

## **Study on the effect of green pit viper venom (*Trimeresurus albolabris*) on platelet morphology by flow cytometry**

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### Abstract:

The incidence of venomous snake bites, especially by green pit viper, has been increasing every year in Thailand. The bite of green pit viper causes bleeding because of thrombin-like property of the venom and it has been reported that the mean platelet volume decreases in those bitten by this snake. The objective of this study was to study the effect of green pit viper venom (*Trimeresurus albolabris*) on platelet volume (MPV), number of platelets and platelet morphology in vitro. The test was carried out by washing platelet in phosphate buffer pH 7.2 so as to get rid of fibrinogen, then the washed platelets were mixed with green pit viper venom. The mean platelet volume and number of platelets were determined by flow cytometry. The results showed that there was a decrease in the number of platelets ( $216 \pm 101 \times 10^9/l$  and  $78.1 \pm 43.4 \times 10^9/l$ ;  $P < 0.05$ ) and also the MPV ( $8.9 \pm 1.2 fl$  and  $4.8 \pm 1.3 fl$ ,  $P < 0.05$ ). The platelet size was smaller than normal, ranging from 1.1-1.2 micrometers. In conclusion, the green pit viper venom had a direct effect on platelet morphology, especially by decreasing platelet volume and numbers.

**Key Words:** Green pit viper, Venom, Platelet

### Introduction:

The green pit viper (*Trimeresurus albolabris* and *Trimeresurus macrops*) is a common venomous snake in Thailand and incidence of its bites has increased dramatically up to 73.58%. [1] Its venom has thrombin-like effect in vitro and causes a defibrination syndrome in vivo [2] and the clinical features of this venomous snake bite vary from asymptomatic to fatal bleeding. The venom of *Trimeresurus albolabris* can increase fibrinolytic activity by shortening euglobulin time. [3] A recent study of a group of patients who had been bitten by green pit viper (*Trimeresurus albolabris* and *Trimeresurus macrops*) found that fibrinolytic system activation was very common as indicated by low plasminogen, low antiplasmin and elevated fibrin-fibrinogen degradation products (FDPs). [4] Significantly decreased total platelet count and mean platelet volume (MPV) were demonstrated in envenomous blood. The changes might be partly due to the effect of green pit viper toxin on platelet morphology. [5] In this study we performed the in vitro study by mixing

green pit viper venom and platelet rich solution. Then the changes on MPV and platelet number were measured by flow cytometry, while the platelet morphological changes were observed by SEM.

### Materials and Methods:

1. Lyophilized crude venom (*Trimeresurus albolabris*) was obtained from snake farm of Thai Red Cross. One milligram of crude venom was dissolved in normal saline solution (NSS) as described in an earlier study. [6]
2. Fibrinogen-free platelet was prepared by using 10ml of EDTA blood mixed with 150ml of 0.1 M phosphate buffer pH 7.2 (40.5 ml of 0.2M dibasic sodium phosphate and 9.5 ml of monobasic sodium phosphate then added equal volume of distilled water). [7] The solution was then centrifuged in refrigerated centrifuge at 3,000 x g for 15 minutes. The supernatant was discarded and added another 145 ml. Gentle agitation was performed so as to disperse clumping platelets. The solution was re-centrifuged at 50 x g for 10 minutes. The platelets in supernatant were used to measure the MPV and platelet count by flow cytometry (Technicon H\*3). The number of platelets subjected to this experiment was not less than  $100 \times 10^9/L$ . Small amount of red cells could be found in the supernatant.
3. Morphological changes after exposure to green pit viper venom were determined. Two hundred microlitres of fibrinogen-free platelet concentrate were mixed with 100  $\mu$ l of venom, then incubated at 37°C at different periods of time (1-30 minutes), after which the MPV and platelet counts were determined by the Technicon H\*3. This process was repeated twenty times.

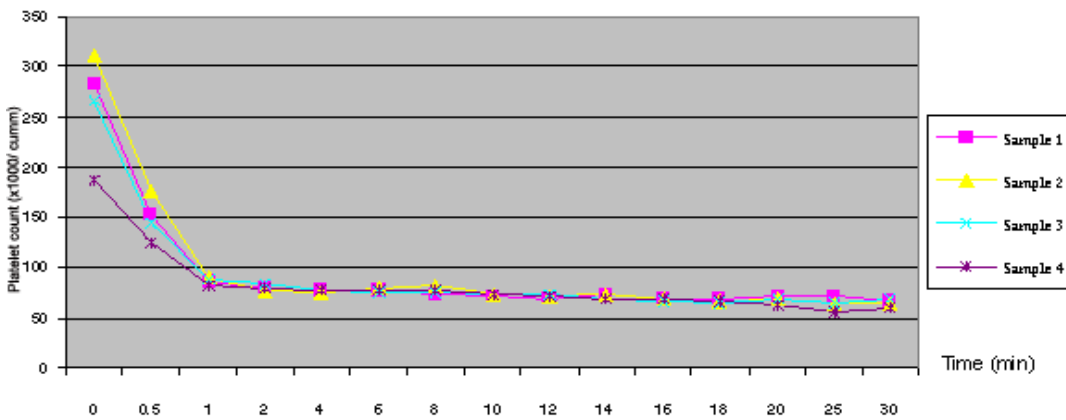
**Results:**

Before treatment, the platelets were within normal limits at  $216 \pm 101 \times 10^9/L$  while the MPV was  $8.9 \pm 1.2 fl$ . By electron micrograph, the platelets appeared regular shape with smooth surface, ranging from 1.4-2.0 micrometers. The red cells that still existed in the supernatant had smooth surface, round, disc-like sphere, measuring ranged from 5-6 micrometers in diameter. After addition of green pit viper venom to the platelet solution at one minute, the red

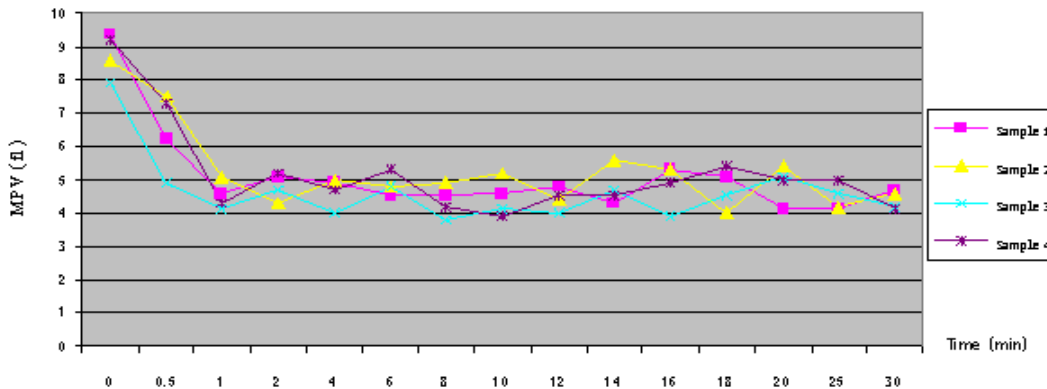
blood cells were irregular with multiple cytoplasmic projections. Most red cells showed shrinkage with the diameter ranging from 3-4 micrometers. The number of platelets decreased dramatically from  $216 \pm 101 \times 10^9/L$  to  $78.1 \pm 43.4 \times 10^9/L$  ( $P < 0.05$ ). The MPV also decreased from  $8.9 \pm 1.2 fl$  to  $4.8 \pm 1.3 fl$ , difference being significant ( $P < 0.05$ ), as shown in Table 1. The decrease in the number of platelets and that of MPV occurred at the same time as seen in Fig. 1 and Fig. 2.

**Table 1. Changes occurred after treatment of the platelets with green pit viper venom**

Parameters	Before treatment	Post treatment	P value
Platelets	$216 \pm 101 \times 10^9/l$	$78 \pm 43.4 \times 10^9/l$	$<0.05$
MPV	$8.9 \pm 1.2 (fl)$	$4.8 \pm 1.3 (fl)$	$<0.05$



**Fig. 1: Platelet number after addition of green pit viper venom (1mg/ml in normal saline solution) to platelet rich solution for 30 minutes**



**Fig.2: The Mean Platelet Volume (MPV) after addition of green pit viper venom (1mg/ml in normal saline) for 30 minutes**

### Discussion:

This study found that the numbers of platelet decreased after the exposure to green pit viper venom. The decrease in platelets *in vivo* might be in part the result of direct reaction of venom and partly due to consumption by clot formation. This study supports the view that the decreased MPV *in vivo* might be due to the direct effects of the snake venom.[5] The same may be the cause for decreased MCV also, as suspected in a previous report.[8] This study found that the red cell morphology treated with green pit viper venom have morphologically changed very much like those treated with Russell's viper venom.[9] However, Russell's viper causes a significant increase in hematocrit value. Such altered morphology was observed immediately at 1 minute and reached maximum at 30 minutes.[6,9] The green pit viper venom might have some properties different from that of the Russell's viper, even though both could cause spherocytosis. The decrease of platelets at the first minute might be due to cell lysis, but some of them could tolerate and persist in toxic environment, thus beyond that time, both graphs were constant. However, further research is necessary on this issue.

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