รายงานวิจัย

Effect of Paraquat on Skeletal Contractile Responses in Albino Rats

Chaiyong Rujjanawate and Amphawan Apisariyakul
Department of Pharmacology, Faculty of Medicine, Chiang Mai University.

Paraquat, a member of the group of compounds designated quaternary bipyridyls, is a widely used herbicide. It is also quite toxic to man and animals. Paraquat dichloride is a quaternary ammonium salt which is hygroscopic, nonvolatile and extremely soluble in water.[1] It can be reduced to a stable radical cation by a one-electron transfer process and this property of paraquat is believed to contribute to its herbicidal activity.[2] Since the discovery of its phytotoxic property in 1950s, paraquat has become widely used as a herbicide. It rapidly desicates all green plant tissues with which it comes in contact. Paraquat which is available to farmers in Thailand is marketed in the form of concentrated aqueous solution under the name Gramoxone®, Combozone®

Paraquat intoxication may occur accidentally or suicidally. In both cases, ingestion of concentrated paraquat solution causes damages to many organs but the most fatal effect is in the lung. The relation between paraquat concentration in the lung and the degree of its toxicity was demonstrated in rats.[3] Studies of lung tissue with electron microscope after a single oral dose of paraquat indicated that the first discernible changes were pulmonary edema, swelling of the epithelium and increase in collagen.[4] The mechanism underlying lung toxicity caused by paraquat was proposed to be lipid peroxidation in pulmonary tissue.[5]

The initial symptoms of paraquat poisoning in man include burning of the mouth and throat followed often by nausea and vomiting.[6, 7] Paraquat may directly elicit contraction of the gastrointestinal smooth muscle or skeletal muscle, but these effects have not been confirmed in experimental models. Van den Heede[8] reported that a male patient who ingested an alcoholic drink, containing Gramoxone® showed an extreme dyspnoea, and died, and the high paraquat concentrations were expected to be found in all tissues of visceral organs. Further, it has been shown that paraquat was to be toxic in the lung mainly through the mechanism of inhibition of acetylcholinesterase, it must be assumed that the compound is concentrated 100-fold in a compartment containing acetylcholinesterase.[9]

The purpose of this study is to examine the toxic effect of paraquat on skeletal contractile responses. Experiments were carried out both in vivo and in vitro preparations in order to study the effects of paraquat on neuromuscular junction in rats.

MATERIALS AND METHODS

Experimental animals

Albino rats of either sex weighing about 150-300 grams were used in this investigation both in vitro and in vivo experiments. The method was based on modification of the technique of Bülbring[10] and Apisariyakul.[11] The resting tension of the hemidiaphragm was

2 g. Isometric recordings were measured by a Grass 79D polygraph (Grass Instrument Co., Quincy, Mass., U.S.A.). The preparation was left in the tissue bath for 30 minutes to reach equilibrium before the experiment commenced.

Single repeated supramaximal nerve stimulation

Nerve stimulation was elicited by rectangular pulses of supramaximal voltage of 0.6 msec duration and at a frequency of 0.4 Hz.

Train-of-four nerve stimulation

A short train of four supramaximal electrical stimuli was applied to the phrenic nerve at a frequency of 2 Hz. The duration of each rectangular pulse was 0.6 msec. Each train was repeated every 20 seconds.

Drug administration into the tissue bath

Muscle twitch in response to single repeated stimulation or train-of-four stimulation was recorded before and after adding the drug into the tissue bath. The preparation was washed three times with Krebs' solution and left 30 minutes before starting the next experiment.

Determination of acetylcholinesterase activity in the presence of Paraquat

This experiment was performed to study the effect of paraquat on acetylcholinesterase activity in vitro. The reference drug which was treated in the same way as paraquat was neostigmine, a reversible cholinesterase inhibitor. The colorimetric method used was as described by Augustinsson et al.[12] with some modification.

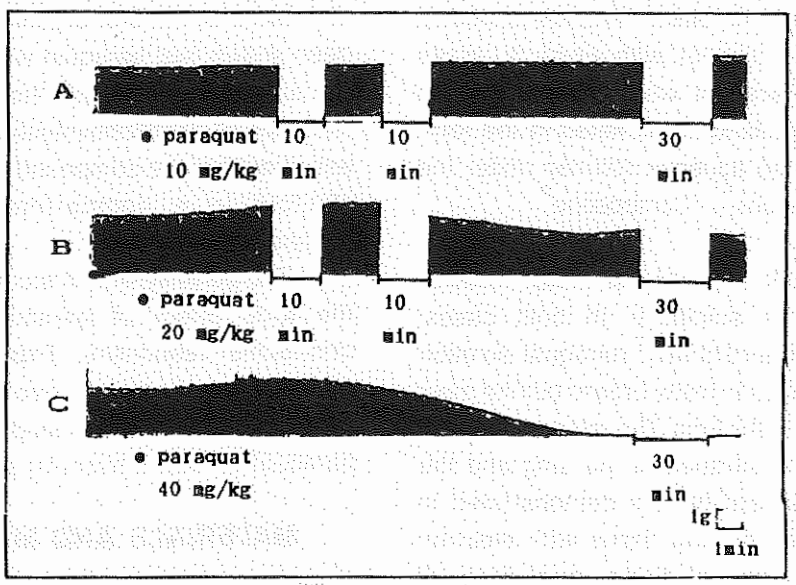

Figure 1 The effect of paraquat on neurally-evoked twitch in ra sciatic nerve-gastrocnemius preparation, in situ.
A Paraquat 10 mg/kg body weight produced a slight potentiation of the neurally-evoked twitch.
B Paraquat 20 mg/kg body weight produced twitch potentiation followed by twitch depression.
C Paraquat 40 mg/kg body weight produced twitch potentiation followed by complete neuromuscular blockade.

RESULTS

The results of the effect of paraquat on skeletal muscle contraction were divided into 3 parts as follows:

The effect of paraquat on neurally-evoked twitch

In the isolated rat phrenic nerve-hemidiaphragm preparation, paraquat in the doses of 1.00×10^{-3} M, 0.25×10^{-2} M, and 1.00×10^{-2} M caused twitch depression 18.5 ± 2.7, 47.1 ± 12.4, and 82.4 ± 12.2 per cent respectively.

The effect of paraquat on neurally-evoked twitch in rat sciatic nerve-gastrocnemius preparation was shown in Figure 1. It was found that paraquat in the doses of 10, 20, and 40 mg/kg body weight produced a slight potentiation of the neurally-evoked twitch. This twitch potentiation was followed by a gradual twitch depression when the doses of paraquat were 20 and 40 mg/kg body weight. Paraquat in the dose of 40 mg/kg body weight produced a complete neuromuscular blockade within 20 minutes.

Paraquat in the doses of 1.00×10^{-3} M, 0.25×10^{-2} M, and 1.00×10^{-2} M did not produce depression of the directly-evoked twitch of the isolated rat hemidiaphragm preparation (data not shown).

The effect of paraquat on the nerve action potential was studied in the isolated rat sciatic nerve preparation. The per cent amplitude of the action potential in isolated rat sciatic nerve preparation after 10 and 20 minutes of paraquat administration was not significantly different from control (Table 1)

Table 1 Effect of paraquat on the nerve action potential of isolated rat sciatic nerve preparation, determined 10 and 20 minutes after paraquat administration

Dose of paraquat [M]	Per cent amplitude of nerve action potential (mean ± S.E.M., n = 6) 10 minutes after paraquat	20 minutes after paraquat
0 (control)	95.2 ± 4.0	93.1 ± 4.2
1.00×10^{-3}	92.0 ± 13.2	91.3 ± 12.8
0.25×10^{-2}	81.0 ± 15.9	80.0 ± 16.1
1.00×10^{-2}	96.2 ± 5.9	91.9 ± 4.3

The effect of paraquat on muscle twitch procuced by train-of four nerve stimulation

The train-of-four nerve stimulation was applied to the isolated rat phrenic nerve-hemidiaphragm preparation in order to examine the effect of a drug on presynaptic site of the neuromuscular junction. In this study it was found that paraquat in the doses of 1.00×10^{-3} M, 0.25×10^{-2} M did not produce a train-of-four fade while d-tubocurarine in the dose of 1.00×10^{-6} M markedly produced a train-of-four fade as shown in Figure 2. In the rat sciatic nerve-gastrocnemius preparation, in situ, paraquat in the doses of 10, 20, and 40 mg/kg body weight did not produce a train-of-four fade.

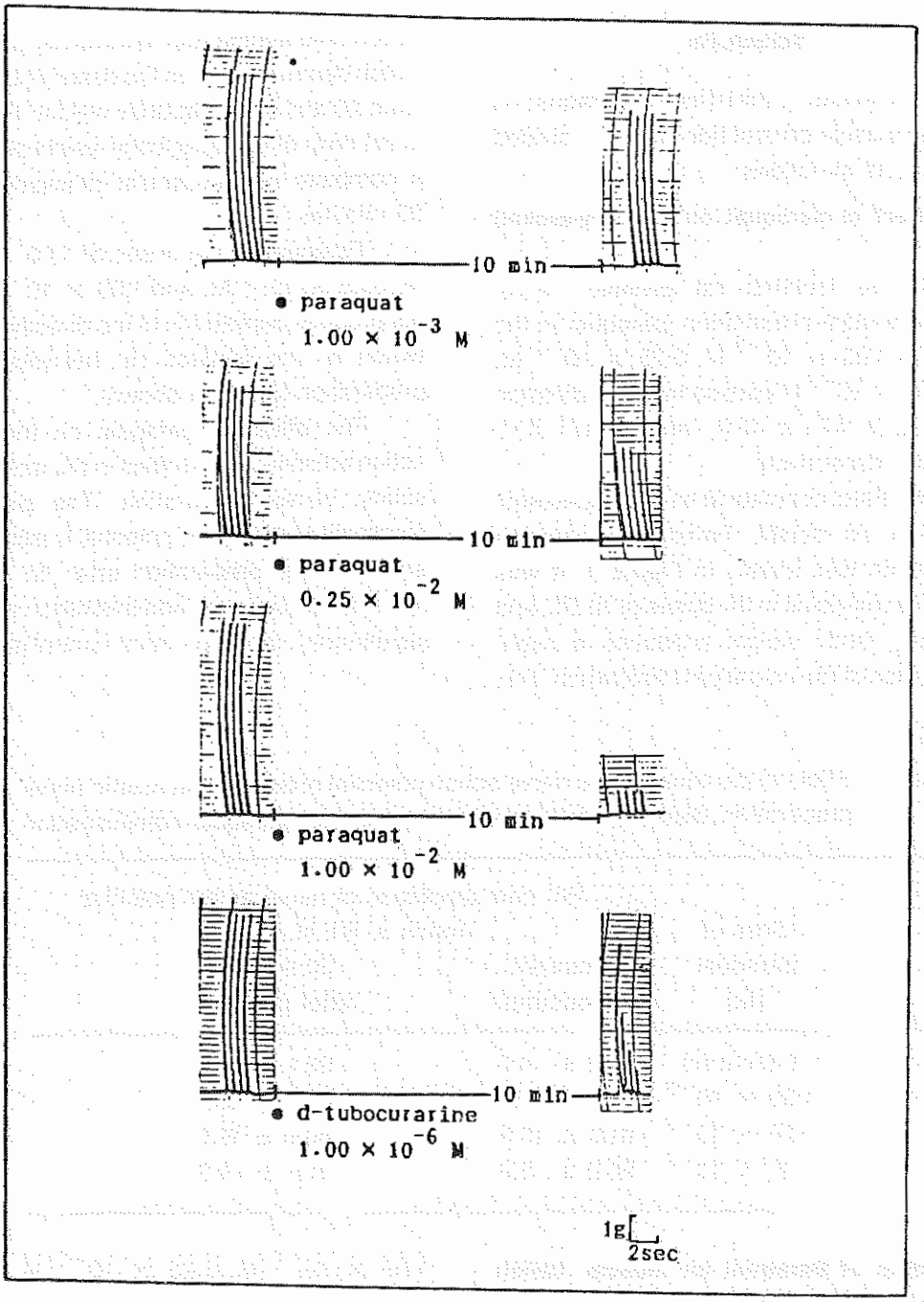

Figure 2 Comparison of the effect of paraquat and d-tubocurarine on muscle twitch produced by train-of-four nerve stimulation in isolated rat phrenic nerve-hemidiaphragm preparation.

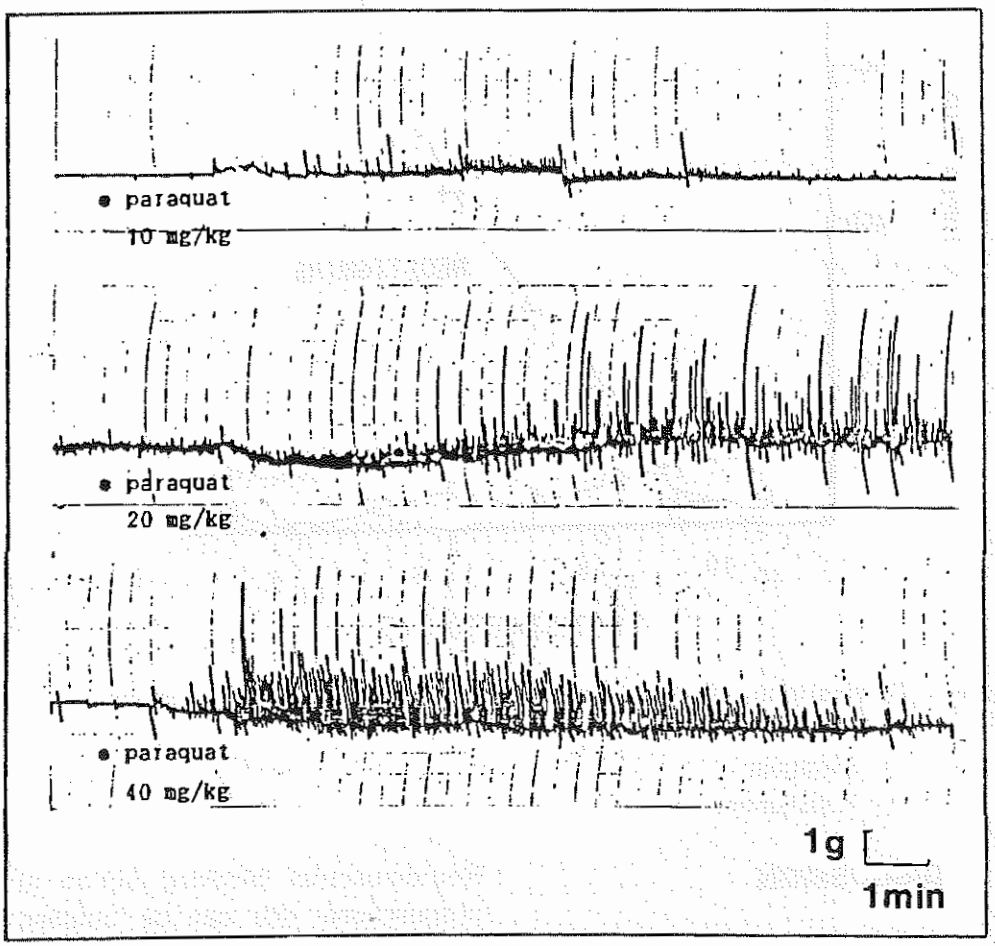

Figure 3 The effect of paraquat on rat denervated gastrocnemius preparation, in situ.

The effect of paraquat on denervated muscle

The left gastrocnemius muscle of the rat was denervated by aseptic technique. After 20 days of denervation, the set up four recording muscle contraction in situ was performed. Paraquat was injected via the right femoral artery and the contractile response of the left gastrocnemius muscle was observed. It was found that paraquat in the doses of 10, 20, and 40 mg/kg body weight produced fasciculation of the denervated muscle as shown by the tracings in Figure 3. The fasciculation produced by paraquat in the doses of 20 and 40 mg/kg body weight was more prominent that that produced by paraquat in the dose of 10 mg/kg body weight.

The effect of paraquat on acetylcholinesterase activity

Acetylcholinesterase activity in the presence of paraquat was assayed invitro by the colorimetric method. It was found that paraquat in the dose range 10^{-10} M to 10^{-3} M could not inhibit the activity of acetylcholinesterase enzyme more than 8 per cent while neostigmine markedly inhibited the enzyme activity (Figure 4).

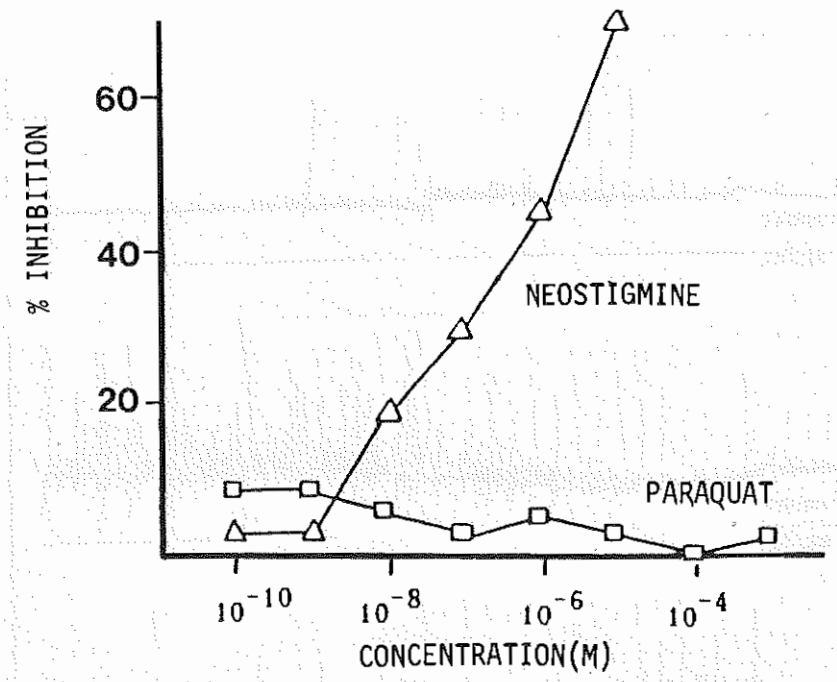

Figure 4 Comparison of the effect of paraquat and neostigmine on acetylcholinesterase activity
□ : paraquat
△ : neostigmine

DISCUSSION

The classical view of physiology of neuromuscular transmission holds that transmitter acetylcholine is released from the nerve endings by nerve impulses, and it acts on the postjunctional membrane of the motor endplate to set in action the chain of events that leads to muscle contraction.[13] According to this view, when extended to pharmacology, there are two possible sites within the neuromuscular junction where a neuromuscular blocker can act. They are prejunctional site and post junctional site. Blocking of the prejunctional site results in diminishing of acetylcholine output and thereby produces neuromuscular depression. Blocking of the postjunctional site interupts neuromuscular transmission and results in depression of the muscle twitch. Neuromuscular blocking action at the postjunctional site can be clasified into two types. They are non-depolarizing block and depolarizing block. In brief, non depolarizing agent acts by combining with the cholinergic receptor sites at the postjunctional membrane and thereby blocks competitively the transmitter action of acetylcholine. Depolarizing agent acts by depolarizing the postjunctional membrane and making it unexcitable by depolarized blockers.

In this study, the method of "train-of-four" nerve stimulation was applied to the isolated rat phrenic nerve hemidiaphragm preparation and rat sciatic nerve-gastrocnemius preparation in situ in order to postulate the site of action of paraquat within the neuromuscular junction. This method is helpful in distinguishing between depolarizing and non-depolarizing drugs. In brief, the train-of-four nerve

stimulation utilizes a short train of supramaximal stimuli applied to the motor nerve at a frequency of 2 Hz. The amplitude of the neurally-evoked twitch is recorded. The ratio of the amplitude of the fourth twitch to the amplitude of the first twitch in the same train indicates the degree of non-depolarizing block. During a non-depolarizing block this ratio is reduced and inversely proportional to the degree of neuromuscular block. During a depolarizing block, all twitch heights are ideally equal, or in other words, no train-of-four fade occurs.

The mechanism underlying the train-of-four fade was considered to be primarily a prejunctional phenomenon.[14] Lee et al.[15] demonstrated that the powerful postjunctional receptor blocker alpha-bungarotoxin did not produce train-of-four fade although it produced marked tension depression. This suggested that the train-of-four fade resulted from a different action to that which produced peak tension depression.

Miyamoto[16] reported that nicotinic chlinergic receptors are present on motor nerve endings as well as on the postjunctional membrane of the motor endplate. Blaber[17] showed that "decamethonium", a depolarizing agent, acted on motor nerve endings to increase the mobilization of acetylcholine transmitter from the reserve to the readily releasable store and this action was blocked by d-tubocurarine. This finding led to the postulation that blocking of the prejunctional chlinergic receptors impaired the mibilization of the transmitter so that the train-of-four fade was produced.[18]

In the present study, paraquat produced no train-of-four fade both in vitro and in vivo experiments. This suggested that paraquat had no prejunctional effect in blocking neurotransmitter release and the neuromuscular block produced by paraquat might be a depolarizing type.

Alternatively, depolarizing of the postjunctional membrane of the motor endplate can be demonstrated by means of denervated muscle. It is known that chronically denervated skeletal muscle is over excitable to acetylcholine and some other chemical agents.[19] In this study, paraquat produced a dose-related fasciculation in chronically denervated rat gastrocnemius muscle. This finding supported the suggestion that the depression action of paraquat on neuromuscular transmission may be due to depolarization at the postjunctional membrane.

Brown and Maling[20] reported that acetylcholinesterase activity of rat lung was inhibited by paraquat. In this study, paraquat did not inhibit acetylcholinesterase activity. This result agrees with the finding of Kohen and Chevion[21] who reported that acetylcholinesterase activity was not inhibited by paraquat. Thus, it might be suggested that the neuromuscular depression produced by paraquat may be due to its depolarizing action at the postjunctional membrane rather than its anticholinesterase action.

REFERENCES :

1. Windholz M. The Merck Index, 10th edition, New Jersey : Merck & Co Inc., 1983.
2. Ledwith A. Electron transfer reactions of paraquat. in : Autor AP.Biochemical Mechanisms of ParaquatToxicity. New York, Academic Press 1977,pp. 21-37.
3. sharp cw, Ottolenghi A, Posner HS. Cor-ation of paraquat taxicity with tissue concentrations and weight loss of the rat. Toxicol Appl Pharmacol 1972 ; 22 : 241 - 51.

4. Kimbrough RD and Gaines TB. Toxicity of paraquat to rats and its effects on rat lungs. Toxicol Appl Pharmacol 1970 ; 17 : 679-690.
5. Bus JS, Cagen SZ, Olgard M and Gibsor JE. A mechanism of paraquat toxicity in mice and rats. Toxicol Appl Pharmacol 1976 35 : 501-513.
6. Bullivant, C.M., Accidental poisoning by paraquat : report of two cases in man.Br Med J 1966 ; 1 : 1272-1273.
7. Matthew H, Logan A, Woodruff M F A and Heard B. Paraquat poisoning lung transplantation. Brit Med J 1968 ; 3 : 759 -763.
8. M. Van den Heede J, Cordonnier L, Van Bever and Heyndrickx A. Routine metho dology for the toxicological determination paraquat in case of Gramoxone poisoning in man. Med Fac Landbouww Rijksuiv Gent, 47/1, 1982.
9. Brandenburge E A. and Malin H M.Effect of paraquat and related herbicides on acetylcholinesterase of rat lung.Biochem Pharmacol 1980 , 29 : 465-466.
10. Bulbring E. Observation on the isolated phrenic-nerve diaphragm preparation of the rat,Brit J Pharmacol 1946 ; 1 : 38.
11. Apisariyakul A C. Pharmacology of Neuromuscular Synapse.Department of Pharma cology, Faculty of Medicine, Chiang Mai University 1984.
12. Augustinsson KB. Clinica Chim Acta 89, 239-252,1978.
13. Taylor P. Neuromuscular blocking agents. in : Goodman LS and Gilman A. The Pharmacological Basis of Therapeutics, 7th edition, New York : Macmillan Publishing Company, 1985.
14. Bowman WC, Gibb AJ and Marshall IG. Prejunctional and postjunctional effects of Vecuronium : in Clinical Experiences with Norcuron., Amsterdam : Excerpta Medica, 1983.
15. Lee CC and Katz RL. Characteristics of non-deporalizing neuromuscular block post-junctional block by alpha-bungarotoxin. Can Anest Soc J 1977 ; 24: 212.
16. Miyamoto M. The actions of cholinergic drugs on motor nerve terminals. Pharmacol Rev 1978 ; 29 : 221.
17. Blaber LC. The effect of facilitatory action of decamethonium on the storage and release of transmitter at the neuromuscular junction in the cat. J Pharmacol Exp Ther 1970 ; 175 : 664-672.
18. Bowman WC. Prejunctional and postjunctional cholinoceptors at the neuromuscular junction. Anesth Anal 1980;59:935-943.
19. Eyzaguirre C and Fidone SJ. Physiology of the Nervous System, Year Book Medical Publishers Incorporated, 1975.
20. Brown E A B and Mailing HM. Effects of paraquat and related herbicides on acetylcholinesterase of rat lung. Biochem Pharmacol 1980 ; 29 : 465-466.
21. Kohen R and Chevion M. Paraquat toxicity is enhanced by iron and reduced by desferrioxamine in laboratory mice. Biochem Pharmacol 1985 ; 34 : 1841-1843.