The effects of aqueous extract of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaves on rat blood pressure

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DOI: 10.4103/0974-8490.99086

Sir,

The genus *Cleistanthus* (Euphorbiaceae) comprises about 140 species which includes *Cleistanthus collinus*, *Cleistanthus patulus, Cleistanthus schlechteri*, and *Cleistanthus gracili*.[1] *Cleistanthus collinus* (Roxb.) Benth. ex Hook. f. (Euphorbiaceae) is known as a toxic plant and found in Africa, India, Sri Lanka, and Malaysia.[2] In India, this plant is commonly used as a suicidal poison. All parts of the plant are reported to be toxic and used as suicidal, homicidal, cattle and fish poison and for inducing criminal abortion.[3] In patients, the clinical manifestations of *C. collinus* poisoning include hyperthermia, hypokalemia, ECG abnormalities (prolongation of QT interval, ST depression, and flat P wave), and elevation of hepatic enzymes such as alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT).[2] In some clinical cases, a water decoction of *C. collinus* causes fall in blood pressure.[2] Hence, this study was planned to determine the effects of aqueous extract of *C. collinus* leaves on rat blood pressure using an invasive procedure.

The taxonomically identified *C. collinus* (Roxb.) Benth. ex Hook. f. (Euphorbiaceae) plant parts were collected in the regions of Puducherry, India, rural parts of Villupuram, Cuddalore districts of Tamil Nadu, India, and certified by the Botanical Survey of India (BSI), Coimbatore (BSI/SC/5/23/08-09/Tech. 241). Leaves of *C. collinus* were collected in the month of February–April of every year. Voucher specimen of the plant is kept in the Department of the Pharmacology, JIPMER, for further reference.

Healthy, adult, male albino rats of Wistar strain, weighing 170–190 g, were obtained from Animal House, JIPMER, Puducherry, India. The rats were housed under 25 ± 2°C temperature, 40–60% humidity, and 12:12 ± 1 h light dark cycle. The animals were fed with water and rat pellets *ad libitum*. The rat pellets were supplied by Amrut laboratory animal feeds, Sangli, India. The experimental protocol was approved by the Institute Animal Ethics Committee (IAEC) of JIPMER, Puducherry, India. All the animal experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

The fresh *C. collinus* leaves were cleaned in running water and manually ground with help of a mortar and a pestle. The leaves were ground with sufficient quantity of water and warmed (30–45 °C) for 45 min. The crude material was filtered through a muslin cloth, and the filtrate was concentrated and used within 15 days.

C. collinus leaf extract was weighed and dissolved in warm distilled water (qs). Acetylcholine, histamine, and isoprenaline were dissolved in distilled water. The catecholamines such as adrenaline, noradrenaline were dissolved in 1% w/v ascorbic acid solution. Heparin was diluted with normal isotonic saline.

A fasted male rat (8–10 h) was anesthetized using urethane 1200 mg/kg. The animal was placed on a surgical platform, and the skin on the ventral side of the neck, right hind leg, chest region were exposed and disinfected. A small incision was made in the right thigh, the femoral vein was identified, cannulated with a 26 G × 1/2" needle and flushed with normal saline. The neck region of the animal was cleaned, opened, and tracheostomy was carried out. The carotid arteries were identified along with the vagus nerve on both sides of the trachea. Carotid artery on one side of the trachea was cleaned, separated from the vagus nerve, and cannulated using a cannula pre-filled with heparinised 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, Australia). The femoral vein was flushed with heparinized normal saline to avoid blood clotting at the puncture site and inside the cannula.[4,5]

The data acquisition system was pre-calibrated using a mercury sphygmomanometer. The preparation was allowed

to stabilise for 15 min, and the basal blood pressure was recorded for 10 min to confirm the stability and accuracy of the experimental preparation. The cholinergic, adrenergic and histaminergic agents, the aqueous extract of *C. collinus* leaves were injected through the femoral vein, and the blood pressure was recorded. The desired amount of the drug was administered in 0.1 ml of the vehicle and flushed with 0.1 ml of normal saline.

The effect of aqueous extract of *C. collinus* leave, adrenergic and cholinergic agents are presented in Table 1 and Figure 1. The aqueous extract of *C. collinus* leaves *per se* induced hypotension and did not influence the effects of adrenergic and dopaminergic agents on blood pressure. *C. collinus* extract at 2 and 4 µg also induced hypotension followed by death [Figure 2a]. In one animal *C. collinus* (2 µg) injections followed by acetylcholine (1 µg) injection caused mortality. Acetylcholine-induced hypotension did not revert to normal in that animal [Figure 2b]. It significantly increased the duration of acetylcholine-induced hypotension in four out of five animals. Acetylcholine action is evanescent due to metabolism of acetylcholine by acetylcholinesterase in blood.[6] Hence a sharp decline in blood pressure induced by acetylcholine recovers immediately unless the acetylcholinesterase is inhibited. The fact that the duration of acetylcholine action is prolonged by *C. collinus* raises a doubt whether the acetylcholinesterase activity could probably be inhibited by *C. collinus*. Further investigations are required for determining the actual mechanism involved in the increased duration of acetylcholine-induced hypotension by the *C. collinus* extract.

The earlier reports suggest that the aqueous extract of *C. collinus* leaves exerts α-receptor blocking activity. The extract inhibited the α-receptor action of phenylephrine on guinea pig vas deferens and aortic strip in a dose-dependant manner.[7] However in this study, the aqueous extract of *C. collinus* leaves did not block the hypertensive action of adrenaline, noradrenaline, and dopamine. This may be due to variations in the phytochemicals present in the extract. However, the constituents of *C. collinus* isolated using the acetone extract exhibited a significant α-receptor inhibition property and antagonized the α-receptor action of noradrenaline and dopamine.[8] This study concludes that the aqueous extract of *C. collinus* leaves *per se* causes hypotension and does not affect the catecholamines-induced-hypertension.

Table 1: The effects of the aqueous extract of C. collinus leaves on the rat blood pressure

Drug	Basal response		Response after treatment with *C. collinus* extract	
	Percentage change in blood pressure (mmHg)	Duration of response (s)	Percentage change in blood pressure (mmHg)	Duration of response (s)
Adrenaline 1 µg	50.05 ± 7.47	149 ± 12.49	54.72 ± 7.58	150 ± 13.03
Noradrenaline 1 µg	55.09 ± 12.14	150 ± 13.03	50.78 ± 8.28	144 ± 21.53
Dopamine 1 µg (D1 receptor action)	−18.94 ± 1.95	120 ± 17.60	–	120 ± 12.24
Dopamine 1 µg (α-receptor action)	42.37 ± 4.18		40.86 ± 5.08	
Acetylcholine 1 µg	−43.61 ± 2.54	58 ± 8.74	−47.38 ± 5.50	126 ± 9.27*
Histamine 1 µg	−31.90 ± 3.17	29 ± 1.87	−32.81 ± 4.46	46 ± 4.00
Aqueous extract *C. collinus* leaves 1 µg	−28.84 ± 6.53	35 ± 8.94	–	–

Values represent mean ± SEM ($n = 5$); *$P < 0.05$ as compared to basal response (one-way ANOVA followed by Tukey's test)

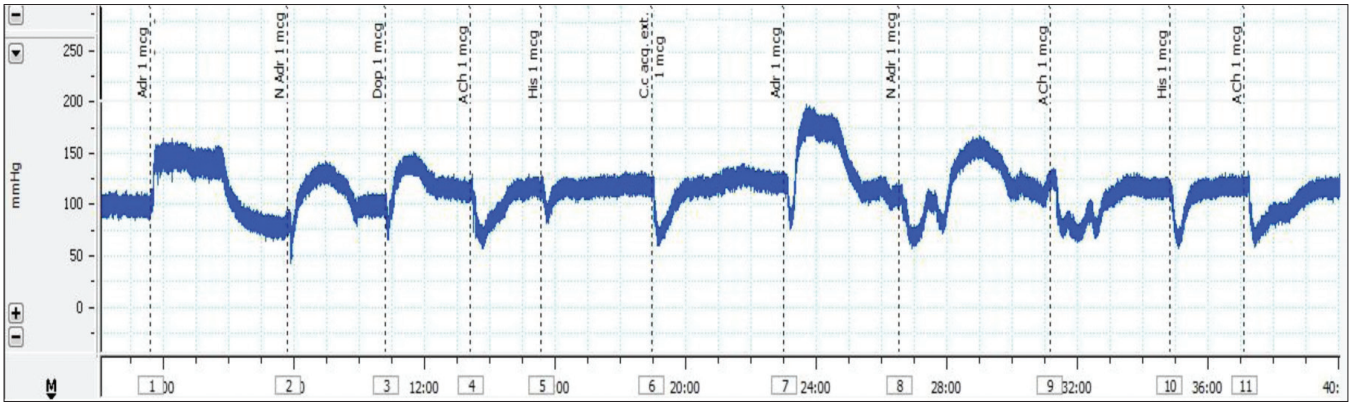

Figure 1: The effects of aqueous extract of *C. collinus* on rat blood pressure and its interaction with cholinergic and adrenergic agents. Adr = Adrenaline, N Adr = Noradrenaline, Dop = Dopamine, ACh = Acetylcholine, His = Histamine, C.c acq ext = Aqueous extract of *C. collinus* leaves and Mcg = Microgram

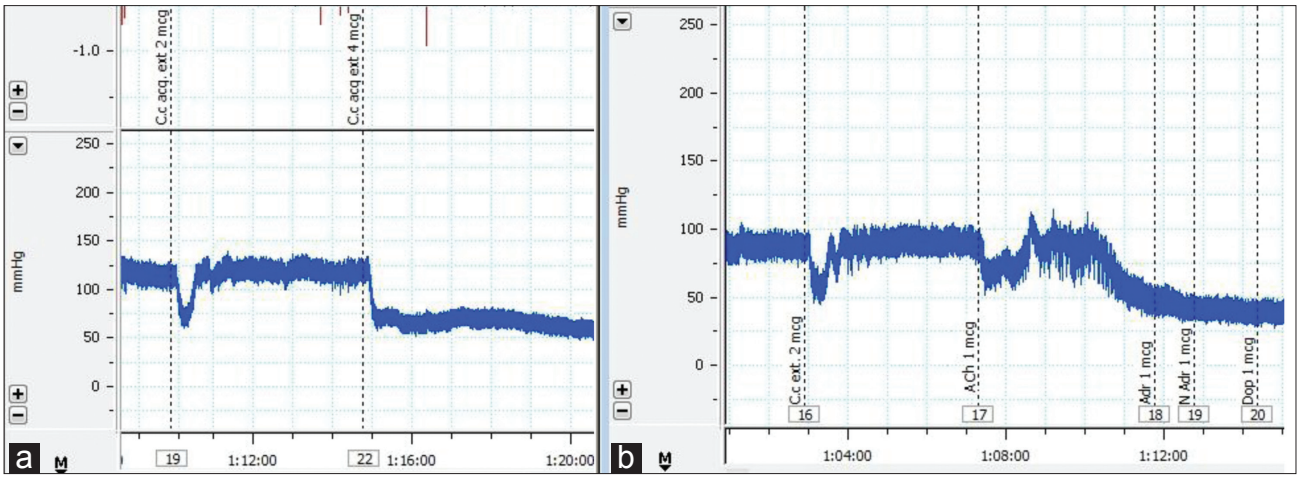

Figure 2: (a) Lethal effect of *C. collinus* (4 µg) leaves aqueous extract. (b) Lethal effect of *C. collinus* (2 µg) injections followed by acetylcholine (1 µg) injection. C.c acq ext: Aqueous extract of *C. collinus* leaves, ACh: Acetylcholine, Adr: Adrenaline, N Adr: Noradrenaline, Dop: Dopamine and Mcg: Microgram

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