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CARDIOVASCULAR EFFECTS OF AQUEOUS LEAF EXTRACT OF *ARISTOLOCHIA BRACTEATA LAM.* ON ISOLATED PERFUSED FROG HEART PREPARATION

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ABSTRACT

Aristolochia bracteata lam. is known for many medicinal properties. The present study was undertaken to evaluate the cardiotonic activity of the aqueous extract of leaves of *Aristolochia bracteata* on isolated perfused frog heart preparation. The aqueous extract of *Aristolochia bracteata* produced positive results for alkaloids, glycosides terpenoids saponins, flavanoids, phenols, volatile oils. The aqueous extract of *Aristolochia bracteata* produced negative results for carbohydrates, proteins, gums and mucilage. Ringer solution without calcium was used as a vehicle for administration of aqueous extract as test and Digoxin as standard. The incremental dose of aqueous extract of *Aristolochia bracteata* produced positive ionotrophic and negative chronotrophic effect on isolated frog heart and is dose dependent. The test extract had not produced cardiac arrest even at a dose of 10mg/ml, 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. The mechanism involved in the positive ionotrophic action of extract has been studied. In the present study Theophylline increase the dose response curve of *Aristolochia bracteata* aqueous extract. *Aristolochia bracteata* aqueous extract induced positive ionotrophic effect were completely blocked by Propranolol. The data suggests that *Aristolochia bracteata* aqueous leaf extract induce positive ionotrophism involved any adrenergic receptor mediated action.

Key words: Cardiotonic activity, Digoxin, Calcium free Ringer's solution, Isolated frog heart.

INTRODUCTION

Cardiovascular diseases are increasingly becoming one of the leading diseases causing morbidity and mortality in India It is estimated that cardiac disease will emerge as single largest contributor to morbidity in India accounting for nearly one third of total deaths in near future. Ethnographic evidence suggests that these diseases are often first managed by indigenous and related herbs before patients are referred for allopathic forms of management. The use of herbal preparations for therapeutic remedies is as old as man himself. In many parts of India this tradition is still being practiced, mainly as a first choice form of medication before the patient is referred for management by the "western type" of medicine. In addition, there is a strong global trend for the revival of interest in the traditional system of medicines. In line with this trend, anecdotal evidence suggests that a number of plant products have been used traditionally for the management of cardiovascular diseases. *Aristolochia bracteata.lam* is globally distributed in Tropical Africa, Arabia, Sri Lanka, Pakistan and India. Within India, it is found in northern and central India from Haryana to West Bengal and southwards to Tamil Nadu and Kerala. It is common in dry areas, particularly on black cotton soil, usually growing as a weed. It has been reported to produce Antimicrobial, Ant allergic, Snake bite, tedious labor, Intestinal worms, Constipation, Inflammation. The present study is to evaluate the cardiotonic effect of aqueous leaf extract of *Aristolochia bracteata lam* on isolated perfused frog heart preparation.

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MATERIALS AND METHODS

Chemicals

Propranolol, Verapamil, Theophylline, Sildenafil and Lignocaine were used.

Animal

Frogs belonging to the species of *Rana hexadactyla* of either sex weighing 100 -150 gms were used.

Preparation of extract

The fresh leaves of *Aristolochia bracteata* were collected from Nellore district in A.P and authenticated by Dr.S.M.Khasim, M.Sc.Ph.D., department of botany in Acharya Nagarjuna University, Guntur, Andhra Pradesh. The washed leaves are dried in room temperature (25 - 35˚c), the dried leaves were powdered and passed through sieve number 80, the dried powdered leaves were extracted with water using soxhlet extractor and dried at 45˚c in an oven and it was used for further studies (Harborne JB, 1998). The aqueous extract of *Aristolochia bracteata* produced positive results for alkaloids, glycosides, terpenoids, saponins, flavanoids, phenols and volatile oils. The aqueous extract of *Aristolochia bracteata* produced negative results for carbohydrates, proteins, gums and mucilage.

Exponential design

Frogs were stunned by blow on the head and then pithed. The heart was quickly exposed and isolated by a method of burn (Burn JH, 1952), syme´s cannula was inserted in to inferior venacava and the heart along with syme´s cannula was mounted. It was perfused with Ringer's solution which consists of (mM), NaCl 111.11; KCl, 1.88; $CaCl_2$ 1.08; $NaHCO_3$ and glucose 11.11 and continuously bubbled with air to room temperature. The perforate was delivered to the heart at 7cm water filling pressure. The heart was allowed to stabilize for about 15min prior to administration of drugs the response to various drugs were recorded on a student's kymograph by attaching on one end of thread to the apex of the heart and the other end of the thread to the Sterling's heart lever. In the first series experiments after 15 min of stabilization graded doses of *Aristolochia bracteata* aqueous extract were added and the responses in terms of force of contraction .The next higher dose was administered only after the recovery from the preceding dose. The extract was dissolved in distilled water to obtain appropriate concentrations of 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12 mg/ml.

The isolated frog heart perfusion technique was used as a model to evaluate the activity of various concentration of aqueous extract of *Aristolochia bracteata*

The experiment was carried out by using Ca^{++} free ringer solution (Burn JH, 1952). The basal cardiac contraction was recorded on a kymograph after the administration of calcium free ringer solution. The average basal heart rate and the contraction amplitude were 52 beats per min and 1 mm respectively. The responses of *Aristolochia bracteata* at various concentrations were recorded on kymograph and their cardiac activity in terms of heart rate (HR) and height of force of contraction (HFC) was noted. The effects obtained with test extract of *Aristolochia bracteata* were shown in table 1. The frog heart was washed with Ringer's solution after every administration of test extract till it was brought to normal state.

Fig 1. Showing various conc. viz, a-0.25, b-0.5, c-1,d-2,e-4,f-6, (mg/ml)

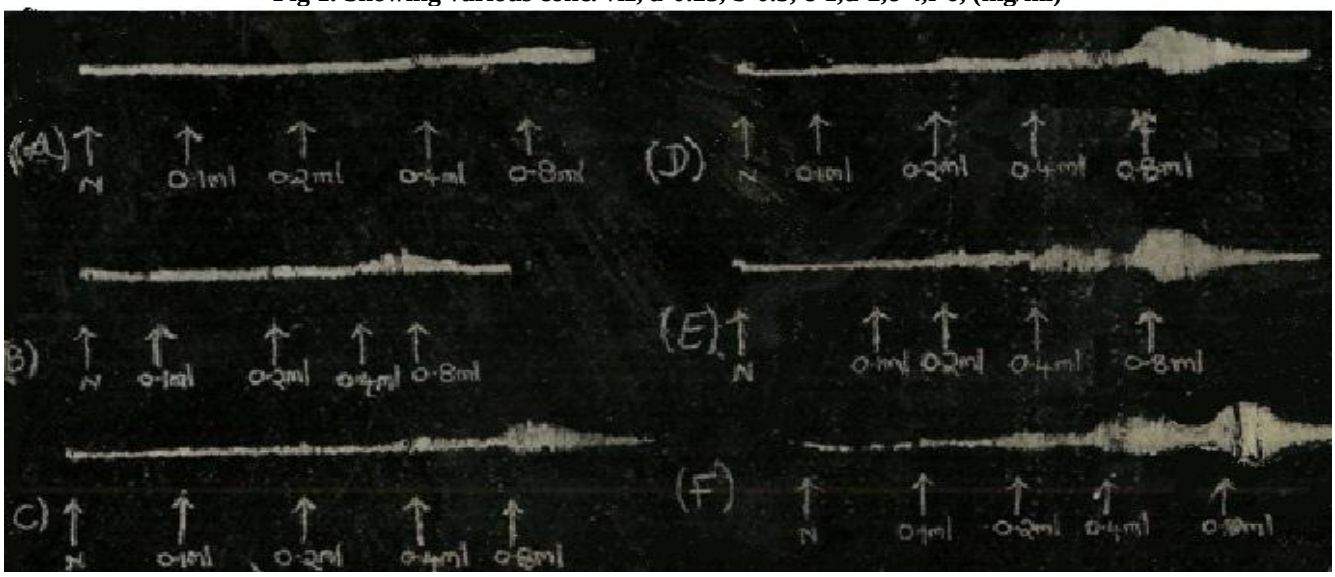


Fig 2. Showing various conc. viz, j-8, k-10, l-12, (mg/ml)

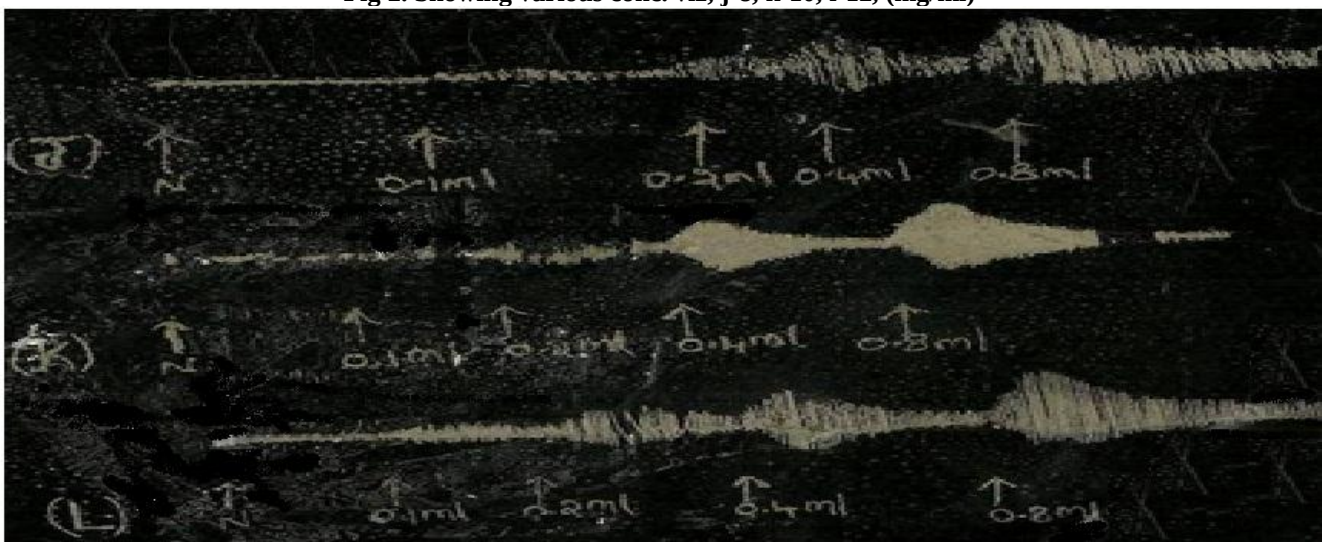


Fig 3. Showing cardiogram, measures are: N-Normal, 2-Verapamil, 3-Theophylline.

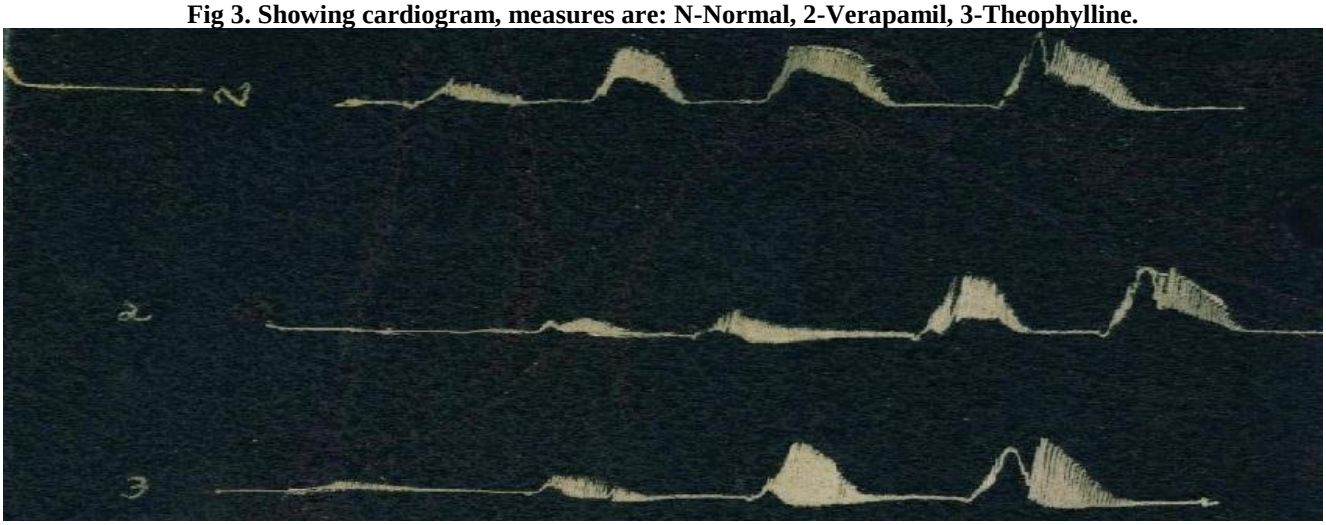
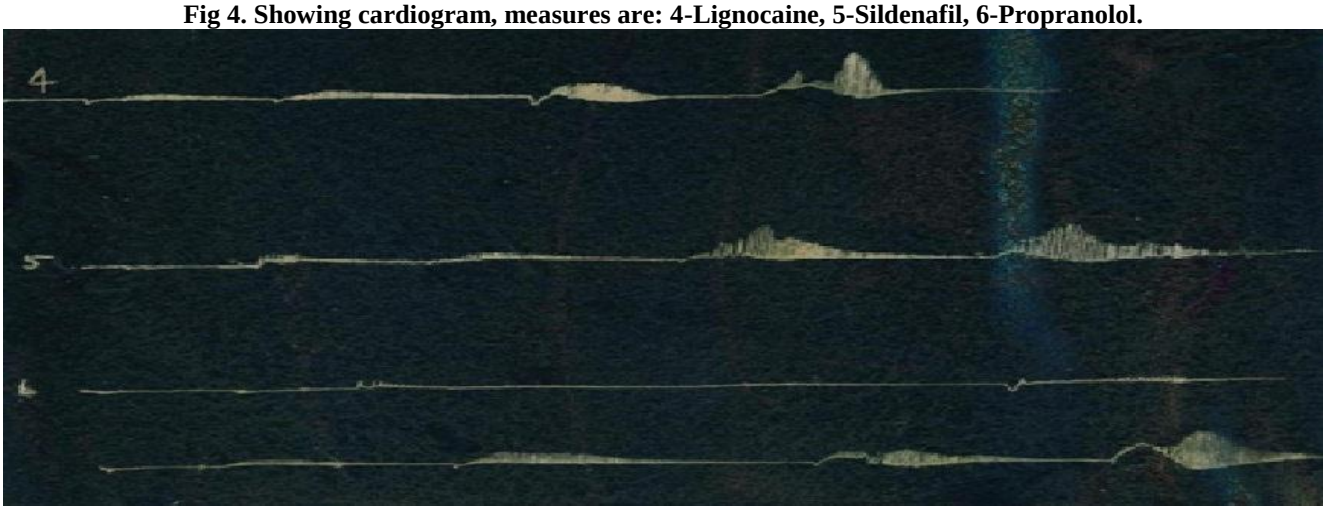


Fig 4. Showing cardiogram, measures are: 4-Lignocaine, 5-Sildenafil, 6-Propranolol.



To study the interaction of extract with other agents, heart was perfused with frog ringer containing adrenoreceptor blocker, Propranolol (1 x 10^{-6}M), Calcium channel blocker, Verapamil(1 x 10^{-7}M), Sodium channel blocker Lignocaine(1 x 10^{-4}M), Phosphodiesterase inhibitor, Theophylline (1 x 10^{-4} M) and specific Phosphodiesterase type 5(PDE5) inhibitor, Sildenafil(1 x 10^{-6} M) for 15min and the responses to *Aristolochoia bracteata* were recorded as earlier. In second series of experiments, to study the effect of extra cellular ion concentration on positive inotropic effect induced by *Aristolochoia bracteata*, heart was perfused with frog Ringer containing half NaCl (55mM) or 1.5 times NaCl(165mM), half $CaCl_2$ (0.79mM) or double $CaCl_2$ (3.17mM) and half KCl (0.9mM) or double KCl (3.6mM). The equimolar concentration of glucose was added to maintain isoosmolality while lowering the sodium chloride concentration. When glucose level is reduced sodium chloride concentration was increased in the perfusion fluid, for the osmotic adjustment. The heart was perfused for 15min with these solutions and the responses to *Aristolochoia bracteata* were obtained as earlier.

RESULTS

Table 1. Effect of aqueous extracts from *Aristolochia bracteata* on isolated frog heart perfusion

S.no	Conc of extract (mg/ml)	Dose (ml)	Conc in diff doses (mg)	HR	HFC (mm)
1.	0.25	Control	-	-	01
		0.1	0.025	52	02
		0.2	0.05	51	03
		0.4	0.1	50	03
		0.8	0.2	49	04
2.	0.5	Control	-	-	01
		0.1	0.025	55	02
		0.2	0.05	54	03
		0.4	0.1	52	03
		0.8	0.2	50	05
3.	1	Control	-	-	01
		0.1	0.025	54	02
		0.2	0.05	52	03
		0.4	0.1	48	04
		0.8	0.2	47	07
4.	2	Control	-	-	01
		0.1	0.025	50	02
		0.2	0.05	49	03
		0.4	0.1	47	09
		0.8	0.2	46	09
5.	3	Control	-	-	01
		0.1	0.025	49	02
		0.2	0.05	47	03
		0.4	0.1	45	06
		0.8	0.2	43	10
6.	4	Control	-	-	01
		0.1	0.025	48	02
		0.2	0.05	47	04
		0.4	0.1	46	06
		0.8	0.2	44	10
7.	5	Control	-	-	02
		0.1	0.025	46	02
		0.2	0.05	44	04
		0.4	0.1	43	08
		0.8	0.2	41	12
8.	6	Control	-	-	02
		0.1	0.025	45	03
		0.2	0.05	43	05
		0.4	0.1	41	11
		0.8	0.2	40	14

9.	7	Control 0.1 0.2 0.4 0.8	- 0.025 0.05 0.1 0.2	- 44 42 41 39	02 04 07 11 15
10.	8	Control 0.1 0.2 0.4 0.8	- 0.025 0.05 0.1 0.2	- 42 40 39 38	02 02 05 10 14
11.	9	Control 0.1 0.2 0.4 0.8	- 0.025 0.05 0.1 0.2	- 40 38 37 35	02 03 08 12 15
12.	10	Control 0.1 0.2 0.4 0.8	- 0.025 0.05 0.1 0.2	- 38 36 35 33	02 04 08 13 15
13.	Digoxin	Control 0.1 0.2 0.4 0.8	- 0.025 0.05 0.1 0.2	- 44 42 40 39	02 02 03 09 14

DISCUSSIONAND CONCLUSION

Effect was studied by using calcium free ringer solution and isolated frog heart perfusion technique. Incremental dosage of aqueous extract of *Aristolochia bracteata* produced positive inotropic and negative chronotropic effect on isolated frog heart, and is dose dependent. The cardiotonic action was studied by the effect of aqueous extracts shown in kymogram (fig.1& 2).

The responses to *Aristolochia bracteata* were neither affected by Lignocaine ($1x10^{-4}$) nor by verapamil ($1x10^{-7}$M).sildenafil ($1x10^{-6}$ M). Also failed to produce any change in *Aristolochia bracteata* induced responses. The responses to *Aristolochia bracteata* were potentiated by Theophylline ($1x10^{-4}$M) and inhibited by Propranolol ($1x10^{-6}$M).

The mechanisms involved in the positive ionotropic action of various drugs (Vasavada BH, 1910, Satoskar RS, 2005, Thripati KD, 2002) may be direct stimulation of adrenergic receptors as seen with catcholamines or indirect of NA seen DA (Gold berg LI, 1972), fatty acids (Patel SB and verma SC, 1981), diuretic (Chandiramani BR and verma SC, 1981) or through direct action on the muscle. *Aristolochia bracteata* aqueous extract (ABAE) induced positive inotropic effects were not altered by the sildenafil, a cGMP, PDE-5 possibility of involvement of cGMP and nitric oxide related mechanism. High KCl concentration activates where as low KCl concentration inhibits Na^+/K^+ ATP as pump (Beauge LA *et al*., 1979). ABAE induced positive Ionic effects were not altered by increase or decrease in K^+ concentration in ringer, which rules out the possible involvement of Na^+/K^+ ATPase pump as that of cardiac glycosides.

Verapamil, which prevents the entry of calcium ions, also did not alter the responses to ABAE, it indicates that positive ionotropic action is not by directly increasing the availability of $Ca^{++}$from extracellular sites (Patel VG and Goyal RK, 1983). Intracellular sodium promotes the influx of calcium ions in increasing the calcicum concentration at the cellular sites. The responses ABAE were not altered when sodium ion concentration in ringer was reduced or increased. Further Lignocaine, which prevents the entry of Na^+ ions also, did not alter the responses to ABAE. It therefore rules out the possibility that ABAE induced positive ionotropic effects are not due to the increase in Na influx and thereby causing greater availability of Ca^{++} intracellulary. cAMP, is involved as the second messenger in the positive ionotropic responses to various drugs. In the present study theophylline, PDE inhibitor increases the dose response curve of ABAE. ABAE induced positive ionotropic effects were completely blocked by propranolol, β-receptor antagonist. The data suggest that ABAE induced positive ionotropism involve any adrenergic receptors (fig 3& 4).

The incremental doses of aqueous extract of *Aristolochia bracteata* produced positive ionotropic and negative chronotropic effect on isolated frog heart and are

dose dependent. The possible mechanism of action of aqueous extract induced positive ionotropism may be involved any adrenergic receptor.

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REFERENCES

Beauge LA, Glynn IM and Richrards DE. The biphasic action of external potassium ions on Na^+/ATPase (proceedings). *Journal physiol lond*, 295, 1979, 88.

Burn JH. Perfused heart preparations, Practical Pharmacology, Black Wel Science Publications, Oxford, New Delhi, 1952, 1-30.

Chandiramani BR and verma SC. Positive ionotrophic responses to furosemide on isolated frog heart. *Indian J of Exp Biol*, 19, 1981, 558.

Gold berg LI. Cardio vascular and renal actions of dopamine: potential clinical applications. *Pharmacol Rev*, 24, 1972, 1.

Harborne JB. Methods of extraction and isolation. In: Phytochemical Methods. Chapman & Hall, London, 1998, 60-66.

Patel SB and Verma SC. Cardiotonic activity of selective fatty acids in amphibian heart. *Indian J of pharmacy*, 13, 1981, 271.

Patel VG and Goyal RK. Influence of extracellular ions on cardiotonic effects of BDPU [I - (Butyl – 3 (1 – (6, 7 dimethoxy quinazoline 4-yl) urea)]. *Indian J of Exp Biol*, 21, 1983, 448.

Satoskar RS, Bandarkar SD and Nirmala N Rege. Handbook of Experimental Pharmacology & Pharmacotherapeutics, revised 19th edition, Popular Prakashan Pvt Ltd Popular Press Bldg Mumbai, 2005, 435.

Thripati KD. Cardiac glycosides and drugs for CHF, essentials of medical pharmacology, 4th edition (Jayapee Brothers Medical Publishers Pvt Ltd Newdelhi) 2002, 497.

Vasavada BH, Mehtha DD, Santhani DD and Goyal RK. Mechanism of action of various ionotropic agents: a search for digitalis substitute. *Indian J of pharmacy*, 22, 1910, 199.