Chronotropic and Inotropic Effects of *Piper longum* Linn

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Abstract: Extract of the plant *Piper longum* Linn (Family: Piperaceae) is used as folk medicine in India and China. Roots of the plant *Piper longum* are reported for the treatment of heart diseases in ancient literature of East Asia. However, no data is available on exact role of fractions of plant in heart diseases. In the present study we isolated four major fractions namely F, G, H and I and the activity of the fractions was studied on isolated frog heart. Fraction F has produced negative inotropic and negative chronotropic effect on isolated frog heart at 200 µg mL⁻¹. Fraction F shows maximum antagonistic activity on beta-receptors by decreasing heart rate and force of contraction. Actions of Adrenaline are blocked by fraction F and in the presence of propanolol, it shows continuous decrease in heart rate and force of contraction. In the present study, it was observed that fraction F shows antagonistic activity on beta adreno-receptors.

Key words: Chronotropic, inotropic, beta- blocking, *Piper longum*

Introduction

Medicinal plants have shown tremendous potential for the development of new drug molecules for various serious diseases. Many plant derived products have found to play an important role in various cardiovascular diseases (Charismsitha and Stanza, 1984). *Piper longum* Linn. (Piperaceae) has been used as a therapeutic agent in the treatment of various pathological conditions (Kirtikar and Basu, 1984 ). Fruits of the plant have been used as thermogenic, stomachic, aphrodisiac, carminative, expectorant, laxative, digestive, emollient, anti-giddiness, anti-amoebic, anti-asthmatic, antiseptic and in combination with Butea Monosperma the plant showed immuno-stimulatory activity in mice (Warrier et al., 1995; Agarwal et al., 1997; Goswal et al., 1996; Reddy et al., 1984). Dry fruits of the *Piper longum* have been used in the prevention of recurrence of asthma (Dahamnikar and Karandikar, 1984). The benzene extract of *Piper longum* when mixed with methanol extract of the Embelia ribes berries and administered to female rats inhibited pregnancy in 80% of the animals indicating Antifertility activity of the fruits (Khodkute et al., 1979).

Alkaloidal amides of the plant shows enhancement in the bioavailability of therapeutically important drugs (Khajuria et al., 1998). *Piper longum* in combination with the extract of *Zingiber officinalis* and *Ferula* species are found to be effective in gastric ulceration (Agarwal et al., 2000). As a fraction of Brahmi Rasayana *Piper longum* shows Anti-inflammatory activity (Jain et al., 1994). Root of the plant have been used traditionally by Indians for regularizing the heart rate and it is used as tonic for heart diseases (Charismsitha and Stanza, 1984).

Phytochemical investigation of the plant showed presence of pipertumurine, pipertumurinidine, piperman, sesamin, 3,4,5-trimethoxyximamine, beta-sitoster, aristolactum, piperyline, piplartine and hexacosanoic acid isobutyl amide (Dutta et al., 1977; Shankaracharya et al., 1997; Desai et al., 1989; Kouli et al., 1988).

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The diverse pharmacological properties of the *Piper longum* has indicated its vital role as a therapeutic agent in many diseases. Roots of the plant have shown good therapeutic potential. However proper qualification and quantitative analysis of the plant is very essential for exploring the exact role of fractions in serious cardiac diseases. Literature survey shows that no data is available on the cardiovascular properties of fractions of *Piper longum*. Since heart diseases has posed a great challenge in front of us it has become essential to discover plant based drugs which are safe and very useful in cardiac diseases. Fruit and roots of different species of piper like *Piper aduncum* (Orjala et al., 1993), *Piper betle* (Nair and Brake, 1990) and *Piper sarmentosum* have been used as a food material in Asian countries which indicate its safety.

The root of the plant shows beneficial role in the treatment of cardiac disease however no data is available on the exact role of the different fractions of *Piper longum*. Hence, the aim of present study was to isolate different fractions of *Piper longum* by column chromatographic technique (Wu et al., 2004) and to screen the exact cardiac activity of different isolated fractions. Dried roots of *Piper longum* were powdered and hexane was used as eluent for the isolation of different fractions. The isolated fractions were subjected for studying their cardiac effect on isolated frog heart (Satoskar and Bhandarkar, 1985).

### Materials and Methods

#### Isolation

Dried roots of *Piper longum* Linn. were purchased from local supplier and authenticated from botanical survey of India, Pune (Voucher No. 2005/13). The dried roots were powdered and then thin layer chromatographic analysis was carried out in different solvents such as water, benzene, acetone, ether, ethyl acetate, methanol, hexane and chloroform. Depending upon R\textsubscript{f} values and number of spots on TLC plate the solvent which extracts the maximum number of the fractions was selected. Hexane has shown presence of maximum ten spots on TLC plate and hence was selected for isolation (Table 1). TLC plates were observed under Ultra-violet light chamber and iodine chamber. The dried powdered roots were extracted with hexane at room temperature for 48 h. This extract was then filtered and evaporated under reduced pressure to obtain a viscous mass (4 g). This material was chromatographed over silica gel using hexane as eluent (Lee et al., 2002; Yang et al., 2002).

Total four major Fractions, namely F, G, H and I along with some minor fractions were isolated. The compounds obtained were purified with crystallization and no further column chromatography was needed since on gas chromatography fractions showed single fraction.

#### Cardiac Activity

The study involves screening of large number of compounds of *Piper longum*, which are not screened earlier for cardiac activities. Roots of *Piper longum* have been used as an active extract in various ayurvedic formulations and preparations. Due to the beneficial role of pharmacological actions of *Piper longum* in cardiac diseases, we have selected roots of the plant for the isolation and cardiac studies of different fractions. On TLC plate total ten fractions were observed. In order to study the cardiac activity of such large numbers of fractions we have selected isolated frog heart experiment.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>No. of constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>02</td>
</tr>
<tr>
<td>Benzene</td>
<td>03</td>
</tr>
<tr>
<td>Acetone</td>
<td>03</td>
</tr>
<tr>
<td>Ether</td>
<td>07</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>03</td>
</tr>
<tr>
<td>Methanol</td>
<td>03</td>
</tr>
<tr>
<td>Hexane</td>
<td>10</td>
</tr>
<tr>
<td>Chloroform</td>
<td>05</td>
</tr>
</tbody>
</table>

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Frogs (*Rana tigrina*), weighing 150-200 g were pithed (Guide-Guina et al., 1995; Bhanusli et al., 1987; Tripathi and Das, 1983). The heart was exposed and isolated from body after cannulation of the inferior vena cava using symes cannula. The cannula was connected to the reservoir containing frog's ringer solution. The solution was continuously bubbled with air, at room temperature. The composition of frog ringer in mM was Na⁺ 110.7, Cl⁻ 114.2, K⁺ 1.2, Ca²⁺ 1.10, HCO₃⁻ 2.8, H₂PO₄⁻ 0.1 and glucose 11.1 (pH 7.6-8.0).

The heart was stabilized for fifteen minutes prior to the administration of drugs. Responses were recorded on a smoked drum using a starting heart lever. The study was conducted in six different groups of animals. The fractions were prepared in different doses like 0.5, 10, 20, 30, 40, 50, 100 and 200 µg mL⁻¹. The activity of fractions was tested in the presence of Adrenaline and Propranolol. The crude hexane extract which is a mixture of all the fraction was also prepared in similar doses as that of the individual fraction and was subjected to cardiac studies on isolated frog heart. Similar condition is maintained in all the experiments to compare the activity of different fractions. This experiment is very reproducible, economic and can be used as a cardiac activity marker.

**Results and Discussion**

The results were analyzed statistically using students 't' test. Heart rate and force of contraction are mentioned in terms of percentage (Table 2). Initially the normal responses of isolated frog heart were recorded on smoked drum. Adrenaline was administered in doses of 0.5, 10, 20, 40 and 100, 200 µg mL⁻¹, the increase in heart rate and force of contraction indicate beta adrenergic agonist activity of adrenaline (Satokar and Bhanderkar, 1985). When Fraction F was administered in presence of adrenaline, in doses like 5, 10, 20, 30, 40, 100 and 200 µg mL⁻¹. There was a dose dependent decrease in heart rate and force of contraction. As the dose of the fraction was increased the heart rate and force of contraction decreases which indicates that the maximum inhibition is observed at a higher dose. At 200 µg mL⁻¹ maximum inhibition of adrenaline activity was observed (Fig. 1). Since adrenaline is a agonist of beta receptor in order to confirm the beta antagonistic activity, of the fraction F, atropine was administered in similar doses as that of fraction F, to verify the action of fraction F on cholinergic receptor. It was observed that in presence of Atropine the actions of Fraction F remains unaffected which indicates that the actions of Fraction F are mediated through beta receptor. The crude extract which is a combination of all the fractions was comparatively less effective on the heart rate and force of contraction. The extract shows maximum inhibition of heart rate and force of contraction at a increasing dose of 500 µg mL⁻¹. The finding suggests that the fraction F acts as antagonist of beta-receptor. Propranolol, which is a beta-blocking agent was used as a standard drug for comparison of beta antagonistic activity. Propranolol was also administered in a doses like 5, 10, 20, 30, 40, 50, 100 and 200 µg mL⁻¹. It has been observed that the fraction F in presence of propranolol shows synergic action and decreases the heart rate and force of contraction. The fraction F thus shows antagonistic activity on beta receptors and could be used as beta blocking agent.

**Table 2: Cardiac activity of fraction F and crude extract on isolated frog heart**

<table>
<thead>
<tr>
<th>Dose (µg mL⁻¹)</th>
<th>Fraction F (Presence Adrenaline)</th>
<th>Fraction F+Propranolol</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (%)</td>
<td>FC (%)</td>
<td>HR (%)</td>
</tr>
<tr>
<td>0.5</td>
<td>94</td>
<td>61</td>
<td>99</td>
</tr>
<tr>
<td>10</td>
<td>96</td>
<td>50</td>
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</tr>
<tr>
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<td>70</td>
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<td>54</td>
</tr>
<tr>
<td>200</td>
<td>65</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>500</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Each value was obtained from six experimental groups. Each group consisted of five animals. p<0.01. (HR% = Heart Rate in Percentage average, FC% = Force of contraction in Percentage average; ND = Not Done)*
Fig. 1: effect of fraction F on isolated frog heart

Beta blockers has been established as a standard first line therapy for both stable and unstable angina pectoris. Several studies and clinical trials have shown that beta blockers is used to reduce mortality when used in patients after myocardial infarction and in heart failure. In a recent literature, it has been pointed out that the currently available beta-blockers have deleterious effects on two aspects of clinical relevance to ischemic heart diseases, namely coronary collateral flow under controlled occlusion of the coronary artery and the impact on ischemic and stunned myocardium (Sato et al., 2004; Billinger et al., 2004; Monnet et al., 2004).

Thus there is a urgent need to find out new, safe and efficient drug molecules as beta blocking agents to fight against heart diseases. The existing synthetic Beta blockers are having certain adverse reaction like indigestion, nausea, diarrhoea. Since, Piper species are used as food material they might not show adverse reactions as that of present synthetic beta blocking agents. The data indicate that the isolated fractions can be used for their beta antagonistic activity in cardiac diseases.

Similar studies were carried out on fractions G, H and minor fractions however they have not produced any significant effects on isolated frog heart. The study of Fraction I is under progress. The spectral analysis of the fraction F is IR ν, cm⁻¹: 2941, 1683, 1620, 1132. ³H NMR (CDCl₃): δ: 5.90 (9H), δ: 4.1 (2H br), δ: 1.6 (6H), δ: 1.4 (10H), δ: 0.9 (2H), δ: 0.8 (3H). MS m/z (Rel. Int.): 238 (M⁺), 237, 146, 119, 92. The spectral data shows that one of the fraction F is having structural similarity as that of Piperine.

Conclusions

Fraction F produces negative inotropic and negative chronotropic effect on isolated frog heart. Fraction F blocks the actions of adrenaline completely at 200 meg mL⁻¹. The heart rate and force of contraction decreases in a dose dependent manner. Fraction F acts as antagonist of beta adrenergic receptors. It blocks the actions of Adrenaline in a dose dependant manner. The hexane extract of root of Piper longum was comparatively found to be less active than fraction F. The isolated fraction was thus found to have more activity and the present work can serve as a platform for studying the beta antagonistic activity of the isolated fractions of the Piper longum. The biochemical role of the fraction can be investigated in future in animals like, mice and rat which may explore the role of the fraction F as a beta blocking agent.

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