Effect of potassium channel modulators on toxicity of *Cleistanthus collinus*

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Received 16 June 2002; revised 31 July 2003

The study was conducted to determine the effects of boiled extract of *Cleistanthus collinus* on rats by observing ECG changes and electrolyte levels in serum and urine. Influence of minoxidil and glibenclamide on *Cleistanthus collinus* induced toxicity was determined. ED₅₀ for arrhythmia, changes in contractility and heart rate were recorded using the isolated frog heart. *Cleistanthus* at low doses caused transient tachycardia and increase in contractility and at high dose caused arrhythmia and cardiac arrest in rat. LD₅₀ was found to be 1690 mg/kg. Minoxidil potentiated cardiac toxicity, whereas glibenclamide did not produce any significant change. High concentration of potassium in *Cleistanthus* extract hindered comparison of its levels. There was excretion of sodium even in the presence of hyponatraemia. *Cleistanthus* at low dose caused transient tachycardia and increase in contractility and at high dose caused arrhythmia and cardiac arrest in isolated frog heart. ED₅₀ for arrhythmia was found to be 1406 mg/kg. Acute toxicity was mainly due to depressive cardiac activity of *Cleistanthus*. It also caused renal failure. Potassium channel modulators did not have important role in acute cardiac toxicity treatment. Probably in chronic toxicity, electrolyte level changes are involved and potassium channel modulators might have a role.

Key words: Acute toxicity, *Cleistanthus collinus*, Contractility, ECG, Electrolyte, Heart rate, Oduvanthalai

*Cleistanthus collinus*, belonging to Euphorbiaceae is a poisonous tree that causes mortality when consumed in fresh form or as extract in water¹-⁴. Treatment protocol for *Cleistanthus* poisoning includes stomach wash, activated charcoal, intravenous fluids and potassium replacement, cardiac pacing, atropine and the required supportive measures. The principle of treatment includes decreasing the absorption of *Cleistanthus*, maintaining normal potassium level, preventing bradycardia and providing supportive measures. No specific antidote is available till now.

In *Cleistanthus* poisoning, most of the deaths occur when hypokalemia is at its peak³ and is mostly due to arrhythmia. Adenosine triphosphate sensitive potassium channel (KATP) modulators can modify cardiac function by altering potassium level⁵-⁶ and thus it may alter the toxic effect of *Cleistanthus* on heart.

It has been observed that boiled extract of *Cleistanthus* is more toxic to human beings as assessed by mortality rate⁷. An animal study done earlier used fresh ground extract. It had minute particles that could obstruct the small blood vessels when given intravenously and also its colour changed with time, as observed earlier in our laboratory⁷. Effect of boiled extract has not yet been validated in animal models. Effects observed on patients following *Cleistanthus* poisoning is simulated maximum by the use of boiled extract for the experiment rather than isolated active constituents. Hence, this study was aimed at determining the effects of boiled extract of *Cleistanthus* on the isolated perfused frog heart and to compare ED₅₀ for arrhythmia of pH adjusted with pH unadjusted extract. If comparable, the pattern of cardiac and electrolyte level changes after *in vivo* administration of extract to rat was also observed, since these were the usual effects observed in the poisoned patients. Influence of KATP modulators on *Cleistanthus*-induced cardiac toxicity in rat was determined and compared.

Materials and Methods

Animal care and use—Frogs weighing 40 to 60 g and Wistar albino rats of either sex weighing 250 to 350 g were used for the study. The rats were housed under standard laboratory conditions with 12 hr dark and light cycle. They were provided food and water *ad libitum*.

To detect the effect on isolated heart, six frogs were used in each group. For the determination of
changes in electrocardiogram (ECG), serum and urine electrolyte values, six rats were used in each of the groups. The Christian Medical College animal ethical committee sanctioned the use of the above number of animals for the study.

Drugs and chemicals—Pentobarbitone sodium (Loba-Chemie Indoasramal Co, Bombay), minoxidil sulphate (Dr Reddy’s Laboratory, Hyderabad), glibenclamide (Nicholas Piramal India Ltd, Mumbai), heparin (Gland Pharma Limited, Hyderabad), double distilled water, 0.9% saline, and chemicals like sodium hydroxide, sodium chloride, potassium chloride, sodium bicarbonate, sodium dihydrogen phosphate, calcium chloride and D glucose from standard laboratory suppliers were used.

Preparation of extract—Fresh Cleistanthus leaves identified by a botanist was collected and used for the study. Double distilled water (300 ml) was taken in a porcelainine dish (Porcelainine dish of 15 cm dia) and kept for boiling. Freshly collected Cleistanthus leaves (30 g) were added to the above boiling distilled water. Boiling was continued for 15 min. Then it was left to cool and the leaves removed. About 150 ml of yellowish brown extract with a characteristic odour and pH (Systronics, India) at 3.2 to 3.4 was obtained. It was adjusted at 7.34-7.44 by titration and addition of 1 N sodium hydroxide. The volume of the extract was adjusted to 300 ml with distilled water, filtered and used for the study as the pH adjusted extract and without pH adjustment extract was also made up to 300 ml to obtain pH unadjusted extract. The above solution was calculated to have 100 mg of leaf extract in 1 ml of distilled water. Conventionally patients use similar boiled preparation, therefore the same method was followed for leaf extract preparation.

In vitro experiment on frog heart—Frog was pithed and pinned to frog board, thoracic cavity opened, pericardium removed, a thread passed below the inferior venacava and then nicked, Symes cannula introduced and tied. It was separated, and perfused with frog Ringer solution to obtain an isolated frog heart preparation. Increasing doses of Cleistanthus were added to the isolated frog heart set up and the heart rate and force of contraction recorded till arrhythmia started appearing. ED₉₀ for arrhythmia was determined and compared between the pH adjusted and unadjusted extracts.

Changes in ECG of rat heart after administration of extract—Rats were anaesthetised with pentobarbitone sodium (50 mg/kg; ip). The external jugular vein and trachea were cannulated. For artificial ventilation when required, the tracheal cannula was connected to artificial respirator (500 ml/kg/min). Heparin solution (0.5 ml of 500 IU/ml) was injected through venous cannula to prevent interference due to coagulation of blood in the tube.

The extract was given iv at the rate of 1ml in 3 min. Limb lead II ECG recording was done every min for 3-5sec using the polygraph (Polyrite, Chandigarh). The time taken for absence of P wave, for heart rate to decrease by 50%, and for cardiac asystole were determined. In some animals, occasional further transient contractions and electrical activities were observed after an initial cardiac arrest. Hence voltage recordings of less than 3 mV were considered as cardiac inactivity. The LD₅₀ was also determined.

Pre-treatment groups were given glibenclamide (5 mg/kg; ip) or minoxidil (1.6 mg/kg; ip) 15 min prior to the experiment undertaken.

Measurement of electrolyte level—Sodium and potassium levels in the boiled extract, and serum and urine of rat were estimated. Blood was collected from the inferior venacava after the death of the rat. Urine samples were collected by supra pubic puncture.

Statistical method—Heart rate and contractility were compared using repeated measure ANOVA test followed by Dunnett multiple comparison test. Time changes in heart rate were compared using one-way ANOVA test followed by Tukey-Kramer multiple comparison test. Electrolyte levels were compared using Kruskal-Wallis test followed by Dunns test. Kaplan Meier survival curve, log rank and Cox regression analysis were also performed. The data was analysed using Graph Pad Instat Program and survival curve by SPSS. The dose causing arrhythmia in 50% of animals was determined by graphical method of Miller and Tainter.

Results

Effect on isolated frog heart—ED₉₀ for arrhythmia with Cleistanthus leaves pH-unadjusted extract on frog heart was found to be 753±174µl and for pH-adjusted extract was 703±186µl which was not significantly different.

Isolated frog heart rates, after exposure to various concentrations of pH adjusted boiled extract were compared (Fig. 1). Extract (1.6 ml) exposed heart rate, showed significant decrease from pre-treatment heart rate (Table 1).

Concentration of the extract to which the frog's heart was exposed was compared with contractility (Fig. 1). Initial contractility was considered as 100%
and subsequent contractility were determined comparing it with initial contractility. There was significant increase at 0.4 ml of extract and decrease noted at 1.6 ml of extract, when compared with pre-treatment contractility (Table 1).

In vivo effect of extract on rat heart — ECG changes were studied in 6 rats. Initial tachycardia was noted in 5 rats. Abnormal P waves and absence of P wave were observed in all the rats. Other changes included junctional escape in 4, atrioventricular block in 2, bundle branch block in 3, evidence of multiform wide QRS escape beat in 1, tall T wave in 1 and transient polymorphic ventricular tachycardia in 1 rat. Finally all the rats developed bradysystolic cardiac arrest. Similar ECG changes were observed in minoxidil and glibenclamide pre-treated rats.

There was no significant difference between the three groups for time taken for 50% heart rate and for absence of P wave when compared between KATP modulator pre-treated and untreated rats (Table 2).

Kaplan Meier survival curve was done to compare the survival of rats in different groups (Fig. 2). There was significant difference between the groups noted by Log rank statistics. Risk for asystole was 3.9 times for minoxidil group and 1.3 times for glibenclamide group when compared to Cleistanthus alone group by univariate Cox regression analysis. Survival curve with glibenclamide was found to cross Cleistanthus survival curve at certain points. LD₅₀ with boiled Cleistanthus extract in rat was 1690 mg/kg.

In vivo study on serum and urine sodium and potassium levels — Sodium and potassium levels were compared with control values. Cleistanthus alone treated rats, those pretreated with glibenclamide and minoxidil. Cleistanthus extract contained potassium (11.57 ± 3.11 milliequivalent per ml) and no detectable sodium. In serum of Cleistanthus, glibenclamide and minoxidil treated group, sodium values were significantly low compared to control values. In serum of glibenclamide and minoxidil treated group, potassium was high compared to control values (Table 3). In urine of Cleistanthus, glibenclamide and minoxidil treated group, sodium levels were significantly high compared to control values whereas

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**Fig. 1** — Effect of increasing doses of boiled extract of Cleistanthus on isolated frog heart preparation

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Heart rate (min)</th>
<th>Contractility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>57.67±10.99</td>
<td>100±0</td>
</tr>
<tr>
<td>0.1</td>
<td>59.17±9.68</td>
<td>111.20±10.54</td>
</tr>
<tr>
<td>0.2</td>
<td>60.33±11.15</td>
<td>122.03±9.66</td>
</tr>
<tr>
<td>0.4</td>
<td>58.67±12.37</td>
<td>155.60±32.90**</td>
</tr>
<tr>
<td>0.8</td>
<td>51.50±12.39</td>
<td>119.21±63.96</td>
</tr>
<tr>
<td>1.6</td>
<td>37.33±12.53**</td>
<td>30.42±25.97**</td>
</tr>
</tbody>
</table>

**Table 1** — Effect of Cleistanthus on isolated frog heart [Values are mean ± SD of 6 animals]

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**Table 2** — Time taken for changes in the heart rate in rat [Values are mean ± SD of 3 replications]

<table>
<thead>
<tr>
<th>Group (min)</th>
<th>Time for rate to fall below 50%</th>
<th>Time for disappearance of P wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleistanthus</td>
<td>21.00±7.32</td>
<td>21.33±6.86</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>15.00±10.18</td>
<td>17.17±9.45</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>10.00±6.60</td>
<td>10.17±6.49</td>
</tr>
</tbody>
</table>

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**Fig. 2** — Kaplan Meier survival curves showing time (min) to asystole for Cleistanthus alone, minoxidil and glibenclamide groups. Values are significant at P<0.05 noted between the groups by Log rank statistics.
in urine of *Cleistanthus* and glibenclamide treated group, potassium values were high compared to control values (Table 3).

**Discussion**

The pH of the extract had to be adjusted to 7.4±0.04 with sodium hydroxide since acidic substances can cause decrease in contractility. Since pH adjustment caused precipitation of certain proteins, the effects of pH adjusted and unadjusted extracts were compared. There was no significant difference noted for ED50 to produce arrhythmia and hence the effects of the two extracts were comparable.

In isolated perfused frog heart preparation, *Cleistanthus* extract produced transient insignificant tachycardia, significant increase in contractility followed by arrhythmia and significant decrease in heart rate, finally leading to cardiac arrest at higher dose. Similar findings have been noted earlier using ground extract. ED50 for arrhythmia was 703±186 µl (Dose of pH-adjusted extract was equivalent to 1406 mg/kg).

When *Cleistanthus* extract was given intravenously to anaesthetised rats, it produced changes in ECG. These effects can be summarised as due to initial stimulation followed by gradual inhibition of electrical activity of the heart, first affecting the pacemaker then gradually affecting the lower centres, finally causing total asystole. An earlier study done using oral ground extract in rat has shown a decrease in heart rate and arrhythmias, which are mainly junctional in origin, but does not show the initial tachycardia.

Increase in contractility and arrhythmia may be due to different glycosides in *Cleistanthus* extract. Direct toxicity of *Cleistanthus* might also be partly responsible for this, considering the immediate cardiac effect noted. Cardiac glycosides by inhibiting sodium-potassium ATPase leads to increased availability of calcium leading to increase in contractility and arrhythmia. ECG changes noted in patients are tachycardia, bradycardia, flat P wave, prolonged QT interval, ST segment depression and inversion of T wave. Our study showed evidence of bradyarrhythmias, proving clinical benefit of pacing.

There was no evidence of hypokalemia in the rat serum following extract administration. The earlier oral toxicity study using ground extract also does not show evidence of hypokalemia. A concrete conclusion on serum electrolyte level could not be made out due to high potassium and low sodium content in the extract. Hyperkalemia as the cause of cardiac arrest needs to be excluded since it was systolic cardiac arrest.

Urine examination of rat showed significant increase in excretion of sodium and potassium with extract treatment. Considering the fact that administering extract (3-5 ml) to blood (6-12 ml) of rat will cause a huge change in electrolyte make up and blood volume, the variation in electrolyte excretion has to be viewed with caution. Significant sodium excretion at normal, and low sodium level in serum is due to nephrotoxicity of *Cleistanthus* as has been reported in 37% of patients. There are evidences of urinary potassium loss and absence of intracellular shift of potassium in patients. In 72% patients, peak hypokalemia has been noted between 3rd and 5th day and the peak mortality coincides with this period. From the observation in animals and patients, hypokalemia seems to be chronic rather than an acute cause. This is likely due to steroid-like structure of glycoside or secondary to activation of renin-angiotensin-aldosterone axis since it requires 3 to 4 days. In the present study pre-treatment drugs did not produce any characteristic alteration in ECG pattern compared to *Cleistanthus* group.

None of pretreatment drugs was protective. The significant toxicity of minoxidil observed in the survival curve might be due to exaggeration of cellular

### Table 3 — Serum and urine electrolyte levels in rat

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum sodium (meq/l)</th>
<th>Serum potassium (meq/l)</th>
<th>Urine sodium (meq/l)</th>
<th>Urine potassium (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>146.50±3.27</td>
<td>4.8±0.28</td>
<td>18.50±9.7</td>
<td>219.33±29.17</td>
</tr>
<tr>
<td><em>Cleistanthus</em></td>
<td>85.83±8.80**</td>
<td>21.18±3.28</td>
<td>281.67±80.67**</td>
<td>491.50±144.09**</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>90.50±13.56*</td>
<td>23.52±5.29**</td>
<td>258.83±132.64**</td>
<td>484.67±204.04*</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>89.83±12.34*</td>
<td>24.65±3.72**</td>
<td>259.83±232.94*</td>
<td>447.01±179.19</td>
</tr>
<tr>
<td>KW (df)</td>
<td>13.081(3,20)</td>
<td>13.991(3,20)</td>
<td>15.336(3,20)</td>
<td>9.144(3,20)</td>
</tr>
</tbody>
</table>

**P value**

- **P**<0.01, *P*<0.05 compared to control group values.
hypokalemia due to opening of potassium channel. Survival curve for glibenclamide seems to overlap with that of *Cleistanthus* though a significant difference was not noted. A bigger sample size might be needed if at all a protective effect for glibenclamide is to be demonstrated. LD50 in rat was 1690 mg/kg.

It can be concluded that acute toxicity was mainly due to cardiac activity of *Cleistanthus*, that can also cause nephrotoxicity. Potassium channel modulators did not have important role in acute toxicity treatment.

Acknowledgement

The authors express their gratitude to Mr V Shankar, for helping in statistical analysis and Dr Ashish, for helping in ECG reading. We also express our sincere thanks to the Christian Medical College research committee for providing grant for the project. Thanks are also due to Mr. Raju John T., Department of Botany, Saint Aloysius College, Elthuruth, Thrissur, Kerala 680 011 for identification of the plant.

References