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จากการเกิดเนื้องอกตาย ในผู้ป่วยที่ถูกงูเห่ากัด และงูกะปะกัด
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**PREVALENCE, SEVERITY, DETERMINANTS AND
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ENVENOMED BY *Naja kaouthia* (THAI COBRA) AND
Calloselasma rhodostoma (MALAYAN PIT VIPER) AND ITS
PREVENTION IN AN EXPERIMENTAL MODEL**

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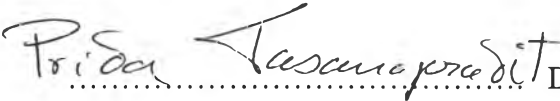
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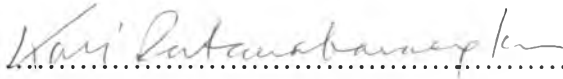
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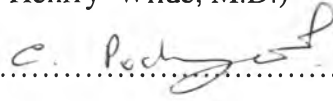
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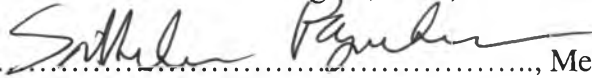

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นวน้อง วงศ์ทองคำ : การศึกษาภาวะความชุก ความรุนแรง บั๊จจัย และผลที่เกิดขึ้นจากการเกิดเนื้อตายในผู้ป่วยที่ถูกงูเห่า และงูกะปะกัด และการศึกษาการป้องกันภาวะเนื้อตายที่เกิดขึ้นในสัตว์ทดลอง.

(PREVALENCE, SEVERITY, DETERMINANTS AND CONSEQUENCES OF TISSUE NECROSIS AMONG VICTIMS ENVENOMED BY *Naja kaouthia* (THAI COBRA) AND *Calloselasma rhodostoma* (MALAYAN PIT VIPER) AND ITS PREVENTION IN AN EXPERIMENTAL MODEL)

อาจารย์ที่ปรึกษา : ศาสตราจารย์นายแพทย์ จิตร สิทธิอมร

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การถูกงูพิษกัดในประเทศไทย ยังคงเป็นปัญหาที่สำคัญทางการแพทย์โดยเฉพาะงูเห่า และงูกะปะ ซึ่งพิษสามารถก่อให้เกิดผลทั่วร่างกายและผลเฉพาะที่ ในผู้ป่วยที่มีอาการรุนแรงอาจเสียชีวิตหรืออาจเสียอวัยวะที่ถูกกัดอย่างถาวร หรืออาจต้องตัดอวัยวะส่วนนั้นทิ้งและก่อให้เกิดความพิการ พยาธิสภาพบริเวณที่ถูกกัด เกิดจากการทำงานร่วมกันของเอนไซม์ phospholipase A₂ (PLA₂), ซึ่งสามารถทำลายกล้ามเนื้อ, เอนไซม์ metalloproteinase ซึ่งทำให้เกิดภาวะเลือดออก และเอนไซม์ hyaluronidase ซึ่งช่วยทำให้พิษงูแพร่กระจายรวมทั้งสารพิษอื่น ๆ ในพิษงู

การวิจัยครั้งนี้แบ่งออกเป็น 2 ส่วน คือ ก) การศึกษาทางด้านระบาดวิทยา ทั้งส่วนการศึกษาไปข้างหน้า และการศึกษาย้อนหลัง ในผู้ป่วยที่ถูกงูเห่า หรืองูกะปะกัด ข) การทดลองเพื่อดูประสิทธิภาพของตัวยับยั้งเอนไซม์ metalloproteinase และ PLA₂ โดยมีจุดมุ่งหมายเพื่อหาวิธีลดภาวะการเกิดเนื้อตายบริเวณที่ถูกกัด และลดการเกิดพิษทั่วร่างกาย

ผลการศึกษาทางด้านระบาดวิทยาพบว่า อุบัติการณ์ของการเกิดเนื้อตายบริเวณที่ถูกงูกะปะ และงูเห่ากัดสูงถึง 94.50 % และ 91.10 % ตามลำดับ โดยส่วนใหญ่จะพบภาวะเนื้อตายที่มีรุนแรงและจะแสดงออก ในช่วง 12 ชั่วโมงแรกของการเข้ารับการรักษา การรักษาผู้ป่วยส่วนใหญ่เป็นการทำแผลเพื่อป้องกันและลดการติดเชื้อ มีผู้ป่วยที่ถูกงูเห่ากัด เพียง 1 รายที่ต้องถูกตัดนิ้ว

ผลการศึกษาในการทดลอง พบว่า 2 mM Na₂EDTA หรือ 20 mM N-Phenylglycine สามารถยับยั้งผลของเอนไซม์ metalloproteinase และ PLA₂ ตามลำดับได้อย่างสมบูรณ์ ทั้งในพิษงูเห่า และพิษงูกะปะ การทดลองในสัตว์ทดลอง พบว่า Na₂EDTA (93.05 ไมโครกรัม/หนู) หรือ N-phenylglycine (37.80 ไมโครกรัม/หนู) เมื่อทำการ preincubate กับพิษงูก่อนที่จะฉีดในหนู พบว่าสามารถลดการเกิดภาวะการบวม และการเกิดเนื้อตายอันเกิดจากพิษงูได้อย่างมีนัยสำคัญทางสถิติ ส่วน 'Inhibitor mixture' ที่ประกอบด้วย N-phenylglycine (37.80 ไมโครกรัม/หนู) Na₂EDTA (93.05 ไมโครกรัม/หนู) และตัวยับยั้งเอนไซม์ hyaluronidase คือ sodium aurothiomalate (195 ไมโครกรัม/หนู) พบว่าสารผสมนี้สามารถลดการเกิดเนื้อตายเฉพาะที่เมื่อฉีดภายในเวลา 1, 3 และ 10 นาที หลังการฉีดพิษงู และยังคงพบว่าผลของตัวยับยั้งเอนไซม์ metalloproteinase และ PLA₂ สามารถยืดระยะเวลาตายของหนูที่ฉีดพิษงูเห่า และงูกะปะ ในขนาดที่ทำให้หนูตาย ผลการทดลองนี้บ่งชี้ว่าสารผสม 'Inhibitor mixture' มีประสิทธิภาพดีในการลดภาวะการเกิดเนื้อตาย ถ้าฉีดทันทีที่บริเวณที่ถูกกัด และลดความเป็นพิษที่ออกฤทธิ์ทั่วร่างกายของพิษงูเห่า และงูกะปะ

สาขาวิชา การพัฒนาระบบสาธารณสุข.....
ปีการศึกษา.....2546.....

ลายมือชื่อนิสิต
ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

PH : 001222 : MAJOR HEALTH SYSTEMS DEVELOPMENT PROGRAMME
 KEYWORD : TISSUE NECROSIS / SNAKE SEVERITY SCORE / 'INHIBITOR
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 VICTIMS ENVENOMED BY *Naja kaouthia* (THAI COBRA) AND *Calloselasma*
rhodostoma (MALAYAN PIT VIPER) AND ITS PREVENTION IN AN
 EXPERIMENTAL MODEL

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The majority of venomous snakebites in Thailand are caused by *Naja kaouthia* and *Calloselasma rhodostoma*. The venom of these snakes induces systemic and local toxicities. In severe cases of envenomation, these local effects may lead to permanent tissue loss, disability or amputation. The local pathogenesis is mainly due to the concomitant actions of myotoxic phospholipase A₂ (PLA₂), hemorrhagic metalloproteinase, hyaluronidase and membrane active toxins.

The research work is divided into 2 parts: a) epidemiology study including prospective and retrospective studies of victims of *Calloselasma rhodostoma* and *Naja kaouthia* bites and b) experimental models carried out to find potent inhibitors of metalloproteinase and PLA₂ with the aim of using them to reduce local tissue damage and systemic symptoms caused by the venoms.

The results of epidemiology studies showed that the incidences of tissue necrosis at bite sites from *C. rhodostoma* and *N. kaouthia* were 94.50 % and 91.11 %, respectively. Most of the cases showed mild local clinical manifestation, especially at 12 hours after hospitalization. Almost all patients needed wound care to prevent or control infection. Only one case required amputation; the victim was bitten by *N. kaouthia* and required amputation of a thumb.

The experimental models revealed that 2 mM Na₂EDTA, 20 mM N-phenylglycine completely inhibited metalloproteinase and PLA₂ activities, respectively, of both venoms. In *in vivo* experiments, Na₂EDTA (93.05 µg/mouse) or N-phenylglycine (37.80 µg/mouse) when preincubated with each of the venoms, has been shown to significantly decrease local toxicity (edema and myonecrosis). An 'Inhibitor mixture' containing N-phenylglycine (37.80 µg/mouse), Na₂ EDTA (93.05 µg/mouse) and an inhibitor of hyaluronidase sodium aurothiomalate (195 µg/mouse) has been shown to significantly reduce tissue necrosis when locally injected 1,3 or 10 min after venom injection. Most metalloproteinase and PLA₂ inhibitors prolonged the survival time of mice injected with lethal doses of *N. kaouthia* or *C. rhodostoma* venom. These results indicate that the 'Inhibitor mixture' was effective if injected immediately at the bite site in reducing the local and systemic toxicities of *N. kaouthia* or *C. rhodostoma* venom.

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ABBREVIATION

NK	<i>Naja kouthia</i>
CR	<i>Calloselasma rhodostoma</i>
PLA ₂	Phospholipase A ₂
SSS	Snake Severity Score
VCT	Venous Clotting Time
CPK	Creatine Phosphokinase
LI	Desferiprone
DFO	Desferrioxamine
TEPA	Tetraethylenepentamine
EDTA	Ethylenediamine tetraacetic acid
p-BPB	para-bromophenacyl bromide