Effects of Cleistanthins A and B on Blood Pressure and Electrocardiogram in Wistar Rats

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Z. Naturforsch. **66 c**, 581–587 (2011); received February 16/October 12, 2011

We have studied the effects of cleistanthin A and cleistanthin B, phytoconstituents isolated from the leaves of *Cleistanthus collinus* Roxb. (Euphorbiaceae), on blood pressure, electrocardiogram, and barium chloride-induced arrhythmia in Wistar rats. The two compounds were isolated by column chromatography and their identity was confirmed spectroscopically. A healthy, male Wistar rat was used to record the invasive blood pressure and electrocardiograph. The antiarrhythmic effects of cleistanthins A and B were studied using the barium chloride model. Both cleistanthin A and cleistanthin B showed a dose-dependent hypotensive effect. Both compounds reduced the mean blood pressure significantly although the dose required for the effect was higher in the case of cleistanthin B. In the electrocardiogram, cleistanthins A and B significantly altered the electrical activity of the heart, the changes were transient and of no further consequence. Intravenous injection of 64 μg or more of cleistanthins A and B caused a sudden respiratory depression without affecting the electrocardiogram. Cleistanthins A and B did not display any antiarrhythmic effect against barium chloride-induced arrhythmia. In conclusion, both cleistanthin A and cleistanthin B exert a hypotensive effect and have no antiarrhythmic effect against barium chloride-induced arrhythmia in Wistar rats.

Key words: Cleistanthins A and B, Blood Pressure, Electrocardiogram

Introduction

Cleistanthin A and cleistanthin B are phytoconstituents of the leaves of *Cleistanthus collinus* Roxb. (Euphorbiaceae), which is widely distributed in Southeast Asian countries. In India this plant has been identified in the southern part of the country. The leaves of the plant are widely used to commit suicide. The effects of *Cleistanthus collinus* leaf poisoning include a drop in blood pressure, changes in the electrocardiogram, and respiratory paralysis. Cleistanthins A and B were reported to be toxic substances present in the leaves of *Cleistanthus collinus* (Annapoorani *et al.*, 1984). Apart from cleistanthins A and B, *Cleistanthus collinus* leaves contain more than 30 phytochemicals (Parasuraman *et al.*, 2009). A structure-activity relationship prediction carried out for cleistanthins A and B indicated antihypertensive, antitumour, and neurotrophic factor enhancement properties, respectively. Toxicity prediction studies did not suggest that cleistanthins A and B had any serious toxic effects. The present study was conducted to investigate the effects of cleistanthin A and cleistanthin B on cardiovascular parameters including blood pressure, electrocardiogram, and barium chloride-induced arrhythmia in Wistar rats.

Material and Methods

Animals

The study was approved by the Institute Animal Ethics Committee, and all experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Healthy adult male Wistar rats (170–190 g) were obtained from the central animal house at JIPMER, Pondicherry, India. The rats were housed at (25 ± 2) °C, 55–65% humidity, and a (12 ± 1) h/(12 ± 1) h light/dark cycle. The animals were fed with standard rat pellets (Hindustan Liver Ltd., Bangalore, India) and water *ad libitum*.

Isolation of cleistanthins A and B

The leaves of *Cleistanthus collinus* were collected near Pondicherry, India. The plant was identified and certified (BSI/SC/5/23/08-09/Tech.241) by the Botanical Survey of India, Southern Circle, Coimbatore. Cleistanthins A and B were isolated from the leaves using chromatographic techniques, and the chemical structure of each compound was confirmed spectroscopically.

Air-dried leaves of the plant were powdered and defatted with *n*-hexane and subsequently extracted with acetone. The acetone extract was dissolved in benzene and passed through a neutral alumina column. Fractions collected with benzene, benzene/ethyl acetate (4:1), benzene/ethyl acetate (1:1), and methanol/chloroform (9.5:0.5) were subjected to thin layer chromatography and spectroscopic analysis. Cleistanthins A and B were obtained from the benzene/ethyl acetate (1:1) and methanol/chloroform (9.5:0.5) fractions, respectively (Govindachari *et al.*, 1969; Parasuraman and Raveendran, 2011).

Preparation of cleistanthins A and B solution

Cleistanthins A and B were insoluble in water, but soluble in benzene, acetone, acetic acid, and 95% ethanol. The two compounds were dissolved in a minimal volume of 95% ethanol, and the volume was made up with sufficient quantity of sterile water.

Effects of cleistanthins A and B on blood pressure and electrocardiogram

A fasted rat (8–10 h) was anaesthetized using ketamine [80 mg/kg body weight, intraperitoneal (i.p.)] and xylazine (16 mg/kg body weight, i.p.). The animal was placed on a surgical platform, and the skin on the ventral side of the neck, right hind leg, and chest region was carefully shaved and disinfected. A small incision was made in the right thigh, the femoral vein was identified, cannulated with a 26G × 1/2″ needle, and flushed with normal saline. The neck region of the animal was opened, and tracheostomy was carried out. The carotid artery was identified along with the vagus nerve on both sides of the trachea. One side of the carotid artery was cleaned, separated from the vagus nerve, and cannulated using a cannula prefilled with heparinized normal saline (0.5 IU/ml). The cannula was connected to a pressure transducer, and the blood pressure of the animal was recorded using a data acquisition system (Power Lab, AD Instruments, Bella Vista, Australia). The femoral vein was flushed with heparinized normal saline to avoid blood clotting at the puncture site and inside the cannula (Vogel, 2002).

The positive, negative, and reference electrocardiogram electrodes were connected to the left fore leg, right fore leg, and left thigh, respectively, to record electrocardiograms. The data acquisition system was pre-calibrated using a mercury sphygmomanometer. The preparation was allowed to stabilize for 15 min, and the basal blood pressure was recorded for 7–10 min to confirm the stability and accuracy of the experimental preparation. Cleistanthin A and cleistanthin B were injected through the femoral vein, and the blood pressure was recorded and electrocardiograms were obtained (Ordodi *et al.*, 2005).

Effects of cleistanthins A and B on arrhythmia

A male rat was anaesthetized using 1200 mg/kg body weight urethane and placed on a temperature-controlled surface. The femoral vein was cannulated for drug administration, and tracheostomy was carried out. The carotid artery was cannulated next for monitoring the blood pressure. The positive, negative, and reference electrocardiogram leads were fixed on the animals to obtain electrocardiograms. Cleistanthin A or cleistanthin B (2 μg) was injected 5 min before induction of arrhythmia using 1 ml/kg body weight of 30 mg/ml barium chloride solution (Marona *et al.*, 2008).

Statistical analysis

The mean and SEM values were calculated for each parameter. Significant differences between the groups were determined using repeated ANOVA measures followed by Bonferroni comparison of the groups. A P value less than 0.05 was considered significant.

Results

At the resting level, the mean blood pressure values of group I (cleistanthin A-administered group) and group II (cleistanthin B-administered group) animals were (66.23 ± 10.1) and (65.44 ± 6.2) mm Hg, respectively. In general, cleistanthin A and cleistanthin B, when adminis-

tered individually, elicited a dose-dependent drop in the mean blood pressure in rats (Fig. 1).

The effects of cleistanthin A and cleistanthin B on blood pressure are presented in Tables I and II. Cleistanthin A produced significant changes in the maximum pressure (↓), minimum pressure (↓), end diastolic pressure (EDP) (↓), mean blood pressure (↓), systolic duration (↓), diastolic duration (↓), and cycle duration (↓) at doses of 16 μg onwards. Similar changes were observed with cleistanthin B at a dose of 128 μg except for the diastolic duration, which was not significantly affected. Both cleistanthin A and cleistanthin B did not produce any significant difference in the heart rate, contractility index, isovolumetric relaxation period (IRP), time constant of ventricular relaxation (tau), and pressure time index.

The effects of cleistanthin A and cleistanthin B on the electrocardiogram are presented in Tables III and IV. In the electrocardiogram, cleistanthin A and cleistanthin B caused a dose-dependent increase in the RR intervals and reduced the heart rate, but these effects were not significant except at 128 μg. Cleistanthin A produced significant changes in the R wave amplitude (increased at 4 and 8 μg), S wave amplitude (increased at 8, 16, 32, and 64 μg), ST height (decreased at 64 and 128 μg), and T wave amplitude (decreased at 64 and 128 μg). Cleistanthin B did not produce any significant changes in the amplitudes of the R, S, and T waves. Cleistanthin B produced a significant decrease in the ST height at 64 and 128 μg. Both cleistanthin A and cleistanthin B did not produce any significant changes in the PR, QRS, QT, and JT intervals. Both compounds proved to be lethal to rats at doses above 64 μg, causing sudden respiratory paralysis. There were no changes in the electrocardiogram.

Neither cleistanthin A nor cleistanthin B demonstrated any significant antiarrhythmic effect against barium chloride-induced arrhythmia. However, cleistanthin B reduced the duration of barium chloride-induced arrhythmia in Wistar rats (Table V). Neither cleistanthin A nor cleistanthin B induced arrhythmia in normal rats.

Discussion

Cleistanthin A and cleistanthin B produce a dose-dependent hypotensive effect in Wistar rats. Cleistanthin A reduces the mean blood pressure at doses of 16 μg and above, whereas the reduction caused by cleistanthin B is significant only at a dose of 128 μg. This might indicate that cleistanthin B is less potent than the other compound in reducing blood pressure. The crude extract of *Cleistanthus collinus* was shown to block phenylephrine-induced contraction of the guinea pig aorta, and the effect was attributed to alpha-adre-

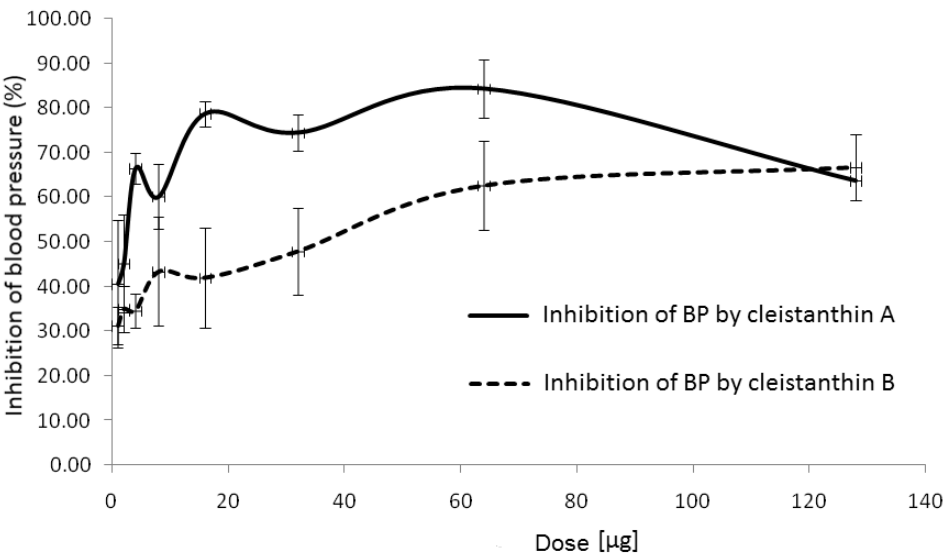

Fig. 1. Dose-dependent percentage inhibition of rat blood pressure by intravenous cleistanthins A and B. The values are mean values, and the error bars represent the SEM (n = 5).

Table I. Effect of cleistanthin A (administered i.v.) on blood pressure.

Group	Normal	Cleistanthin A							
		1 μg	2 μg	4 μg	8 μg	16 μg	32 μg	64 μg	128 μg
Max. pressure (mm Hg)	104.10 ± 9.41	85.23 ± 10.26	96.85 ± 8.52	85.71 ± 7.22	90.21 ± 1.97	78.15 ± 3.80	70.26 ± 6.38*	65.28 ± 5.34**	63.64 ± 0.68***
Min. pressure (mm Hg)	86.63 ± 7.79	57.33 ± 9.70	67.44 ± 7.43	56.79 ± 7.20	61.86 ± 2.88	51.56 ± 4.05*	43.76 ± 5.73***	40.12 ± 5.07***	38.54 ± 0.64***
EDP (mm Hg)	82.78 ± 4.83	59.22 ± 10.16	69.57 ± 8.02	58.88 ± 7.37	63.60 ± 2.18	52.46 ± 3.97	44.78 ± 6.01**	40.78 ± 5.16**	39.09 ± 0.65***
Mean pressure (mm Hg)	100.50 ± 6.04	79.0 ± 9.13	90.16 ± 6.04	80.02 ± 8.20	85.92 ± 5.29	74.29 ± 5.03	65.71 ± 5.64*	58.98 ± 5.23***	58.13 ± 0.46***
Max. – min. pressure (mm Hg)	47.33 ± 16.10	27.90 ± 1.58	29.42 ± 1.13	28.93 ± 1.0	28.35 ± 1.04	26.58 ± 0.49	26.50 ± 0.73	25.16 ± 0.28	25.10 ± 0.04
Systolic duration (s)	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.13 ± 0.0	0.14 ± 0.0
Diastolic duration (s)	0.06 ± 0.02	0.10 ± 0.02	0.07 ± 0.0	0.09 ± 0.01	0.10 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.17 ± 0.01**	0.21 ± 0.0***
Cycle duration (s)	0.12 ± 0.04	0.16 ± 0.03	0.14 ± 0.02	0.15 ± 0.02	0.17 ± 0.03	0.19 ± 0.04	0.20 ± 0.04	0.28 ± 0.01*	0.32 ± 0.0***
Pressure time index (mm Hg/s)	7.46 ± 1.77	6.85 ± 1.82	7.06 ± 1.52	6.60 ± 1.59	7.14 ± 1.22	6.70 ± 1.50	6.0 ± 1.17	7.64 ± 0.60	8.83 ± 0.04

Max., maximum; Min., minimum; EDP, end diastolic pressure.
The values are means ± SEM; $n = 5$ except for 128 μg cleistanthin A, where $n = 4$; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared with normal.

Table II. Effect of cleistanthin B (administered i.v.) on blood pressure.

Group	Baseline	Cleistanthin B							
		1 μg	2 μg	4 μg	8 μg	16 μg	32 μg	64 μg	128 μg
Max. pressure (mm Hg)	78.09 ± 4.17	79.94 ± 7.27	83.77 ± 7.66	80.32 ± 7.04	82.20 ± 8.93	86.74 ± 5.23	74.61 ± 5.10	68.30 ± 7.53	40.99 ± 4.34*
Min. pressure (mm Hg)	55.51 ± 5.19	55.78 ± 7.51	59.32 ± 7.83	55.73 ± 6.95	57.32 ± 8.83	61.68 ± 5.18	49.92 ± 4.88	42.75 ± 6.79	17.0 ± 4.48**
EDP (mm Hg)	50.84 ± 3.33	56.31 ± 7.45	59.80 ± 7.82	56.30 ± 7.03	58.01 ± 8.83	62.47 ± 5.14	50.57 ± 4.90	43.56 ± 6.95	17.48 ± 4.54*
Mean pressure (mm Hg)	73.15 ± 7.89	73.47 ± 9.45	80.17 ± 10.39	79.65 ± 7.69	84.17 ± 14.32	82.61 ± 4.63	70.56 ± 4.86	65.39 ± 9.65	33.02 ± 4.53*
Max. – min. pressure (mm Hg)	36.66 ± 12.70	24.16 ± 0.30	24.44 ± 0.51	24.58 ± 0.71	24.87 ± 1.04	25.06 ± 0.93	24.69 ± 0.29	25.55 ± 0.85	23.99 ± 0.15
Systolic duration (s)	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.03	0.13 ± 0.01	0.20 ± 0.06	0.14 ± 0.03	0.15 ± 0.04	0.15 ± 0.02	0.22 ± 0.06
Diastolic duration (s)	0.27 ± 0.07	0.24 ± 0.05	0.24 ± 0.06	0.22 ± 0.04	0.38 ± 0.17	0.26 ± 0.20	0.26 ± 0.12	0.21 ± 0.04	0.46 ± 0.19
Cycle duration (s)	0.37 ± 0.08	0.34 ± 0.06	0.34 ± 0.07	0.32 ± 0.05	0.52 ± 0.20	0.37 ± 0.13	0.38 ± 0.14	0.32 ± 0.05	0.63 ± 0.24
Pressure time index (mm Hg/s)	13.88 ± 3.13	13.03 ± 2.41	14.22 ± 3.08	11.93 ± 1.58	21.66 ± 11.18	16.62 ± 6.72	10.96 ± 2.51	9.82 ± 2.26	9.13 ± 3.88

Max., maximum; Min., minimum; EDP, end diastolic pressure.
The values are means ± SEM; $n = 5$. **$P < 0.01$, *$P < 0.05$ compared with normal.

Table III. Effect of cleistanthin A (administered i.v.) on electrocardiogram.

Group	Normal	Cleistanthin A							
		1 μg	2 μg	4 μg	8 μg	16 μg	32 μg	64 μg	128 μg
n →	5	5	5	5	5	5	5	4	3
RR interval (s)	0.19 ± 0.02	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.0	0.22 ± 0.01	0.29 ± 0.05	0.28 ± 0.02	0.42 ± 0.01***
Heart rate (BPM)	319.82 ± 25.23	315.62 ± 21.75	314.32 ± 20.29	295.64 ± 8.97	295.80 ± 7.69	273.14 ± 9.18	249.90 ± 18.45	256.22 ± 3.73	193.0 ± 4.35***
P amplitude (mV)	0.04 ± 0.01	0.02 ± 0.0	0.04 ± 0.02	0.03 ± 0.01	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.0	0.01 ± 0.0	0.01 ± 0.0
Q amplitude (mV)	–0.03 ± 0.02	–0.04 ± 0.03	–0.05 ± 0.04	–0.09 ± 0.04	–0.11 ± 0.03	–0.05 ± 0.01	–0.02 ± 0.01	–0.03 ± 0.01	–0.01 ± 0.0
R amplitude (mV)	0.49 ± 0.02	0.52 ± 0.01	0.50 ± 0.02	0.58 ± 0.02*	0.61 ± 0.03**	0.55 ± 0.01	0.58 ± 0.01*	0.52 ± 0.0	0.45 ± 0.0
S amplitude (mV)	–0.04 ± 0.01	–0.07 ± 0.02	–0.10 ± 0.02	–0.17 ± 0.02	–0.20 ± 0.05*	–0.22 ± 0.04**	–0.20 ± 0.03*	–0.24 ± 0.02**	–0.16 ± 0.0
ST height (mV)	0.0 ± 0.01	–0.01 ± 0.01	–0.02 ± 0.01	–0.08 ± 0.01	–0.10 ± 0.02	–0.08 ± 0.04	–0.05 ± 0.04	–0.14 ± 0.034*	–0.15 ± 0.0*
T amplitude (mV)	0.09 ± 0.02	0.10 ± 0.01	0.13 ± 0.02	0.12 ± 0.02	0.10 ± 0.02	0.03 ± 0.03	0.04 ± 0.04	–0.06 ± 0.02**	–0.09 ± 0.0***

BPM, beats per minute.
The values are means ± SEM. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared with normal.

Table IV. Effect of cleistanthin B (administered i.v.) on electrocardiogram.

Group	Normal	Cleistanthin B							
		1 μg	2 μg	4 μg	8 μg	16 μg	32 μg	64 μg	128 μg
n →	5	5	5	5	5	5	4	4	3
RR interval (s)	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.22 ± 0.03	0.20 ± 0.01	0.24 ± 0.03	0.42 ± 0.02***
Heart rate (BPM)	326.84 ± 22.22	325.64 ± 13.70	321.50 ± 12.35	310.08 ± 12.12	311.16 ± 12.95	299.70 ± 20.51	304.95 ± 9.03	272.70 ± 22.37	190.37 ± 14.59***
P amplitude (mV)	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.0	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.0
Q amplitude (mV)	–0.01 ± 0.01	–0.0 ± 0.01	–0.03 ± 0.01	–0.02 ± 0.01	–0.02 ± 0.02	–0.03 ± 0.02	–0.02 ± 0.0	–0.05 ± 0.03	–0.03 ± 0.0
R amplitude (mV)	0.58 ± 0.11	0.58 ± 0.11	0.57 ± 0.10	0.58 ± 0.10	0.60 ± 0.10	0.55 ± 0.09	0.57 ± 0.09	0.54 ± 0.11	0.60 ± 0.06
S amplitude (mV)	–0.08 ± 0.03	–0.09 ± 0.03	–0.10 ± 0.03	–0.10 ± 0.03	–0.10 ± 0.05	–0.10 ± 0.02	–0.20 ± 0.04	–0.22 ± 0.04	–0.25 ± 0.04
ST height (mV)	0.01 ± 0.01	0.0 ± 0.01	–0.0 ± 0.0	–0.01 ± 0.01	–0.04 ± 0.03	–0.03 ± 0.02	–0.09 ± 0.04	–0.12 ± 0.04*	–0.14 ± 0.05*
T amplitude (mV)	0.06 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.03	0.05 ± 0.02	–0.01 ± 0.05	–0.01 ± 0.05	–0.06 ± 0.05

The values are means ± SEM. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared with normal.

Table V. Antiarrhythmic effect of cleistanthins A and B.

Group	Time taken for induction of arrhythmia after barium chloride injection [s]	Lethality after barium chloride injection [s]
Control	11 ± 1.44	39.8 ± 7.20
Cleistanthin A (2 μg, i.v.)	8.5 ± 1.51	74 ± 11.76
Cleistanthin B (2 μg, i.v.)	5 ± 1.0*	27.75 ± 10.52

*$P < 0.05$ compared with control; $n = 5$.

nergic receptor blockade (Kumar *et al.*, 2011). Since cleistanthins A and B are the major constituents of the *Cleistanthus collinus* extract, it is possible that these compounds reduce the blood pressure by blocking the alpha-adrenergic receptors.

There was no change in the difference between the mean blood pressure and the end diastolic pressure, both of which were altered by cleistanthins A and B. A constant difference was maintained irrespective of the effects produced by different doses of these compounds. Digitalis, which is a cardiac glycoside, reduces the end diastolic pressure (Rahimtoola, 2004): a similar effect was observed with cleistanthin A and cleistanthin B as well.

In the electrocardiogram, the amplitudes of the R and S waves were elevated and the ST height and T wave amplitude were decreased by cleistanthin A. Cleistanthin B also produced a decrease in ST height. Not much importance can be attached to these transient changes since they occur only with a few doses and disappear at higher doses. Both compounds do not have a significant antiarrhythmic effect against barium chloride-induced arrhythmia in rats although cleistanthin B, and not cleistanthin A, reduces the time taken for barium chloride to induce arrhythmia. It may be inferred that cleistanthin B sensitizes the heart to barium chloride. Further, both compounds do not induce arrhythmia in normal animals.

Poisoning by *Cleistanthus collinus* leaves in humans causes hypotension, non-specific ST-T changes and QTc prolongation in the electrocardiogram, metabolic acidosis, hypoxia, hypokalaemia, neuromuscular weakness, renal failure, and dilated pupils (Thomas *et al.*, 1987; Subrahmanyam *et al.*, 2003; Eswarappa *et al.*, 2003; Benjamin *et al.*, 2006). The present study on rats also found hypotension and changes in the electrocardiogram induced by cleistanthins A and B, which are the major phytoconstituents of *Cleistanthus collinus*. Hence it is ascertained that the hypotension seen in patients with *Cleistanthus collinus* poisoning is due to cleistanthins A and B.

Maneksh *et al.* (2010) reported that there was no evidence of cardiotoxicity in animals administered an extract of *Cleistanthus collinus* leaves. The findings of the present study are in agreement with this. It was found that the animals developed sudden respiratory depression after the administration of 128 μg of cleistanthin A and cleistanthin B, and no cardiotoxicity was seen in our study either.

Cleistanthins A and B exert a hypotensive effect. Though cleistanthins A and B alter the electrical activity of the heart, these compounds do not demonstrate an antiarrhythmic effect against barium chloride-induced arrhythmia in Wistar rats.

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