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Short communication

Effect of *Trimeresurus albolabris* (green pit viper) venom on mean corpuscular volume, osmotic fragility and red blood cell morphology: A preliminary report

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An *in vitro* study was conducted by mixing small amounts of green pit viper venom with blood and observing changes. At a concentration of 10 µg crude venom, red blood cells (RBC) osmotic fragility slightly increased. RBC morphology changed to spherical shape which was compatible with what was observed in scanning electron microscope (SEM). However, there was no change in mean corpuscular volume ($p > 0.05$).

Key words: Green Pit Viper, Mean Corpuscular Volume (MCV), Osmotic Fragility.

INTRODUCTION

Local and systemic bleeding are frequent complications in human victims of *Trimeresurus albolabris* (green pit viper). Such symptoms appear to result from thrombin-like property of the venom. The venom can activate clot formation and cleave fibrinogen into fibrinopeptide A (Wiwanitkit, 2004). A thrombin-like enzyme has been isolated from the venom of *Callosellasma rhodosmama* as well as from other pit-viper venoms (Esnouf and Tunnah, 1967; Morse et al., 1967; Damus et al., 1972). A previous report mentioned the effect of Russel's viper venom on red cell morphology, changing from disk like to echinocyte and increasing the hematocrit somehow (Nopathorn et al., 1998). Also, decreased in mean corpuscular volume (MCV) in those who had been bitten by green pit viper has been reported (Wiwanitkit and Suwansaksri, 2001). However, the data in this report were obtained *in vivo*, but some victims might be thalassemic carriers because Thailand is the endemic area. However, there is no report of green pit viper venom on red blood cell morphology. The aim of this work is to study the *in vitro* effect of green pit viper venom on red blood cell morphology. In this study, *in vitro* tests on red blood cell characteristics such as mean corpuscular volume (MCV), osmotic fragility test (OF), morphology, and scanning electron micrograph (SEM) were conducted.

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MATERIALS AND METHODS

The mixture of defibrinated blood and green pit viper venom at 1 h was used to determine the MCV by hematology analyzer Technicon H∗3.

To study the effect of green pit venom on red cell morphology, 10 mg of lyophilized venom in 1.0 ml of PBS pH 7.4 was further diluted to 100 µgm/ml. The diluted venom was kept at -20°C until required. One hundred microliter (100 µl) of diluted venom was mixed with 900 µl of defibrinated blood. The mixture was made so that the venom in the solution ranged from 0.5 - 100 µgm/ ml of blood. Incubation was performed in water bath at 37°C for 1 h, after which a thin smear was made and then stained with Wright's stain. Four samples were prepared. Similar protocol for testing of green pit viper toxin effect on platelet has been published in our previous work (Soogarun et al, 2006).

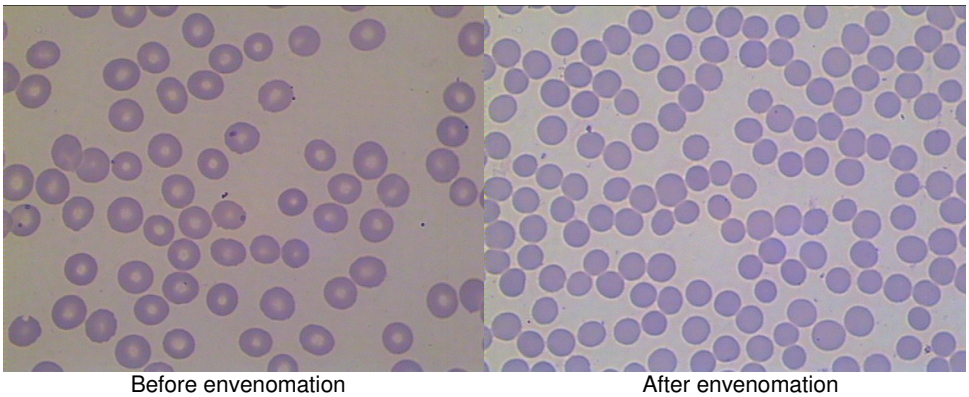

Figure 1. Spherocytosis at the concentration of 10 µg crude venom. Red blood cell morphology changes to spherical shape (Wright's stain; x1,000).

Table 1. Hemolysis of each concentration of buffered saline solution with control and envenomous blood of the same blood sample.

Buffered saline	Control (n = 4)		*T. albolabris* (n = 4)	
	A 450	% hemolysis	A 450	% hemolysis
Distilled water	2.865	100	2.833	100
0.30	2.851	99.511	2.790	98.482
0.35	2.704	94.380	2.725	96.188
0.40	1.620	56.545	1.734	61.207
0.45	0.180	6.283	0.532	18.799
0.50	0.064	2.234	0.155	5.471
0.90	0.050	1.745	0.108	3.812

Osmotic fragility was determined according to the method described by Beutler (1995) with some modifications. Briefly, 50 µl of envenomous blood was transferred to 5 ml of buffered saline solution at different concentrations of saline 0.5, 0.45, 0.35 and 0.3% and complete hemolysis was made by adding the defibrinated blood into distilled water. After the suspensions were left at room temperature for 30 min, the optical density readings were made at 540 nm.

To study red blood cell morphology study after envenomation, the mixture of red cells and green pit viper venom at concentration 10 µgm/ml of blood was fixed with 2.5% glutaraldehyde for 4 - 6 h. The morphology was observed by scanning electron microscope (SEM), with observation was made before and after envenomation.

Descriptive statistical analysis was used in this study. Comparison between the MCV by non-envenomous and envenomous blood was performed using unpaired student t-test.

RESULTS AND DISCUSSION

A concentration of 10 µg of venom with 1 h of incubation period can cause spherocytosis (Figure 1) compared with non-envenomous blood from the same sample. However, there is no significant difference in MCV (83.84 ± 2.97 fl and 84.39 ±1.86 fl, $p > 0.05$). There was also a tendency of increased fragility in envenomous blood which could be seen by the percent hemolysis in each saline concentration (Table 1). This phenomenon was compatible with the alteration of red blood cell into spherical shape with surface protrusion (echinocytes; Figure 2).

The red cell morphology has changed from normal configuration to spherical shape and had a tendency of osmotic fragility increment. It is of interest that 10 µg or more of the venom can cause such changes. It is possible that this alteration might be useful in determining the amount of venom after the bites. Clinicians can justify whether the patient needs antivenom therapy. This alteration has also been observed with Russell's viper venom (Nopathorn et al, 1998.). A slightly increased osmotic fragility might not cause harmful sequelae in those who had been bitten by this snake since the increment is within normal limits. Nopathorn (1998) indicated that not only the red cell morphology was changed but the hematocrit increased, which is contrary to this study. Ruiz (1980) studied blood cell and metabolic changes from the dogs bitten by Rattlesnake and observed increase in hematocrit, hemoglobin and mean corpuscular volume at 5 min after envenomation. However, these parameters were not significantly different from those of the control. Our study found that mean corpuscular volume were normal, which is totally different from the previous report on the retrospective data on envenomous

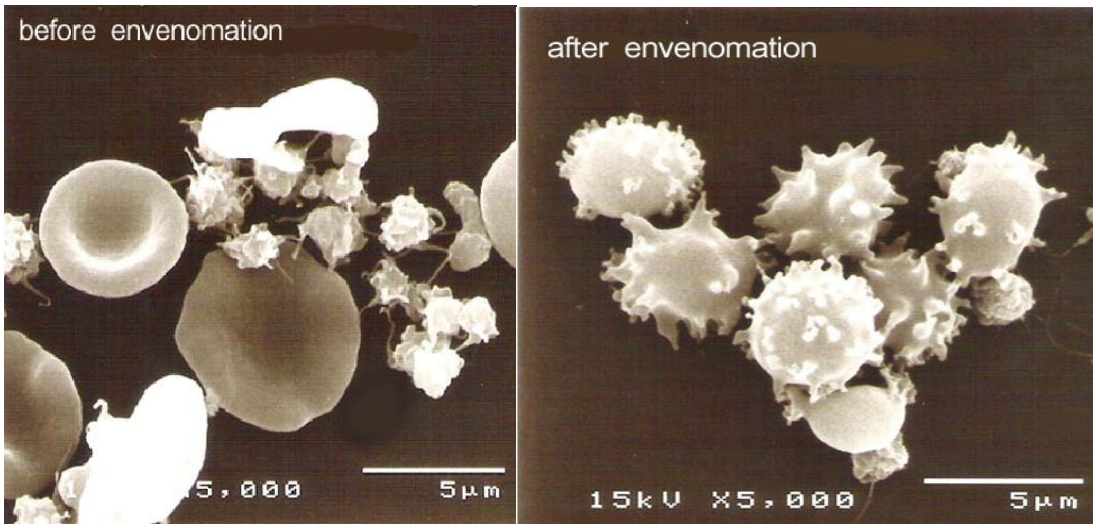

Figure 2. Electron micrograph of red cell morphology before and after envenomation. Red blood cell has changed to spherical shape with surface protrusion (echinocytes).

patients observed by Wiwanitkit and Suwansaksri (2001). There might be actual null effect of green pit viper on red blood cell or there might be some effects which could not be demonstrated by this *in vitro* study. Also osmotic fragility increase in this study was not significantly different from control values. Nevertheless, an *in vivo* study to reflect the actual change would have been much reliable. However, it is impossible to perform such a prospective *in vivo* study due to the ethical aspect on administration of green pit viper toxin on human subjects.

REFERENCES

Beutler E (1995). Osmotic fragility. In William Hematology. 5th edition. Beutler E (eds). Mc Graw-Hill, Inc. N.Y. L. pp. 46-47.

Damus PS, Markland FS, Davison TM,Shanley JD (1972). A purified procoagulant enzyme from the venom of the eastern diamondback rattlesnake *(Crotalus adamanteus): In vito* and *in vitro* studies. J. Lab. Clin. Med. 79: 906-923

Esnouf MP, Tunnah GW (1967). The isolation and properties of the thrombinlike activity from *Anci.strodon rhodostoma* venom. Br. J. Haematol. 13: 581- 590.

Morse EE, Jacsaon DP, Conley CL (1967).Effects of reptilase and thrombin on human blood platelets and observations on the molecular mechanism of clot retraction. J. Lab. Clin. Med. 70: 106-115.

Nopathorn S, Tejachokviwat M, Maneesri S, Kasantikul V (1998). Effects of Russell's viper venom on human erythrocytes *in vitro*. J. Natl. Toxins 7(1):73-85

Ruiz CE, Schaeffer RC, Jr Weil MH, Carlson RW (1980).Hemostatic Changes following Rattlesnake (*Crotalus viridus helleri*) venom in the dog. J. Pharm. Exp. Therapeutics. 213(2):414-417

Soogarun S, Choewbamrungkiat M, Wiwanitkit V, Suwansaksri J, Nopanitaya W, PradniwatP, Palasuwan A, Chanprasert S (2006). Study on the effect of green pit viper venom (*Trimeresurus albolabris*) on platelet morphology by flow cytometry. Online J. Health Allied Scs. 3: 5.

Wiwanitkit V (2004). A review of the hematologic effects of green pit viper venom. Toxin review. J. Toxicol. 23(1): 105-110.

Wiwanitkit V, Suwansaksri J (2001). Effect of green pit viper toxin on red blood cell index (an interim analysis). Toxicol. 164(suppl): 178.