ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 1, 2016

ISSN - 0974-2441

Research Article

EFFECT OF AQUEOUS EXTRACT OF PEEL OF *CITRUS SINENSIS* ON FROG'S HEART

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Received: 18 August 2015, Revised and Accepted: 29 August 2015

ABSTRACT

Objectives: To evaluate the cardiotonic action of aqueous extract of the peel of *Citrus sinensis* and also to know the effect in the presence of propranolol, nifedipine, and verapamil.

Methods: We studied the cardiotonic effect of citrus peel extracts on isolated frog heart perfusion technique alone and also with a continuous infusion with calcium channel blockers and a beta blocker. The aqueous extract obtained by drying the peel of citrus and then adding the powder form in distilled water.

Results: Aqueous extract produced positive ionotropic action on frog heart and its effect antagonized by propranolol but neither by nifedipine nor verapamil.

Conclusion: Aqueous extract of peels of *C. sinensis* has cardiotonic activity and the cardiotonic effect produced by citrus may be due to having beta adrenergic action.

Keywords: Digoxin, Cardiac stimulant, Isolated frog's heart, *Citrus sinensis*.

INTRODUCTION

Cardiovascular diseases (CVDs) are the most common cause of death worldwide [1]. Many drugs are used in the therapy of congestive heart failure, but cardiac glycosides and catecholamines are the most important therapeutic agents. The safety and efficacy of cardiac glycosides is a matter of concern [2,3]. Literature has shown that synthetic catecholamines could cause oxidative stress in the myocardium through free radical formation [4]. Hence, there are many types of research going on the medicinal herbs having good cardiac activity.

The major medicinal properties of orange include antibacterial [5], anti-diabetic [6], cardioprotective [7], anti-thyroidal [8], anti-hyperglycemic [8], anti-hypertensive [9], etc. With the purpose of determining the cardiotonic activity of *Citrus sinensis* we carried out this study in our set up. We also evaluated the cardiotonic effect of citrus in the presence of propranolol, a beta blocker and nifedipine and verapamil which are calcium channel blockers to know the site and mechanism of action of *C. sinensis*.

METHODS

This study was done in the Department of Pharmacology of Kalinga Institute of Medical Sciences. This study was approved by the Institutional Animal Ethics Committee of the institute.

Preparation of extract

The fruits of *C. sinensis* were purchased from local market Bhubaneswar, Odisha, India. It was authenticated by a botanist. The fruits were washed several times using clean water; peels were separated, cut into small pieces, dried under shade, and powdered in a blender. 1 g of powder was mixed with 100 ml distilled water with the help of magnetic stirrer for ½ hr. The material was filtered through Whatman filter paper No. 40, and the filtrate was collected. The prepared infusion was diluted with the help of distilled water in varying proportion and labeled as follows:

- E1 - Undiluted filtrate
- E2 - 1:1 (filtrate: distilled water)
- E3 - 1:2 (filtrate: distilled water)
- E4 - 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 were determined.

Preparation of digoxin solution

Digoxin ampoules were purchased from a local pharmacy. Various different dilutions were made with distilled water and labeled as follows: S1 - 25 µg/ml, S2 - 50 µg/ml.

Preparation of ringer solution

Ringer solution was prepared by using standard method (Kulkarni S K, 1993).

Evaluation of cardiotonic activity

After obtaining ethical clearance from the Institute Animal Ethics Committee, Kalinga Institute of Medical Sciences, KIIT University the frog of species *Rana tigrina* was pithed and pinned 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 frog ringer was put over the heart. The inferior vena cava was traced, and a thread was put around it and a small cut was made to insert the venous cannula. The cannula was inserted in the vein and the thread was tied to assure the cannula in

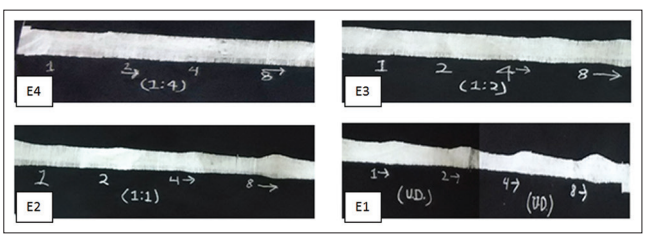

Fig. 1: Effect of juice of peels of *Citrus sinensis* on (E4, E3, E2, E1) ringer solution perfused heart

place which is in turn connected to a Marriott's bottle containing frog ringer solution. A small cut in one of the aorta was given for the ringer to come out. The heart was isolated and attached to the stand with a 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, 1993; Kale, 2003). The experimental animals served as self-control, i.e. same heart used for both controls as well as test. First the normal heart contraction was noted without exposing to extract. All test samples that is E1, E2, E3, E4, S1, and S2 were administered in different doses *viz*. 1 drop to 8 drops, respectively. The rate and force of heart contraction were noted as given in the following Figs. 1-4 and Tables 1-4. The effect of the extract was compared with that of digoxin solution.

To know the site and mechanism of cardiotonic action we studied the effect of citrus in the presence of a beta blocker, i.e. propranolol and calcium channel blockers, i.e. nifedipine and verapamil. The frog heart was perfused with propranolol solution (2 µg/ml) [10] for 10 minutes. This was followed by administration with increasing doses of aqueous extracts and response was recorded. Similar procedure was followed for nifedipine solution (0.3 µg/ml) [10] and verapamil (0.3 µg/ml) [10] and response was recorded.

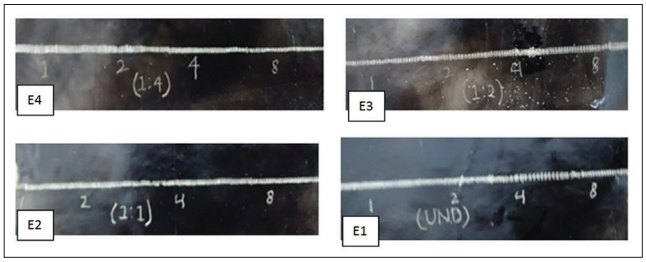

Fig. 2: Effect of extract (E4, E3, E2, E1) on heart in presence of propranolol

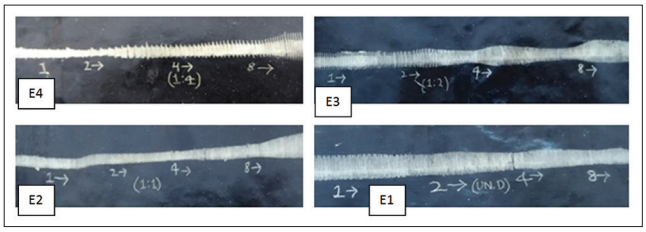

Fig. 3: Effect of extract (E4, E3, E2, E1) on heart in presence of nifedipine

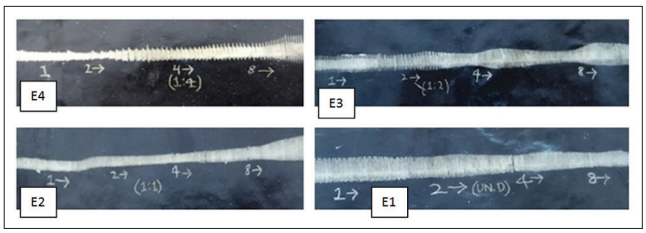

Fig. 4: Effect of extract (E4, E3, E2, E1) on heart in presence of verapamil

Table 1: Effect of juice of peels of *C. sinensis* on (E4, E3, E2, E1) ringer solution perfused heart

Drug	Dose (drops)	Change in rate	Change in force
Control	0	Normal	Normal
E4	1	Transient decrease	-
E4	2	Transient decrease	-
E4	4	Decrease	Mild increase
E4	8	Decrease	Moderate increase
Control	0	Normal	Normal
E3	1	Decrease	Moderate increase
E3	2	Decrease	Mild increase
E3	4	Decrease	Moderate increase
E3	8	Decrease	Significant increase
Control	0	Normal	Normal
E2	1	Decrease	Moderate increase
E2	2	Decrease	Moderate increase
E2	4	Decrease	Significant increase
E2	8	Decrease	Significant increase
Control	0	Normal	Normal
E1	1	Decrease	Significant increase
E1	2	Decrease	Significant increase
E1	4	Decrease	Significant increase
E1	8	Decrease	Significant increase

C. sinensis: Citrus sinensis

Table 2: Effect of extract (E4, E3, E2, E1) on heart in presence of propranolol

Drug	Dose (drops)	Change in rate	Change in force
Control	0	Normal	Normal
E4	1	No change	No change
E4	2	No change	No change
E4	4	No change	No change
E4	8	No change	No change
Control	0	Normal	Normal
E3	1	No change	No change
E3	2	No change	No change
E3	4	No change	No change
E3	8	No change	No change
Control	0	Normal	Normal
E2	1	No change	No change
E2	2	No change	No change
E2	4	No change	No change
E2	8	No change	No change
Control	0	Normal	Normal
E1	1	No change	No change
E1	2	No change	No change
E1	4	No change	No change
E1	8	No change	No change

Table 3: Effect of extract (E4, E3, E2, E1) on heart in presence of nifedipine

Drug	Dose (drops)	Change in rate	Change in force
Control	0	Normal	Normal
E4	1	No change	No change
E4	2	Decrease	Mild increase
E4	4	Decrease	Mild increase
E4	8	Decrease	Significant increase
Control	0	Normal	Normal
E3	1	Decrease	Significant increase
E3	2	Decrease	Significant increase
E3	4	Decrease	Significant increase
E3	8	Decrease	Significant increase
Control	0	Normal	Normal
E2	1	Decrease	Mild increase
E2	2	Decrease	Mild increase
E2	4	Decrease	Mild increase
E2	8	Decrease	Significant increase
Control	0	Normal	Normal
E1	1	Decrease	Moderate increase
E1	2	Decrease	Moderate increase
E1	4	Decrease	Moderate increase
E1	8	Decrease	Moderate increase

Table 4: Effect of extract (E4, E3, E2, E1) on heart in presence of verapamil

Drug	Dose (drops)	Change in rate	Change in force
Control	0	Normal	Normal
E4	1	Decrease	No change
E4	2	Decrease	No change
E4	4	Decrease	Mild increase
E4	8	Decrease	Moderate increase
Control	0	Normal	Normal
E3	1	Decrease	No change
E3	2	Decrease	Mild increase
E3	4	Decrease	Moderate increase
E3	8	Decrease	Significant increase
Control	0	Normal	Normal
E2	1	Decrease	No change
E2	2	Decrease	Moderate increase
E2	4	Decrease	Moderate increase
E2	8	Decrease	Significant increase
Control	0	Normal	Normal
E1	1	Decrease	Mild increase
E1	2	Decrease	Moderate increase
E1	4	Decrease	Significant increase
E1	8	Decrease	Significant increase

RESULT AND DISCUSSION

A pilot study was conducted on normal ringer solution perfused toads heart with graded doses of the freshly prepared juice of peels of *C. sinensis* showed a cardiostimulant effect preceded by a brief period of cardiac arrest, which mimicked the action of digitalis. The force of cardiac contraction by the juice of peels of *C. sinensis* was more prominent than digoxin in our study.

Effect of increasing doses of aqueous extract of citrus on frog heart is shown in Fig. 1. Change in heart rate and force of cardiac contraction is shown in Table 1. The cardiotonic activity of citrus was blocked by propranolol (Fig. 2 and Table 2) but, was not blocked by nifedipine (Fig. 3 and Table 3) nor by verapamil (Fig. 4 and Table 4). After infusion with nifedipine, the cardiotonic action of citrus existed. The significant increase in the force of contraction may be due to the action of citrus as well as additional reflex sympathomimetic action due to nifedipine (as it causes vasodilation). After infusion of verapamil, the cardiotonic activity of citrus also existed. High dose of undiluted extract (16 drops) caused some arrest in cardiac activity when the heart was on verapamil infusion, and this may be due to blocking the action of verapamil. We can conclude here that the cardiotonic activity of citrus does not involve the calcium channels. Nevertheless, β adrenergic receptors may be involved in the action of the extract.

CONCLUSION

It is suggestive from the data's that aqueous extracts of peels of *C. sinensis* fruits has cardiotonic activity, and this action may be due to the involvement of beta-adrenergic receptors. Hence, this can be useful in treating CVD. More researches should be done regarding molecular mechanism of action.

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