ผลของพิษงูทะเล (*Lapemis hardwickii*) ต่อการทำงานของไตในสุนัข

นางสาวเกษศิรินทร์ ศักดิ์วิวัฒกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสรีรวิทยาการสัตว์ ภาควิชาสรีรวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2542

ISBN 974-334-744-5

EFFECTS OF SEA SNAKE VENOM (*Lapemis hardwickii*) ON RENAL FUNCTIONS IN DOGS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Animal Physiology
Department of Physiology
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 1999

ISBN 974-334-744-5

Thesis title	Effects of sea snake venom (*Lapemis hardwickii*) on renal functions in dogs
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เกษศิรินทร์ ศักดิ์วิวัฒกุล : ผลของพิษงูทะเล (*Lapemis hardwickii*) ต่อการทำงานของไตในสุนัข (EFFECTS OF SEA SNAKE (*Lapemis hardwickii*) VENOM ON RENAL FUNCTIONS IN DOGS) อ. ที่ปรึกษา: ศ.น.สพ.ดร. ณรงค์ศักดิ์ ชัยบุตร; อ. ที่ปรึกษาร่วม: ศ.นพ.ดร. วิศิษฏ์ สิตปรีชา, 53 หน้า. ISBN 974-334-744-5

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของพิษงูทะเลต่อระบบหมุนเวียนเลือด และการทำงานของไตในสุนัขหลังจากฉีดพิษงูเข้ากล้ามเนื้อ โดยแบ่งสัตว์ทดลองออกเป็น 3 ชุด ชุดแรกแบ่งออกเป็น 2 กลุ่มสัตว์ทดลอง กลุ่มละ 5 ตัว แต่ละกลุ่มได้รับพิษงูอย่างเดียวในขนาดน้อย 0.16 ม.ก.ต่อน้ำหนักตัว 1 กก. (กลุ่มที่ 1) และขนาดมาก 0.34 ม.ก.ต่อน้ำหนักตัว 1 กก. (กลุ่มที่2) ชุดที่ 2 แบ่งออกเป็น 2 กลุ่มสัตว์ทดลอง กลุ่มละ 5 ตัว ทำการทดลองเหมือนชุดแรก แต่มีการให้สารละลายโซเดียมไบคาร์บอเนต ($NaHCO_3$) ขนาด 4.2 กรัมเปอร์เซ็นต์ตลอดเวลา เพื่อทำให้ปัสสาวะเป็นด่างล่วงหน้าก่อนฉีดพิษงู 30 นาที ทั้งในขนาดน้อย(กลุ่มที่ 3) และ ขนาดมาก(กลุ่มที่ 4) ชุดที่3 แบ่งออกเป็น 2 กลุ่มสัตว์ทดลอง กลุ่มละ 5 ตัว ทำการทดลองเหมือนชุดที่1 แต่สัตว์จะได้รับการฉีดสารละลายโซเดียมไบคาร์บอเนต ($NaHCO_3$) ขนาด 4.2 กรัมเปอร์เซ็นต์เพื่อทำให้ปัสสาวะเป็นด่างภายหลังจากฉีดพิษงู 60 นาทีทั้งในขนาดน้อย(กลุ่มที่ 5) และ ขนาดมาก(กลุ่มที่ 6) ทำการศึกษาระบบหมุนเวียนเลือดทั่วไป ระบบหมุนเวียนเลือดที่ไต และการทำงานของไตเป็นเวลา 3 ชั่วโมงหลังจากได้รับพิษงู

กลุ่มที่1 ไม่พบการเปลี่ยนแปลงอย่างมีนัยสำคัญของปริมาณเลือดที่ออกจากหัวใจในหนึ่งนาที(CO) อัตราการเต้นของหัวใจ(HR) ความต้านทานที่หลอดเลือดส่วนปลาย(TPR) และปริมาตรเม็ดเลือดแดงอัดแน่น(PCV) ในขณะที่ความดันเลือดแดงเฉลี่ย(MAP) เพิ่มขึ้นอย่างมีนัยสำคัญที่120 และ180 นาที หลังจากฉีดพิษงูในขนาดน้อยอย่างเดียว กลุ่มที่2 ไม่พบการเพิ่มขึ้นของ MAP แรงดันชีพจร(PP) และ PCV ในขณะที่ HR ลดลงอย่างมีนัยสำคัญที่180 นาที($P<0.05$) แต่ปริมาตรเลือดที่ออกจากหัวใจหนึ่งครั้ง(SV) เพิ่มขึ้นอย่างมีนัยสำคัญที่180 นาที($P<0.05$) หลังจากได้รับพิษงูอย่างเดียว กลุ่มที่1 ไม่มีการเปลี่ยนแปลงของอัตราการไหลของปัสสาวะ(V) อัตราการกรองผ่านกลอเมอรูลัส(GFR) และความต้านทานของหลอดเลือดแดงที่ไต(RVR) อัตราการไหลของพลาสมาที่ผ่านไต(ERPF) และอัตราการไหลของเลือดที่ผ่านไต(ERBF) ลดลงอย่างมีนัยสำคัญ แต่สัดส่วนการกรอง(FF) เพิ่มขึ้นอย่างมีนัยสำคัญ($P<0.05$) กลุ่มที่2 ค่า V และ GFR ลดลงอย่างมีนัยสำคัญที่180 นาที($P<0.05$) ส่วนค่า ERPF และ ERBF ลดลงอย่างมีนัยสำคัญที่ 180 นาทีหลังจากได้รับพิษ ทั้งกลุ่มที่1 และกลุ่มที่2 ค่าของมายโอโกลบินในปัสสาวะเพิ่มขึ้นอย่างมีนัยสำคัญที่180 นาที($P<0.05$) เอนไซม์แลคเตตดีไฮโดรจีเนสในพลาสมาเพิ่มขึ้นอย่างมีนัยสำคัญที่60($P<0.05$) และ180 นาที($P<0.01$) เอนไซม์ครีเอตินฟอสโฟไคเนสในพลาสมาเพิ่มขึ้นอย่างมีนัยสำคัญที่60($P<0.05$) และ180 นาที($P<0.05$) กลุ่มที่3 และกลุ่มที่4 ไม่มีการเปลี่ยนแปลงของระบบหมุนเวียนเลือดหลังจากได้รับพิษงูทั้งในขนาดน้อยและมาก ยกเว้น HR เพิ่มขึ้นอย่างมีนัยสำคัญที่ 60 นาที($P<0.05$) หลังจากได้รับพิษงูในขนาดน้อย กลุ่มที่3 ไม่มีการเปลี่ยนแปลงของค่า GFR, ERPF, ERBF และ RVR ในขณะที่ค่า V และ FF เพิ่มขึ้นอย่างมีนัยสำคัญที่120 และ 180 นาที($P<0.05$) กลุ่มที่4 ไม่มีการเปลี่ยนแปลงของ GFR, ERPF, ERBF และ FF แต่มีการเพิ่มขึ้นอย่างมีนัยสำคัญของค่า V ที่120 นาที($P<0.05$) ค่าของมายโอโกลบินในปัสสาวะ เอนไซม์แลคเตตดีไฮโดรจีเนส และเอนไซม์ครีเอตินฟอสโฟไคเนสในพลาสมาของกลุ่มที่3 และกลุ่มที่4 เพิ่มขึ้นอย่างมีนัยสำคัญตลอดการทดลอง กลุ่มที่5 ไม่มีการเปลี่ยนแปลงของ MAP, PCV และ TPR ตลอดการทดลอง แต่ HR เพิ่มขึ้นอย่างมีนัยสำคัญที่60 นาที($P<0.05$) ส่วน PP, CO และ SV ลดลงอย่างมีนัยสำคัญที่60 นาที($P<0.01$) กลุ่มที่6 ไม่มีการเปลี่ยนแปลงของ SV และ TPR ตลอดการทดลอง ในขณะที่ MAP เพิ่มขึ้นอย่างมีนัยสำคัญที่120 นาที($P<0.05$) แต่ PP และ CO ลดลงอย่างมีนัยสำคัญที่60 นาที($P<0.05$) ทั้ง HR และ PCV ลดลงอย่างมีนัยสำคัญที่120($P<0.05$) และ180($P<0.05$) นาที ไม่มีการเปลี่ยนแปลงของ ERPF, ERBF และ FF ในกลุ่มที่5 ในขณะที่ ค่า V, GFR และ RVR เพิ่มขึ้นอย่างมีนัยสำคัญที่120 และ 180 นาที($P<0.05$) กลุ่มที่6 ไม่มีการเปลี่ยนแปลงของ GFR, ERPF, ERBF และRVR ส่วนค่า V และ FF เพิ่มขึ้นอย่างมีนัยสำคัญที่120 นาที($P<0.05$) ทั้งกลุ่มที่5 และ6 ค่าของมายโอโกลบินในปัสสาวะ เอนไซม์แลคเตตดีไฮโดรจีเนส และเอนไซม์ครีเอตินฟอสโฟไคเนสในพลาสมาเพิ่มขึ้นอย่างมีนัยสำคัญที่60 ($P<0.05$) และ180 นาที($P<0.05$)

การทดลองครั้งนี้แสดงให้เห็นว่าการให้สารละลายโซเดียมไบคาร์บอเนตเพื่อทำให้ปัสสาวะเป็นด่างทั้งก่อนและหลังการฉีดพิษงูสามารถป้องกันไม่ให้เกิดความผิดปกติของการทำงานของไต

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3970150631: MAJOR ANIMAL PHYSIOLOGY
KEYWORDS : SEA SNAKE VENOM / RENAL FUNCTIONS / DOGS

KEDSIRIN SAKWIWATKUL: EFFECTS OF SEA SNAKE VENOM (*Lapemis hardwickii)* ON RENAL FUNCTIONS IN DOGS. THESIS ADVISOR: PROF. NARONGSAK CHAIYABUTR, Ph.D.; THESIS CO-ADVISOR: PROF. VISITH SITPRIJA, Ph.D. 53 pp. ISBN 974-334-744-5

The present study was undertaken to clarify the mechanism responsible for the sea snake venom (SSV) action on cardiovascular and renal functions in experimental dogs after intramuscular injection of the venom. Three series of experiments were carried out in anesthetized dogs. The first series was performed on two groups of five animals each, which the animals were given either a single low dose of SSV (0.16 mg/kg. of lyophilized venom in distilled water, Gr. I) or a single high dose of SSV 0.34 mg/kg. (Gr. II). The second series was conducted on two groups of five animals each. A similar protocol was applied as in the first series except the animals were pretreated with sustaining infusion of 4.2 gm % $NaHCO_3$ solution to develop the alkalinized urine 30 minutes before envenomation in either low dose (Gr. III) or a high dose (Gr. IV). The third series was conducted on two groups of five animals each. A similar protocol was applied as in the first series except the animals received sustaining infusion of 4.2 gm % $NaHCO_3$ solution to develop the alkalinized urine which was given 1 hr. after injection of SSV in either low dose (Gr. V) or a high dose (Gr. IV). Measurements of general circulation, renal hemodynamics and renal functions were carried over 3 hr. after envenomation.

In group I dogs, there were no significant changes in cardiac output (CO), heart rate (HR), total peripheral resistance (TPR) and packed cell volume (PCV) whereas mean arterial blood pressure (MAP) significantly increased at 120 and 180 minutes after a low dose of SSV injection alone. In group II animals, there were no significant changes in MAP, PP, and PCV whereas HR significantly decreased at 180 minutes ($P<0.05$). Stroke volume (SV) significantly increased at 180 minutes ($P<0.05$) after SSV injection alone. No significant changes in the urine flow rate (V), glomerular filtration rate (GFR) and renal vascular resistance (RVR) were apparent in group I. Effective renal plasma flow (ERPF) and effective renal blood flow (ERBF) significantly decreased while a significant increase in filtration fraction (FF) was noted. In group II, V and GFR significantly decreased at 180 minutes ($P<0.05$). There were significant decreases in ERPF and ERBF in group II animals at 180 minute after envenomation. In both groups I and II, urinary myoglobin (U_{Mb}) significantly increased at 180 minutes ($P<0.05$), plasma lactate dehydrogenase (P_{LDH}) significantly increased at 60 minutes ($P<0.05$) and 180 minutes ($P<0.01$) and plasma creatine phosphokinase (P_{CPK}) significantly increased at 60 minutes ($P<0.05$) and 180 minutes ($P<0.05$). In groups III and IV of the animals pretreated with $NaHCO_3$, there were no significant changes of the general circulation after given either a low dose or a high dose of SSV throughout the period of study except the HR increased significantly at 60 minutes ($P<0.05$) in animals given a low dose of SSV. There were no changes in GFR, ERPF, ERBF and RVR in group III dogs, whereas V and FF significantly increased at 120 and 180 minutes ($P<0.05$). In group IV dogs given a high dose of SSV, there were no significant changes in GFR, ERPF, ERBF and FF whereas V significantly increased at 120 minutes ($P<0.05$). Animals in groups III and IV showed significantly increased of U_{Mb}, P_{LDH} and P_{CPK} after envenomation. In group V animals, there were no significant changes of the MAP, PCV and TPR throughout periods of study whereas the HR increased significantly at 60 minutes ($P<0.05$). PP, CO and SV significantly decreased at 60 minutes ($P<0.01$). In group VI, there were no significant changes of SV and TPR throughout periods of study while MAP significantly increased at 120 minutes ($P<0.05$) but PP and CO significantly decreased at 60 minutes ($P<0.05$). HR and PCV significantly decreased at 120 ($P<0.05$) and 180 minutes ($P<0.05$). No changes in ERPF, ERBF and FF were apparent in group V dogs, whereas V, GFR and RVR significantly increased at 120 and 180 minutes ($P<0.05$). In group VI dogs showed no significant changes in GFR, ERPF, ERBF and RVR whereas V and FF significantly increased at 120 minutes ($P<0.05$). In both groups of V and VI, marked elevation of U_{Mb}, P_{LDH} and P_{CPK} appeared at 60 and 180 minutes $P<0.05$).

The present results indicate that infusion of $NaHCO_3$ for alkalinuric conditions either before or after SSV administration has a protective role against dysfunction of renal functions.

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ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Professor Dr. Narongsak Chaiyabutr, and my co-advisor, Professor Dr. Visith Sitprija for their kind advice, guidance, writing assistance and their constant encouragement this study.

My thanks are also expressed to the thesis committee of Department of Physiology, Faculty of Veterinary Science, Chulalongkon University.

I would like to thank the Queen Saovabha Memorial Institute of the Thai Red Cross Society for the donation of sea snake venom. In addition, I would like to warmly thank Dr. Lawan Chanhome for her kind suggestion, especially identification of the species of sea snakes.

It is my pleasure to acknowledge the kind assistance Miss Siripen Komolvanich for her kindness and provision of the facilities used in experimental work and laboratory technique. I am grateful, too, to Miss Hathaitip Parkinsee for her help, sincerity and friendship.

This study was supported in part by a fund from the Faculty of Graduate Studies, Chulalongkorn University.

Finally, I would like to express my extreme gratitude to my parents for their kind encouragement throughout the entire period of study. My gratitude to them is beyond description.

And if I have missed anyone involved with this project, please accept my apology, I have not overlooked them in my heart.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABBREVIATION

B	=	Blood
C	=	Clearance
C_{H2O}	=	Free Water Clearance
CO	=	Cardiac Output
Ds	=	diastolic pressure
Sp	=	systolic pressure
ERBF	=	Effective renal blood flow
ERPF	=	Effective renal plasma flow
FE	=	Fractional excretion of electrolyte
FF	=	Filtration fraction
GFR	=	Glomerular filtration rate
gm%	=	gram/100 milliliter
HR	=	Heart rate
In	=	Inulin
IV	=	Intravenous infusion
kg.	=	kilogram of body weight
µg	=	microgram
mg.	=	milligram
mg%	=	milligram/100 milliliter
ml.	=	milliliter
MAP	=	Mean arterial blood pressure
NSS	=	Normal saline solution
Osm	=	Osmolality
P	=	Plasma
PAH	=	P - Aminohippuric acid
PCV	=	Packed cell volume
PP	=	Pulse pressure

$NaHCO_3$	=	Sodium bicarbonate
RF	=	Renal fraction
RVR	=	Renal vascular resistance
SSV	=	Sea snake Venom
SV	=	Stroke volume
TPR	=	Total peripheral resistance
U	=	Urine
UV	=	Urinary excretion
V	=	Urine flow rate

CHAPTER I

INTRODUCTION AND AIMS

There are many varieties of sea snakes in the Gulf of Thailand. The most common sea snakes in the Gulf of Thailand are *Lapemis hardwickii*, which accounts for 81% of all sea snakes (Tu, 1987). It is well established that in most animals species, the main cause of death due to sea snake venom poisoning is respiratory paralysis (Tu *et al.*, 1976; Yang and Lee, 1976).

The chief pathological lesions of sea snake bite poisoning in man are in skeletal muscle, kidney, and liver (Marden and Reid, 1961). Renal damage often results from sea snake envenoming as happened to other types of myonecrosis and other types of envenoming (Reid, 1975b). Myoglobinuria appears within 3 hr. after the bite. Myoglobin released from damaged skeletal muscle can cause renal failure. Sitprija and co-workers (1971) studied two patients with severe poisoning and renal failure. In both patients, biopsies confirmed tubular necrosis in the kidneys and myonecrosis. In victims dying 48 hrs latter after the bite, acute renal failure appeared to be the immediate cause of death (Reid, 1975b). Experimentally, Bywater and Stead (1944) showed that myoglobin injected into healthy rabbits caused no renal damage unless the urine was acidified. It is therefore unlikely that the myoglobin released in sea snake poisoning is solely responsible for the renal lesions. Muscle pains and myoglobinuria are the most characteristic symptom in human sea snake bite victims. Muscular pains are associated with myoglobinuria, muscular paralysis. Acute renal failure occurred in the majority of patients (Barsoum and Sitprija, 1997).

Although the clinical aspects of the sea snake bites are well described, little is known about the sequential pathophysiological changes of the effects of myoglobinuria in the kidney. The mechanisms that produce these alterations in renal function after envenomation are not fully explained. In addition, no experimental

evidence supports the role of alkalinuric condition on renal function in animals given sea snake venom. Thus, the purpose of the present study was conducted to obtain information for possible effects of crude venom from *Lapemis hardwickii* on renal function and general circulation. Experiments have been investigated in dogs in order to determine, whether the changes of renal function are resulting from the systemic changes of cardiovascular system, and whether changes in renal function are due to the direct effect of venom during acidified urine after envenomation.

CHAPTER II

BACKGROUD INFORMATION

Sea snake venom (SSV)

The sea snake is a reptilian in the family Elapidae. Among the species of sea snake, those of *Hydrophis cyanocinctus*, *Enhydrina schistosa*, *Microcephalophis gracilis* and *Pelamis platurus* have been commonly found. *Lapemis hardwickii* is one of the poisonous snake that can be mostly found throughout Thailand (Tu, 1987). Sea snake bites occur mainly among marine fishermen working in the tropical waters of the Indian and Pacific oceans, while snakes are being removed from the nets (Mackessy and Tu, 1993). Particularly dense populations of sea snakes have been found in the coastal waters of the Philippines and Thailand (Minton, 1975) and the Western Pacific coasts of Central America (Tu, 1976). Sea snakes in general are not particularly aggressive, so bites are rare even in these circumstances (Mackessy and Tu, 1993).

The venom of *Lapemis hardwickii* is clear and colorless. There is still some dispute over the exact nature of the toxic effect of the venom. In animal experiments, sea snake venom seems to have a paralyzing effect at the junction between the nerves and the muscles, leading to a paralysis of the diaphragm and death from suffocation. In humans, Reid (1975a,b) has concluded that the direct damage to the muscles occurred. The appearance of the muscle protein myoglobin in the dark colored urine of poisoned patients is an evidence of muscle damage.

Action of sea snake venom on the muscle

Damage to the muscle has been shown by from myoglobinuria in sea snake poisoning. The postmortem examination of a sea snake poisoned patient revealed a widespread of hyaline necrosis in the skeletal muscle (Karunaratne and Panabokke, 1972). Myoglobinuria in human victims was also a common observation (Reid, 1973, 1975a, b). The components responsible for muscle damage are not neurotoxins, but it is myotoxin acting by myotoxic phospholipase A (Tu *et al.*, 1970; Tu and Passey, 1972; Lind and Eaker, 1981).

The *E. schistosa* venom itself caused the swelling of mitochondria and a decrease in oxygen uptake by mitochondria (Taub and Elliott, 1964). Intramuscular injection of the venom caused the damage of mitochondria of the end-plate region (Toh *et al.*, 1975). Myotoxic phospholipase A from *E. schistosa* venom inhibited Ca^{2+} uptake into sarcoplasmic recticulum (Ng and Howard, 1980). The myotoxic effect of *E. schistosa* envenomation was also reflected by an elevation of the plasma creatine kinase level (Sutherland *et al.*, 1981).

Clinical features of sea snake poisoning

Clinical symptoms following snakebite are quite variable and depend on the amount of venom injected, the site of injected, the physical state of the victim, and several other factors. Local reactions at the site of the bite are usually minor or asymptomatic and the punctured wounds are small and often not visible without careful inspection.

The major manifestations of sea snake envenomation result from the action of the predominant neurotoxins including muscle pain, paralysis (local and general), and respiratory arrest (Tu, 1987). Respiratory arrest is frequently the most immediate life–threatening symptom of sea snakebite. The diaphragm/phrenic nerve endplate is

blocked by neurotoxin binding (Carey and Wright, 1961; Karunaratne and Pannabokke, 1972).

Renal failure and myoglobinuria may result in cause of severe poisoning, presumably from the myotoxic action of phospholipase A_2. The whole venom from *Laticauda semifasciata* did not appear to affect the cells of the proximal tubule of the kidney of experimental envenomed mice (Schmidt *et al.*, 1976), though localized intracellular swelling of visceral epithelium was noted. A widespread hyaline myonecrosis of the skeletal muscles was noted at postmortem in a patient bitten by a sea snake, and myoglobinuria was also a common observation in human victims (Karunaratne and Panabokke, 1972; Reid, 1973). Purified phospholipase A_2 was shown to induce myonecrosis in experimental animals (Tu and Passey, 1972; Lind and Eaker, 1981) and was probably the causative agent of myonecrosis in human sea snakebite victims.

Cardiovascular effects of sea snake venoms

Although the primary cause of death after envenomation by sea snakes is due to the peripheral respiratory paralysis in many species of animals (Lee, 1972), this venom also produces cardiovascular changes. Since the sea snake venoms have either very little or no proteolytics enzyme activity, the cardiovascular changes produced by this venoms is in general not as profound as those produced by crotalid or viperid venoms.

Sea snake venoms are in general highly toxic and produce muscular paralysis and respiratory failure in animals, like venoms of land elapid snakes. These effects have been attributed to the neurotoxic components, especially the curarimimetic toxics, present in the these venoms. Besides neurotoxic symptoms, muscle pain, myoglobinuria, and necrosis of the skeletal muscle have been reported in human victims bitten by some species of sea snake. Unlike cobra and some other elapid

venoms, no cardiotoxin (cytotoxin)-like component has been found in sea snake venoms (Lee and Lee, 1979).

The cardiovascular changes produced by sea snake venoms are in general not pronounced, but the victim may succumb from hyperkalemic cardiac arrest or acute renal failure in protracted poisoning. Tu (1967) reported that intravenous injection of 0.1-0.2 mg/kg of *Laticauda laticauduta* venom into rabbits produced a transient fall followed by a gradual rise in arterial blood pressure along with respiratory depression. The blood pressure fell again after respiration had ceased. Cutting of the vagi and the administration of atropine prior to the venom injection did not alter the venom action. In the isolated frog heart, the venom produced an augmentation of the ventricular contraction. No inhibitory effect was found in cases of perfusion of up to 0.01 % of venom solution. The rabbit heart was not affected by the venom. Phillips (1972) also showed that *Laticauda semifasciata* venom (0.1 mg/kg) essentially caused no immediate hemodynamic changes other than an initial blood pressure fall in dogs. Yang and Lee (1978) also obtained similar results with the venom of *Hydrophis cyanocinctus* in rats and cats. While most sea snake venoms so far studied have produced an initial fall in the systemic arterial pressure following an intravenous administration, the venom of *Pelamis platurus* did not produce any changes in blood pressure, heart rate, and electrocardiogram until asphyxia became apparent when the respiration was severely depressed (Tu *et al.*, 1976). The cause of such differences has not been identified but may be related to the content of some constituents of the venom, such as phospholipase A (Lee, 1971).

Renal effects of sea snake venom

Little is known about the effect of sea snake venom on the renal function, although it has been reported in mice by a single subcutaneous injection of the whole venom of *Aipysurus laevis* (0.075 mg/kg) causing acute renal tubular degeneration and proliferative gromerulonephritis (Zimmerman *et al.*, 1992). The tubular changes appeared within 1 hour and remained for at least 14 days. Mesangial proliferative

glomerulonephritis developed with 3-10 days, and it characterized by mild mesangial proliferation, mesangial and glomerular basement membrane deposits. This sea snake venom has been shown to have a direct nephrotoxic effect.

Renal toxicity of myoglobin

The major life-threatening complication of myoglobinuria is acute tubular necrosis. The mechanism of tubular damage by myoglobin has not yet been clarified (Koppel, 1989). Precipitation of myoglobin and blockade of tubules is favored by acidosis and dehydration, which has been demonstrated in animal models (Bywaters and Stead, 1944). Acute tubular necrosis with pigment in renal tubules has been observed in acidotic and dehydrated rabbits treated with human myoglobin. Additionally, myoglobin probably causes a decrease in renal blood flow and glomerular filtration rate (Ayer *et al.* 1971; Campion *et al.* 1972).

Although the clinical aspects of sea snake bites are well described, little is known about the sequential pathologic changes of the effects of myoglobinuria in the kidney. Tubular obstruction by myoglobin occurs very early before tubular necrosis and alkalinization of the urine by sodium bicarbonate has been known to prevent this obstruction. Protection of renal function and morphologic changes by the role of tubular obstruction appears to be crucial in the pathology of acute renal failure resulting from snake venom. Forced diuresis with sodium bicarbonate probably protects the kidney function from acidosis and precipitation of myoglobin in tubules. (Koppel, 1989).

CHAPTER III

MATERIALS AND METHODS

Animals Preparation

Experiments were carried out on thirty adult male mongrel dogs, weighing 12-17 kilograms. The animals were deprived of food but not of water for 12 hours prior to the study. On the day of the experiment, the animals were anesthetized with sodium pentobarbital (Nembutal) 30 mg/kg, intravenously injection initially, and supplemented with the subsequent doses of 1-2 mg/kg. when necessary to maintain anesthetic condition throughout the experiment.

The animals were tracheotomized then endotracheal tube was inserted to free airways. Two femoral veins were cannulated with polyethylene tubes (PE 180) for infusion of the clearance solution and for urea solution injection in the experiments. The carotid artery was cannulated with polyethylene tube (PE 200) for blood collection and connected to the pressure transducer (PE 23 AA, Statham Instruments) and a recorder (Polygraph Model 79, Grass instruments Co.) which allowed continuous monitoring of the arterial blood pressure and the heart rate. Abdomen was explored via a paracostal incision, and the left ureter was cannulated with polyvinyl catheter (PV 190) for urine collection.

Before clearance studies, the fluid replacement was done with 0.9% NaCl in the volume of 10 ml/kg. The clearance study was started by the priming injection solution containing p-aminohippuric acid (PAH) (Sigma chemical company) 1.2 gm% and inulin (In) (Sigma chemical company) 5 gm% in normal saline solution administered at the dose of 0.5 ml/kg. The sustaining solution composed of PAH 0.12 gm% and inulin 0.5 gm% which was infused with a constant peristaltic infusion

pump (Eyela Model 3) at the rate of 1.5 ml/min for 45 minutes continuously to stabilize the plasma inulin and the PAH concentration.

After the equilibrium period, urine collections along with arterial blood samplings at the midpoint of the urine collection were done. The experiment was divided into six 30-minute periods. Blood samples (3 ml) were taken from each period, except the period that was determined for the cardiac output. For cardiac output determination, 15 ml of blood was collected in each measurement. Collected blood was replaced by 0.9% NaCl and made volume equal to the blood collection in each period.

Investigations for the minimal lethal dose 50 percent (LD_{50}) of the sea snake (*Lapemis hardwickii*) venom in mices.

The experiments were performed on 4 mices/dose, weighing between 18-20 gm. It was found that, fifty percent of mices died within 24 hr. when received sea snake venom at a dosage of 0.341 mg/kg intravenously injection. Therefore, sea snake venom was injected intramuscularly to experimental dog at a high dosage of 0.34 mg/kg, corresponding to 0.34 mouse LD_{50}/kg. Then this dose of sea snake venom was diluted for 2 times as a low dose.

Experimental Design

The experiments were carried out into 3 series with 2 groups of each series.

Series A

Group I: The animals received the low dose (0.16 mg/kg.) of SSV alone.

Five dogs were used in this group. After two 20-minute observations as the control period. The animal was intramuscularly injected with 0.16 mg/kg. of sea

snake venom (lyophilized venom in 2 ml of distilled water. After venom injection, blood and urine samples were collected every 30 minutes for 3 hour.

Group II: The animals received the high dose (0.34 mg/kg.) of SSV alone.

Five dogs were used in this group. The protocol of the experiment was the same as in group I except the animal was intramuscularly injected with 0.341 mg/kg. of lyophilized sea snake venom in 2 ml of distilled water. After venom injection, blood and urine samples were collected every 30 minutes for 3 hour.

Series B

Group III: The animals were pretreated with sodium bicarbonate ($NaHCO_3$) and received the low dose of SSV.

Five dogs were used in this group. Blood and urine samples were collected during the control period, which was followed by the pretreated period with intravenous injection of 10 ml of 10 gm% $NaHCO_3$ as a priming dose via the left femoral vein. After that, the $NaHCO_3$ solution (4.2 gm% in combination with the sustaining solution) was infused at a rate of 1.5 ml/min throughout the experiment period. One 30 minutes in the pretreated period with sodium bicarbonate injection was performed. After the pretreatment with sodium bicarbonate for 30 minutes, SSV was injected intramuscularly in a single dose of 0.16 mg/kg. Six of the 30-minute observations throughout experimental period were carried out after envenomation.

Group IV: The animals were pretreated with $NaHCO_3$ and received the high dose of SSV.

Five dogs were used in this group. The protocol of the experiment was started similar to that of the group II except the high dose of SSV at 0.34 mg/kg was injected.

Series C

Group V: The animals were given sodium bicarbonate after the administration of the low dose of SSV.

Five dogs were used in this group. The protocol of the experiment was similar to that of group I and the solution of 10gm% sodium bicarbonate at the same dose as that in group III was given 1 hr after intramuscular injection of SSV.

Group VI: The animals were given sodium bicarbonate after the administration of the high dose of SSV.

Five dogs were used in this group. The protocol of the experiment was similar to that of group II and the solution of 10 gm% sodium bicarbonate at the same dose as that in group V was given 1 hr after intramuscular injection of SSV.

Determination of the cardiac output

The cardiac output (CO) was measured by the dye dilution technique, urea was used instead of Evans blue dye (T-1824), as described by Chaiyabutr *et al.* (1980). A bolus dose of 1 ml of urea solution (10-gm/100 ml 0.9 % NaCl) was injected into the femoral vein. The series of blood samples were collected from the carotid artery immediately within 3-5 seconds after urea injection. Serial samples of arterial blood were collected 1 ml/second for a period of 10-15 seconds by means of the peristaltic pump. The amount of urea in each fraction of blood was determined

using spectrophotometer. The cardiac output was calculated as described by Hamilton *et al.* (1948).

Parameter determined from the blood and urine samples

Inulin concentration in plasma and urine were determined by the anthrone method, which modified the method of Young and Raise (1952). The determination of PAH concentration in plasma and urine were carried out by the method of Bratton and Marshall (1939) as described by Smith (1962).

The composition of electrolytes in plasma and urine were measured as following: sodium and potassium concentration by flame photometer (Clinical flame photometer 410C, Corning Ltd.) and chloride concentration by chloridometer (Chloride Analyzer 925, Corning Ltd.). The osmolality of plasma and urine was measured by the freezing point osmometer (The Advanced osmometer model 3D3).

Plasma lactate dehydrogenases (LDH), plasma creatine kinases (CK) and urinary myoglobin were measured by immunoturbidimetric assay (Hitachi model 711, Boehringer Mannheim Diagnostic Indianapolis, IN.). Blood and urine samples were determined for the bicarbonate concentration by the Van Slyke manometric method (Natelson Microgasometer model # 600, Scientific Industries, INC.)

Calculations

Mean arterial blood pressure (MAP) = $P_d + 1/3\ (P_s - P_d)$

Glomerular filtration rate (GFR) = $\frac{U_{In}V}{P_{In}\dot{V}}$

Effective renal plasma flow (ERPF) = $\frac{U_{PAH}V}{P_{PAH}}$

Effective renal blood flow (ERBF) = $\frac{ERPF \times 100}{(100\text{-}PCV)}$

Filtration fraction (FF) = $\frac{GFR \times 100}{ERPF}$

Renal vascular resistance (RVR) = $\frac{MAP}{ERBF}$

Filtered load of electrolyte = $GFR \times P_e$

Urinary electrolyte excretion = $U_e \times V$

Fractional excretion of electrolyte (FE_e) = $\frac{U_e \times V/P_e \times 100}{GFR}$

Fractional water excretion = $V/GFR \times 100$

Osmolar clearance (C_{osm}) = $U_{osm} \times V/P_{osm}$

Free water clearance (C_{H2O}) = $V - C_{osm}$

Renal fraction (RF) = $\frac{ERBF \times 100}{CO}$

Statistical Analysis

All data are presented as means ± SD. The paired t-test was used to estimate the statistical significance between value obtained from the control period and from each period of the experiment in the same group. The unpaired t-test was used to estimate the statistical significance of difference between value obtained from group of animals given a low dose and a high dose of SSV in the same series.

CHAPTER IV

RESULTS

The effects of sea snake venom (SSV) on changes in general circulation and renal function were carried out in animals given SSV alone (Series A), animals received sea snake venom with the pretreatment of an intravenous infusion of $NaHCO_3$ (Series B) and animals received an intravenous infusion of $NaHCO_3$ which was given 1 hr. after SSV (Series C). Two groups of animals in each series were given either a low dose (0.16 mg/kg.). or a high dose of SSV (0.34 mg/kg.)

Effects of sea snake venom on general circulations.

Effects of sea snake venom on general circulation during given venom alone (Series A) (Table 1 and Table 2).

Animals given a low dose of SSV alone caused significantly increased in mean arterial blood pressure (MAP) by average from 116.3 $\pm$ 5.7 to 131.0 $\pm$ 9.0 and to 139.6 $\pm$ 15.7 mmHg at 120 minutes and 180 minutes after envenomation respectively. There were no significant changes in pulse pressure (PP), heart rate, packed cell volume, cardiac output, stroke volume and total peripheral resistance after envenomation.

Animals given a high dose of SSV, there were no significant increases in MAP, PP and packed cell volume. In the periods of 180 minutes after venom injection, heart rate fell from the control value of 151 $\pm$ 36 to 104 $\pm$ 8 beats/min ($P < 0.05$). There were gradual increases in stroke volume from 27.8 $\pm$ 7.8 ml/beat of the control value to 31.1 $\pm$ 7.9 and 47.8 $\pm$ 13.4 ml/beat in 120 and 180 ($P<0.05$) minutes after envenomation respectively. Total peripheral resistance showed no increase throughout the experimental periods.

Effects of sea snake venom on general circulation during given venom in animals pretreated with sodium bicarbonate (Series B) (Table 1 and Table 2).

An intravenous infusion of sodium bicarbonate before envenomation produced slight increases in MAP and PP that were no significantly different as compared to animals in series A. When animals were given a low dose of SSV, heart rate increased significantly from 173 $\pm$ 19 beats/min to 207 $\pm$ 12 beats/min at 60 minutes after envenomation combining with $NaHCO_3$ infusion. There were no significant changes of packed cell volume, cardiac output, stroke volume and total peripheral resistance in animals given a low dose of SSV and pretreated with sodium bicarbonate.

An intravenous infusion of sodium bicarbonate alone before given a high dose of SSV produced slight decrease in MAP as compared to those of the control period of animals given a high dose of SSV in Series A and after that it increased along to the .experimental periods. There were no significant changes in pulse pressure, heart rate and packed cell volume. Cardiac output, stroke volume and total peripheral resistance did not significantly change throughout the experimental periods

Effects of sea snake venom on general circulation in animals received sodium bicarbonate which was given 1 hr after intramuscular injection of venom (Series C).

An intravenous infusion of sodium bicarbonate solution after 1 hr. of administration of a low dose of SSV had no changes in any of the measurements made. As shown in table 1, there was no significant decrease in mean arterial blood pressure that coincided with significant decrease in PP at 60 minute after SSV administration. No significant change in heart rate was observed, except in the periods of 120 minutes after venom injection combining with $NaHCO_3$ infusion, it increased from the control value of 152 $\pm$ 27 to 170 $\pm$ 28 ($P<0.05$). There were no

changes in packed cell volume and total peripheral resistance along the experimental periods. Envenomation alone either a low and a high dose in the first 60 minutes caused significant decrease in cardiac output from 3.443 $\pm$ 0.668 to 2.729 $\pm$ 0.633 L/min ($P<0.01$). After that, the decrease of cardiac output was gradually increased nearly to the control level. In the first 60 minutes, stroke volume significantly decreased from 23.17 $\pm$ 5.77 to 17.62 $\pm$ 5.96 ml/beat ($P<0.01$) in a group given a low dose of SSV alone. After that, it gradually increased to the control level.

At the periods of 120 minutes after a high dose of venom injection combining with $NaHCO_3$ infusion, MAP increased from the control value of 119.2 $\pm$ 16.7 to 122.5 $\pm$ 14.8 mmHg ($P<0.05$). At 60 minutes after envenomation without $NaHCO_3$ infusion, PP and cardiac output decreased significantly from 31 $\pm$ 3 to 22 $\pm$ 5 ($P<0.05$) mmHg and from 3.365 $\pm$ 0.636 to 2.871 $\pm$ 0.525 L/min respectively as compared to the control value (Table 1 and Table 2). Heart rate decreased significantly from 145 $\pm$ 27 to 124 $\pm$ 36 ($P<0.05$) and 107 $\pm$ 15 beats/min ($P<0.05$) at 120 and 180 minutes respectively (Table 1). The significant decrease in packed cell volume was noted in 120 minutes after envenomation from 40 $\pm$ 9 to 37 $\pm$ 10 % ($P<0.05$). There were no significant changes in stroke volume and total peripheral resistance after given SSV along the experimental periods.

Effects of sea snake venom on renal hemodynamics.

The results of changes in renal hemodynamics in dogs given sea snake venom are shown in Table 3 and Table 4.

Effects of sea snake venom on Renal Hemodynamics during given venom alone (Series A) (Table 3 and Table 4).

Renal vascular resistance (RVR) showed no significant increases in stepwise in animals given either a low dose or a high dose of SSV along the experimental periods whereas filtration fraction (FF) significantly increased by 32.37 $\pm$ 4.05 and

32.33 ± 6.10 % of control value at 120 and 180 minutes after given a low dose of SSV respectively. There was significant decrease in effective renal plasma flow (ERPF) from the control value 6.3 ± 1.2 to 4.9 ± 1.5 ($P<0.05$) and 4.5 ± 1.9 ml/min/kg. ($P<0.01$) and effective renal blood flow (ERBF) from 9.5 ± 1.4 to 7.4 ± 2.0 ($P<0.05$) and 6.7 ± 2.4 ml/min/kg. ($P<0.01$) at 60 minutes and 120 minutes after given a low dose of SSV respectively. There were decreases in renal fraction (RF) throughout the experimental periods after envenomation. No significant changes in glomerular filtration rate (GFR) and urine flow rate (V) were observed after envenomation.

There were significant decreases in ERPF and ERBF from 5.76 ± 1.54 and 9.24 ± 1.60 ml/min/kg. of the control value to 2.83 ± 2.68 and 4.61 ± 4.18 ml/min/kg. ($P<0.05$) at 180 minutes of envenomation with a high dose of SSV respectively. RF markedly decreases at 120 minutes and 180 minutes throughout the experimental period after given a high dose of SSV alone. Significant decreases in GFR and the V were observed as compared to the control value at 180 minutes after given a high dose of SSV.

Effects of sea snake venom on renal hemodynamics in animals pretreated with sodium bicarbonate (Series B) (Table 3 and Table 4).

Animals received an intravenous infusion of sodium bicarbonate before envenomation showed no changes in renal vascular resistance (RVR) after given either a low or a high dose of SSV. FF significantly increased by approximately from 20.63 ± 5.87 to 27.78 ± 5.17 % ($P<0.05$) at 120 minutes and to 31.27 ± 6.36 % ($P<0.05$) at 180 minutes of the control value in animals given a low dose of SSV. There were no significant changes in ERPF, ERBF and RF. GFR showed no significant increase while V significantly increased by approximately from the control value 34.8 ± 37.3 to 59.8 ± 35.9 µl/min/kg. ($P<0.01$) at 120 minutes and 80.3 ± 41.9 µl/min/kg. ($P<0.001$) at 180 minutes. FF slightly increased after envenomation in a high dose of SSV. ERPF slightly decreased from the control value 5.34 ± 0.39 to

4.66 ± 0.42, 4.14 ± 1.70 and 3.73 ± 0.13 ml/min/kg. at 60 minutes, 120 minutes and 180 minutes respectively after given a high dose of SSV. ERBF showed slight decrease along periods of study. There was no significant change in RF along the experimental periods. GFR showed no significant decrease, whereas V significantly increased from the control value 44.5 ± 54.6 to 72.9 ± 52.2 µl/min/kg ($P<0.05$) at 120 minutes after a high dose of envenomation.

Effects of sea snake venom on renal hemodynamics in animals received sodium bicarbonate, which was given 1 hr. after intramuscular injection venom (Series C) (Table 3 and Table 4).

Renal vascular resistance (RVR) significantly increased at 180 minutes after given a low dose of SSV with $NaHCO_3$ loading. FF showed a tendency to increase in stepwise along periods of study. There were no changes in ERPF, ERBF and RF throughout the experimental periods. GFR showed significant increase at 120 minutes and at 180 minutes as compared to the control value whereas V increased after envenomation.

RVR showed no significant increase during given a high dose of SSV with $NaHCO_3$ loading while a marked increase in FF at 180 minutes after envenomation was apparent. There were no significant changes in ERPF, ERBF and RF throughout the experimental periods. GFR showed no significant decrease whereas V increased by approximately two folds of the control value at 120 and 180 minutes after envenomation.

Effects of sea snake venom on plasma electrolyte concentrations, urinary electrolytes excretion and fractional electrolytes excretion.

Results of changes in plasma electrolyte concentrations, urinary electrolytes excretion and fractional electrolytes excretion in dogs given sea snake venom are shown in Table 5 - Table 7.

Effects of sea snake venom on plasma electrolytes concentration, urinary electrolytes excretion and fractional electrolytes excretion during given venom alone (Series A) (Table 5 - Table 7).

After a low dose of sea snake venom administration alone, there were no differences in plasma concentration of sodium, potassium and chloride ion as compared to the control value. There were no significant changes for $U_{Na}V$, FE_{Na}, U_KV, FE_K, $U_{Cl}V$ and FE_{Cl} after given a low dose of SSV alone.

After a high dose of sea snake venom administration, plasma sodium concentration, urinary sodium excretion and fractional excretion showed no significant changes while plasma potassium concentration significantly decreased at 60 minutes ($P<0.05$) and at 120 minutes ($P<0.01$) after envenomation. Urinary potassium excretion and fractional excretion of potassium showed no significant changes throughout the experimental periods. Sea snake venom produced a significant raise in plasma chloride concentration ($P<0.05$) in the periods of 180 minutes after given a high dose of venom. Urinary chloride excretion and fractional excretion of chloride showed no significant changes throughout the experimental periods.

Effects of sea snake venom on urinary electrolyte excretion during given venom in animals pretreated with sodium bicarbonate (Series B) (Table5-Table7).

After sea snake venom administration, there were no differences in plasma concentrations of potassium and chloride from the control value. At the period of 180 minutes after a low dose of venom injection, the plasma sodium concentration increased from the control value of 129.4 $\pm$ 2.3 to 136.0 $\pm$ 3.5 mEq/L ($P<0.05$). Urinary sodium excretion showed significant increase in stepwise during $NaHCO_3$ infusion accompanying SSV injection at 60 minutes, 120 minutes ($P<0.01$) and at 180 minutes. Fractional excretion of sodium significantly increased by approximately 4.95 $\pm$ 2.33, 6.09 $\pm$ 1.35 and 7.91 $\pm$ 2.28 % of the control value at 60 minutes, 120 minutes ($P<0.01$) and 180 minutes ($P<0.001$) respectively after

envenomation and $NaHCO_3$ loading. Urinary potassium excretion and fractional excretion of potassium increased throughout the experimental periods which significantly increased at 120 minutes ($P<0.05$) and at 180 minutes ($P<0.01$) after envenomation and $NaHCO_3$ loading. Urinary chloride excretion and fractional excretion of chloride significantly increased at 180 minutes after envenomation and $NaHCO_3$ loading.

A high dose of sea snake venom administration showed no significant changes in plasma sodium concentration, urinary sodium excretion and fractional excretion of sodium as compared to the control value. Plasma potassium concentration decreased while urinary potassium excretion and fractional excretion of potassium showed no significant increase throughout the experimental periods. Plasma concentration of chloride significantly decreased at 60 minutes and gradually increased at 120 and at 180 minutes. Urinary chloride excretion and fractional excretion of chloride showed no significant changes throughout the experimental periods.

Effects of sea snake venom on Urinary Electrolyte Excretion in animals received sodium bicarbonate which was given 1 hr. after SSV injection (Series C) (Table 5, Table 6 and Table 7).

After a low dose of sea snake venom administration, there were no significant differences in plasma concentration of sodium, potassium and chloride from the control value. Urinary excretion and fractional excretion of sodium significantly increased at 120 minutes ($P<0.05$) and at 180 minutes ($P<0.05$). Urinary excretion of potassium and fractional excretion of potassium significantly increased at 180 minutes (Table 6). Fractional excretion of chloride significantly increased at 60 minutes ($P<0.05$) whereas urinary excretion of chloride showed no significant increase in comparison to the control value.

Plasma concentration of sodium significantly increased ($P<0.05$) at the first 60 minutes after given a high dose of SSV. Urinary sodium excretion and fractional

excretion of sodium significantly increased at 120 minutes ($P<0.05$). Plasma concentration of potassium significantly decreased at120 ($P<0.05$) and 180 ($P<0.05$) minutes after envenomation. Urinary potassium excretion and fractional excretion of potassium showed no significant changes throughout experimental periods. There were no significant changes in plasma concentration of chloride, urinary excretion of chloride and fractional excretion of chloride along the experimental periods.

Effects of sea snake venom on plasma osmolality, osmolar clearance and free water clearance.

The results of changes in plasma osmolality (P_{osm}), osmolar clearance (C_{osm}) and free water clearance (C_{H2O}) in dogs given sea snake venom are shown in Table 8.

Effects of sea snake venom on P_{osm}, C_{osm} and C_{H2O} during given venom alone (Series A) (Table 8).

P_{osm} significantly increased at 60 minutes ($P<0.05$) and gradually decreased at 120 and 180 minutes after envenomation. C_{osm} and C_{H2O} showed no significant increased throughout the experimental periods.

Animals given a high dose of SSV showed no significant changes in P_{osm}, C_{osm} and C_{H2O}.

Effects of sea snake venom on P_{osm}, C_{osm} and C_{H2O} during given venom in animals pretreated with sodium bicarbonate (Series B) (Table 8).

An intravenous infusion of sodium bicarbonate before given a low dose of SSV produced slight changes in P_{osm}, C_{osm} and C_{H2O} and there were no significant differences as compared to similar period in Series A. When animals were given a low dose of SSV, P_{osm} increased significantly from 302.7 ± 5.6 to 308.4 ± 9.0 mOsm/kg.H_2O and to 316.4 ± 12.4 mOsm/kg.H_2O at 60 minutes and at 180 minutes

after envenomation accompanying with $NaHCO_3$ infusion. Osmolar clearance gradually increased and it significantly increased at 120 minutes ($P<0.01$) and 180 minutes ($P<0.001$) whereas free water clearance slightly decreased.

An intravenous infusion of sodium bicarbonate alone before given a high dose of SSV caused increase in C_{osm} (Table 8). There was significant increase in P_{osm} at 120 minutes ($P<0.001$) in animal given a high dose of SSV. C_{osm} slightly increased but no significance whereas C_{H2O} showed to increase after envenomation.

Effects of sea snake venom on P_{osm}, C_{osm} and C_{H2O} in animals received sodium bicarbonate which was given 1 h after intramuscular injection of venom (Series C) (Table 8).

An intravenous infusion of sodium bicarbonate solution after a low dose of SSV administration 1 hr. showed no significant changes in P_{osm}. C_{osm} increased significantly increased at 180 ($P<0.05$) minutes whereas C_{H2O} significantly decreased at 180 minutes ($P<0.05$).

At the periods of 120 minutes after a high dose of venom injection combining with $NaHCO_3$ infusion, P_{osm} increased from the control value of 288.4 ± 3.7 to 300.5 ± 5.5 mOsm/kg.H_2O ($P<0.05$). C_{H2O} did not change throughout the experimental periods whereas C_{osm} slightly increased.

Effects of sea snake venom on blood urea nitrogen (BUN) concentration, urinary bicarbonate (U_{HCO3}) concentration and urinary pH (U_{pH}).

Results of changes in the blood urea nitrogen (BUN) concentration, urinary bicarbonate (U_{HCO3}) concentration and urinary pH (U_{pH}) in dogs given sea snake venom are shown in Table 9.

Effects of sea snake venom alone on BUN, U_{HCO3} and U_{pH} (Series A) (Table 9).

Animals given a low dose of SSV alone caused no significant change in the BUN concentration whereas U_{HCO3} showed no significant changes along experimental periods. No apparent changes were observed for U_{pH} after envenomation alone.

After a high dose of SSV administration, there were significant increase in BUN from 11.90 $\pm$ 4.26 mg% of the control value to 15.65 $\pm$ 4.35 ($P<0.01$), 14.11 $\pm$ 4.06 ($P<0.001$) and 14.42 $\pm$ 3.40 mg% ($P<0.01$) at 60 minutes, 120 minutes and 180 minutes respectively. U_{pH} and U_{HCO3} showed no significant change as compared to the control value after envenomation.

Effects of sea snake venom on BUN, U_{HCO3} and U_{pH} during given venom in animals pretreated with sodium bicarbonate (Series B) (Table 9).

An intravenous infusion of $NaHCO_3$ alone before given either a low dose of or a high dose of SSV produced no changes in BUN, U_{HCO3} and U_{pH}. Envenomation in a low dose SSV at the first 60 minutes causes significant increase in BUN from 8.20 $\pm$ 3.03 mg% to 13.77 $\pm$ 2.81 mg% ($P<0.05$). U_{HCO3} significantly increase by approximately 10.23 $\pm$ 3.94 mmol/L ($P<0.05$) at 120 minutes and 16.64 $\pm$ 4.22 mmol/L ($P<0.05$) at 180 minutes of the control value while there was no significant increase in U_{pH}.

BUN slightly increased in animals given a high dose of SSV. Animals given a high dose of SSV showed an increase in U_{HCO3} from the control value (3.92 $\pm$ 3.13) to 11.09 $\pm$ 3.97 mmol/L ($P<0.05$) and 11.29 $\pm$ 3.35 mmol/L ($P<0.05$) at 60 minutes and 180 minutes respectively. U_{pH} showed significant increase from 7.30 $\pm$ 1.26 of the control value to 8.99 $\pm$ 0.63 ($P<0.05$) at 60 minutes after envenomation.

Effects of sea snake venom on BUN, U_{HCO3} and U_{pH} in animals received sodium bicarbonate which was given 1 h after intramuscular injection venom (Series C) (Table 9)

BUN showed no significant increase after given a low dose of SSV and $NaHCO_3$ loading. U_{HCO3} significantly increased from the control value from 0.32 $\pm$ 0.27 to 6.74 $\pm$ 5.21 mmol/L ($P<0.05$) at 120 minutes and 9.11 $\pm$ 4.67 mmol/L ($P<0.05$) at 180 minutes after given a low dose of SSV. U_{pH} significantly increased by approximately 8.06 $\pm$ 1.06 ($P<0.05$) at 120 minutes and 8.54 $\pm$ 1.17 ($P<0.05$) at 180 minutes of the control value.

After given a high dose of SSV, BUN showed significant increase ($P<0.05$) at 60 minutes. There were no significant increases in U_{HCO3} throughout experimental periods while U_{pH} increased significantly in comparing to the control value from 7.45 $\pm$ 1.05 to 9.06 $\pm$ 0.52 ($P<0.01$) at 120 minutes and to 9.47 $\pm$ 0.16 ($P<0.05$) at 180 minutes.

Effects of sea snake venom on plasma creatine phosphokinase (P_{CPK}), plasma lactate dehydrogenase (P_{LDH}), urinary myoglobin (U_{Mb}) concentrations and urinary myoglobin excretion ($U_{Mb}V$)

The results of changes in plasma creatine phosphokinase (P_{CPK}), plasma lactate dehydrogenase (P_{LDH}), urinary myoglobin (U_{Mb}) concentrations and urinary myoglobin excretion ($U_{Mb}V$) of animals given sea snake venom are shown in Table 10 and Table 11.

Effects of sea snake venom on plasma creatine phosphokinase (P_{CPK}), plasma lactate dehydrogenase (P_{LDH}) and urinary myoglobin (U_{Mb}) concentration and urinary myoglobin excretion ($U_{Mb}V$) during given a venom alone (Series A) (Table 10 and Table 11).

There were slightly increased in P_{CPK}, P_{LDH} and $U_{Mb}V$ along the experimental periods but U_{Mb} concentration significantly increased from 0.46 $\pm$ 0.49 to 17.60 $\pm$ 13.97 at 180 ($P<0.05$) minutes after given a low dose of SSV.

A marked increases in P_{CPK}, P_{LDH}, U_{Mb} and $U_{Mb}V$ at 120 minutes and at 180 minutes ($P<0.05$) were also observed after given a high dose of SSV alone.

Effects of sea snake venom on plasma creatine phosphokinase (P_{CPK}), plasma lactate dehydrogenase (P_{LDH}) and urinary myoglobin (U_{Mb}) concentration and urinary myoglobin excretion ($U_{Mb}V$) during given a low dose of venom in animals pretreated with sodium bicarbonate (Series B)(Table 10 and Table 11).

There were slight increases in P_{CPK}, P_{LDH}, U_{Mb} and $U_{Mb}V$ along the experimental periods during $NaHCO_3$ loading with a low dose of SSV administration.

Intravenous infusion of sodium bicarbonate caused no changes in any of the measurements made. P_{CPK} increased in stepwise at 60 minutes and at 180 minutes nearly nine folds as compared to the control value. P_{LDH} increased by mean average from 67.40 $\pm$ 28.35 U/L of the control value to 119.00 $\pm$ 56.48 U/L at 60 minutes and 96.50 $\pm$ 26.16 U/L at 180 minutes after envenomation. U_{Mb} markedly increased from 2.77 $\pm$ 1.05 ng/ml to 58.13 $\pm$ 54.79 ng/ml ($P< 0.05$) at 60 minutes and to 50.57 $\pm$ 13.39 ng/ml at 180 minutes after envenomation. $U_{Mb}V$ significantly increased at 60 minutes and 180 minutes ($P<0.01$) after envenomation.

Effects of sea snake venom on plasma creatine phosphokinase (P_{CPK}), plasma lactate dehydrogenase (P_{LDH}) and urinary myoglobin (U_{Mb}) concentration and urinary myoglobin excretion ($U_{Mb}V$) in animals received sodium bicarbonate, which was given 1 hr. after intramuscular injection of venom (Series C) (Table 10 and Table 11).

There were increases in P_{LDH} and $U_{Mb}V$, whereas P_{CPK} and U_{Mb} significantly increased from 80.00 $\pm$ 18.01 U/L to 124.00 $\pm$ 38.66 U/L ($P< 0.05$) and 5.00 $\pm$ 5.20 ng/ml to 73.80 $\pm$ 39.36 ng/ml ($P< 0.05$) respectively after given a low dose of SSV with $NaHCO_3$ loading.

P_{CPK} showed significant increase in stepwise at 60 minutes and at 180 minutes ($P<0.05$) after given a high dose of SSV with $NaHCO_3$ loading. P_{LDH} significantly increased from 56.60 $\pm$ 10.38 to 95.80 $\pm$ 28.38 U/L ($P<0.05$) and to 85.00 $\pm$ 14.94 U/L ($P<0.01$) at 60 minutes and 180 minutes after envenomation respectively.

Table1. Changes in means arterial pressure (MAP), pulse pressure (PP), heart rate (HR), packed cell volume (PCV) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after Venom injection	MAP (mmHg)		PP (mmHg)		HR (beats/min)		PCV (%)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)								
Control	116.3± 5.7	129.1±20.4	30± 5	23± 6	140±23	151±36	34±5	39±7
60 min. after SSV	119.9±12.3	122.9±21.4	28± 9	19± 6	164±42	151±28	35±6	39±6
120 min. after SSV	131.0± 9.0*	131.1±22.7	32± 7	24± 8	136±40	122±31	35±6	38±5
180 min. after SSV	139.6±15.7*	142.5±31.3	41±10	41±18	122±39	104±8*	38±4	42±6
Series B (pretreated with $NaHCO_3$)								
Control with $NaHCO_3$	141.0±27.5	122.6±14.2	43± 9	31±7	173±19	150±30	33±6	37±3
60 min. after $NaHCO_3$ + SSV	126.3±19.2	130.0± 8.1	45±16	27±7$^+$	207±12*	143±26$^+$	36±8	36±3
120 min. after $NaHCO_3$ + SSV	138.3±20.1	127.9± 6.8	45±10	26±12$^+$	204±19	125±33$^+$	35±5	36±3
180 min. after $NaHCO_3$ + SSV	138.6±21.4	134.2± 5.4	42±13	26±7	205±26	144± 6$^+$	35±6	35±4
Series C (given $NaHCO_3$ after SSV)								
Control	128.7±22.0	119.2±16.7	34±5	31±3	152±27	145±27	33± 9	40±9
60 min. after SSV	117.4±28.1	111.9±12.8	25±5*	22±5*	162±37	140±32	32±10	39±10
120 min. after SSV + $NaHCO_3$	133.1±21.6	122.5±14.8*	33±5	24±7	170±28*	124±36*	30± 8	37±10*
180 min. after SSV + $NaHCO_3$	132.9±20.7	107.0±6.4	30±3	37±20	152±40	107±15*	31± 8	38±12

Values are means ± SD. Abbreviation: SSV, sea snake venom (mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^+$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

Table 2. Changes in cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	CO (L/min)		SV (ml/beat)		TPR (mmHg ×min/L)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	3.298±1.692	4.034±0.639	22.56± 7.68	27.81±7.88	42.16±18.06	32.50±4.90
60 min. after SSV	2.681±1.449	3.469±0.502	16.31± 7.77	23.83±6.68	53.27±20.55	30.04±4.90
120 min. after SSV	3.979±2.349	3.604±0.355	30.29±17.23	31.17±7.92	42.19±21.57	36.76±7.48
180 min. after SSV	2.390±0.509	4.283±0.510^{+}	20.75± 5.48	47.88±13.46*$^{+}$	60.77±16.73	33.59±8.02
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	3.374±1.260	2.833±0.987	19.56± 7.50	20.35±10.21	40.30±15.81	50.14±25.59
60 min. after $NaHCO_3$ + SSV	3.656±1.736	2.652±0.700	17.38± 7.19	18.68±5.29	39.11±15.23	37.03±12.47
120 min. after $NaHCO_3$ + SSV	3.502±1.319	2.856±0.809	16.86± 5.02	24.48±9.80	43.02±12.34	47.96±15.28
180 min. after $NaHCO_3$ + SSV	4.744±3.442	2.759±1.344	22.02±13.00	19.36±10.09	38.44±17.40	54.63±24.64
Series C (given $NaHCO_3$ after SSV)						
Control	3.443±0.668	3.365±0.636	23.17±5.77	24.22±8.28	35.04±14.08	36.12±7.62
60 min. after SSV	2.729±0.633**	2.871±0.525*	17.62±5.96**	21.56±7.02	45.47±17.84	33.64±8.25
120 min. after SSV + $NaHCO_3$	4.126±2.003	3.011±0.413	26.07±17.85	24.55±9.50	37.11±15.06	40.07±7.46
180 min. after SSV + $NaHCO_3$	3.491±0.946	2.796±0.389	24.36±9.26	24.40±8.35	39.75±10.24	39.47±8.07

Values are means ± SD. Abbreviation: SSV, sea snake venom (mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

Table 3. Changes in urine flow rate (V), glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and effective renal blood flow (ERBF) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	V(µl/min/kg.)		GFR(ml/min/kg.)		ERPF(ml/min/kg.)		ERBF(ml/min/kg.)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)								
Control	37.4±9.6	13.6±8.0	1.45±0.31	1.27±0.22	6.3±1.2	5.76±1.54	9.5±1.4	9.24±1.60
60 min. after SSV	40.1±33.4	10.7±6.3^{+}	1.37±0.39	1.05±0.37*	4.9±1.5*	5.43±1.98	7.4± 2.0*	7.21±2.98
120 min. after SSV	41.8±34.6	12.6±8.0^{+}	1.40±0.44	1.19±0.46^{+}	4.5±1.9**	5.25±1.67	6.7±2.4**	8.47±2.85
180 min. after SSV	40.0±31.4	9.6±2.4*$^{+}$	1.44±0.32	0.71±0.58 *	4.7±1.1	2.83±2.68*	6.8±2.2	4.61±4.18*
Series B (pretreated with $NaHCO_3$)								
Control with $NaHCO_3$	34.8±37.3	44.5±54.6	1.02±0.38	1.56±0.15	4.9±0.9	5.34±0.39	7.4±1.5	8.47±0.78
60 min. after $NaHCO_3$ + SSV	52.6±32.8	64.2±43.6	1.17±0.33	1.62±0.26^{+}	5.1±2.0	4.66±0.42	8.0±2.8	7.29±0.85
120 min. after $NaHCO_3$ + SSV	59.8±35.9**	72.9±52.2*	1.21±0.22	1.46±0.29	4.4±0.6	4.14±1.70	6.8±0.8	6.49±2.70
180 min. after $NaHCO_3$ + SSV	80.3±41.9***	97.1±63.7	1.36±0.15	1.20±0.24	4.5±0.9	3.73±0.13	6.8±1.0	5.75±0.58
Series C (given $NaHCO_3$ after SSV)								
Control	11.4±5.8	13.6±9.3	0.90±0.61	1.51±0.18	4.5±2.9	6.32±1.18	7.9±2.5	10.53±2.00
60 min. after SSV	13.2±6.9	15.9±10.9	1.04±0.69	1.61±0.15	4.8±3.4	5.94±1.25	8.1±3.9	9.93±2.20
120 min. after SSV + $NaHCO_3$	28.4±17.2	32.7±24.8	1.27±0.76*	1.43±0.20	4.8±2.7	5.81±0.49	7.8±3.1	9.36±1.52
180 min. after SSV + $NaHCO_3$	33.6±21.3	36.7±27.4	1.50±0.76*	1.28±0.34	4.4±3.0	6.86±1.41	7.5±3.4	9.68±0.64

Values are means ± SD. Abbreviation: SSV, sea snake venom (mg/kg.).

Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$, *** $P<0.001$ when compared to the control value in the same group.

Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

Values received from the left kidney.

Table 4. Changes in renal fraction (RF), filtration fraction (FF) and renal vascular resistance (RVR) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	RF (%)		FF (%)		RVR (mmHg×min×kg./ml)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	15.4±2.2	15.0±5.7	24.36±4.70	22.11±5.69	12.36±1.50	14.94±4.95
60 min. after SSV	13.0±4.1	13.9±7.5	28.58±2.66	24.98±6.04	17.31±4.89	19.88±8.99
120 min. after SSV	11.0±3.4	12.0±4.3	32.37±4.05**	22.40±2.68^{+}	21.77±8.64	16.81±6.29
180 min. after SSV	12.5±3.1	9.1±2.9	32.33±6.10*	27.92±7.95*	23.46±12.41	19.55±9.73
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	14.7±5.3	16.1±4.0	20.63±5.87	29.54±4.25	19.23±2.80	15.08±3.06
60 min. after $NaHCO_3$ + SSV	14.2±4.2	16.7±3.2	24.53±8.34	35.29±8.09^{+}	17.42±6.12	17.95±1.78
120 min. after $NaHCO_3$ + SSV	14.5±5.4	15.7±2.9	27.78±5.17*	40.45±10.5^{+}	20.44±3.91	23.14±11.08
180 min. after $NaHCO_3$ + SSV	13.6±5.2	15.1±2.3	31.27±6.36*	32.35±7.62	20.66±4.64	23.40±1.42
Series C (given $NaHCO_3$ after SSV)						
Control	14.9±4.1	17.8±2.2	20.98±5.60	24.40±2.59	17.64±5.77	11.80±2.89
60 min. after SSV	15.6±4.0	16.7±1.9	24.61±6.64	28.06±6.28	18.61±12.66	11.86±3.52
120 min. after SSV + $NaHCO_3$	14.2±2.6	16.6±3.5	29.49±9.48	28.04±5.88	20.17±11.68	13.23±1.65
180 min. after SSV + $NaHCO_3$	14.9±3.4	18.0±3.5	30.27±8.08	32.42±5.73*	20.03±6.71*	10.75±0.07

Values are means ± SD. Abbreviation: SSV, sea snake venom (mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.
Values received from the left kidney.

Table 5. Changes in the plasma sodium concentration (P_{Na}), urinary sodium excretion ($U_{Na}V$)and fractional excretion of sodium (FE_{Na})in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	P_{Na} (mEq/L)		$U_{Na}V$ (mEq/min/kg.)		FE_{Na} (%)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	129.7±3.6	134.2±2.6	5.90±2.03	4.54±2.88	3.22±1.28	2.64±1.48
60 min. after SSV	127.0±4.4	129.4±5.4	6.66±5.88	3.25±2.10	3.94±3.73	2.34±1.43
120 min. after SSV	128.0±4.3	131.8±4.4	5.92±5.03	4.03±2.08	3.43±3.06	2.91±1.07
180 min. after SSV	132.6±7.1	135.8±4.1	6.54 ±4.40	3.19±0.89	3.34±2.04	2.13±0.31
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	129.4±2.3	140.0±3.2	3.91±2.71	10.48±11.99	3.19±0.71	4.40±4.50
60 min. after $NaHCO_3$ + SSV	130.8±5.1	140.2±2.6^{+}	8.04±4.86	15.36±10.12	4.95±2.33	6.67±4.47
120 min. after $NaHCO_3$ + SSV	134.6±4.6	142.8±4.2^{+}	9.95±2.53**	11.01±3.20	6.09±1.35**	5.51±2.07
180 min. after $NaHCO_3$ + SSV	136.0± 3.5*	135.0±0.0	14.40±3.26	13.39±3.83	7.91±2.28**	8.19±0.72
Series C (given $NaHCO_3$ after SSV)						
Control	132.6±6.5	132.8±1.6	2.11±1.87	3.65±2.88	1.31±0.68	1.92±1.77
60 min. after SSV	131.6±8.4	135.0±1.4*	3.41±2.74	3.45±2.55	1.82±1.04	1.59±1.19
120 min. after SSV + $NaHCO_3$	131.4±5.3	132.8±5.6	7.67±5.16*	7.21±3.64	3.62±1.50	4.27±1.76*
180 min. after SSV + $NaHCO_3$	135.8±6.9	136.5±3.9	9.43±5.46	7.71±1.06	4.37±1.48	3.77±2.34

Values are means ± SD. Abbreviation: SSV, sea snake venom (mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.
Values received from the left kidney.

Table 6. Changes in the plasma potassium concentration (P_K), urinary potassium excretion (U_KV), fractional excretion of potassium (FE_K) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	P_K (mEq/L)		U_KV (mEq/min/kg.)		FE_K (%)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	2.73±0.22	3.05±0.27	1.12±0.55	1.20±0.83	28.03±12.19	32.18±19.94
60 min. after SSV	2.86±0.30	3.02±0.27	1.09±0.30	0.87±0.51	28.13±6.48	29.81±17.01
120 min. after SSV	2.86±0.33	3.12±0.13	1.06±0.39	1.00±0.33	26.31±5.03	35.69±10.39
180 min. after SSV	3.08±0.24*	3.73±0.61	1.22±0.50	0.77±0.14	27.47±7.67	23.41±0.04
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	2.65±0.06	3.08±0.25	0.70±0.42	1.79±1.99	29.04±21.50	36.76±38.03
60 min. after $NaHCO_3$ + SSV	2.56±0.15	2.68±0.20*	1.55±0.55	3.35±3.09	53.27±16.28	81.71±83.74*
120 min. after $NaHCO_3$ + SSV	2.64±0.17	2.58±0.22*	1.93±0.51*	1.41±0.34	60.87±15.71*	38.80±13.22$^+$
180 min. after $NaHCO_3$ + SSV	2.60±0.10	2.35±0.07*$^+$	2.23±0.38*	1.67±0.20$^+$	62.30±5.92*	59.83±6.53$^+$
Series C (given $NaHCO_3$ after SSV)						
Control	2.95±0.28	3.08±0.33	0.55±0.40	0.90±1.07	16.79±6.66	20.10±26.31
60 min. after SSV	2.78±0.24	3.08±0.29	0.72±0.46	0.74±0.65	20.50±11.01	14.86±13.41
120 min. after SSV + $NaHCO_3$	2.82±0.22	2.88±0.25*	1.42±0.69*	0.95±0.59	34.04±15.61*	28.04±19.32
180 min. after SSV + $NaHCO_3$	2.80±0.27	2.83±0.37*	1.46±0.65*	1.21±0.42	35.21±11.45	26.43±23.23

Values are means ± SD. Abbreviation : SSV, sea snake venom(mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^+$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.
Values received from the left kidney.

Table 7. Changes in the plasma chloride concentration (P_{Cl}), urinary chloride excretion ($U_{Cl}V$), fractional excretion of chloride (FE_{Cl}) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	P_{Cl} (mEq/L)		$U_{Cl}V$ (mEq/min/kg.)		FE_{Cl} (%)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	115.5±4.1	114.4±2.1	4.01±2.63	2.51±1.72	2.51±1.88	1.67±0.82
60 min. after SSV	115.8±2.8	113.8±5.5	7.96±6.46	1.47±0.95	3.14±4.42	1.19±0.79
120 min. after SSV	115.6±4.2	116.0±4.2	4.28±5.16	2.18±1.88	2.72±3.43	1.67±1.11
180 min. after SSV	113.2±3.9	119.4±3.3^{+}	4.62±4.49	1.67±0.99	2.59±2.64	1.26±0.59
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	114.4±6.4	127.2±4.7	1.40±1.46	4.99±5.19	0.97±0.85	2.34±2.11
60 min. after $NaHCO_3$ + SSV	114.6±5.6	122.6±4.2*$^{+}$	1.94±1.96	3.56±2.22	1.33±1.30	1.73±0.93
120 min. after $NaHCO_3$ + SSV	114.2±6.1	125.4±5.4	1.97±1.61	3.85±2.23	1.37±1.07	2.28±1.70
180 min. after $NaHCO_3$ + SSV	117.2±8.5	125.5±0.7	2.99±2.10*	3.90±3.33	1.96±1.42*	2.42±1.74
Series C (given $NaHCO_3$ after SSV)						
Control	118.6±5.6	119.2±3.6	1.09±1.09	2.20±1.81	0.71±0.56	1.27±1.10
60 min. after SSV	122.8±2.1	118.6±3.9	2.43±2.93	2.34±1.67	1.23±1.35*	1.22±0.87
120 min. after SSV + $NaHCO_3$	119.8±2.9	121.3±3.5	4.14±5.04	3.89±2.26	1.84±1.96	2.34±1.28
180 min. after SSV + $NaHCO_3$	121.0±2.9	125.3±7.9	5.15±5.53	3.33±0.93	2.25±1.94	1.84±1.41

Values are means ± SD. Abbreviation : SSV, sea snake venom(mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.
Values received from the left kidney.

Table 8. Changes in plasma osmolality (P_{osm}), osmolar clearance (C_{osm}), and free water clearance (C_{H2O}) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	P_{osm} (mOsm/kg.H_2O)		C_{osm} (µl/min/kg.)		C_{H2O} (µl/min/kg.)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	290.3±4.5	294.8±5.2	53.2±18.0	44.4±17.4	-15.9±15.4	-30.8±11.0
60 min. after SSV	298.0±5.0*	292.5±7.9	62.3±49.9	32.7±17.5	-22.1±22.6	-23.7±12.3
120 min. after SSV	294.6±10.5	290.4±9.5	54.7±39.8	45.2±17.4	-13.0±19.4	-32.6±11.9
180 min. after SSV	291.6±9.8	300.5±7.8	61.4±33.4	36.4±11.9	-22.5±16.6	-27.2±8.7
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	302.7±5.6	291.6±8.3	35.4±21.0	116.8±153.0	-0.6±25.3	-70.1±102.0
60 min. after $NaHCO_3$ + SSV	308.4±9.0*	300.6±11.2	59.9±31.7	139.2±125.8	-7.3±35.4	-75.0±90.8
120 min. after $NaHCO_3$ + SSV	305.6±8.5	305.4±5.2***	74.5±19.0**	82.1±16.1	-14.7±27.8	-31.0±17.4
180 min. after $NaHCO_3$ + SSV	316.4±12.4*	303.0±11.3	99.2±23.1***	96.6±10.2	-18.9±29.8	-39.0±12.3
Series C (given $NaHCO_3$ after SSV)						
Control	295.2±5.3	288.4±3.7	29.1±14.8	42.3±36.0	-17.6±13.4	-28.6±31.3
60 min. after SSV	296.2±7.1	295.2±6.8	42.4±27.7	39.0±30.9	-29.2±23.0	-23.0±25.2
120 min. after SSV + $NaHCO_3$	298.2±8.6	300.5±5.5*	73.6±46.2	60.2±33.0	-45.1±29.5	-25.6±30.1
180 min. after SSV + $NaHCO_3$	300.2±9.4	296.3±12.7	83.8±51.8*	64.2±15.4	-50.1±33.3*	-28.7±19.5

Values are means ± SD. Abbreviation : SSV, sea snake venom (mg/kg.).

Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$, *** $P<0.001$ when compared to the control value in the same group.

Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

Values received from the left kidney.

Table 9. Changes in the blood urea nitrogen (BUN) concentration, urinary bicarbonate (U_{HCO3}) concentration and urinary pH (U_{pH}) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	**BUN (mg%)**		**U_{HCO3} (mmol/L)**		**U_{pH}**	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)						
Control	8.51±2.78	11.90±4.26	0.82±0.24	0.82±0.19	6.80± 0.39	6.63± 0.59
60 min. after SSV	13.07±3.22	15.65±4.35**	0.88±0.27	0.90±0.25	7.88±1.15	7.20 ± 0.61
120 min. after SSV	12.13±6.24	14.11±4.06***	0.89±0.19	0.92±0.15	7.57± 1.1	7.56± 0.70
180 min. after SSV	10.73±3.47	14.42±3.40**	0.84±0.18	0.82±0.19	7.27±1.13	7.43± 0.80
Series B (pretreated with $NaHCO_3$)						
Control with $NaHCO_3$	8.20±3.03	12.07±2.03	2.87±2.87	3.92±3.13	7.56±1.50	7.30±1.26
60 min. after $NaHCO_3$ + SSV	13.77±2.81*	11.99±1.90	7.99±5.28	11.09±3.97*	8.73±0.68	8.99±0.63*
120 min. after $NaHCO_3$ + SSV	10.20±2.01	16.04±8.49	10.23±3.94*	11.29±3.35*	9.16±0.48	8.76±0.27
180 min. after $NaHCO_3$ + SSV	11.62±2.36	13.56±0.83	16.64±4.22*	12.97±3.48	8.84±0.16	9.68±0.11^{+}
Series C (given $NaHCO_3$ after SSV)						
Control	8.42±1.06	9.96±2.52	0.32±0.27	0.91±1.18	6.12±1.18	7.45±1.05
60 min. after SSV	8.47±2.27	12.52±2.51*$^{+}$	2.24±2.38	2.28±1.51	7.09±1.50	7.96±0.61
120 min. after SSV + $NaHCO_3$	9.30±1.15	11.70±3.01	6.74±5.21*	7.52±5.48	8.06±1.06*	9.06±0.52**
180 min. after SSV + $NaHCO_3$	9.69±2.06	12.20±2.21	9.11±4.67*	8.09±5.15	8.54±1.17*	9.47±0.16*

Values are means ± SD. Abbreviation : SSV, sea snake venom (mg/kg.); ND, not determine.

Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$, *** $P<0.001$ when compared to the control value in the same group.

Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

Table 10. Changes in plasma creatine phosphokinase (P_{CPK}) and plasma lactate dehydrogenase (P_{LDH}) concentrations in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	P_{CPK} (U/L)		P_{LDH} (U/L)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)				
Control	95.20±12.66	88.00±26.3	103.00±24.63	77.40±23.01
60 min. after SSV	102.20±27.85	166.00±66.90*	171.40±40.10	116.40±36.00*
120 min. after SSV	ND	ND	ND	ND
180 min. after SSV	130.80±41.75	357.40±18.51*	162.60±98.21	158.80±44.70**
Series B (pretreated with $NaHCO_3$)				
Control with $NaHCO_3$	46.80±8.64	72.00±30.18	91.00±37.85	67.40±28.35
60 min. after $NaHCO_3$ + SSV	66.20±16.71	83.20±21.03	136.80±40.87	119.00±56.48
120 min. after $NaHCO_3$ + SSV	ND	ND	ND	ND
180 min. after $NaHCO_3$ + SSV	105.40±47.51	106.00±55.15	160.20±72.97	96.50±26.16
Series C (given $NaHCO_3$ after SSV)				
Control	80.00±18.01	89.80±22.68	141.60±48.39	56.60±10.38
60 min. after SSV	110.00±41.76	136.60±41.05	205.40±139.50	95.80±28.38*
120 min. after SSV + $NaHCO_3$	ND	ND	ND	ND
180 min. after SSV + $NaHCO_3$	124.00±38.66*	144.75±13.82*	178.00±102.46	85.00±14.94**

Values are means ± SD. Abbreviation : SSV, sea snake venom (mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

Table 11. Changes in urinary myoglobin (U_{Mb}) concentrations and urinary myoglobin excretion ($U_{Mb}V$) in response to sea snake venom (SSV) administration in six groups.

Elapsed time after venom injection	U_{Mb} (ng/ml)		$U_{Mb}V$ (ng/min/kg.)	
	Low (0.16SSV)	High (0.34SSV)	Low (0.16SSV)	High (0.34SSV)
Series A (given SSV alone)				
Control	0.46±0.49	0.66±0.84	0.01±0.01	0.01±0.00
60 min. after SSV	8.62±15.90	18.03±29.45	0.14±0.20	0.13±0.22
120 min. after SSV	ND	ND	ND	ND
180 min. after SSV	17.60±13.97*	200.00±248.28*	0.52±0.45	1.84±2.28
Series B (pretreated with $NaHCO_3$)				
Control with $NaHCO_3$	0.86±1.04	2.77±1.05	0.06±0.09	0.17±0.25
60 min. after $NaHCO_3$ + SSV	0.84±0.79	58.13±54.79*	0.06±0.08	5.11±8.06**$^{+}$
120 min. after $NaHCO_3$ + SSV	ND	ND	ND	ND
180 min. after $NaHCO_3$ + SSV	38.00±31.05	50.57±13.39	2.38±1.58	2.76±0.37**
Series C (given $NaHCO_3$ after SSV)				
Control	5.00±5.20	2.30±0.50	0.04±0.02	0.03±0.02
60 min. after SSV	27.26±44.67	31.18±17.73*	0.26±0.37	0.42±0.20
120 min. after SSV + $NaHCO_3$	ND	ND	ND	ND
180 min. after SSV + $NaHCO_3$	73.80±39.36*	31.73±12.09*	2.54±2.33	1.22±0.72

Values are means ± SD. Abbreviation : SSV, sea snake venom (mg/kg.).
Significant difference values using paired t-test are indicated by * $P<0.05$, ** $P<0.01$ when compared to the control value in the same group.
Significant difference values using unpaired t-test are indicated by $^{+}$ $P<0.05$ when compared between animals given a low and a high doses of SSV in each series.

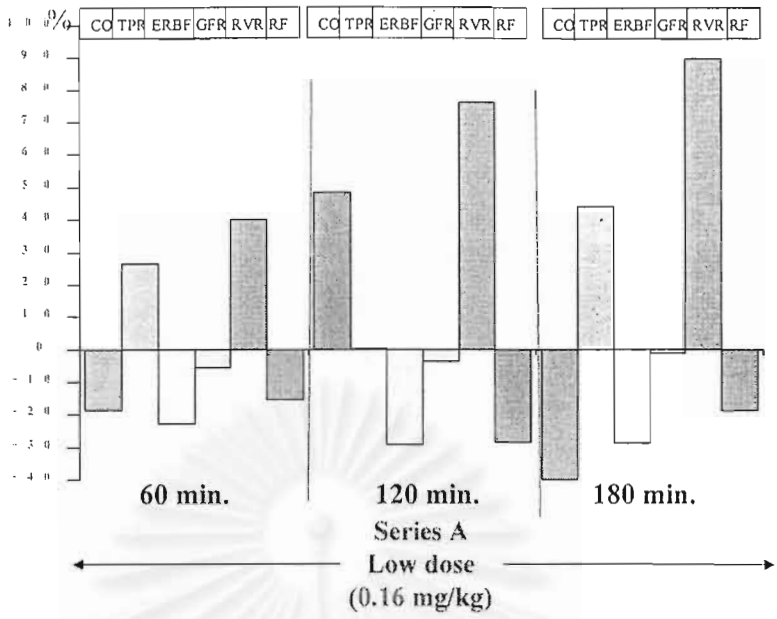

Figure 1. Percentage of changes of cardiac output (CO), total peripheral resistance (TPR), effective renal blood flow (ERBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and renal fraction (RF) in dogs given a low dose of SSV alone (Series A)

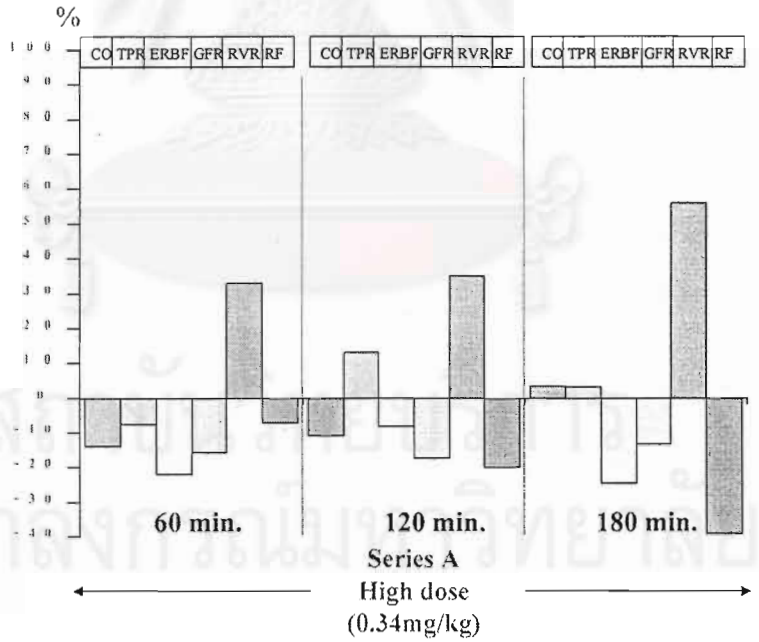

Figure 2. Percentage of changes of cardiac output (CO), total peripheral resistance (TPR), effective renal blood flow (ERBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and renal fraction (RF) in dogs given a high dose of SSV alone (Series A)

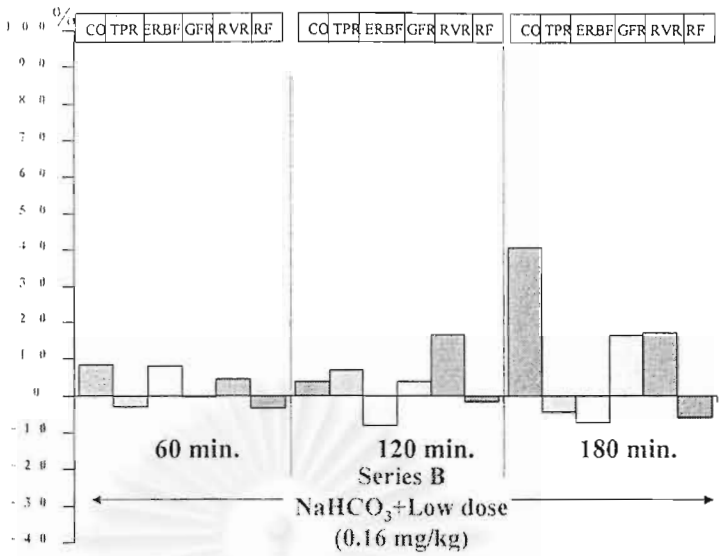

Figure 3. Percentage of changes of cardiac output (CO), total peripheral resistance (TPR), effective renal blood flow (ERBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and renal fraction (RF) during envenomation with a low dose of SSV in dogs pretreated with an intravenous infusion of NaHCO3 (Series B)

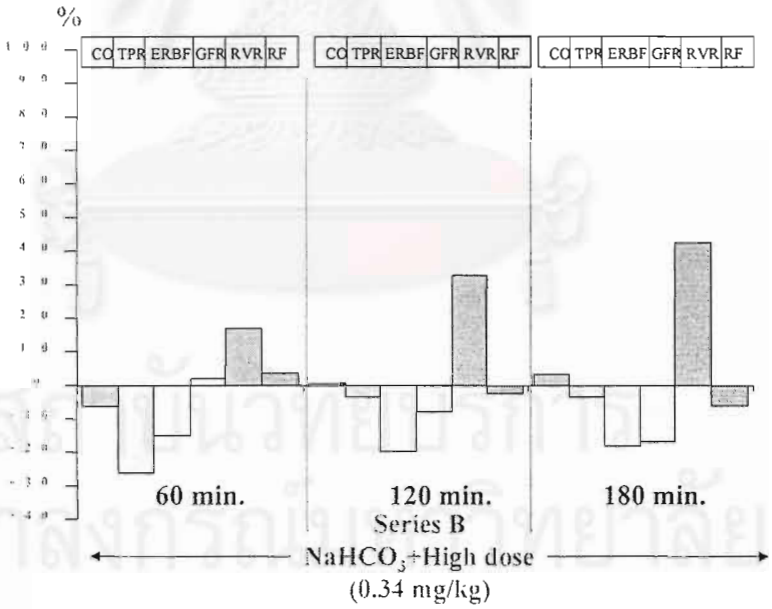

Figure 4. Percentage of changes of cardiac output (CO), total peripheral resistance (TPR), effective renal blood flow (ERBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and renal fraction (RF) during envenomation with a high dose of SSV in dogs pretreated with an intravenous infusion of NaHCO3 (Series B)

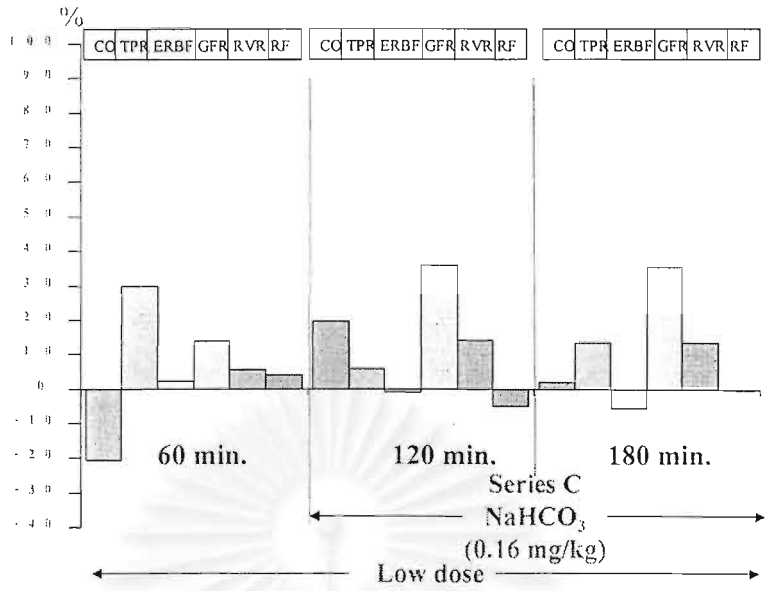

Figure 5. Percentage of changes of cardiac output (CO), total peripheral resistance (TPR), effective renal blood flow (ERBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and renal fraction (RF) in dogs given of intravenous infusion of NaHCO3 1 hr. after a low dose of SSV (Series C)

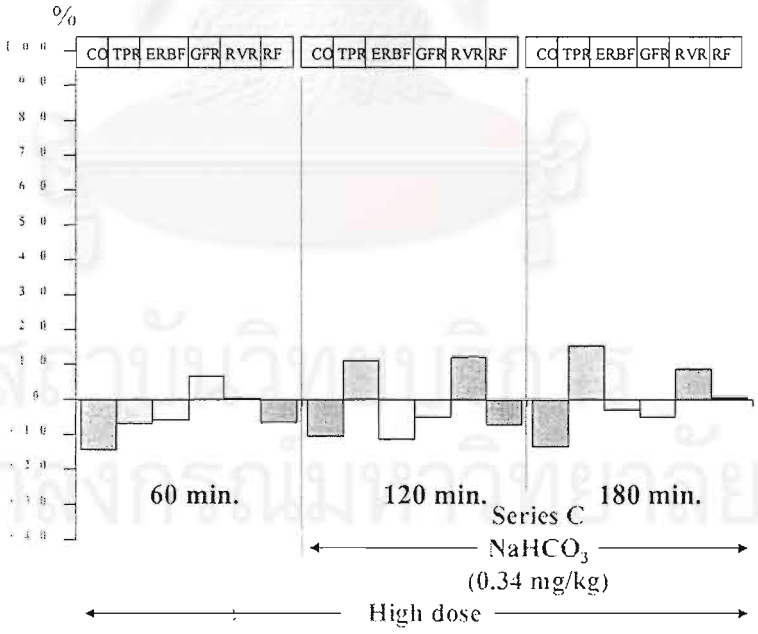

Figure 6. Percentage change of cardiac output (CO), total peripheral resistance (TPR), effective renal blood flow (ERBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and renal fraction (RF) in dogs given of intravenous infusion of NaHCO3 1 hr. after a high dose of SSV (Series C)

CHAPTER V

DISCUSSION

In the present study, intramuscular injection of either a low dose or a high dose of SSV alone caused slight increases in mean arterial blood pressure and pulse pressure. These changes were similar to the results in rabbit after intravenous administration with 0.1-0.2 mg/kg. of *Laticauda laticaudata* venom showing a transient fall followed by a gradual rise in arterial blood pressure (Tu, 1967). Changes in arterial blood pressure would relate to a slight increase in total peripheral resistance without alteration of cardiac output after envenomation alone. It indicates that a compensatory mechanism occurs for maintenance of blood pressure during envenomation. An increase in total peripheral resistance after envenomation was probably due to vasoconstriction during initial hypotension that might be caused by activation of sympathetic activity or other compensatory mechanisms (Ganong, 1977). During envenomation, no hemolysis was apparent. It seem likely that a tendency increase in packed cell volume was due to sympathetic activity causing spleenic contraction after envenomation (Ganong, 1977). A marked increase in renal vascular resistance after envenomation was also apparent which would be due to the renal vasoconstriction. The magnitude of an increase in renal vascular resistance appeared to be more than an increase in total peripheral resistance throughout the experimental periods, thereby leading to reductions of both glomerular filtration rate and effective renal plasma flow after envenomation alone. A disproportionate decrease in effective renal plasma flow and glomerular filtration rate gave the significant increase in filtration fraction after envenomation. From the present results, it revealed that the SSV could affect both cardiovascular system and renal hemodynamics, which resulted a reduction of renal fraction (ERBF/CO) after given a high dose of SSV alone. Thus, venom actions given alone exhibit a reduction of the rate of urine flow. At the 180 minutes of the experimental period in both groups of animals given SSV alone, the renal vascular resistance was still maintained in a high

level and the effective renal blood flow was still decreased when compared with the control period. The reduction of renal tubular function might be expected to appear if animals were observed for a longer period of time.

Plasma concentration, urinary excretion and fractional excretion of sodium ion and chloride ion showed no significant changes after SSV administration alone. Urinary potassium excretion was not increased throughout the experiment as compared with the control. However, plasma concentration of potassium increased in both groups at 180 minutes after SSV administration alone. It is possible that potassium may release from muscle which was alarming in myotoxic effect of sea snakes venom (Hood and Johnson, 1975; Marsdan and Reid, 1961; Sitprija *et al.*, 1971). Plasma CPK and LDH concentrations showed significant increases throughout the experimental periods. The level of urinary myoglobin also increased, which indicates muscular damage from myotoxic action of SSV. In the present results, plasma levels of CPK, LDH and urinary myoglobin have been shown to higher the degree of muscle cell destruction in animals treated with a high dose of SSV. An increase in urinary myoglobin probably causes a decrease in renal blood flow and glomerular filtration rate (Ayer *et al.*, 1971; Campion *et al.*, 1972). Acute renal failure has been generally reported to occur as a consequence of myonecrosis due to the myolytic action of the sea snake poisoning (Chugh, 1989). Myoglobinuria occurred throughout experimental periods after envenoming alone. It is possible that intraluminal cast obstruction, renal vasoconstriction and direct myoglobin–induced cytotoxicity would contribute to the reduction of renal hemodynamics (Zager, 1996). Secondary renal changes resulting of myoglobinuria and blockade the renal tubular lumen by myoglobin casts have been noted in myotoxic snake bite (Sitprija and Chaiyabutr, 1999).

In the present study, administration of SSV in animals accompanying with $NaHCO_3$ infusion (Series B and C) showed no significant effect on MAP, HR, PP, CO and TPR. An intravenous administration of $NaHCO_3$ after or before treated in either a low dose of SSV injection or a high dose of SSV injection showed increases in urine flow, urinary excretion and fractional excretion of sodium ion throughout

experimental periods. PCV was also not affected by SSV administration with $NaHCO_3$ loading. It is possible that $NaHCO_3$ loading would induce a shift of fluid from the intracellular compartment to the extracellular compartment to maintain osmotic equilibrium (Rose, 1994). The regulatory response was sufficient for volume expansion resulting on reduction of sympathetic activity (Rose, 1994).

Administration of $NaHCO_3$ to animals given SSV caused increases in urine flow, fractional excretion of electrolytes, urinary sodium excretion, urinary concentration of bicarbonate and urinary pH. An increase in the urine flow rate coincided with an increase in fractional excretion of sodium during infusion of $NaHCO_3$, reflecting a reduction of renal tubular reabsorption of sodium ion. The present data agree with those of other studies that urine pH increased after administration of $NaHCO_3$ to animals (Lloyd and Rose, 1995). The increase in urine pH after $NaHCO_3$ loading would be the result of a regulatory renal response by which bicarbonate reabsorption in the proximal tubule is limited and more secretion in the cortical collecting duct (Rose, 1994). During metabolic alkalosis, the excess bicarbonate ions would be excreted in the urine (Pitts *et al.*, 1949). Reduced reabsorption of Na in the proximal tubule after $NaHCO_3$ administration would be mediated by direct inhibition of Na^+/H^+ antiporter during metabolic alkalosis and by expansion of the extracellular fluid volume that follows hypernatremia (Rose, 1994). Administration of $NaHCO_3$ to animals had no effect on GFR, which was similar to the result in horse by Rivas *et al.* (1997). During Sodium bicarbonate loading with SSV administration, a tendency of an increase in urinary potassium and chloride excretions was apparent. The decrease in the level of plasma potassium was also noted in animals of both Series B and C. This result could account for an increase of urine pH which was concomitant with an increase of potassium excretion. Such changes can be explained by the well know fact that kidney play a significant role in acid-base regulation by an attempt to ensure hydrogen ion with a reciprocal secretion of potassium ions (Johnson and Selkurt, 1966). Thus, the pattern of electrolyte excretion during SSV administration with $NaHCO_3$ loading would show the tendency of hypoalkalemic alkalosis. However, the present study indicates that

animals given $NaHCO_3$ either before or after SSV injection did not depress GFR and ERBF.

The measurements of plasma enzyme and urinary myoglobin concentration from the effect of sea snake venom indicates that increases of both CPK and LDH and urinary myoglobin were still apparent in both Series B and C. These results indicate a damage of skeletal muscle due to myolytic effect could not recovery to normal state by $NaHCO_3$ loading. A number of studies indicate that the release of large amounts of myoglobin into the circulation can ultimately lead to the development of ARF (Grossman *et al.*, 1974). Pigment casts in the lumina of tubules causing tubular obstruction would be a major factor both in the present of myoglobin in serum and urine which indicates dysfunction of a major muscle, hypotension, and volume depletion. (Hamilton *et al.*, 1989). This hypothesis receives indirect support from experimental studies on myoglobinulic ARF induced by glycerol, in which volume expansion within 3 to 6 hours restores glomerular filtration rate and renal plasma flow to normal (Reineck, *et al.*, 1980). An increase in intratubular pressure and overcome tubular obstruction would relate to the ARF (Tanner and Steinhausen, 1976). Tubular obstruction leads to increase proximal tubular uptake of filtered, reabsorbable nephrotoxins. Thus, it is likely that myoglobin cast formation tubular obstruction, provoked by aciduria, acutely increases proximal tubular cell myoglobin uptake, helping to induce tubular necrosis (Zager, 1989).

In the present study, the decrease in renal hemodynamics in animals given SSV could be reversed by infusion of $NaHCO_3$ that was similar to the result of Ron *et al.* (1984) and Eneas *et al.* (1979) that suggested that prompt and aggressive volume expansion, alkalinization of the urine may prevent the development of the acute tubular necrosis. Treatment protocols for myoglobinuria include administration of sodium bicarbonate, increased fluid intake has been suggested to prevent myoglobin precipitation within the renal tubules (Eneas *et al.*, 1979). It is possible for the present study in both series B and C that urinary myoglobin excretion showed a marked increase in animals after SSV injection accompanying with $NaHCO_3$ infusion.

Although the mechanism for myoglobin induced ARF has not yet been elucidated. It is suggested that large amounts of myoglobin present in the renal tubules can precipitate, particularly under acidic conditions, resulting in increased intratubular pressure and subsequently decreased hemodynamics including GFR and ERBF. An infusion of $NaHCO_3$ for alkalinuric conditions both before and after could be a protective role against depression of renal function following sea snake venom administration.

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