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Hypocholesterolaemic potential of Russell's viper (*Daboia russelii siamensis*) venom in experimental mice

**Win Aung, **Shwe Zin Lin & ***Khin Maung Maung*

*Blood Programming Division
Department of Medical Research (Lower Myanmar)
**Department of Zoology, University of Yangon
***Radiation Toxicology Research Division
Department of Medical Research (Lower Myanmar)

With an aim to determine the hypocholesterolaemic potential of Russell's viper venom (RVV), changes in serum cholesterol levels of experimental mice, envenomed with different dosages of RVV were studied at various time intervals after envenomation, and were compared with changes in serum total protein and albumin. This *in vivo* study demonstrated that the serum cholesterol levels in these mice significantly reduced at half an hour after administration of RVV, obtaining its lowest level at one hour after envenomation. Although the degree of hypocholesterolaemia did not directly associate with respective dosage of RVV administered, the rates of fall in serum cholesterol level within first 24 hours were found to be apparently correlated with the relapsing time intervals after envenomation. Besides, RVV was found to have a direct lowering action on serum cholesterol in *in vitro* test. It could be concluded that the development of hypocholesterolaemia in experimental mice at early hours after administration of RVV indicates the sign of envenomation, and RVV also seems to be promising in cholesterol lowering action which could be used as a diagnostic indicator of envenomation.

INTRODUCTION

Being a national health problem in Myanmar, much research has been conducted on Russell's viper (*Daboia russelii siamensis*) venom with special inference to purification, characterization and biological activities of the different components for many decades [1]. However, little is known about the effects of RVV on serum lipid profiles in Russell's viper bite victims. In recent years, Pattnaik *et al.* reported that phospholipase A_2 (PLA_2) of *Crotalus adamanteus* snake venom was found to have an action on human serum high density lipoprotein (HDL) [2]. Sun *et al*. also showed that extract of snake venom of *Agkistrodon hylas* has little effect on the regression of atherosclerosis but it prolongs blood clotting and lowers serum cholesterol in experimental Japanese quail [3]. Winkler *et al.* reported that venom of *Vipera palaestinae* induced decrease in serum cholesterol level in human victims and determination of total serum cholesterol may serve as an indicator of the severity of clinical syndrome [4]. These findings prompt us to conduct this study with an aim to investigate the effects of RVV of Myanmar on serum total cholesterol levels of experimental mice being administered with different dosages of RVV at various time intervals after envenomation.

MATERIALS AND METHODS

Desiccated crude RVV was purchased from Myanmar Pharmaceutical Factory (MPF), Yangon. All chemicals used in this experiment were of analytical grade from Sigma Chemical Co. Ltd., U.S.A. Adult albino mice

of both sexes, Institute of Cancer Research (ICR) strain, weighing 20 ± 2 gm were obtained from Laboratory Animals Services Division, Department of Medical Research (Lower Myanmar), Yangon. After determining the LD_{50} of RVV on mice, *in vivo* effect of RVV on serum cholesterol, protein and albumin level at various time intervals after envenomation and in different dosages on mice were carried out. Finally, *in vitro* direct effect of RVV on these parameters was carried out. The detailed procedures were as follows:

Determination of intramuscular (IM) median lethal dose (LD_{50}) of RVV on mice

Six groups of mice each consisting of six animals were envenomed with different concentrations of RVV ranging from 5 to 160 ug/0.1ml. Death of mice within 24 hours was noted. The I.M LD_{50} of RVV on mice was calculated by Spearman-Karber method, 1981 [5].

Effect of RVV on mice at various time intervals after envenomation (in vivo test 1)

Six groups of mice each consisting of five animals were injected intramuscularly into the inner aspect of thigh with calculated LD_{50} dose of RVV. They were then sacrificed at 0.5, 1, 2, 4, 6 and 24 hours post envenomation. The mice of the control group received the same amount of normal saline only. Their blood samples were taken out through the opened cardiac puncture.

Effect of RVV in different dosages on mice (in vivo test 2)

A total of 25 mice, equally divided into five groups were tested. They were administered with I.M RVV in different dosages i.e. 2.5, 5, 10, 15 (i.e. LD_{50}) and 20 ug/0.1 ml into the inner aspect of the thigh. They were sacrificed at one-hour post envenomation and their blood samples were taken out through the opened cardiac puncture. The control mice were injected with the same volume of normal saline only.

Direct effect of RVV on serum of mice (in vitro test)

In this experiment, blood samples of unenvenomed mice were taken transcardially after being anesthetized and sera were separated out and collected. Then 0.1 ml of serum was mixed with 0.1 ml of RVV in concentration of LD_{50} dose. Another 0.1ml of serum was also mixed with same volume of normal saline. These two types of mixture were incubated in a water bath at 37°C for half an hour with gentle shaking. Determinations of cholesterol, total protein and albumin in these sera and mixtures obtained above were carried out by using the standard assay procedures [6, 7, 8].

RESULTS

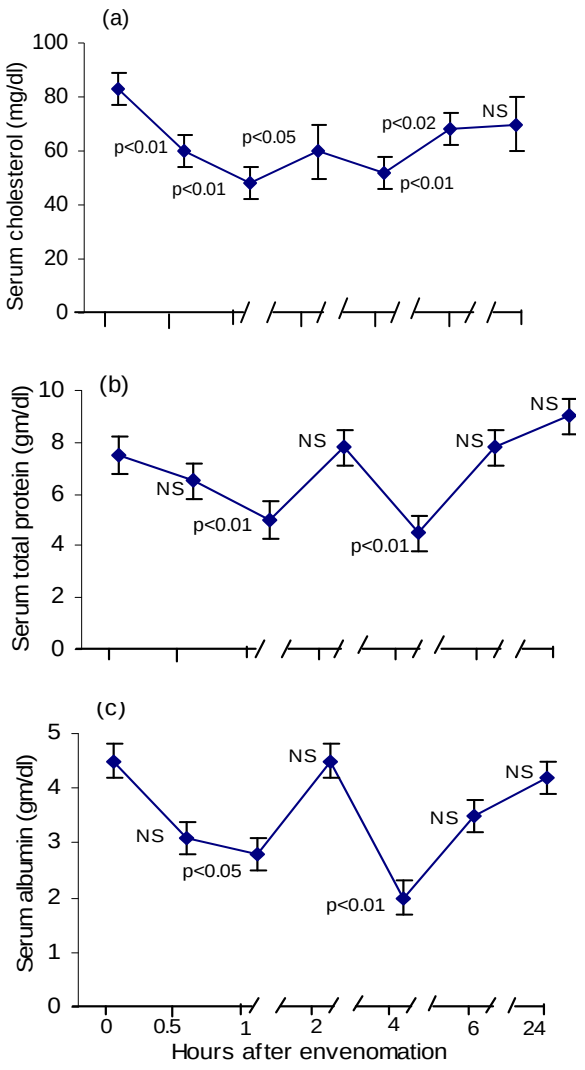
The I.M LD_{50} of RVV on mice was calculated to be 15ug/0.1ml (i.e 0.75ug/gm or 15ug /20gm mouse) (Table 1).

Table 1. Determination of intramuscular median lethal dose (LD_{50}) of RVV on mice

Concentrations of RVV	Deaths of mice within 24 hr
160 ug/0.1ml	6/6
80 ug/0.1ml	6/6
40 ug/0.1ml	6/6
20 ug/0.1ml	5/6
10 ug/0.1ml	0/6
5 ug/0.1ml	0/6
Normal saline (control)	0/6

Numerator indicates number of mice died and denominator indicates numbers of mice used.
Calculated LD_{50}=15 ug/0.1ml (i.e 15 ug/ 20gm mouse or 0.75 ug/ gm body weight)

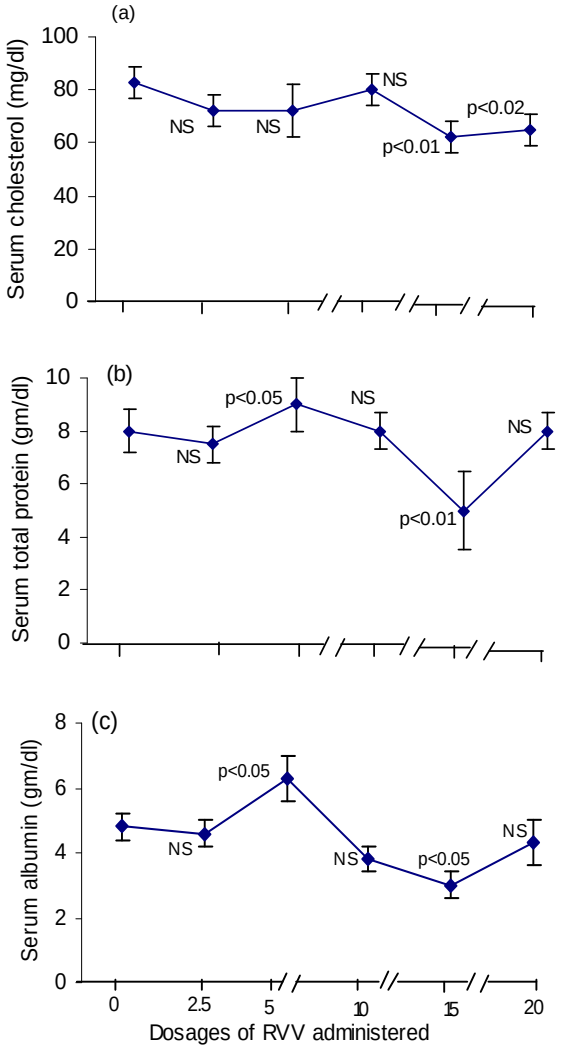
Fig. la shows the effect of RVV on serum cholesterol levels of mice at different time intervals after being administered with LD_{50} dose of RVV. It was found that serum cholesterol level started to decline significantly at half an hour after envenomation and reached its lowest level at one hour. The persistence of an apparent reduction in serum cholesterol level was observed till four hours. Then, it started to rise again at six hours and retain its initial level at 24 hours after envenomation. Changes in serum total



Each vale represents Mean ± SEm (n=5).
'p' value was calculated compared to the respective initial level, using Student's unpaired 't' test.
'NS' means not significant at the 5% probability level.

Fig 1. Changes in serum cholesterol, total protein and albumin levels of mice at various time intervals after administration with RVV in LD_{50} dose

protein levels of envenomed mice at various time intervals after envenomation were clearly illustrated in Fig.1b. Its levels showed a slight reduction at 0.5 hour, and significant reduction at one hour and four hours after envenomation. Total protein concentration retained its nearly initial level at two hours and six hours, and reached beyond the initial level at 24 hours after envenomation. Similarly, changes in serum albumin levels of mice at various time intervals after envenomation were clearly visualized in Fig.1c. The significant decrease in levels was observed only at one hour and four hours. Serum albumin level regained its initial concentration at two hours and 24 hours after envenomation.



Each value represents Mean± SEm (n=5)
'p' value was calculated compared to the respective initial level, using Student's unpaired 't' test.
'NS' means not significant at the 5% probability level.

Fig 2. Changes in serum cholesterol, total protein and albumin levels of mice at one hour after administration with RVV in different doses

Fig. 2a demonstrates the changes in serum cholesterol levels of mice at one hour after administration of RVV in different dosages. The apparent reduction in serum cholesterol levels was found after envenomation with each and every dose. The significant decrease

in levels was observed at the RVV in dosage of 15 ug/0.1ml (i.e. LD_{50} dose). Changes in serum total protein and albumin levels of mice after being envenomed with different dosages of RVV can be observed in Fig. 2b & 2c respectively. Although the obvious reduction was seen at some dosages of envenomation, the significant rise in total protein and albumin levels were observed at RVV in dosages of 5 ug/0.1ml unexpectedly.

Table 2 summarizes the direct effect of RVV on serum concentration of cholesterol, total protein and albumin. These parameters were found to be significantly reduced after incubation with RVV, thus indicating that RVV had a direct lowering (i.e. destructive) effect on cholesterol, protein and albumin in the sera.

Table 2. Determination of the direct effects of RVV on serum cholesterol, total protein and albumin (*in vitro* test 1)

Parameters	Serum concentrations (mg/dl)		Significance 'p' value
	without RVV	with RVV	
Cholesterol	70.4 ± 6.6	48.0 ± 5.1	<0.05
Total protein	8.4 ± 0.67	4.8 ± 0.85	<0.05
Albumin	3.9 ± 0.51	2.5 ± 0.12	<0.05

Each value represents Mean ± SEm (n=5)
Amount of RVV used = 15 ug/0.1ml (i.e. LD_{50})
NS means not significant at the 5% probability level
'p' value was calculated using Student's unpaired 't' test

DISCUSSION

In our clinical practice in the management of Russell's viper bite victims, presence and/or development of RVV evenomation is an indication for administration of anti-snake venom therapy and is manifested only by appearance of incoagulable (non-clotted) blood and clinical proteinuria in Russell's viper bite victim. These parameters are detected before the appearance of clinical features by using 20-minute whole blood clotting test and bed side boiling test respectively [9, 10]. These parameters usually show the abnormalities about 2 to 6 hours after bite. Therefore, it is necessary to detect the presence of envenomation in Russell's viper bite victims at the early hours by using a more rapid and simple procedure or parameter which could be available in district and township hospitals where most of the Russell's viper bite cases are admitted at the early hours after bite.

From our findings, it is strongly evident that RVV induced the significant lowering of blood cholesterol level within 24 hours after evenomation and presence of evenomation in animal victims could be detected as earliest as half an hour after evenomation at which a significant degree of hypocholestrolaemia was apparently detected (Fig.1a.). In addition, different degrees of hypocholesterolaemia in accordance with RVV in different dosages were also observed in animal victims after being administered with RVV (Fig. 2a).

Although an apparent reduction of serum total protein and albumin were observed only at one hour after en-venomation and their degrees of reduction were not consistently and directly related to the time elapsed. The fluctuation in serum total protein and albumin levels were observed in first 24 hours after evenomation (Fig.1b & 1c). It may be due to the prompt and compensatory synthesis of protein and albumin by liver. Besides, the degree of hypoproteinaemia and hypoalbuminuria did not totally reflect the amount of RVV administration since both total protein and albumin levels unexpectedly increase after administration of RVV in some doses (Fig. 2b & 2c).

The mechanism of the apparent lowering effect of RVV on serum cholesterol, total protein and albumin may be through the direct effect (Table 2). Since the presence of phospholipase A_2 (PLA_2) enzyme in RVV, it seemed to induce a direct destruction of cholesterol resulting in a significant hypocholesterolaemia. The RVV also contains many proteolytic enzymes that are supposed to directly destroy the serum protein and albumin, inducing a significant lowering of

their concentrations in sera. Another possibility for hypocholesterolaemic action of RVV may be the indirect mechanism, in which several toxic components of RVV such as proteolytic enzymes, hyaluronidase, phospholipase, phosphodiesterase cause capillary endothelial damage in victims, resulting in extravasation of plasma proteins.

The decrease in cholesterol levels may be due to lipoprotein leakage through the damaged endothelium. However, since the dimensions of lipoprotein particles are considerably greater than that of the albumin molecule, capillary leakage of lipoprotein alone would not be sufficient explanation for hypocholesterolaemia that was encountered earlier than hypoproteinaemia and hypoalbuminemia in our study.

Besides, changes in cholesterol levels in envenomed mice were not apparently correlated with changes in total protein and albumin levels in both various time intervals and different dosages of RVV. Therefore the hypocholesterolaemia encountered in envenomed mice is probably due to the direct destructive action of PLA $_2$ on serum cholesterol rather than an indirect action through the endothelial leakage. With recovery from envenomation, the cholesterol pool can be released into the blood stream by the liver in the form of newly synthesized very low-density lipoprotein (VLDL). Therefore, hypocholesterolaemic effect of RVV was transient and it restored to normal level at 24 hours after envenomation.

Our study showed a totally time-dependent and a partially dose-dependent hypocholesterolaemia in animal victims envenomed with RVV. Therefore, prompt and simple measurement of serum cholesterol in RVV bite victims at early hours of clinical course may indicate the presence of envenomation. This may facilitate the decision on early administration of anti-snake venom (ASV) to the victims before the development of the abnormalities of other parameters for envenomation and severe clinical features. It may also reduce the untoward side effects of unnecessarily administering ASV (i.e. horse antiserum) such as pyrogenic reaction and anaphylactic reaction which occurred about 100% and 13% respectively of victims receiving ASV [11].

Based on our findings, it could be concluded that RVV has a definite lowering effect on blood cholesterol levels in experimental mice and development of hypocholesterolaemia in victims after Russell's viper bite can be considered as a simple indicator for early detection of envenomation. The hypocholesterolaemic potential of RVV on serum cholesterol level in human victims needs to be studied in the near future for possible use in clinical practice.

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