Introduction

*Ocimum basilicum* Linn. (Labiatae), popularly known as “Sweet Basil” is used in both Ayurvedic and Unani systems of Medicine. It is a small perennial, tropically growing shrub of Asian origin. It has antipyretic, antiemetic, diuretic and cardiotonic properties.

Despite continuing advances in understanding the basic pharmacology of cardiac glycosides, digitalis intoxication remains a common clinical problem. It necessitates research for new nature based drugs which increase cardiac muscle contractility with a broad therapeutic index. As a part of the screening for a suitable natural drug we have chosen *Ocimum basilicum* and evaluated its cardio active potential and its mechanism of action.

Material and Methods

Preparation of extracts

*Ocimum basilicum* Linn. was collected from the Arignar Anna Hospital for Indian Medicine, Arumbakkam, Chennai, after being duly identified by a Botanist, Prof. R. Rangasamy, University of Madras. The aerial part of the plant was cleaned with water and dried in the shade until a constant weight was obtained. It was extracted with 95% ethanol (Total alcoholic extract = TAE) and then with double distilled water (Total aqueous extract = TAQ). The extracts were concentrated over a water-bath maintained at 55°C until a semi solid concentrate masses were obtained. The yields were for TAE, 4.8% and for TAQ, 9.3% of the plant material. For subsequent pharmacological and biochemical studies, 1% of TAE was suspended in 5% gum acacia, as it was not soluble in water and TAQ was water soluble hence an aqueous solution was used.

Drugs

Adrenaline, digoxin, propranolol and nifedipine. (Sigma Chemical Co, St.Louis, MI, USA).

Animals

Frogs of *Rana hexadactyla* species maintained in the animal house and male Wistar albino rats (150 to 200 g) housed...
in cages at 27°C ± 2°C on a 12 h light / dark cycle were used for the studies. The animals were fed with food and water ad libitum. The animals were maintained as per the norms of CPCSEA and the experiments were cleared by CPCSEA and the local ethics committee.

Frog heart in situ preparation

Frogs were pithed and the heart exposed. The inferior vena cava was cannulated for perfusing the heart with the frog’s Ringer solution. (The composition of the frog Ringer solution in millimoles: NaCl-110; KCl-1.9; CaCl₂-1.1; NaHCO₃-2.4; NaH₂PO₄-0.06; Glucose-11.1). The basal cardiac contraction was recorded on a smoked kymographic drum after the administration of frog Ringer’s solution and gum-acacia (5%). The administration of gum acacia was done to see that it did not contribute to the effects of TAE. The drugs and extracts were administered through the cannula. The average basal heart rate and the contraction amplitude were 70 beats/min and 18 mm respectively. The effects obtained with the drugs and extracts were transposed to the respective percentage of the basal values. Graded dose-response was recorded for each extract (0.5, 1 and 1.5 mg) and the dose which caused the maximum effect was chosen as the experimental dose. The frog heart was washed with the Ringer solution after every administration of extracts and drugs till it was brought back to the normal state.

The frog heart was perfused with propranolol, a β-adrenergic blocker at 3 X 10⁻⁵ M concentration in frog Ringer solution for 60 seconds followed by the administration of extracts and the recording were noted. Nifedipine, a calcium-channel blocker at 2.88 X 10⁻⁵ M concentration in frog Ringer solution was administered for 60 seconds followed by extracts and the recordings were noted.

Biochemical studies

Wistar albino rats were divided into 3 groups of 6 animals each. Group I received with 5% gum acacia suspension which served as control; Group II and Group III were treated with TAE and TAQ at a dose of 100 mg/kg (approximately 1/10 of the LD₅₀) body weight i.p. for 7 days. On the 8th day the animals were sacrificed and the heart and tissue were collected and the serum was separated from the blood. The heart was washed in ice-cold saline and about 100 mg of tissue was weighed and homogenized in chilled 0.1 M Tris-HCl Buffer in Patter-Elvejem tellion homogenizer: The serum and homogenized samples were assayed for clinical marker enzymes like CPK, LDH and transaminases AST and ALT. Heart homogenate samples were also assayed for Na⁺, K⁺ATPase, Ca⁺⁺ATPase and Mg⁺⁺ATPase.

Table 1

<table>
<thead>
<tr>
<th>Extracts / drugs</th>
<th>Frog ringer</th>
<th>Frog ringer + propranolol (3x10⁻⁴M)</th>
<th>Frog ringer + nifedipine (2.88x10⁻⁵M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR%</td>
<td>FC%</td>
<td>HR%</td>
</tr>
<tr>
<td>Digoxin (1.28x10⁻⁴M)</td>
<td>76.5 ± 2.47*</td>
<td>306.0 ± 10.99*</td>
<td>59.5 ± 2.67*</td>
</tr>
<tr>
<td>Adrenaline (2.5x10⁻⁴M)</td>
<td>158.6 ± 5.05*</td>
<td>258.2 ± 8.12*</td>
<td>116.6 ± 3.40*</td>
</tr>
<tr>
<td>TAE (1 mg/ml)</td>
<td>76.0 ± 2.68*</td>
<td>261.0 ± 10.83*</td>
<td>74.0 ± 2.56*</td>
</tr>
<tr>
<td>TAQ (1 mg/ml)</td>
<td>121.5 ± 3.67*</td>
<td>132.8 ± 4.25*</td>
<td>86.0 ± 5.10*</td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>120.54</td>
<td>66.71</td>
<td>92.51</td>
</tr>
<tr>
<td>df</td>
<td>3.36</td>
<td>3.36</td>
<td>5.54</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Control values: HR: 70.0 ± 1.25 (100%), FC: 18.2 ± 6.06 (100%). Values with different superscript in a column are significantly different from each other at P<0.05, n=10. Values are mean±SEM. TAE: Total alcoholic extract. TAQ: Total aqueous extract.

Statistical analysis

The frog heart in situ experiment and biochemical parameters obtained were subjected to one-way ANOVA followed by Tukey’s multiple comparison test. P value < 0.05 was considered significant.

Results

Total alcoholic extract (TAE) produced significant positive inotropic and negative chronotropic actions similar to that of digoxin on frog heart. This cardiotonic action was not antagonized by propranolol (Table 1). Nifedipine pretreatment significantly reduced the cardiotonic activity of the extracts. There was a significant decrease in membranous Na⁺, K⁺ATPase, and Mg⁺⁺ATPase and an increase in Ca⁺⁺ATPase (Figure 1). Total aqueous extract produced a significant increase in the force of contraction as reflected by an increase in the amplitude and heart rate. Both propranolol and nifedipine antagonized the effect of the extract TAQ (Table 1). No significant change was observed in Na⁺, K⁺ATPase, Mg⁺⁺ATPase and Ca⁺⁺ATPase of the heart (Figure 1).

Both the TAE and TAQ did not produce any significant changes in the levels of AST, ALT, LDH and CPK in heart and in serum samples when compared with the control animals (Table 2).

Discussion

Cardiac glycosides and catecholamines have been used as the main therapeutic drugs in the treatment of congestive cardiac failure. However, the dangers of cardiac glycoside intoxication are well documented and doubts have been expressed...
pressed about their long-term effectiveness. The use of catecholamines is limited by their insufficient differentiation between positive ionotropic and chronotropic actions, their potential arrhythmogenic properties and tachyphylaxis due to receptor down-regulation.11

Total alcoholic extract elicited a powerful cardiotonic effect, which was characterized by positive ionotropic and negative chronotropic actions. This effect was not significantly blocked by propranolol whereas nifedipine, the calcium channel blocker antagonized the effect significantly.

The cardiac enzyme profile indicates that TAE exhibited cardiotonic like activity which manifested as a result of general decrease in the activity of Na+K+ATPase, and Mg2+ ATPase and an increase in Ca2+ATPase. This inhibition of Na+K+ATPase is similar to the action of cardiac glycosides.13 Cardiac glycosides are specific and unique inhibitors of Na+K+ATPase at normal concentrations (10⁻⁹ to 10⁻⁸M).14

Na+K+ATPase inhibition by cardiac glycosides leads ultimately to increase intracellular Ca2+ concentrations through Na⁺/Ca²⁺ exchange and an associated increase in slow inward Ca²⁺ current16 as well as in transient Ca²⁺ current15. Ca²⁺ induced Ca²⁺ release is a general mechanism that most cells use to amplify Ca²⁺ signals.16 In heart cells, this mechanism is operated between voltage-gated L-type calcium channels (LCCs) in the plasma membrane and calcium release channel, commonly known as ryanodine receptors in the sarcoplasmic reticulum.17 Nifedipine is a LCC antagonist.18 Since nifedipine blocks the cardiotonic action of TAE significantly, the extract might have produced its action by opening the voltage sensitive slow Ca²⁺ channel. In connection with the cardiotonic effects observed one could see a relationship that exists between the inhibitory levels of the activities of Mg²⁺ATPase and Na⁺K⁺ATPase. The significant rise in the level of activity of Ca²⁺ ATPase might be due to the rise of cytosolic Ca²⁺.19

The total aqueous extract (T AQ) produced positive chronotropic and ionotropic effects similar to that of adrenaline by stimulating the β-adrenergic receptors.

### Table 2
Effects of the extracts from Ocimum basilicum Linn. on the marker enzymes in rats

<table>
<thead>
<tr>
<th>Marker enzymes</th>
<th>Group I (Control)</th>
<th>Group II (T AE)</th>
<th>Group III (TAQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Serum</td>
<td>Heart</td>
</tr>
<tr>
<td>AST</td>
<td>0.193 ± 0.007</td>
<td>0.512 ± 0.021</td>
<td>0.203 ± 0.007</td>
</tr>
<tr>
<td>ALT</td>
<td>0.0952 ± 0.006</td>
<td>0.614 ± 0.034</td>
<td>0.0978 ± 0.007</td>
</tr>
<tr>
<td>LDH</td>
<td>2.92 ± 0.09</td>
<td>5.220 ± 0.179</td>
<td>3.01 ± 0.08</td>
</tr>
<tr>
<td>CPK</td>
<td>0.56 ± 0.04</td>
<td>8.560 ± 0.320</td>
<td>0.61 ± 0.04</td>
</tr>
</tbody>
</table>

N = 6, values are expressed as mean ± SEM, NS: not significant. Enzyme units: Aminotransferases: (AST, ALT) µmoles x 10⁻² of pyruvate liberated/min/mg protein. LDH: µmoles x 10⁻¹ of pyruvate liberated/min/mg protein. For Heart CPK: µmoles of phosphorus liberated/min/mg protein. For Serum CPK: µmoles x 10⁻¹ of phosphorus liberated/min/mg protein.

### References
13. Akera T, Broddy TM. The role of Na⁺ K⁺ATPase in the ionotropic action of digi-
17. Fabiato A. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J Gen Physiol 1985;85:247-89.

ICMR INTERNATIONAL FELLOWSHIPS
INDIAN COUNCIL OF MEDICAL RESEARCH

The Indian Council of Medical Research (ICMR), the premier national agency for the formulation, promotion and conduct of biomedical research in India invites applications from Indian biomedical scientists for the above fellowship awards for the year 2004-05.

Applications Form & details are available at ICMR website.

Applications should be submitted to:

Chief
International Health Division, Indian Council of Medical Research,
V. Ramalingaswami Bhawan, Post Box No. 4911, Ansari Nagar, New Delhi -110029, India.

Last date of receipt of applications : 31st July 2004