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Confirmatory *in vivo* Electrodiagnostic and Electromyographic Studies for Neuromuscular Junctional Blocking Action of *Cleistanthus collinus* Leaf Extract in Rat

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Detailed electromyographic and electrodiagnostic studies were made on sciatic nerve–anterior tibialis muscle preparations in rat *in vivo* for the first time to confirm the neuromuscular junctional (NMJ) blocking action of *Cleistanthus collinus* leaf extract (CCLE) and the transient reversal of NMJ blockade by neostigmine. A sublethal dose of 175 mg dry leaf powder/kg body weight of rat caused NMJ blockade confirmed by a decremental response in nerve evoked compound muscle action potentials (NCMAPs). The study suggests the action of the toxic plant extract on acetylcholine (ACh) receptor sites. Muscle evoked compound muscle action potentials (MCMAPs) showed negligible decremental response suggesting the action at the NMJ level only and not on muscle excitation and contractility. The CCLE poisoning revealed a specific diagnostic EMG pattern which might be of help to clinicians in diagnosing *Cleistanthus* poisoning. The studies confirm that the leaf extract contains a proven and promising new NMJ blocker.

Keywords: Cleistanthus collinus; neuromuscular blockade; electrodiagnosis; compound muscle action potentials; sciatic nerve–anterior tibialis muscle; neostigmine; d-tubocurarine.

INTRODUCTION

Cleistanthus collinus is a toxic plant whose leaves have been used for homicidal and suicidal purposes in rural areas of some parts of Southern India (Modi, 1977; Chopra *et al.*, 1965; Nanda Kumar *et al.*, 1988; 1989; Vijayalakshmi *et al.*, 1994). The neuromuscular blocking action of *Cleistanthus collinus* crude leaf extract (CCLE) as well as the semi-purified extract in rat *in vitro* (Nanda Kumar *et al.*, 1989; Vijayalakshmi *et al.*, 1994) and mouse (Nanda Kumar *et al.*, 1989) phrenic nerve–diaphragm preparation was reported for the first time. The above studies were confined to *in vitro* models only. The present elaborate investigation is undertaken for confirmatory electromyographic (EMG) and electrodiagnostic studies on sciatic nerve–anterior tibialis muscle *in vivo* in rat administered intraperitoneally with whole CCLE for the first time and evaluation of the mechanism of action by comparing with established neuromuscular junctional (NMJ) blocking agents such as d-tubocurarine chloride as well as neostigmine which is administered clinically for reversing NMJ blockade. Diagnostic electromyographic patterns by recording nerve evoked compound muscle action potentials (NCMAPs) are observed when NMJ blocking agents are clinically administered or in neuromuscular disorders (Drachman *et al.*, 1976; Grob and Namba, 1976; Ozdemir and Young, 1976; Pagala, 1987; Pagala *et al.*, 1990; Stalberg, 1991). It is suggested that *Cleistanthus collinus* poisoning might also show a diagnostic EMG pattern due to NMJ blockade which might be of use to clinicians. These studies were made by recording NCMAPs, MCMAPs and muscle tension at different frequencies of stimulation and time intervals with intraperitoneal administration of whole CCLE, d-tubocurarine and neostigmine. Though various types of organic compounds are reported in *Cleistanthus collinus* (Evans, 1987; Govindachari *et al.*, 1969; Anjaneyulu *et al.*, 1977) the whole leaf extract is consumed and hence whole CCLE has been administered and tested.

MATERIAL AND METHODS

Cleistanthus collinus leaves were dried in shade and were blended to obtain a fine powder. Appropriate amounts of dry powder extracts were prepared (w/v) in mammalian ringer and used for intraperitoneal administration in rats. A sublethal amount of 175 mg/kg was administered for studying the electromyographic (EMG) pattern (Nanda Kumar and Vijayalakshmi, 1996). Rats were anaesthetized with sodium thiopentone (Rhonw-Poulene Ltd. Bombay, India) 40 mg/kg intraperitoneally. Maintenance doses of sodium thiopentone of 20 mg/kg (I.P) were given when required to keep the rat anaesthetized during the course of the experiment. From the sciatic nerve–anterior tibialis muscle preparation NCMAPs were recorded by the procedure described by Nanda Kumar and Vijayalakshmi (1996); Pagala *et al.* (1992) employing a Tektronix 5131 Oscilloscope (Tektronix, Oregon, USA) for recording traces and a

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Grass P15 Preamplifier and Grass S88 Stimulator (Grass Instrument Company, Quincy, USA) for amplification and delivering single and supramaximal repetitive stimulations of various frequencies respectively through a Grass SIU5 Stimulus Isolation Unit. The anterior tibialis muscle distal tendon was secured to an isometric Force Transducer (Harvard apparatus (UK) model—529503). The signal from the transducer was coupled to a Tektronix Storage Oscilloscope for recording electromyograms.

The per cent decrement in the train of nerved evoked CMAPs was calculated by measuring the mean amplitudes of CMAPs in a train of experimental over control and also by calculating the per cent change or decrease in amplitude of the last action potential compared with the first action potential, as described by Pagala (1987), Pagala *et al.* (1990), Sanders *et al.*, (1976), Grob and Namba (1976) and Nanda Kumar *et al.* (1988).

d-Tubocurarine (Sigma, USA) was dissolved in mammalian ringer and 140 μg/kg body weight rat was administered intraperitoneally and EMG recordings were made.

Neostigmine (Sigma, USA) was appropriately dissolved (w/v) in mammalian ringer and 0.3–0.5 μg/kg body weight rat was administered intraperitoneally at different time intervals and the EMG recordings were made to show reversal after 2 h of administration of CCLE.

RESULTS AND DISCUSSION

Electromyographic (EMG) recordings were made in the rat (normal) prior to the intraperitoneal injection of a sublethal dose of *Cleistanthus collinus* leaf extract (CCLE) and the same rat served as experimental after the i.p. injection of CCLE. In the control rat, on nerve stimulation the single CMAP showed a peak amplitide of 27.00 mV and after i.p. administration of CCLE the CMAPs showed a progressive decrease namely 22.00, 20.00, 16.00 and 8.00 mV after 15, 30, 60 and 120 min respectively. However, there was no significant change in the duration of a single CMAP. On lower frequencies of repetitive nerve stimulation (RNS) namely at 10 Hz/s in the control there was no decrement. Whereas in the mean CMAP amplitude (parameter A') in the 10 Hz train showed a progressive decrement from 18.20% to 76.32% after 15 to 120 min of administration of CCLE (Table 1). The per cent decrement between the first and last CMAP (parameter 'B') in the 10 Hz train also showed a gradual decrement from 6.25% to 33.33%. On higher frequencies of stimulation namely 30, 50 and 100 Hz the following decremental responses were observed. The decremental response on 30 Hz stimulation was 13.51% to 75.68% from 15 to 120 min and 16.23% to 44.98% from 15 to 60 min when analysed by parameters 'A' and 'B' respectivley (Fig. 1, Table 1). The results revealed that the per cent decrement observed in parameter 'B' was relatively less in all exposure times compared with parameter 'A', through parameter 'B' was used most often in NMJ disorders (Grob and Namba, 1976; Drachman *et al.*, 1976; Ozdemir and Young, 1976). In reality the decrement observed was high when the EMG pattern was observed. Due to the high decrement in both the first and last potential equally, in parameter 'B' the % decrement or the ratio (last potential/first potential) observed would be relatively less (Fig. 1). In such situations the mean CMAP values gives better analytical insight and is recommended. At 50 and 100 Hz higher frequencies of RNS also showed a progressive increase in decremental response from 15 to 120 min on administration of CCLE (Table 1, Fig. 1).

The above experimental results seem to suggest the action of CCLE on NMJ namely at the ACh receptor sites. The ingredients of the extract seem to block the ACh receptor sites resulting in decreased polarization demonstrated by decremental response of NCMAPs. With an increase in the duration of exposure to CCLE more receptor sites seem to be blocked resulting in a gradual increase in

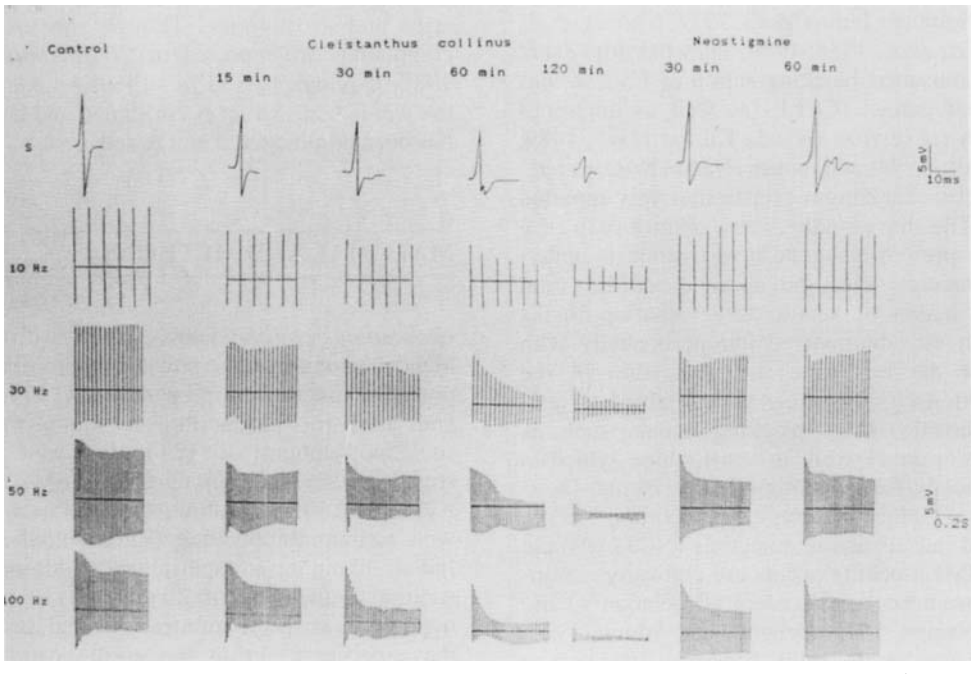

Figure 1. Compound muscle action potentials recorded from anterior tibialis muscle of rat *in vivo* with single and repetitive sciatic nerve stimulation without and with intraperitoneal administation of sublethal dose (175 mg/kg body weight) of *Cleistanthus collinus* whole leaf extract and reversal after administration of neostigmine (300 μg/kg body weight) at different time intervals and at different frequencies of repetitive supramaximal nerve stimulation.

Table 1. Effect of intraperitoneal administration of *Cleistanthus collinus* leaf extract followed by neostigmine on sciatic nerve evoked anterior tibialis muscle action potentials in rat *in vivo*

Frequency of stimulation	EMG parameter meter	Normal	*Cleistanthus collinus* 15	30	60	120	Neostigmine 30	60 min
Single			18.52	25.93	40.74	70.37	22.22	11.11
			±1.04	±1.25	±1.96	±5.96	±1.12	±0.72
			<0.001	<0.001	<0.001	<0.005	<0.001	<0.001
	A		18.20	35.53	59.41	76.32	24.16	21.05
			±1.49	±3.25	±5.42	±7.30	±2.20	±2.00
			<0.001	<0.001	<0.005	<0.005	<0.001	<0.001
10 Hz	B	0.0	6.25	7.69	25.74	33.33	0.0	9.38
			±0.53	±0.67	±2.33	±3.16		±0.91
			<0.001	<0.001	<0.001	<0.001		<0.001
	A		13.51	27.36	43.09	75.68	17.87	18.92
			±1.13	±2.31	±4.10	±7.28	±1.42	±1.51
			<0.001	<0.001	<0.005	<0.005	<0.001	<0.001
30 Hz	B	3.68	16.23	27.54	44.98	20.00	10.61	0.0
		±0.33	±1.39	±2.32	±4.28	±2.00	±1.03	
			<0.001	<0.001	<0.005	<0.001	<0.001	
	A		25.60	28.68	47.24	80.56	23.11	33.33
			±2.26	±2.53	±4.30	±8.02	±2.10	±3.16
			<0.001	<0.005	<0.005	<0.005	<0.005	<0.005
50 Hz	B	11.24	30.25	37.91	53.11	0.0	28.79	36.36
		±1.05	±2.98	±3.30	±5.10		±2.58	±3.38
			<0.001	<0.001	<0.005		<0.001	<0.001
	A		29.74	27.67	49.76	82.14	21.78	21.43
			±2.64	±2.34	±4.59	±8.14	±2.10	±2.09
			<0.001	<0.005	<0.005	<0.005	<0.001	<0.001
100 Hz	B	32.53	44.56	61.78	72.86	33.33	21.43	27.59
		±3.07	±4.23	±6.01	±7.01	±3.16	±2.09	±2.32
			<0.005	<0.005	<0.005	<0.001	<0.001	<0.001

Values are mean of 8 observations; ± indicate SD of mean.
Single normal NCMAP amplitude: 27 mV.
A; Mean amplitude % decrement in experimental over normal.
B; % decrement in last potential over the first potential of the same train.

decremental response. The results can be compared to some neuromuscular disorders where the ACh receptor sites are blocked. For example in neuromuscular disorders like myasthenia gravis, a gradual diagnostic decrement in the train from first to last potential, either in the lower frequencies of stimulation (10 Hz) or in the higher frequencies of nerve stimulation (30–100 Hz) *in vivo* was observed by Grob and Namba (1976), Ozdemir and Young (1976), Drachman *et al.* (1976), Synder-Mackler and Robinson (1989), Stalberg (1991) and *in vitro* by Pagala *et al.* (1990). A similar diagnostic EMG pattern with established NMJ blockers like d-tubocurarine was observed by Drachman *et al.* (1976), Grob and Namba (1976) and Pagala (1987) both in *in vivo* and *in vitro* experiments respectively. It is also a well established fact that d-tubocurarine is a non-depolarizing neuromuscular blocking drug which primarily acts as a competitive antagonist of acetylcholine at the recognition site of the end plate cholinergic receptor (Colquhoun and Sheridan, 1980; Colquhoun, 1980; 1981; Standaert, 1982). It may be presumed that the ingredients of *Cleistanthus collinus* might also be acting at the NMJ level, namely on the ACh receptor sites. Further confirmation of NMJ blocking effects of CCLE can be visualized from the EMG pattern with administration of neostigmine as given in Fig. 1. With CCLE, the per cent decrement observed in the mean action potential amplitude (parameter 'A') over the control at 30, 50 and 100 Hz stimulation was 43.09%, 47.24% and 49.76% after 1 h of CCLE administration respectivley. Whereas after administration of neostigmine the per cent decrement was only 18.92%, 33.33% and 21.43% at the above frequencies of nerve stimulation after 60 min (Table 1). This shows that there was a reversal of more than 50% to 75% in depolarization as evidenced by the increase in the peak to peak amplitude in the train after the administration of neostigmine. Neostigmine is an established anticholinesterase agent (Grob *et al.*, 1956; Taylor, 1980) which causes depolarization due to accumulation of acetylcholine at the receptor site (Grob and Namba, 1976; Nastuk and Wolfson, 1976). Corroborative results were obtained by Grob and Namba (1976) and Drachman *et al.* (1976), where the following EMG pattern was observed: a progressive decrease in muscle action potentials evoked by two or more nerve stimuli; a more rapid decrease in the muscle action potentials evoked by a train of stimuli delivered after tetanic stimulation and inhibition of the depolarization and its reversal by anticholinesterase compound. Thus neuromuscular impairment caused by CCLE can also be detected and confirmed by combined electromyographic analysis and administration of specific pharmacological agents. For further confirmation d-tubocurarine, an established NMJ blocker and inhibitor of polarizing action of acetylcholine transmitter on the end-plate, was also studied. Figure 2 shows a progressive increase in decremental response at 10, 30, 50 and 100 Hz frequencies of nerve stimulation which ranged from 0.0 to 94.87% (Fig. 2). It is likely that the CCLE may be

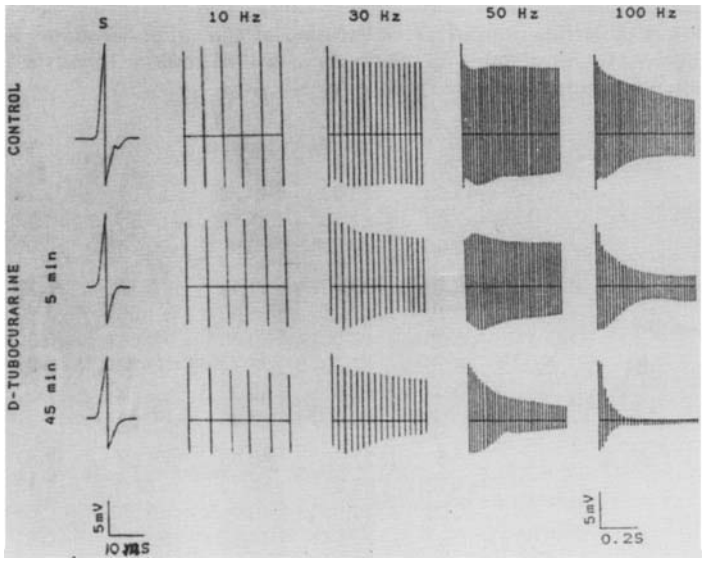

Figure 2. Recordings of CMAPs from anterior tibialis muscle of rat in *in vivo* with single and repetitive nerve (sciatic) stimulation without (control) and with intraperitoneal administration of d-tubocurarine. Recordings were made after 5 and 45 min.

competing with ACh receptor sites like d-tubocurarine. On the contrary muscle evoked CMAPs on single or on repetitive direct muscle stimulation (Table 2) showed least or no decremental response. This is a significant observation and implies that the leaf extract markedly inhibits excitability of nerve and muscle by blocking neuromuscular transmission without affecting excitation–contraction coupling (Pagala *et al.*, 1990; Lambert *et al.*, 1976) or muscle contracture.

The above experimental results are suggestive of the neuromuscular blocking action of CCLE on the ACh receptor sites causing impairment in neuromuscular transmission. The unknown pharmacological agent may be of use similar to various neuromuscular blockers (Taylor, 1980) in clinical physiology and research work is under progress. The results also help clinicians in India in the electrodiagnosis of *Cleistanthus* poisoning.

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Table 2. Responses of muscle action potentials (mean amplitude) on direct stimulation of muscle before (normal) and after (experimental) intraperitoneal administration of sublethal dose of *Cleistanthus collinus* leaf extract (175 mg/kg body weight). See there is no decrement or change in mean amplitude indicating least effect on muscle contractility

Frequency of stimulation	Normal	*Cleistanthus collinus* 2.5 h Amplitude (mV)
Single	40.20	40.2
	±0.92	±0.81
10 Hz	40.00	40.05
	±1.10	±1.10
30 Hz	39.80	39.91
	±0.90	±0.81
50 Hz	39.01	39.81
	±0.85	±0.91
100 Hz	38.98	39.81
	±0.81	±0.71
% Decrement	Nil	Nil

% Decrement is percent change in last potential over first potential.
Values are mean of 6 observations; ± indicate SD of mean.

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