EVALUATION OF CARDIOTONIC EFFECT OF PEELS OF CITRUS MEDICA IN CONGESTIVE HEART FAILURE

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ABSTRACT
Congestive heart failure is a clinical syndrome caused by the inability of the heart to pump sufficient blood to meet the metabolic needs of the body. Cardio tonics are useful in low output failure and digoxin are useful in high output failure. Citrus medica can increase the Force of contraction which may potentiate activity on heart muscles, and also Citrus medica gave potential diuretic effect thus it may help to treat CHF. Positive ionotropic effects of drugs were measured by in-vitro Langendorff’s method.

Keywords: Cardiotonics, Congestive heart failure, Citrus medica, digoxin, Langendorff’s method, Positive inotropics.

INTRODUCTION
Heart failure is a clinical syndrome caused by the inability of the heart to pump sufficient blood to meet the metabolic needs of the body. Heart failure can result from any disorder that reduces ventricular filling (diastolic dysfunction) and/or myocardial contractility (systolic dysfunction). The leading causes of heart failure are coronary artery disease and hypertension. The primary manifestations of the syndrome are dyspnea, fatigue, and fluid retention[1].

It can be diagnosed by chest radiograph, echocardiogram, electrocardiogram, serum brain natriuretic peptide, etc. Symptoms of CHF can also serve as tools for diagnosis of CHF. Hemodynamic parameters by cardiac catheterization can be useful for diagnosis of heart failure. The current therapy available for congestive heart failure includes positive inotropic agents like cardiac glycoside (digoxin), adrenergic drugs like dobutamine, phosphodiesterase III inhibitors like amrinone, milrinone and dopaminergic drugs like ibopamine; diuretics like thiazide diuretic (hydrochlothiazide), loop diuretics (frusemide); ACE inhibitors like captopril, enalepril; vasodilators like nitroglycerin etc[2-].
So CHF can be treated by cardio tonics which can increase the forces of contractions of myocytes. In recent scenario, digoxin is choice of drug as a cardio tonic to treat these diseases. Other categories are adrenergic drugs, phosphodiesterase III inhibitors and dopaminergic drugs can also act as cardio tonics.

The major drawbacks of currently available and mostly used cardiotonic – digoxin is its narrow therapeutic window and most severe adverse effects on heart – digitalis toxicity like heart block and arrhythmia and other system. The other synthetic cardiotonics like phosphodiesterase III inhibitors (amrinone, milrinone) or dopaminergic drugs (dopamine, ibopamine) also possess some severe adverse drug effects. Thus there is need to find safer and efficient cardio tonics with least adverse effects.

Modern science has already, accepted the potential of the herbs as a source of new bio-active constituents. There are numerous plants-derived drugs of unknown chemical structure that have been found clinically useful in different alternative system of medicine, including Ayurveda, Homeopathy and Unani system of medicine.

The recent development of the science of phyto-pharmaceuticals has generated new enthusiasm in herbal drug research to discover new medicines. Looking at the need of a new safe and economical cardio tonic molecule, we resolved to investigate beneficial effects of *Citrus medica* Peels which is responsible for its cardio tonic activity\(^5\)-\(^7\).

*Citrus medica* Linn., commonly known as “Bijoru” in Gujarati, belongs to family Rutaceae\(^8\)-\(^11\). It is most important herb with both higher nutritive and therapeutic values. The citron could also be native to India where it borders on Burma, and Pakistan where it is found in valleys at the foot of the Himalayas, and in the Indian Western Ghats. The main chemical constituents *Citrus medica* contains, citroflavonoids consisting of a mixture of hesperidoside (rhamnoglucoside of hesperetol), naringoside and ecryodietyoside (flavanones). Essential oils and vitamin C are also found, in addition to glucosides hesperidin (vitamin P) and rutin \(^{12\text{-}14}\). The citroflavonoids also have an anti-inflammatory, antihistamine and diuretic action and can cause dilatation of the coronaries\(^15\).

**MATERIALS AND METHODS**
The fruit of Citrus medica were collected from Local market of Rajkot. The crude drug was authentified In Christ College Botanical Department Rajkot, gujarat, India. After that Methanolic extract of Peels were prepared using soxhlet apparatus.

![Figure 1](image)

**Figure 1**
Soxhlet Apparatus for Continuous Extraction

**Drugs and dosage**
From the review of literature the Dose of Citrus medica was 400mg/kg in the form of suspension prepared in double distilled water day for 3 weeks.

**Selection of Animals**
Either sex Wistar albino rats of weighing 200-250 gm were used for the present study (2013). The animals were procured from animal house, Department of Pharmacology, School of Pharmacy R.K.University, Rajkot. Animals were house at a temperature of 24±2°C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All the experimental procedures and protocols use in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of School of Pharmacy R.K.University and care of laboratory animals were taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), The animals were use after approval of IAEC protocol by Ministry of Social Justice and Empowerment, Government of India (Protocol no. RKCP/COL/RP/13/35).

**In vitro Cardio tonic Study of Citrus medica Peels Using Langendorff’s Apparatus:** [16-18]
The heart is innervated by both sympathetic and parasympathetic nerves, which alter the basic rhythm of the heart. Blood is supplied to the walls of the heart by coronary arteries. Postganglionic sympathetic axon passes from the main cardiac sympathetic nerves to the coronary vessels. There are no parasympathetic innervations of the vascular supply to the heart. It is probable that α adrenoceptors mediating vasoconstriction are present in the large coronary arteries and β2 receptor mediating vasodilatation exist in the smaller vessels. However, coronary blood vessels are very sensitive to vasodilator metabolites released from the myocardial cells especially under hypoxic condition. This effect in Albino rat heart can be maintained by perfusing coronary artery using method of Langendorff’s In this method, the aorta is cannulated and thus this technique is retrograde perfusion. The pressure of ringer solution closes the aortic valve so that the ringer solution is delivered directly to the mouths of the coronary arteries without passing through the heart.

The aortic valves prevent the left ventricle from filling and the left side of the heart remains empty. The right side received the fluid draining form the coronary sinus and this is expelled through the cut orifices of the inferior venacava or passes through the right ventricle and is pumped out of the cut pulmonary artery. Although there is no real cardiac output in the isolated heart, the rate at which fluid leaves the heart reflects the coronary flow, and will beat myogenically and the force of ventricular contraction can be measured by attaching a thread from the tip of the ventricle through a pulley system to a transducer and can be recorded on Student’s Physiograph[18].

The myocardial contraction of normal heart takes place according to Starling’s law of heart. According to this law, force of systolic contraction is directly proportional to the fiber length in diastole. Since systolic contraction represents cardiac output and the fiber length indicates to diastole venous pressure, the law indicates that cardiac output (Stroke volume) is directly related venous return of venous pressure during diastole. When the cardiac musculature fails to obey this relationship as in failing heart (congestive heart failure), there will be decrease in stroke volume (cardiac output), incomplete emptying of the ventricles during systole and enlargement of heart size due to residual blood in the heart at the end of systolic contraction. When the heart is in this state, i.e. inability to contract to physiologically normal, it is said to be hypodynamic heart. Experimentally
A hypodynamic heart can be produced by perfusing the heart with Ringer containing less quantity of calcium as this bivalent ion is essential for myocardial contraction\textsuperscript{[18]}.

**Experimental conditions\textsuperscript{[19]}:**

- **PSS:** Ringer Locke solution;
- **Temperature:** $37.5^\circ \pm 0.5^\circ \text{C}$;

**List of chemicals:**

- **Anticoagulant:** Heparin (300 IU, i.p.)
- **Anesthetic:** Diazepam (5 mg/kg, i.m.) followed by ketamine (75 mg/kg, i.p.)
- **Drugs:** Digoxin (50 µg/ml – 0.1 ml), Methanolic extract of *citrus medica* Peels (400mg/kg – 0.5 ml),

**Procedure\textsuperscript{[18]}:**

1. Before beginning the experiment, the Langendorff’s apparatus was set and ensured that the perfusion system was in good condition.
2. Heparin (300 IU, i.p.) was injected and after 20 minutes, the wistar rat was anaesthetize with diazepam (5 mg/kg, i.m.) followed after 10 minutes by ketamine (75 mg/kg, i.p.).
3. Thorax cavity was opened immediately to expose and remove the heart along with aorta as rapidly as possible and then the heart was put into ice cold Ringer Locke solution.
4. Heart was cannulated through aorta using artery cannula and mounted on Langendorff’s apparatus by securing firmly in place with bull-dog clamp.
5. The heart was perfused at a constant pressure and maintained at $37^\circ \text{C}$ and pH 7.4. A perfusion rate of 7 ml/minute was maintained before initialization of trial.
6. Thread was attached to the tip of the ventricle with the help of a fine bent pin as shown in Figure 2.
7. The thread was located around a pulley about 4 mm vertically below the heart.
8. End of thread from heart was attached to transducer for recording the contractions of the heart.
9. The contractions were recorded on student physiograph.

**Dosing of drugs:**
The basal cardiac contraction was recorded on Student’s Physiograph after administration of Ringer Locke solution and distilled water (vehicle). The administration of distilled water was done to see that it did not contribute to the effects of extract. Digoxin and methanolic extract of Citrus medica (MECM) Peels were administered through cannula.

**TABLE 1: DOSING SCHEDULE FOR IN VITRO CARDIOTONIC STUDY OF PEELS OF CITRUS MEDICA**

<table>
<thead>
<tr>
<th>Number of Trial</th>
<th>Control</th>
<th>Standard</th>
<th>Test</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>In normal Ringer-locke solution</td>
<td>Drinking water (Vehicle)</td>
<td>Digoxin 25 – 50 µg/ml</td>
<td>Methanolic extract (400 mg/kg)</td>
<td>To determine cardiotonic activity</td>
</tr>
</tbody>
</table>

Parameters to be measured:

Force of contraction (in mm)\(^{[18]}\) and % force of contraction (in mm)\(^{[18]}\).

**RESULTS AND DISCUSSION**

Cardiotonic effect of digoxin and methanolic extract of Citrus medica Peels on isolated rat heart – Langendorff’s assembly – an in vitro study:

When isolated rat heart was mounted on Langendorff’s assembly, baseline force of contraction and % Force of contraction is 32.66 ± 1.54mm, 100 ± 0mm
After administration of digoxin, as a standard cardio tonic drug, at a dose of 50 µg/ml, there were significant increase in force of contraction, % force of contraction viz 53.5 ± 1.78mm, 164±3.96mm (*p<0.001). which indicated that digoxin increased force of contraction and % Force of contraction as compared to normal force of contraction. After allowing heart to attain its normal state, administration of methanolic extract of peels Citrus medica (MECM) 100 mg/ml was done, which results in significant increase in force of contraction, % force of contraction 42.16± 1.07mm,129.5±3.62mm(*p<0.001) as compared to normal. which indicate that MECM increased force of contraction.
**TABLE 2: EFFECTS OF DIGOXIN AND MECM ON FORCE OF CONTRACTION OF ISOLATED RAT HEART – LANGENDORFF’S ASSEMBLY – IN VITRO STUDY (NORMAL HEART)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Force of contraction</th>
<th>% Force of contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>nc</td>
<td>32.66 ±1.54</td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td>std</td>
<td>53.5 ± 1.78***</td>
<td>164 ± 3.96***</td>
</tr>
<tr>
<td>Test</td>
<td>42.16 ±1.07***</td>
<td>129.5 ± 3.62***</td>
</tr>
</tbody>
</table>

**Figure 5**
Effects of Digoxin and MECM on Force of Contraction of Isolated Rat Heart Using Langendorff’s Assembly

**Figure 6**
Effects of digoxin and MECM on % Force of contraction of Isolated rat heart using langendorff’s assembly
Values are expressed as Mean ± S.E.M
nc group- received distilled water
std group- received digoxin (50 μg/ml – 0.2ml)
test group- received methanolic extract of Citrus medica Peels (MECM: 100mg/ml – 0.5ml)
*** indicate significant difference in the data compared to control group and the level of significance was p<0.001 ⇒ highly significant.
So, from the above in-vitro study we confirm that the methanolic extract of peels of Citrus medica was increase the force of contraction of the heart

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