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Cardiovascular Activity of aqueous extracts of leaves of *Annona squamosa.*

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Abstract:

The present study was undertaken to evaluate cardiotonic activity of aqueous extracts of *Annona squamosa* Leaves of this plant species contain flavonoids, tannins, cardiac glycosides which are potent antioxidant and may believed to prevent cardiovascular diseases. A Cardiotonic effect of aqueous extracts of *Annona squamosa* was studied by using isolated frog heart perfusion technique (IFHP). Calcium free ringer solution was used as vehicle for administration of aqueous extracts of leaves of *Annona squamosa* as test sample and digoxin as standard. A significant increase in height of force of contraction and decrease in heart rate was observed with test sample as compare to same dose of standard digoxin. The present result indicates that a significant increase in height of force of contraction with decrease in heart rate observed as the dose of test sample increased.

Key words: Tannins, glycosides, cardiovascular diseases, *Annona squamosa*.

Introduction:

Cardiac disease is an important cause of premature death in industrialized countries. It is estimated that cardiac disease will emerge as single largest contributor to morbidity in India according for nearly one third deaths in near future. Cardiac glycoside and catecholamine have been used as main therapeutic agent in the treatment of congestive cardiac failure[I]. Despite continuing advancement in understanding the basic pharmacology of cardioactive drugs, cardiac glycoside, intoxication with digitalis a narrow therapeutic index drug remains a common clinical problem. Synthetic cathecholamines has been reported to cause a severe oxidative stress in the myocardium through free radical formation[II]. It necessitates research for a new drug and for this aim we have chosen *Annona squamosa and* evaluated its cardio active potential.

Annona squamosa commonly known as sugar apple is a small tropical tree, indigenous to Amazon rain forest, growing up to 20m tall, cultivated both in the plains and on the hills like in tropical South America, Southern Mexico, West Indies, Bahamas, occasionally in Southern Florida and throughout India. In Traditional System of Medicine, the leaf is used as an insecticide, in skin infections, mucosa, laxative, diarrhea, dysentery, pregnancy, antiaborifacients, for treating cancerous tumors[III]. The phytoconstituents isolated so far from leaves are hydroxyl ketone 10-hydroxy-16-hentriacontanone[IV], squamocenin, annotemoyin-2, reticulatain-2[V], benzoquinoline alkaloid samaquasine A[VI] and acetogenins viz., annonacin, annonacin A, annonastatin[VII], bullatacin, bullatacinon and squamone[VIII]. It is reported to posses antidiabetic[IX], analgesic, anti-inflammatory[X] and larvicidal[XI] activities.

In present study, we are targeting cardiotonic activity of aqueous extracts of leaves of *Annona squamosa on* isolated frog heart perfusion (IFHP).

Materials and Methods:

Plant Material

The plant (*Annona squamosa)* was collected from local area of Warananagar and authenticated from Botany department, Shivaji University, Kolhapur. Fresh leaves were isolated for extraction. Digoxin was purchased from Unique biologicals Kolhapur.

Animal:-

Frog of Rana tagrina species from animal house of Tatyasaheb Kore College of Pharmacy, Warnanagar, Kolhapur where used for studies. The animals were fed with food and water *ad libitum.* The animals were maintained as per the norms of CPCSEA.

Preparation of extract:-

Powder of dried leaves of *Annona squamosa* was kept in beaker containing 100 ml of distilled water for extraction. The brown colour aqueous extract was collected after 7days. The product was dried on rotatory evaporator & powdered. The powdered test drug was dissolved in sterile water to obtain appropriate concentrations of 0.25, 0.5 and 1 mg/ml and the pure sample of digoxin (as a reference standard) was dissolved in sterile water to make solutions of concentrations 0.25, 0.5 and 1mg/ml as experimental set up (Table 1).

Table1. Effect of aqueous extracts of leaves of Annona *squamosa* on isolated frog heart perfusion.

Sr. no.	Conc. Of extracts. (mg/ml)	Dose (ml)	Conc. At differ. Doses. (mg)	HR	HFC (mm)
1.	0.25	Control	-	56	6
		0.1	0.025	54	7
		0.2	0.050	52	8
		0.4	0.1	50	8.4
	0.5	Control	-	57	4
		0.1	0.05	55	5
		0.2	0.10	54	7
		0.3	0.20	52	10
	1	Control	-	53	5
		0.1	0.10	47	10
		0.2	0.20	45	13
		0.4	0.40	48	15
	4	Control	-	49	3
		0.1	0.4	38	12
		0.2	0.8	33	18
		0.4	1.6	28	23
2.	Digoxin				
	0.25	Control	-	56	6
		0.1	0.025	54	8
		0.2	0.050	51	9
		0.4	0.10	59	11

	0.5	Control	-	52	7
		0.1	0.050	51	10
		0.2	0.10	53	12
		0.4	0.20	57	13
	1	Control	-	54	8
		0.1	0.10	56	08
		0.2	0.20	63	12

HR – heart rate; HFC – height of force of contraction
Total 6 frogs are used for each dose and the best results are provided.
The statistical analysis was carried out using Students' t test and it was found significant at *P<0.001*

Isolated frog heart perfusion for study of cardiotonic activity of drugs was followed as per described earlier[XII].

Results:

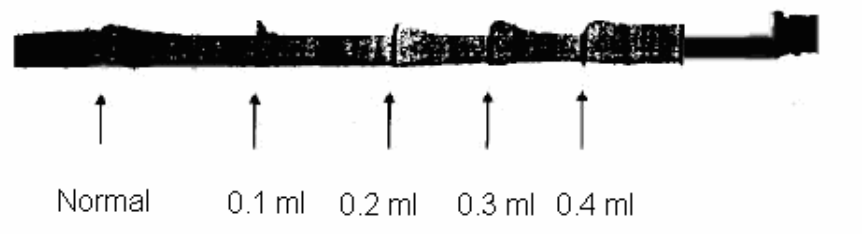
Incremental dosage of aqueous extracts of leaves of *Annona squamosa* produced positive inotropic and negative chronotropic effect on isolated frog heart and is dose dependent. The similar concentration of digoxin produced positive inotropic and negative chronotropic effect. The kymograph obtained indicates that even at lower doses of extract of *Annona squamosa* gives quick and significant increase in height of contraction as compared to digoxin. The dose of digoxin required to produce cardiac arrest was much lower. However, the cardiac arrest with aqueous extract from dried leaves of *Annona squamosa* was observed at high dose level.

Discussion:

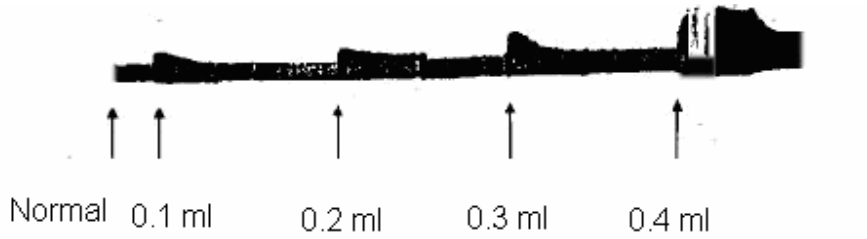
From the observations, it was revealed that at very high dilutions (0.25mg/ml) the aqueous extracts of leaves of *Annona squamosa* showed decrease in heart rate (negative chronotropic) and increase in height of force of contraction (positive inotropic) effect. At concentration of (0.5mg/ml) of test drug showed increase in force of contraction

with negative chronotropic effect. At a concentration of (4mg/ml) the test drug showed cardiac arrest at 0.6 ml dose (fig.1)

A



B



C

D

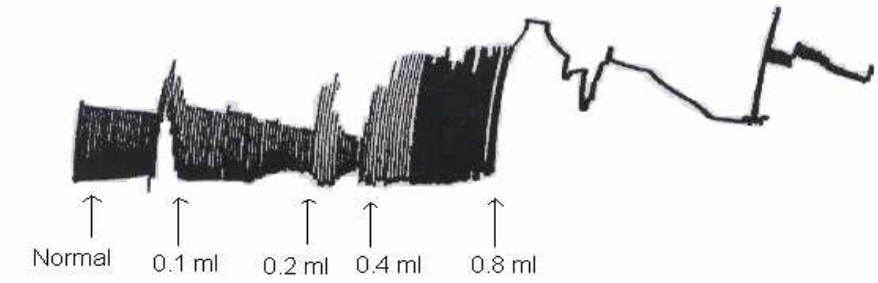


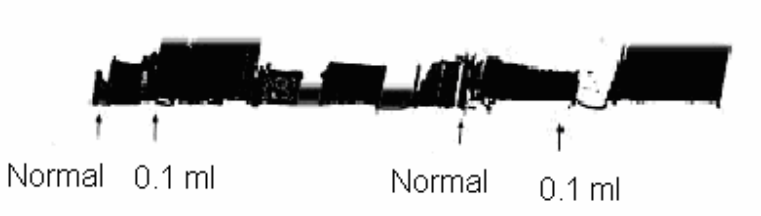
Fig. 1 effect of aqueous extracts of *Annona squamosa* at (A) 0.25 mg/ml (B) 0.50 mg/ml (C) 1 mg/ml and (D) 4 mg/ml.

while at low concentration (0.2mg/ml and 0.5mg/ml) digoxin showed showed increase in height of force of contraction which is 4 times less than height of force of contraction produced by aqueous extracts of leaves of *Annona squamosa*.

The cardiac arrest shown by digoxin was at 1mg/ml (fig.2).

A

B



C

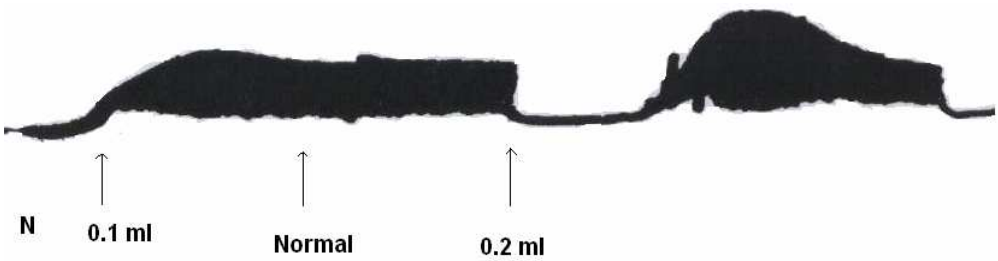


Fig. 2 effect of aqueous extracts of digoxin at (A) 0.25 mg/ml (B) 0.50 mg/ml and (C) 1 mg/ml.

This indicates that aqueous extracts of leaves of *Annona squamosa* have high therapeutic index and high margin of safety as compared to digoxin. It also reveals that both digoxin and leaves of *Annona squamosa* showed dose dependant activity. However aqueous extracts of leaves of *Annona squamosa* produced significant (three fold) positive inotropic action than at the same doses of digoxin.

The result obtained reveal that the therapeutic efficacy of extracts of leaves of *Annona squamosa* is much wider than that of the digoxin. Limitations of using digoxin may overcome by using aqueous extracts of leaves of *Annona squamosa.*

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