0.78
Third	45-51	Purple	0.72
Fourth	67-73	Purple	0.67
Fifth	84-92	Grey	0.53

Table 2: Detection of cardiac glycosides in the eluted compounds

Spot no.	Test for cardiac glycosides		UV (λ_{max} in nm)
	Keller-Killiani's test	Libermann Burchard's test	
First	Negative	Negative	450 nm
Second	Negative	Negative	213 nm
Third	Positive	Positive	220 nm
Fourth	Positive	Positive	253 nm
Fifth	Negative	Negative	371 nm

Table 3: Effect of isolated cardiac glycoside on blocked heart

Drug	Conc. (mg/ml)	Dose (ml)	Conc. at different doses (mg)	HR (heart rate beats/min)	HFC in mm (height of force of contractio)	Cardiac output (HR × HFC)
Digoxin	0.5 mg/ml	0.4	0.2	_	Heart blocked	_
Isolated compound	5 mg/ml	0.05 0.1 0.2	0.25 0.5 1.0	25 36 38	4 7 9	100 252 342

Table 4: Effect of isolated cardiac glycoside on failed heart

Drug	Conc. (mg/ml)	Dose (ml)	Conc. At different doses (mg)	HR (heart rate beats/min)	HFC in mm (height of force of contraction)	Cardiac output (HR × HFC)
Failed heart	_	_	_	_	_	_
Isolated compound	5 mg/ml	0.2 0.4	1.0 2.0	36 32	12 15	432 488

Table 5: Effect of different concentrations of calcium

Drug	Conc. of calcium with respect to it conc. in normal ringer	HR (heart rate beats/ min)	HFC in mm (height of force of contraction)	Change in HF (%)	Cardiac output (HR × HFC)
Ringer	Normal	39	13	100	507
	1/4 th	43	11	84.62	473
	1/2	40	15	115.38	600
	3/4 th	37	18	138.46	666
	Full	35	22	169.23	770

Table 6: Cardiotonic activity of isolated cardiac glycoside

Drug	Conc. (mg/ ml)	Dose (ml)	Conc. at different doses (mg)	HR (heart rate beats/mi n)	HFC in mm (height of force of contraction)	Change in HFC (%)	Cardiac output (HR× HFC)
Isolated compound	5 mg/ml	Control	-	42	8	-	-
		0.05	0.25	41	13	68.42	533
		0.1	0.5	38	17	89.47	646
		0.2	1	36	19	100	684
		0.3	1.5	29	21	110.53	609
		0.4	2	26	25	131.58	650
Digoxin	0.5 mg/ml	0.1	0.05	39	12	63.16	468
		0.2	0.1	37	17	89.47	629
		0.3	0.15	35	21	110.53	735
		0.4	0.2	-	Heart blocked	-	-

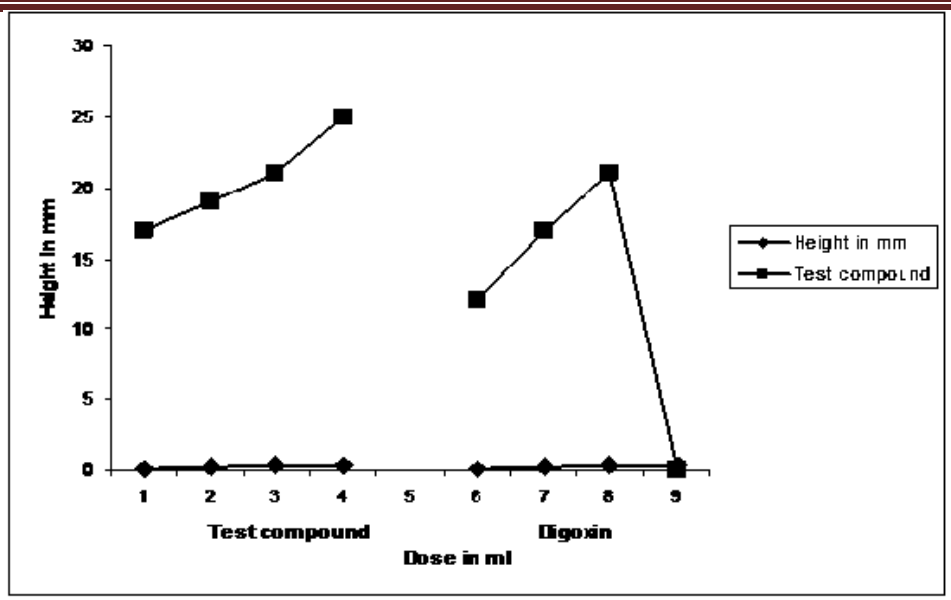

Figure 1: Cardiotonic activity of isolated cardiac glycoside

Source of support: Nil, Conflict of interest: None Declared