

Skeletal muscle relaxant effect of *Chonemorpha macrophylla* in experimental animals

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ABSTRACT

Objective: To study the skeletal muscle relaxant property of *Chonemorpha macrophylla* (CM).

Materials and Method: The skeletal muscle relaxant effect of the alcoholic extract of CM was studied on isolated frog rectus abdominis muscle, isolated rat phrenic nerve diaphragm muscle preparation and in intact young chicks. The parameter studied in the isolated muscle or isolated nerve muscle preparations was the extent of inhibition of acetylcholine or electrically-induced contraction of skeletal muscles. In intact chicks, the drug was administered i.v. in wing veins and the onset, duration and nature of paralysis were recorded. In all the experiments, the effect of the drug was compared with that of gallamine and succinylcholine.

Results: The alcoholic extract of CM reduced the acetylcholine-induced contraction of isolated frog rectus abdominis and electrically stimulated contractions of rat phrenic nerve diaphragm in a dose-dependent manner. In unanaesthetized chicks, it produced spastic type of paralysis with extension of the neck and limbs. The effects were similar to the effects of succinylcholine but different from those of gallamine.

Conclusion: The alcoholic extract of CM possesses skeletal muscle relaxant property. It produces depolarizing type of muscle paralysis similar to that produced by succinylcholine.

KEY WORDS: Nerve muscle preparation, spastic paralysis, gallamine, succinyl choline

Introduction

Chonemorpha macrophylla (CM) is a plant of the Apocyanaceae family. A preliminary report on the pharmacological effects of chloroform extract of a plant of the same family *Ervatamia crispa* revealed skeletal muscle relaxant effect on isolated frog rectus abdominis muscle preparation.^[1] There was no report of a similar study with CM. It was presumed that CM, being a member of same family, may have skeletal muscle relaxant property. There was already a report of the collection of a number of alkaloids of CM by the alcoholic extraction method.^[2] It was therefore considered to use the alcoholic extract for the present work.

Materials and Methods

Preparation of the alcoholic extract of CM

Powdered bark of CM (1.5 kg) was Soxhleted with petroleum ether (B.P. 60–80 °C) for 24 h. The solvent was distilled off on water bath leaving a gummy residue (17 g). The defatted plant material was dried up in air and was percolated with ammonia and rectified spirit for 21 days. The solvent was removed under reduced pressure when a gummy residue (10 g) was obtained. This alcoholic extract was soluble in propylene glycol on little warming. A stock solution of 1 g of alcoholic extract of CM was prepared in 20 ml of propylene glycol to make the concentration 50 mg/ml and was utilized for different

experiments as mentioned below.

The effect of the drug was first studied on isolated frog rectus abdominis muscle by adopting standard procedure.^[3] A portion of frog rectus abdominis muscle was mounted in frog ringer solution in 5ml capacity organ bath maintained at 37±0.5 °C and aerated with oxygen. Each preparation was allowed to equilibrate for 30 min under the resting tension of 1 g to obtain stable basal condition.

Initially, a concentration of acetylcholine (2.5×10^{-7} M) was identified which can produce sub maximal contraction (3.02 cm) of the muscle. Contact time was 90 seconds. The effect of the vehicle propylene glycol (0.1 ml) was noted by adding the vehicle in the bath and allowing to remain in contact with the muscle for 3 min followed by addition of the dose of acetylcholine. Any change in height was recorded. Similarly, the effects of the alcoholic extract of CM (2 mg/ml), succinylcholine (1.25×10^{-7} M) and gallamine (2.5×10^{-7} M) on acetylcholine-induced contractions were recorded. To know the effect of physostigmine (3.3×10^{-6} M) and potassium chloride (3×10^{-1} M) on the antiacetylcholine effect of the alcoholic extract of CM, the respective agent was first administered into the bath 90 sec before administration of the alcoholic extract of CM. Three minutes after administration of the test drug the preparation was then challenged with the same dose of acetylcholine.

Table 1**Effects of different treatments on acetylcholine-induced contraction of isolated frog rectus abdominis muscle preparation**

Drugs (concentration) treatment	Height of contraction (cm) mean \pm SEM		% Change
	Before	After	
Propyleneglycol	3.01 \pm 0.20	3.01 \pm 0.20	--
CM (0.5 mg/ml)	3.02 \pm 0.27	2.20 \pm 0.04*	- 28.60
CM (1.0 mg/ml)	3.00 \pm 0.28	1.90 \pm 0.06**	- 34.50
CM (1.5 mg/ml)	3.00 \pm 0.10	1.70 \pm 0.20***	-43.30
CM (2.0 mg/ml)	3.06 \pm 0.02	1.20 \pm 0.10***	- 60.00
Succinylcholine (1.25 x 10 ⁻⁷ M)	1.90 \pm 0.04	0.95 \pm 0.10***	- 50.00
Gallamine (2.5 x 10 ⁻⁷ M)	2.30 \pm 0.10	1.40 \pm 0.03***	- 39.10
Physostigmine (3.3 x 10 ⁻⁶ M)+ CM (2 mg/ml)	1.90 \pm 0.10	0.70 \pm 0.10***	- 63.50
Physostigmine (3.3 x 10 ⁻⁶ M)+ Succinylcholine (1.25 x 10 ⁻⁷ M)	1.91 \pm 0.02	0.80 \pm 0.10***	- 59.12
Physostigmine (3.3 x 10 ⁻⁶ M)+ Gallamine (2.5 x 10 ⁻⁷ M)	2.25 \pm 0.60	2.30 \pm 0.10	+2.20
Potassium chloride (3 x 10 ⁻¹ M)+ CM (2 mg/ml)	1.80 \pm 0.20	0.70 \pm 0.10***	- 61.10
Potassium chloride (3 x 10 ⁻¹ M)+ Succinylcholine (1.25 x 10 ⁻⁷ M)	1.70 \pm 0.20	0.80 \pm 0.20***	- 52.90
Potassium chloride (3 x 10 ⁻¹ M)+ Gallamine (2.5 x 10 ⁻⁷ M)	3.45 \pm 0.20	3.50 \pm 0.10	+ 1.40

n = 6 in each group; * P <0.05, **P<0.01 and ***P<0.001 as compared to their corresponding values before treatment.

Similarly, the effects of physostigmine and potassium chloride on the antiacetylcholine effect of succinylcholine and gallamine were studied and compared with the test drug.

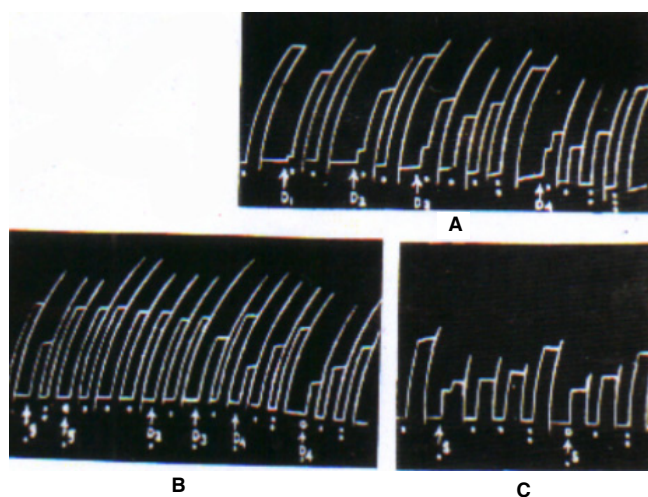
Then the effect of the alcoholic extract of CM on isolated rat phrenic nerve diaphragm preparation was carried out by the method of Bulbring.^[4] A triangular flap of diaphragm muscle was cut out with a portion of phrenic nerve attached to it from an albino rat weighing about 200 g. The muscle was mounted in a large organ bath containing 40 ml of Krebs solution maintained at 37.0 \pm 0.5 °C and aerated with oxygen. The preparation was allowed to equilibrate for 45 min under resting tension of 1-2 g. Contact time for each agent was 3 min.

The preparation was stimulated indirectly (2V) through the phrenic nerve with supramaximal square wave pulse of 0.5 milliseconds duration at 0.5 Hz. The effects of the vehicle and the alcoholic extract of CM in the concentration of 50 and 100 μ g/ml on electrically stimulated contraction of rat diaphragm were recorded.

To know whether physostigmine could modify the inhibitory effect of the test drug on electrically stimulated contraction of diaphragm, a dose of physostigmine (4 x 10⁻⁵ M) was added to the bath following the administration of the test drug (100 μ g/ml) and the effect was recorded for 5 min.

The type of skeletal muscle relaxation produced by CM was studied in unanaesthetized intact young chicks by the method of Battle and Zaimis.^[5] The chicks weighing about 250 g were divided into 5 groups, each group containing 6 chicks. Each group of chicks received either propylene glycol (0.1 ml), alcoholic extract of CM (10 and 20 mg/kg), succinylcholine

Figure 1. A shows the responses of the graded doses of CM on acetylcholine-induced contraction. B and C show the effect of physostigmine on the skeletal muscle relaxant effects of gallamine, CM and succinylcholine on isolated frog rectus abdominis muscle preparation. D₁, D₂, D₃ and D₄ denote 0.5, 1.0, 1.5 and 2.0 mg/ml of bath fluid of CM. • = acetylcholine (2.75 x 10⁻⁷ M), g = gallamine (2.5 x 10⁻⁷ M), O=physostigmine (3.3 x 10⁻⁶ M) and S = succinylcholine (1.25 x 10⁻⁷ M).



(0.05 mg/kg) or gallamine (0.05 mg/kg). All the drugs were administered i.v. through the wing vein of the chicks. The chicks of each group were observed carefully and any difference in the position of the limbs and neck were noted and compared.

Statistical analysis

Student's 't' test was used to evaluate the difference in the height of contraction. The onset and duration of the paralysis of different drugs were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test. A P value <0.05 was considered significant.

Results

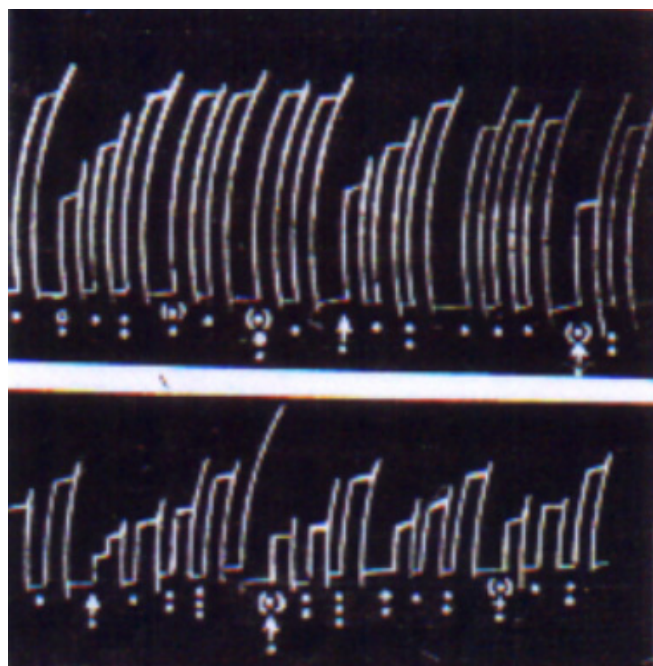
Effect on isolated frog rectus abdominis muscle preparation

The alcoholic extract of CM antagonized acetylcholine-induced contraction in a concentration-dependent manner (Table 1 and Figure 1). Addition of physostigmine (3.3 x 10⁻⁶ M) further enhanced the inhibitory effect of the alcoholic extract of CM (2 mg/ml) by 63.5%. The effect was comparable with the inhibitory effect (58.12%) of the combination of physostigmine (3.3 x 10⁻⁶ M) and succinylcholine (1.25 x 10⁻⁷ M) on acetylcholine-induced contraction (Table 1 and Figure 1). Pretreatment with potassium chloride (3 x 10⁻¹ M) reversed the inhibitory effect of gallamine on acetylcholine-induced contraction of frog rectus abdominis muscle significantly but not that of the alcoholic extract of CM or succinylcholine (Table 1 and Figure 2).

Effects on isolated rat phrenic nerve diaphragm preparation

CM in a concentration of 50 and 100 μ g/ml inhibited the contractile responses of nerve-stimulated rat phrenic nerve diaphragm muscle in a concentration-dependent manner (Table 2). Addition of physostigmine (4 x 10⁻⁵ M) further augmented

Figure 2. Effect of potassium chloride on the skeletal muscle relaxant effects of gallamine, CM (Panel A) and CM, Succinylcholine (Panel B) on isolated frog rectus abdominis muscle.
 • = acetylcholine (2.75×10^{-7} M), 0 = gallamine (2.5×10^{-7} M), (•) = potassium chloride (3×10^{-1} M), • = CM (2 mg/ml) and + = succinylcholine (1.25×10^{-7} M).



the inhibitory effect of CM (Table 2).

Effect on chick

Intravenous administration of CM in unanaesthetized chicks in a dose of 10 and 20 mg/kg body weight produced spastic paralysis with the extension of neck and limbs which was similar to that produced by 0.05 mg/kg dose of succinylcholine; however, the onset was delayed significantly and the duration of paralysis also was significantly shorter than that produced by succinylcholine (Table 3). On the contrary, gallamine produced a more rapid onset with longer duration of flaccid type of paralysis (Table 3).

Table 3

Effects of different treatment on the onset, duration and type of paralysis in unanaesthetized chicks

Drugs (mg/kg) i.v.		Onset of paralysis (min; mean±SEM)	Duration of paralysis (in min; mean±SEM)	% of paralyzed chicks	Mortality if any	Nature of paralysis
Propylene glycol		--	--	Nil	Nil	--
Gallamine (0.05)		12.5±2.0	80.5±8.5	100	Nil	Flaccid
Succinylcholine (0.05)		20.0±2.5	60.0±5.0	100	Nil	Spastic
CM (10.0)		30.0±2.5**	30.0±3.0**	89	Nil	Spastic
CM (20.0)		30.5±3.0*	40.0±5.0*	100	Nil	Spastic
One-way	F	4.88	12.1			
ANOVA	df	2,15	2,15			
	P	0.05	0.01			

n = 6 in each group. *P<0.05 and **P< 0.001 as compared to their corresponding value in the succinylcholine-treated group.

Table 2

Effects of the alcoholic extract of CM and physostigmine on the contractile responses of electrically stimulated isolated rat phrenic nerve diaphragm

Drugs (conc.) treatment	Height of contraction (cm; mean ± SEM)		% of inhibition
	Before	After	
Propylene glycol	4.12±0.12	4.12±0.20	--
CM (50 µg/ml)	4.12±0.13	2.20±0.04*	33.98
CM (100 µg/ml)	5.62±0.26	0.61±0.05**	89.10
Physostigmine (4×10^{-5} M)+ CM (100 µg/ml)	4.12±0.10	1.92±0.80**	53.30

n = 6 in each group; *P< 0.01 and ** P<0.001 as compared to their corresponding values before treatment.

Discussion

Studies on isolated skeletal muscle preparation of frog rectus abdominis muscle reveals that the alcoholic extract of CM can antagonize acetylcholine-induced contraction of the preparation. Pretreatment with potassium chloride reverses the muscle relaxant effect of gallamine but not that of the test drug or succinylcholine. The observation suggests that the alcoholic extract of CM possesses a depolarizing type of neuromuscular blocking action. This is further substantiated by the observation that pretreatment with physostigmine, which antagonizes the inhibitory effect of gallamine, enhances the inhibitory effect of both the alcoholic extract of CM and succinylcholine.

On isolated rat phrenic nerve diaphragm preparation, the alcoholic extract of CM reduces nerve stimulation-induced contraction of diaphragm, which is potentiated by the addition of physostigmine. The observation lends further support to the fact that CM possesses depolarizing type of neuromuscular blocking action.

The effect of CM on intact animals like young chicks further elucidates its mechanism of action. The drug produces extension of the neck and limbs like succinylcholine, a depolarizing blocker, whereas gallamine, a non-depolarizing blocker, produces flaccid type of paralysis.

Thus it is concluded that the alcoholic extract of CM possesses a depolarizing type of skeletal muscle relaxant property. In other words, the drug resembles succinylcholine with a slower onset and shorter duration of action.

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National Workshop on Basic Techniques in Molecular Biology & Bioinformatics in Pharmacogenomics

(Jointly Organized by JIPMER, Pondicherry and VCRC, Pondicherry)

July 27 to August 3, 2005

Pharmacogenomics Lab, Department of Pharmacology, JIPMER, Pondicherry 605006.

Last date for receipt of registration form: 27.6.2005.

Objectives

- ✍ To provide training in basic molecular biology techniques that would be required in pharmacogenetic studies
- ✍ To impart web-based training in basic concepts of bioinformatics
- ✍ To impart theoretical knowledge on some of the recently developed molecular biology tools
- ✍ To create a knowledge base of elementary medical genetics, statistical methods, etc. towards carrying out pharmacogenetic and related research

Target Participants

This workshop is intended for **beginners in molecular biology and bioinformatics**, with focus on 'pharmacogenomics'. A total of 20 participants will be admitted preferably post-graduate students and Faculty members of Departments of Pharmacology and Allied Sciences.

Course Content

- 📖 The duration of the workshop is 8 days, and will include practical demonstrations and hands-on training in molecular biology techniques such as DNA extraction, quality check and quantification of DNA, the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP), gel electrophoresis: horizontal and vertical, extraction of DNA from gel and purification of PCR products for sequencing and RT-PCR.
- 📖 Bioinformatics will include locating the gene sequence of interest, identification of the PCR product, primer design, and selection of the restriction endonucleases and validation of the PCR method by sequencing.
- 📖 Lectures will be held on the elements of medical genetics, organization of a molecular biology laboratory, microarray technology, DNA sequencing, high throughput screening for detection of mutations, elementary statistics for pharmacogenomics etc.

Registration & Accommodation

The registration fee is Rs. 5000/- , which includes:

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| ○ Resource material on the various topics covered | ○ Practical demonstrations |
| ○ Hands - on training in bioinformatics | ○ Lectures on current areas of interest in pharmacogenomics. |
| ○ Accommodation (dormitory/twin sharing will be arranged within the campus). | ○ Breakfast, dinner and working lunch (Vegetarian food Only) |

The registration form may be obtained by sending e-mail to: “**Dr. C. Adithan, Organizing Secretary, National Workshop on Pharmacogenomics, Department of Pharmacology, JIPMER, Pondicherry 605006**”
E-mail: jipgene@yahoo.com Tel: 0413-2272380 – 9 (10 lines) Ext. 3301.

APPLICATIONS MUST REACH ON OR BEFORE 27.06.2005.