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# The protective effects of *Mucuna pruriens* seed extract against histopathological changes induced by Malayan cobra (*Naja sputatrix*) venom in rats

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**Abstract. S**eed of *Mucuna pruriens* (Velvet beans) has been prescribed by traditional medicine practitioners in Nigeria as a prophylactic oral antisnake remedy. In the present studies, we investigated the protective effects of *M. pruriens* seed extract (MPE) against histopathological changes induced by intravenous injection of *Naja sputatrix* (Malayan cobra) venom in rats pretreated with the seed extract. Examination by light microscope revealed that the venom induced histopathological changes in heart and blood vessels in liver, but no effect on brain, lung, kidney and spleen. The induced changes were prevented by pretreatment of the rats with MPE. Our results suggest that MPE pretreatment protects rat heart and liver blood vessels against cobra venom-induced damages.

## INTRODUCTION

Velvet beans (*Mucuna* spp.) are found in Asia (including Malaysia), America and Africa. The beans have been prescribed by traditional practitioners in Nigeria as an oral prophylactic for snakebites. It is claimed that when a few seeds are swallowed intact, the individual is protected for up to one year against snakebite (Guerranti *et al.*, 2001). The prophylactic protective effect of *Mucuna pruriens* aqueous seed extract (MPE) has been demonstrated in mice against *Echis carinatus* (saw-scaled viper) and Africa cobra venoms (Aguiyi *et al.*, 1999, Guerranti *et al.*, 2002). Preliminary studies in our laboratory indicated that pretreatment of rat with MPE protected the animals from the lethal effect of *Naja sputatrix* (Malayan cobra) venom (Sim *et al.*, 2006). *Naja sputatrix* is a medically important snake in South East Asia. In this paper, we report results of our investigations on the effects of pretreatment of MPE against histopathological changes induced by Malayan cobra venom in rats, as part of our effort to understand the mechanism of the prophylactic protective effects of MPE against snake venom.

## MATERIAL AND METHODS

**Materials:**
Male Sprague Dawley rats used were supplied by Central Animal House, Faculty of Medicine, University of Malaya. All animals were handled according to guiding principles given by the Council for International Organization of Medical Sciences (CIOMS) on animal experimentation (Howard-Jones, 1985). Lyophilized *N. sputatrix* venom was obtained from Latoxan (Rosans, France). *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, Nigeria. All chemicals and reagents used in this study were of ACS grade. *Mucuna pruriens* seed extract (MPE) was prepared according to methods described in Aguiyi *et al.* (1999).

**Animal experiments**

Rats were grouped into 4 different groups of 3 animals each. Group 1 (control) consists of rats not injected with *M. pruriens* seed extract (MPE) and not injected with venom; group 2 (NS group) consists of rats injected with saline *i.p.* (once a week for 3 weeks) and then challenged with *N. sputatrix* venom (1.25 µg/g or 1.5 $LD_{50}$, , *i.v.*) at the end of the 3rd week; group 3 (MPE group) consists of rats injected with MPE (once a week for 3 weeks, at 21 mg/kg body weight *i.p.*), group 4 (MPE-NS group) consists of rats injected with MPE (once a week for 3 weeks, at 21 mg/kg body weight, *i.p)* and challenged with *N. sputatrix* venom (1.25 µg/g, *i.v.* or 1.5 $LD_{50}$) at the end of the 3rd week. Rats were monitored for 24 hours post venom injection. Rats in the NS group (group 2, all failed to survive) and in the MPE-NS group (group 4) that survived during the monitoring period were autopsied and their organs (brain, heart, lung, liver, kidney and spleen) were harvested.

**Histological studies**

All internal organs of the rats were fixed in 10% formaldehyde for 72 hours prior to paraffin wax-embedding. The fixed tissues were dehydrated prior to embedding in the paraffin wax. This was carried out in a graded-fashion with increasing ethanol concentration starting from 50%, followed by 70%, 80%, 95% and finally, in absolute ethanol (45 min each). Following ethanol dehydration, the tissues were soaked in 50% cedar wood oil for 40 minutes and transferred to pure cedar wood oil and stored overnight. The next day, the tissues were rinsed in benzene and transferred into a mixture containing equal volumes of benzene and molten paraffin wax and infiltrated for 60 minutes in a 60°C oven. The benzene/wax mixture was discarded and fresh molten wax was added immediately and kept at 60°C for 40 minutes. This was repeated thrice with fresh molten wax and finally, the tissues were transferred with heated forceps into molten wax in an embedding mold and left on a cold plate to harden completely. Wax embedded tissues were sectioned to a thickness of 5 µm and transferred onto silane-coated microscope slides. Different organs of the 12 rats were examined. Slides of rats' organs for light microscopy were stained with hematoxylin and eosin (H&E). Representative images from the slides were photographed. The tissues examined included the followings: lung (blood vessels, air sacs), kidney (renal cells, glomerulus), spleen (white pulp and red pulp), liver (blood vessels, hepatocytes), brain and heart.

## RESULTS AND DISCUSSION

Rats injected with just MPE (group 3) did not show any significant changes in their behavior and all rats in this group as well as group 1 (control) survived till the end of the experiment, and all were healthy. However, all 3 rats injected with *N. sputatrix* venom (group 2) died less than 12 hours after venom injection, while 2 out of 3 rats in group 4 (rats pretreated with MPE for 3 weeks followed by venom injection) survived. This indicated that MPE pretreatment protected rats against *N. sputatrix* venom.

Light microscopic examination of the histological sections of the brain, heart, liver (blood vessels and hepatocytes), lung (blood vessels and air sacs), spleen (white pulp and red pulp) and kidney (renal cells and glomerulus) of rats from group 3 (rat pretreated with MPE) indicated that they were essentially the same as that of the controls (group 1) (Fig. 1, A and C and Fig. 2, A and C. Pictures of brain, liver hepatocytes, lung, spleen and kidney not

shown). Thus, the 3 weeks pretreatment with MPE did not cause any observable damages to these organs.

Injection of 1.5 $LD_{50}$ of *N. sputatrix* venom also did not result in any observable histopathological changes in the brain, lung, spleen and kidney of the survived or dead rats (figures not shown). Light microscopic examinations of the heart sections of rats killed by venom injection, however, revealed a disruption in the striations of the muscle, although the nuclei appeared normal (Fig. 1, B). This may cause irregularities in contraction of the atria and ventricles of the heart, as well as irrhythmical beating of the heart, thus affecting circulation of blood and its contents throughout the body as a consequence. In addition, microscopic examination of the liver sections of the rats killed by venom also revealed shrunken diameter in the liver blood vessels (Fig. 2, B). Obstruction of blood flow in the liver may lead to liver dysfunction. However, since all the hepatocytes remained normal, it appears that the venom caused relatively minor damage to the liver.

On the other hand, microscopic examinations of the heart and liver sections of the survived rats in group 4 (MPE-NS group) revealed normal nuclei, cell structures and striations comparable to the controls (Fig. 1, D and Fig. 2, D). These results indicated that the MPE pretreatment protected the heart and liver of the rats from cobra venom-induced histopathological damages. The results of the present study

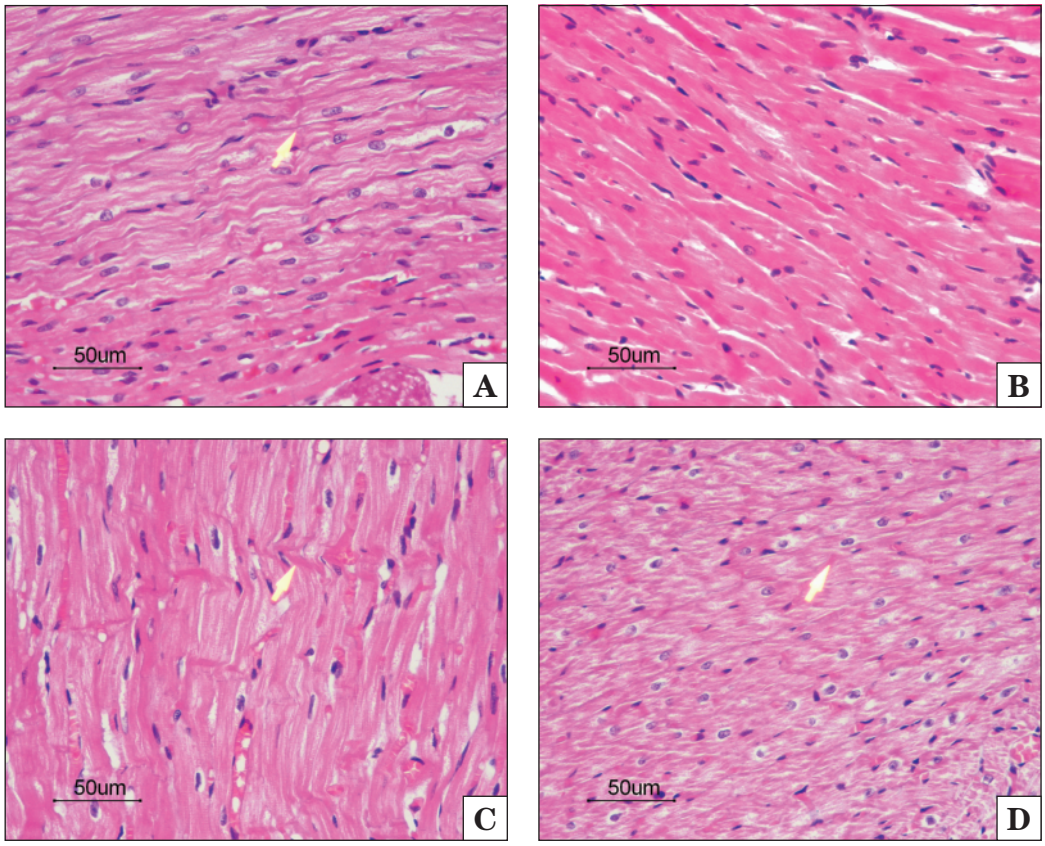


Figure 1: Histopathological changes in the heart of rats treated with *Mucuna pruriens* seed extract and *Naja sputatrix* venom.

A: Control (group 1); B: Rats pretreated with saline for 3 weeks followed by *Naja sputatrix* venom injection (group 2); C: Rats pretreated with *Mucuna pruriens* seed extract for 3 weeks (group 3); D: Rats pretreated with *Mucuna prurines* seed extract for 3 weeks followed by *Naja sputatrix* venom injection (group 4). White arrows in A, C and D indicate normal striation.

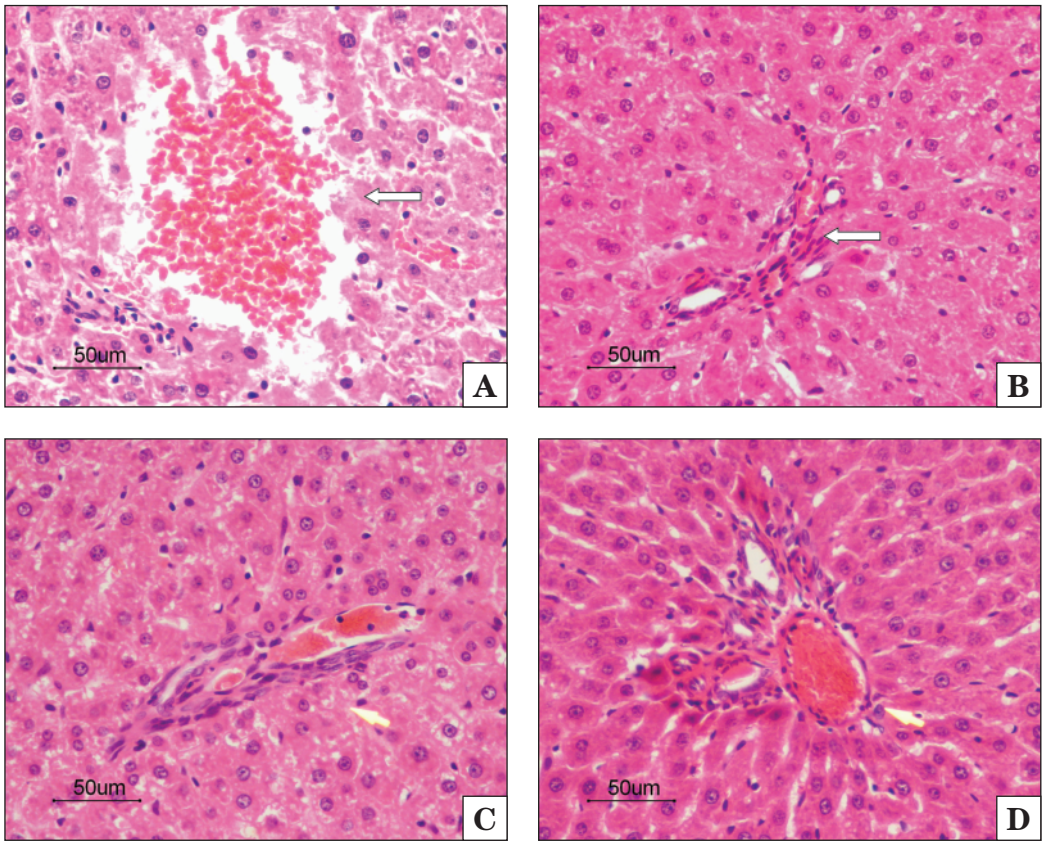


Figure 2: Histopathological changes in the blood vessels of liver of rats treated with *Mucuna pruriens* seed extract and *Naja sputatrix* venom.

A: Control (group 1); B: Rats pretreated with saline for 3 weeks followed by *Naja sputatrix* venom injection (group 2); C: Rats pretreated with *Mucuna pruriens* seed extract for 3 weeks (group 3); D: Rats pretreated with *Mucuna prurines* seed extract for 3 weeks followed by *Naja sputatrix* venom injection (group 4). The blood vessels are indicated by white arrows.

are consistent with our earlier pharmacological studies that pretreatment of rats with MPE protected the animals against cardiovascular depressant effects of *N. sputatrix* venom (Sim *et al.*, 2006).

*Naja sputatrix* venom is known to exhibit both neurotoxic and cardiotoxic effects due to the action of the venom polypeptide neurotoxins and polypeptide cardiotoxins (and possibly phospholipase $A_2$), respectively (Tan, 1991). Cher *et al.* (2005) reported that injection of sublethal dose of *N. sputatrix* venom into mice induced alterations in expression of multiple genes from heart, brain, kidney, liver and lung of the mice. They also reported that of the 203 genes whose expression was altered at least 3-fold in response of the venom injection, 50% were differentially expressed in the heart, indicating that the cardiotoxicity of the cobra venom may be a major cause of death, at least in mice.

Our results appear to be consistent with this observation, as histological examination indicated that in rats killed by injection of *N. sputatrix* venom, the only organ that suffered major damages was the heart, and that in rats protected by the MPE pretreatment, injection of the venom did not induce observable damages to the heart.

Thus, this study not only confirms the earlier report that *M. pruriens* seed extract exhibits prophylactic protection against some snake venoms, it also demonstrates

that the protective mechanism of MPE against *N. sputatrix* venom involves its ability to protect the animal against venom-induced histopathological damages to the heart. Guerranti *et al.* (2002) reported that the protective effect of MPE against *E. carinatus* venom has an immune mechanism. Work is in progress to investigate whether the cardiac-protective effect of MPE against *N. sputatrix* venom involves immune mechanism or a direct protective action.

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