Alpha-adrenergic receptor blocking effect of *Cleistanthus collinus* (Roxb.) Benth. and Hook f. leaf extract on guinea pig isolated smooth muscle preparations

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Received 15 April 2010; revised 10 January 2011

Aqueous extract of *C. collinus* leaves inhibited norepinephrine induced contraction in guinea pig vas deferens and aortic strip in a dose-dependent manner. Inhibition of acetylcholine induced contraction in ileum was dose independent. *C. collinus* extract per se had no effect on isolated guinea pig vas deferens and aortic strip, but inhibited norepinephrine induced contraction in a dose-dependent manner probably by its antagonist action on \(\alpha\)-adrenergic receptor. It had inconsistent effect on guinea pig ileum in vitro preparation.

**Keywords:** \(\alpha\)-adrenergic, Antagonist, *Cleistanthus collinus*, Poisonous

*Cleistanthus collinus* (*C. collinus*) (Roxb.) Benth. and Hook f. (Euphorbiaceae) is a poisonous plant whose leaves are consumed for suicidal purposes. It is known by many vernacular names, garari in hindi, oduvan in Tamil, kadishi in Telugu, karada in Oriya, karlajuri of Pasu in the states of Bengal and Bihar. All parts of the plant are toxic. Leaves are eaten as such or as decoction. *C. collinus* poisoning is reported to be associated with life threatening cardiac, neuromuscular and acid-base disturbances\(^2\)-\(^4\). Drowsiness, decreased muscle power, respiratory failure and uncontrolled hypotension have also been reported following *C. collinus* leaves administration, suggesting involvement of autonomic nervous system\(^5\). However, the exact mechanism of leaves toxicity is not known and there is no suitable antidote available. Therefore the treatment of *C. collinus* poisoning is mainly symptomatic. The leaf extract yielded toxic principles like tannins and glycosides like collinusin, diphyllin, cleistanthin A and ceistanthin B\(^6\)-\(^8\). Though the toxic constituents have been reported to produced DNA strand breaks\(^9\)-\(^12\), oxidative tissue damage\(^13\) and reduced glutathione levels\(^14\), studies exploring the mechanism, which may help in designing antidote are still lacking. Therefore the objective of the study is to explore the role of adrenergic and cholinergic system/receptors by studying the effect of *Cleistanthus collinus* leaf extract on guinea pig isolated vas deferens, aortic strip and ileum.

**Materials and Methods**

*Preparation of leaf extract*—Leaves of *C. collinus* were collected in the month of April in the fields of Karasoor village near Pondicherry. The specimen was identified and authenticated by a botanist. A voucher specimen (V. No. JIP 5233/02) was kept for reference in the departmental library of Pharmacology, JIPMER, Pondicherry.

The leaves were shade dried and powdered using a mixer. Powder (200 g) was mixed in 2 L of water and boiled for 1h and filtered. The filtrate was evaporated using a heating mantle. The brown coloured residue obtained was powdered and stored at room temperature. The yield was 11%. The powdered form of the extract was used by making fresh solution in distilled water prior to each experiment. The concentrations used varied from 0.25 - 4 mg/ml of physiological salt solution.

*Chemicals*—Norepinephrine bitartrate salt was obtained from Sigma Chemical Co., USA and acetylcholine chloride from HiMedia Laboratories, Mumbai. All chemicals and solvents were of analytical grade.

*Smooth muscle preparations*—Male guinea pigs (weighing 400-600 g) were obtained from Central Animal House, JIPMER, Pondicherry. Institute Animal Ethics Committee permission was obtained.
for the study. The animals were maintained under standard laboratory conditions (12:12 L:D cycle and 25° ± 2° C), provided standard food and water ad libitum and housed in separate cages. The guinea pigs were fasted 24 h prior to the study with free access only to water. They were sacrificed by stunning and cervical dislocation. Vas deferens, aorta and ileum were dissected out. Thoracic aorta was dissected out, cut into a spiral strip of 3-5 mm width and 3-4 cm length. The physiological salt solution used for vas deferens and ileum was Tyrode and that for aortic strip was Krebs-Henseleit and was bubbled with carbogen gas (95% O₂ + 5% CO₂). The resting tension of 500 mg was used for vas deferens and ileum and a tension of 2 g was used for aortic strip. A 30 min time interval was allowed for vas deferens and ileum to stabilize after applying the tension whereas aortic strip was allowed to stabilize for 2 h.

Recording of response in smooth muscles—Vas deferens: Cumulative dose response of norepinephrine was recorded initially in two tissues to determine the working dose. The dose which produced a response between 30-70% of maximal response was selected as working dose. After stabilization, the response with the working dose of norepinephrine was taken. Once consistent responses were seen, the curative and preventive effects of the extract were recorded.

(a) Post treatment experiments: A response was taken with the working dose (3 mM/L of bath fluid) of norepinephrine and after 2 min at the peak of contraction, the extract (0.375 mg/ml of bath fluid) was added to the bath. After two min bath was drained of Tyrode solution and two to three washings were given. The procedure was repeated with the same dose of norepinephrine and three higher doses of extract (0.75, 1.5 and 3 mg/ml of bath fluid).

(b) Pre treatment experiments: After baseline became steady, a response was taken with norepinephrine and repeated till consistent response was seen. Then the extract (0.375 mg/ml of bath fluid) was added to the organ tube and allowed a contact period of 5 min. Then norepinephrine response was taken in presence of the extract. After adequate washing, the response with norepinephrine was recorded till recovery. The procedure was repeated with three higher doses of extract (0.75, 1.5 and 3 mg/ml of bath fluid).

Aortic strip: After stabilization, a cumulative dose response was obtained with norepinephrine. Working dose of norepinephrine was selected (1 mM/L of bath fluid) and added to the bath. The extract (0.5 mg/ml of bath fluid) was added after the response reached the peak. After adding the extract, the response was recorded till it reached a steady state. Then organ tube was drained of Krebs solution and refilled with fresh solution. The tissue was washed repeatedly till complete relaxation. After this the procedure was repeated with three more doses of the extract (1, 2 and 4 mg/ml of bath fluid). After the baseline became steady, the extract was added in a cumulative manner and the bath was washed sufficiently to allow the tissue to recover.

The preventive effect of the extract was tried in two tissues using a single dose of extract after a response with norepinephrine was obtained. The extract at a concentration of 2 mg/ml was added to the bath and a contact period of 10 min was allowed. Response of norepinephrine in presence of the extract was taken. The tissue was washed repeatedly and finally a response with norepinephrine alone was taken.

Ileum: After stabilization, a cumulative dose response was obtained with acetylcholine. A dose, which produced a response between 30-70% of maximal response, was selected as working dose. The rest of the procedure was same as for that of isolated vas deferens.

Analysis of responses—Amplitude of responses was measured from kymograph tracing. From this percentage inhibition was calculated. Inhibitory concentration (IC₅₀) was obtained from graph for each individual set of readings and the mean±SE calculated. Student’s t test was used to compare height of responses before and after adding extract. ANOVA (with Bonferroni post test) was used to compare the percentage inhibition with different doses of extract. A P value of < 0.05 was considered statistically significant.

Results

Effect on vas deferens—Curative effect: C. collinus leaf extract relaxed norepinephrine induced contraction at all doses used in curative manner (Table 1). The percentage inhibition varied from 43% at 0.375 mg/ml to 85% at 3 mg/ml of extract concentration by curative method and IC₅₀ of the extract was 0.38 ± 07 mg/ml.
Preventive effect: After incubating the tissue with *C. collinus* extract for 5 min, the amplitude of contraction with norepinephrine was same (or slightly higher at some doses) as that of pre extract level but this contraction was not sustained. The tissue relaxed immediately and reached a lower level of sustained contraction. Amplitude of this sustained contraction decreased significantly with increasing doses of extract (Table 2).

Percentage inhibition varied from 46% at 0.375 mg/ml to 89% at 3 mg/ml of extract concentration by preventive method and the IC$_{50}$ of extract was 0.64± 0.26 mg/ml.

**Effect on isolated guinea pig aortic strip—**

*C. collinus* leaves extract significantly relaxed norepinephrine induced contraction in a dose dependent manner. There was a significant difference in the amplitude of norepinephrine-induced contraction before adding and after adding extract at all doses used (Table 3). There was a significant difference in the percentage inhibition of norepinephrine-induced contraction between doses of extract used. It varied from 17% at 0.5 mg/ml to 73% at 4 mg/ml of extract concentration and the IC$_{50}$ of extract was in this tissue was found to be 2.02 ± 0.42 mg/ml.

**Effect on isolated guinea pig ileum—**

*C. collinus* leaf extract reduced the amplitude of acetylcholine-induced contraction. Of the four doses used, only at 0.5 mg/ml there was a statistically significant reduction in acetylcholine-induced contraction (Table 4).

There was no significant difference in the percentage inhibition of acetylcholine-induced contraction between the doses of extract used.

**Discussion**

The *Cleistanthus collinus* extract relaxed the norepinephrine induced contraction of guinea pig vas deferens and aortic strip in a dose-dependent manner. The extract *per se* did not produce any effect on these tissues. In ileum the extract produced a 20-30% inhibition of acetylcholine induced contraction, which was not dose dependent. Inconsistent effect was seen with extract *per se* in ileum.

In both isolated guinea pig vas deferens and aortic strip, the *C. collinus* extract *per se* did not produce any indicating that it has no agonistic or direct effect on these smooth muscles. The extract

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**Table 1**—Inhibitory effect of *C. collinus* leaf extract on norepinephrine-induced contraction in isolated guinea pig vas deferens

[Values are mean±SE from 6 animals in each group. Figures in parentheses are % inhibition]

<table>
<thead>
<tr>
<th><em>C. collinus</em> extract (mg/ml)</th>
<th>Amplitude of norepinephrine induced contraction(mm)</th>
<th>Before adding extract</th>
<th>After adding extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.375</td>
<td>18.83 ± 3.44</td>
<td>10.67 ± 2.07 (43)</td>
<td></td>
</tr>
<tr>
<td>0.750</td>
<td>25.33 ± 3.91</td>
<td>08.17±2.20 (68)</td>
<td></td>
</tr>
<tr>
<td>1.500</td>
<td>28.33 ± 3.17</td>
<td>05.33 ± 1.23 (81)</td>
<td></td>
</tr>
<tr>
<td>3.000</td>
<td>29.67 ± 2.77</td>
<td>04.50 ± 1.50 (85)</td>
<td></td>
</tr>
</tbody>
</table>

P<0.0001; One-way ANOVA for % inhibition at various concentrations

**Table 2**—Protective effect of *Cleistanthus collinus* leaf extract on norepinephrine-induced contraction in isolated guinea pig vas deferens

[Values are mean±SE from 6 animals in each group. Figures in parentheses are % inhibition]

<table>
<thead>
<tr>
<th><em>C. collinus</em> extract (mg/ml)</th>
<th>Amplitude of norepinephrine induced contraction(mm)</th>
<th>Before adding extract</th>
<th>After adding extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.375</td>
<td>23.75 ±2.59</td>
<td>12.75 ± 2.28 (46)</td>
<td></td>
</tr>
<tr>
<td>0.750</td>
<td>26.17 ±3.09</td>
<td>12.83±2.73 (51)</td>
<td></td>
</tr>
<tr>
<td>1.500</td>
<td>25.33 ±1.80</td>
<td>05.67 ± 1.30 (78)</td>
<td></td>
</tr>
<tr>
<td>3.000</td>
<td>24.83 ±1.62</td>
<td>02.67 ± 0.76 (89)</td>
<td></td>
</tr>
</tbody>
</table>

*For 0.375 mg/ml dose only 4 observations were recordable. P=0.0366 One-way ANOVA for % inhibition at various concentrations

**Table 3**—Effect of *Cleistanthus collinus* leaf extract on norepinephrine-induced contraction in isolated guinea pig aortic strip

[Values are mean±SE from 4 animals in each group. Figures in parentheses are % inhibition]

<table>
<thead>
<tr>
<th><em>C. collinus</em> extract (mg/ml)</th>
<th>Amplitude of norepinephrine induced contraction(mm)</th>
<th>Before adding extract</th>
<th>After adding extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>11.8 ±2.63</td>
<td>9.75 ± 2.39 (17)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>14.5 ±2.22</td>
<td>9.25±1.89 (36)</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>15.5 ±2.96</td>
<td>6.75 ± 1.32 (57)</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>15.0 ±3.1</td>
<td>4.00 ± 0.7 (73)</td>
<td></td>
</tr>
</tbody>
</table>

P=0.0001 One-way ANOVA for % inhibition at various concentrations

**Table 4**—Effect of *Cleistanthus collinus* leaf extract on acetylcholine-induced contraction in isolated guinea pig ileum

[Values are mean±SE from 5 animals in each group. Figures in parentheses are % inhibition]

<table>
<thead>
<tr>
<th><em>C. collinus</em> extract (mg/ml)</th>
<th>Amplitude of acetylcholine induced contraction(mm)</th>
<th>Before adding extract</th>
<th>After adding extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25*</td>
<td>24.75 ±1.32</td>
<td>17.75 ± 3.33 (28)</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>24.40 ± 2.73</td>
<td>19.20±3.34 (21)</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>27.20 ±1.39</td>
<td>22.00 ±3.36 (19)</td>
<td></td>
</tr>
<tr>
<td>2.00*</td>
<td>23.50 ±2.02</td>
<td>16.75±4.42 (29)</td>
<td></td>
</tr>
</tbody>
</table>

*For 0.25 and 2.0 mg/ml doses only 4 observations were recordable. P>0.05 One-way ANOVA for % inhibition at various concentrations.
specifically inhibited the effect of norepinephrine. Since norepinephrine produces contraction in vas deferens and aorta by involving \(\alpha\)-adrenergic receptors, the dose dependent relaxation seen with extract indicates that it has \(\alpha\)-adrenergic receptor blocking activity. Isolated guinea pig vas deferens and aortic strip are, in fact used in the screening of \(\alpha\)-adrenergic drugs\(^{16}\). This supports our explanation that the extract has \(\alpha\)-adrenergic antagonistic property.

Though the extract relaxed the norepinephrine-induced contraction of vas deferens in both curative as well as preventive methods, the initial response of the tissue to norepinephrine was same as that seen before incubation with the extract in preventive method. This initial response was not sustained and the tissue immediately relaxed to a sustained level of contraction which decreased in a dose dependent manner. The reason for initial response of norepinephrine could not be identified. It is speculated that it could be due to sensitization of adrenergic receptors by the extract. The supersensitivity of the receptors leads to exaggerated response, which could not be sustained. Hence there was immediate relaxation.

In isolated guinea pig ileum, when the \(C.\) \(collinus\) extract was added it produced inconsistent effects: in two preparations it induced contraction at higher doses whereas in other preparations it produced a fall in baseline. These effects are difficult to explain on the basis of present experiment. The extract has been reported to produce a generalised irritation of gastrointestinal mucosa\(^ {17}\).

By curative method, the extract inhibited the acetylcholine-induced contraction of ileum by 20-30%. This inhibition was not dependent on dose indicating a probable non-specific action. It is unlikely that \(C.\) \(collinus\) has antagonistic action at muscarinic receptor.

Based on present results, it can be concluded that \(Cleistanthus\) \(collinus\) leaf extract inhibits norepinephrine-induced contraction in isolated guinea pig vas deferens and aortic strip in a dose-dependent manner due to its antagonistic action on \(\alpha\)-adrenergic receptors.

**Acknowledgement**

The authors are thankful to Mr. Parasuraman S for interest and valuable suggestions.

**References**

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