Indian Journal of Experimental Biology
Vol. 49, April 2011, pp.254-259

Mucuna pruriens Linn. seed extract pretreatment protects against cardiorespiratory and neuromuscular depressant effects of *Naja sputatrix* (Javan spitting cobra) venom in rats

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Received 5 August 2010; revised 9 December 2010

Mucuna pruriens has been used by native Nigerians as a prophylactic for snakebite. The protective effects of *M. pruriens* seed extract (MPE) were investigated against the pharmacological actions of *N. sputatrix* (Javan spitting cobra) venom in rats. The results showed that MPE-pretreatment protected against cardiorespiratory and, to a lesser extent, neuromuscular depressant effects of *N. sputatrix* venom. These may be explained at least in part by the neutralisation of the cobra venom toxins by anti-MPE antibodies elicited by the MPE pretreatment.

Keywords: Antivenom effects, Immunology, *Mucuna pruriens* seed, *Naja sputatrix* venom

Many plants have been used by traditional healers as snakebite remedies[1,2]. Hundreds of species of higher plants have been cited as being effective in treatment against snake envenomation[3]. However, few studies have attempted to scientifically validate these claims by investigating the nature, pharmacological properties and potency of the active chemical components.

Mucuna pruriens Linn. (commonly known as velvet beans) is found in Asia, America and Africa. It is a popular medicinal plant in India, where it has long been used in traditional Ayurvedic system of medicine for various diseases including parkinsonism[4]. In Nigeria, the beans have been prescribed by traditional practitioners as an oral prophylactic for snakebites. The protective effect of the aqueous seed extract has been demonstrated in mice against *Echis carinatus* and cobra venom (species unspecified)[5,6], however the mechanism of its protection is not fully understood.

Naja sputatrix (Javan spitting cobra) is a medically important snake indigenous to Southeast Asia. Venom from this cobra contains lethal phospholipase A_2 enzymes, polypeptide cardiotoxins, and neurotoxins[7]. Preliminary studies have shown that anti-*Mucuna pruriens* seed extract (anti-MPE) serum raised from rabbits was able to neutralise the lethalities of several Asiatic cobra venoms in mice, and that the anti-MPE IgG cross-reacted with purified neurotoxin and phospholipase A_2 of *N. sputatrix* venom[8]. The aim of this study is to investigate the protective effect of *M. pruriens* seed extract against the actions of *N. sputatrix* venom on the cardiorespiratory and neuromuscular functions, as part of our attempt to understand the protective action of *M. pruriens* seed against the lethal effect of the venom.

Materials and Methods

Plant material and seed extract—Mucuna pruriens (family: *Fabaceae*, subfamily: *Papilionoideae*) seeds were collected from Rukuba area in Jos, Nigeria, with the aid of a traditional healer. They were authenticated by Prof. S.W.H. Hussini of the Department of Botany, University of Jos. Voucher specimen Number A102 is deposited in the Pharmacy Herbarium of the University of Jos. The *M. pruriens* seed extract (MPE) was prepared according to Aguiyi *et al.*[5]. Seed extract was dissolved in normal saline prior to injection. The extract consists of both proteins and non-protein components[9].

Venom, drug standards and chemicals— Lyophilised *Naja sputatrix* (formerly known as *Naja naja sputatrix)* crude venom was purchased from Latoxan (Rosans, France). Urethane and heparin were

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purchased from Sigma Chemical Company, USA. All chemicals and reagents used in this study were of American Chemical Society (ACS) grade. Stock solutions of all chemicals were prepared using ultrapure water. Dilutions of venom and drugs were made in normal saline.

Animals—Male Sprague Dawley rats (220-300 g) were used. All animals were handled according to the guiding principles given by the Council for International Organisation of Medical Sciences (CIOMS) on animal experimentation[10]. Animals were supplied by the Laboratory Animal Centre of the University of Malaya and the animal experimental protocol was approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Malaya.

Pretreatment of rats—The pretreatment of rats (and dose of MPE chosen) were carried out as modified from Guerranti *et al.*[9]. Rats were divided into two groups of 9 each. The treated group consisted of rats injected with whole seed extract at a dose of 21 mg/kg (*ip*), once a week for 3 weeks. The control (or untreated group) was given a similar volume of saline. After 21 days, the animals were anaesthetised and surgically prepared for the monitoring of cardiorespiratory and neuromuscular functions as described below. There was no sign of toxicity in the pretreated animal and none of the animal died in the course of pretreatment.

Surgical preparation of animal—The rat was anaesthetised with urethane (1.4 g/kg, *ip*). Its trachea was cannulated to facilitate spontaneous respiration. The common carotid artery was cannulated for monitoring of the systemic blood pressure via a Statham pressure transducer connected to a MacLab Data-Acquisition system (version 3.5, Australia). The administration of normal saline and venom was done through a cannulated external jugular vein.

A thread was tied to the skin just below the diaphragm to monitor the respiratory movement via a Grass-compatible isometric force transducer (model FT03C) connected to a MacLab Data-Acquisition system.

The gastrocnemius and its tendon were dissected free from adjacent muscles and connective tissues. A thread was tied onto the Achilles tendon which was then cut to free the separated gastrocnemius. The thread tying the tendon was connected to another channel of the physiograph recorder via another isometric force transducer for the recording of skeletal muscle contractions *in situ*. The contractions of the gastrocnemius were elicited through the stimulation of the isolated sciatic nerve at a frequency of 0.1 Hz, a pulse width of 0.5 milliseconds and at supramaximal voltage of 3-8V. A resting tension of 3-6 g was applied to the gastrocnemius before stimulation commenced.

Monitoring of cardiorespiratory and neuromuscular functions—The mean arterial blood pressure, respiratory movement and nerve-elicited gastrocnemius muscle contractions were monitored simultaneously for at least 30 min to ensure stabilisation of these parameters. The heart rate was estimated from the blood pressure recording. After stabilisation, *Naja sputatrix* venom (½LD_{50}; 0.45 μg/g, this was chosen to enable monitoring of maximum toxic response without causing a quick death of the animal) was administered intravenously into the rats. The blood pressure (BP, mean arterial pressure), heart rate (HR), respiratory rate (RR) and gastrocnemius muscle twitch tension (MTT) were measured at an interval of 1 min for the first 3 min, then at 30 min and thereafter every 30 min until 5 h after venom injection or until the rat died, whichever occurred earlier.

The BP and HR were expressed as mmHg and beats/min (bpm), respectively, whereas both RR (breaths/min) and MTT (g tension) were expressed as percentages of their respective baseline (pre-dose) levels. This was because of the large inter-animal variations in these two latter parameters.

Under normal condition, at ½ LD_{50}, 25% of the rats died within 24 h.

Statistical Analysis—All data are presented as mean ± SE of 9 experiments. Differences in the means between groups were analysed using unpaired Student's *t* test with $P < 0.05$ considered as significant.

Results

N. sputatrix crude venom caused an immediate marked fall in the mean blood pressure (BP) of approximately 30% (from baseline 113.2 ± 7.5 to 82.2 ± 5.0 mmHg, Fig. 1A) within the first minute after intravenous injection. The blood pressure was then maintained for the next 30 min before further decline occurred. This decline continued progressively until the animal died (8 died between 60 and 300 min) or the end of the 300 min monitoring period (1 animal survived). While MPE-pretreatment did not alter the initial depressor response of the crude cobra venom, it significantly prevented the subsequent progressive fall in the blood pressure from 30 min onward until

the end (control, 7.1 ± 7.1; treated, 74.6 ± 12.8 mmHg, $P < 0.01$, Fig. 1A).

Experimental envenomation by *N. sputatrix* in the control animals produced little change to the heart rate (HR) of the anaesthetised rats initially (baseline 360.0 ± 29.6 beats/min), but from 30 min onward, the HR began to decrease progressively until the animal died or the end of the 300 min monitoring period (46.7 ± 46.7 beats/min, Fig. 1B). Pretreatment with MPE significantly attenuated the cardiac depressant effect of the cobra venom (Fig. 1B). At the end of the monitoring period, the HR of the MPE-pretreated animals still retained approximately 73% of the baseline value (baseline, 387.3 ± 22.3; end, 283.3 ± 52.7 beats/min), as compared to approximately 13% of the baseline value for the control animals.

Shortly after injecting the crude cobra venom, the respiratory rate started to decrease, slightly at the beginning (about 10% reduction by 30 min), but from 30 min onward the respiratory rate deteriorated steadily until the end of the monitoring period (about 90% reduction, Fig. 1C) or earlier, if the animal died (usually between 120 and 300 min). MPE-pretreatment greatly attenuated the venom-induced respiratory paralysis (85.2 ± 2.9% of the baseline respiratory rate remained at the end of the experiment).

Depression in the nerve-evoked muscle twitch tension (MTT) occurred soon after injection of the crude cobra venom. This depression in MTT progressed steadily until about 150 min after venom injection, where a residual amount (about 30%) of baseline muscle contraction was maintained to the end of the 300 min monitoring period. While MPE-pretreatment almost totally prevented the respiratory paralysis in the anaesthetised rats, it only partially reversed the venom-induced neuromuscular depressant effect (Fig. 1D). Further, the protection against the blockade of muscular excitability seen in the MPE-pretreated rats did not appear to be lasting.

Representative tracings of the effects of half-LD_{50} of *N. sputatrix* venom on the four parameters measured in MPE-pretreated and untreated (control) rats are shown in Fig. 2.

Discussion

Aguiyi *et al.*[5,11] demonstrated that the aqueous extract of *M. pruriens* seeds (MPE) can protect against the lethal action of some cobra (unknown species) venom and *Echis carinatus* venom. The protection against *E. carinatus* venom was shown to involve inhibition of venom-induced myotoxic, cytotoxic and coagulation activities in rats, as judged

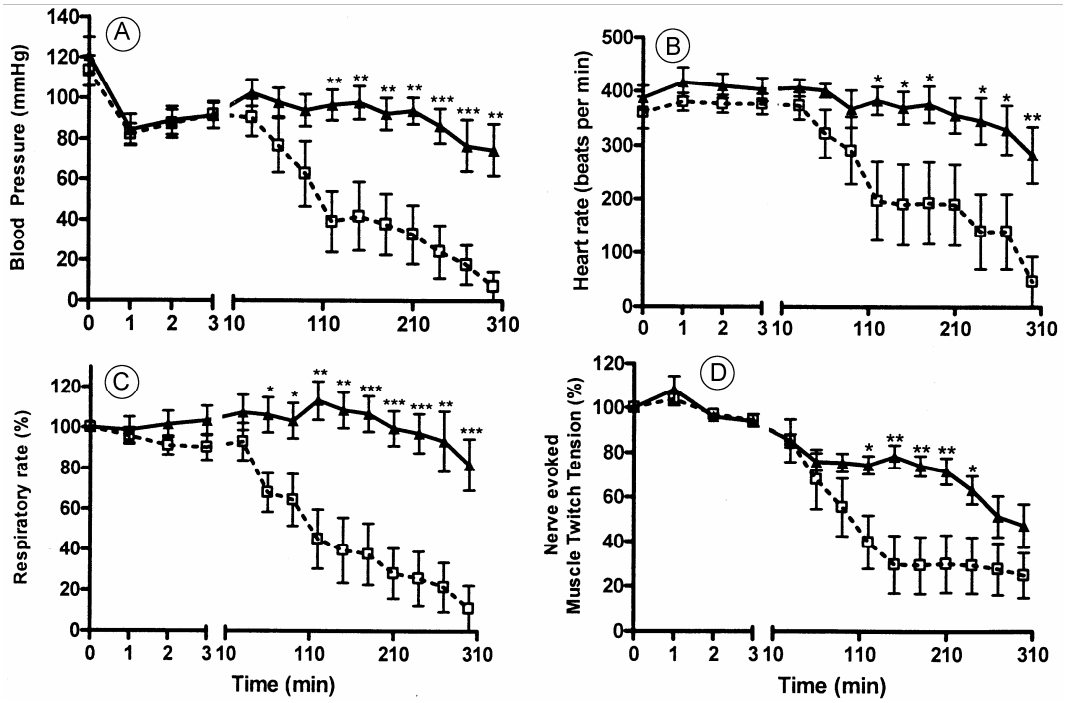

Fig. 1—Effect of half LD_{50} (0.45 mg/kg, *iv*) of crude *N. sputatrix* venom on (A) the mean arterial blood pressure, (B) heart rate, (C) respiratory rate, and (D) nerve-evoked muscle twitch tension of control (untreated;--□--) and MPE-pretreated (—▲—) rats. Values are mean ± SE from 9 animals in each group compared to the untreated animals. Student's *t*-test was used. *P* values: *< 0.05; **< 0.01; *** < 0.001

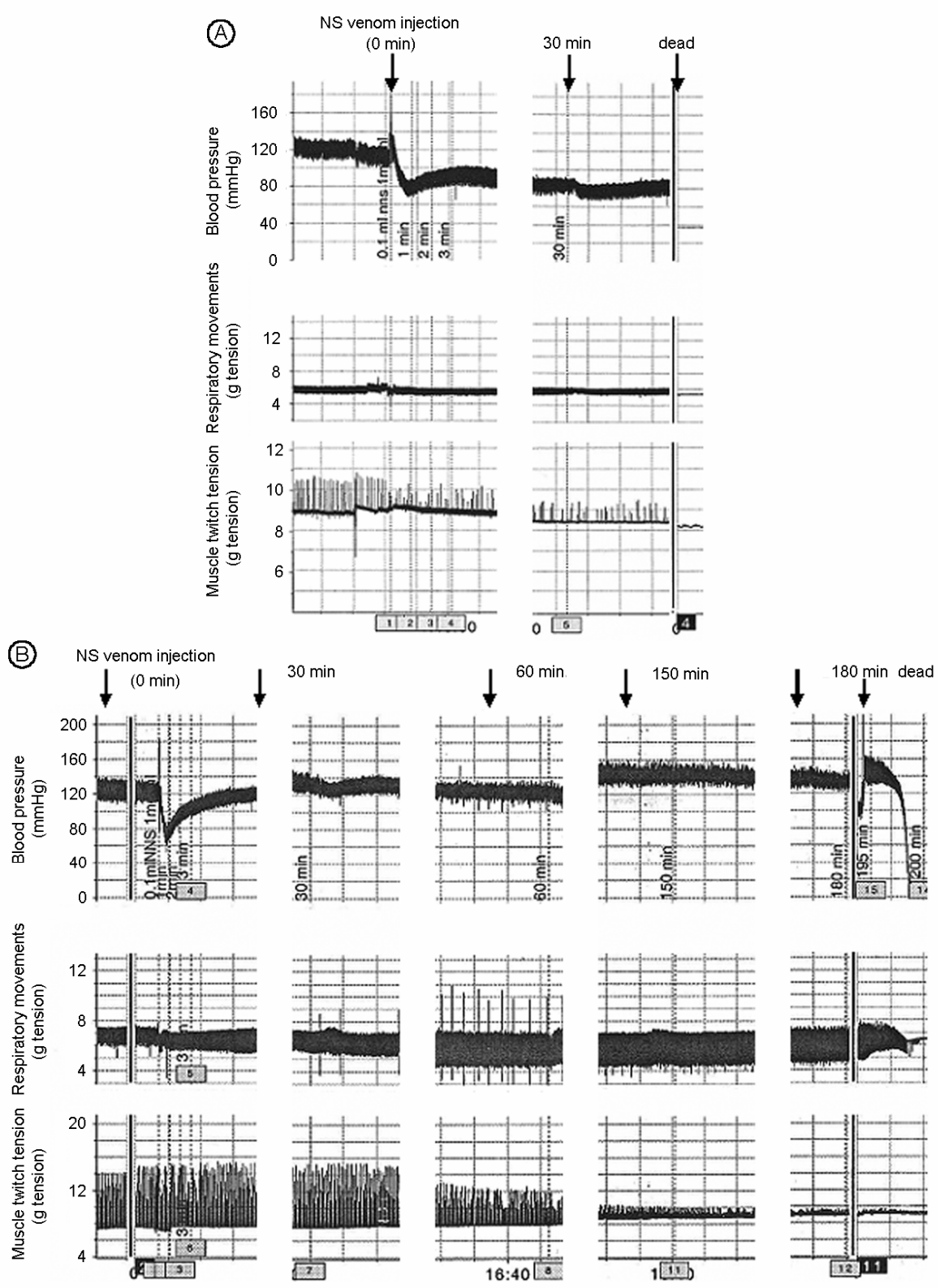

Fig. 2—Representative tracings of the mean arterial blood pressure, respiratory rate and muscle twitch tension in untreated and MPE pretreated rat after injection of half LD_{50} (0.45 mg/kg, *iv*) *N. sputatrix* venom**.** (A) control (untreated) and (B) MPE-pretreated anaesthetised rats

by the suppression of raised serum enzyme markers and coagulation parameters levels in the MPE-pretreated rats[11]. Subsequent studies by Guerranti *et al.*[6] and Tan *et al.*[12], using *in vivo* protection and/or *in vitro* neutralisation assays, demonstrated that the anti-*E. carinatus* venom and anti-*N. sputatrix* venom activities of *M. pruriens* seed extract have an immunological basis. While the protection of *M. pruriens* against the viper venom appears to be directed mainly against the proteins involved in blood coagulation[11,13-14], the protection against the cobra venom seems to be targeting at the heart[15].

In the present study, *M. pruriens* seed extract pretreatment was effective in protecting the animal against the cardiovascular and neuromuscular depressions caused by *N. sputatrix* venom when given at half its median lethal dose (½LD_{50}, 0.45 µg/g). This was evidenced by the significant attenuation in the venom-induced depression of the blood pressure, heart rate, respiratory rate and, to a lesser extent, the nerve-evoked gastrocnemius muscle twitch tension.

The cardiovascular changes seen in the anaesthetised rat following intravenous injection of *N. sputatrix* venom were similar to that observed with *Naja kaouthia* venom in anaesthetised cats[16]. This may be the result of cardiotoxin[16] or phospholipases A_2[17-18] or both acting synergistically.

MPE-pretreatment did not prevent the initial transient hypotensive action of *N. sputatrix* venom or its recovery, but significantly protected against the subsequent more gradual simultaneous fall in blood pressure and heart rate, as well as delayed the onset of cardiac failure. Phospholipase A_2 is known to act synergistically with cardiotoxin on the heart[19]. It is therefore probable that the protection of the seed extract against the cardiotoxic action of cobra venom may be due, at least in part, to the ability of the anti-MPE antibodies to neutralise cobra venom phospholipase A_2 as the anti-MPE antiserum did not cross-react with *N. sputatrux* cardiotoxin[8]. Besides the ability to neutralise *N. sputatrix* venom phospholipase A_2, the anti-MPE IgG was also shown to be able to neutralise the lethality of the cobra neurotoxin[20]. These two neutralisation actions of anti-MPE antibodies may be responsible for the observed protection of the seed extract against the respiratory paralysis and depression of the nerve-evoked gastrocnemius muscle twitch tension. The incomplete protection of *M. pruriens* seed extract against the nerve-evoked muscle twitch tension may be caused by insufficient titre of anti-neurotoxin antibodies in the pretreated rats. However, immune mechanism may not be the only protective mechanism: the MPE pretreatment may also have a direct non-immunological protective action against cobra venom.

Acknowledgement

Thanks are due to Ms. Noor Shafila Shafie for technical assistance. This study was supported by Research University Grant from the University of Malaya (RG022/09HTM) and from the Ministry of Science, Technology and Innovation, Government of Malaysia (02-01-03-SF0153).

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