



THE EFFECTS OF *TAMARINDUS INDICA* SEED
EXTRACT AS ANTI-SNAKE VENOM AGAINST
DABOIA RUSSELLI, *NAJA KAOUTHIA* AND
OPHIOPHAGUS HANNAH IN MICE

BY

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ABSTRACT

Globally, snakebite cases are estimated to be around 5 million annually affecting mainly the residents of poorer counties like Africa and Asia, and in 2009 WHO has categorised it as a 'neglected tropical disease'. Currently the standard treatment for snake envenomation is the use of anti-snake venom (ASV) therapy. However this is expensive and not readily available in smaller hospitals in the developing world. Herbal medicine has been and is still in use in some cultures for the treatment of snakebite and one such plant is *Tamarindus indica*. This plant is found in many countries where snake envenomation is also prevalent. This study was conducted to evaluate the potential of using *T. indica* seed extract (TSE) to inhibit the effects of snake venom of three snakes; namely *Naja kaouthia*, *Ophiophagus hannah* and *Daboia russelli*. The testa of tamarind seed was used and it underwent ethanolic soxhlet extraction to obtain TSE. The inhibition of the activity of the following enzymes i.e phospholipase A₂ (PLA₂), proteinase and phosphomonoesterase (PME) *in vitro* by the three snake venoms with TSE was studied. SDS-PAGE experiment was conducted to observe the effects of TSE on venom proteins. *In vivo* acute subcutaneous (SC) toxicity of TSE in ICR mice was conducted. Study on the inhibition of lethality was conducted on each of the three snake venoms when SC TSE was injected into mice. Venom concentration and site were fixed but TSE concentration, time and site of injection were manipulated. Findings from venom enzymatic inhibition studies showed that, TSE was able to significantly reduce ($p < 0.05$) all three venom enzymatic activities i.e PLA₂, proteinase and PME. SDS-PAGE experiment showed that venom protein bands were disrupted when venom reacted with TSE. No signs of toxicity were observed over a period of 4 weeks when mice were exposed to SC TSE 60 mg/20 g body weight except for skin ulcers. Histological examination on liver, both kidneys and skin at the site of SC injection showed no changes compared to the control group injected with SC distilled water. TSE was able to increase the survival rate of ICR mice when exposed to each of the three snake venoms regardless of the site of injecting SC TSE. Mice injected with *N. kaouthia* or *D. russelli* venom, had increased 24 hour survival rate when SC TSE was given at 15 minutes; and of mice injected with *O. hannah* venom the 24 hour survival rate increased with higher TSE concentration when given sooner. In conclusion, SC TSE was safe to be injected up to 60mg/20 g and has the potential to delay the effects of venom from *N. kaouthia*, *O. hannah* and *D. russelli*.

خلاصة البحث

تقدر حالات لدغ الأفاعي على الصعيد العالمي بحوالي 5 ملايين سنويا، مما يؤثر بشكل كبير على سكان البلدان الفقيرة مثل أفريقيا وآسيا. وقد صنفت منظمة الصحة العالمية عام 2009 لدغ الأفاعي ضمن "أمراض المناطق الاستوائية المهملة". وحاليا فإن المعيار القياسي للعلاج من حالات التسمم من اللدغات السامة يكمن في استخدام ترياق مضاد لسُموم الأفاعي. ولكن هذا أمر مكلف وغير متوفر بسهولة في المستشفيات الصغيرة في العالم النامي. وقد استخدمت الأدوية العشبية في الماضي لعلاج لدغات الأفاعي ومن بينها نبات التمر هندي كأحد هذه الوسائل. وقد تم العثور على هذا النبات في العديد من البلدان حيث التأثير بزغاف الحشرات السامة لا تزال سائدة أيضا. وقد أجريت هذه الدراسة لمعرفة إمكانية استخدام مستخلص بذور التمر هندي لمنع آثار التسمم من ثلاثة ثعابين وهي: الأميوفيجس هانا، والناجاكوثيا والدايبا روسلي. لقد استخدم غلاف بذور التمر هندي والذي تم استخراجة عن طريق السوكسليت المثلي وذلك للحصول على مستخلص التمر هندي. ولقد استخدم في المختبر مستخلص بذور التمر هندي لدراسة تثبيط نشاط انزيمات الفوسفوليبيد 2، بروتينوليتك، والفوسفومونواستيريز وذلك لسُموم ثلاثة أفاعي. وقد أُجري الفصل الكهربائي لهلام متعدد الأكريلاميد لملاحظة تأثير مستخلص التمر هندي على بروتينات سموم الأفاعي. هذا وقد أُجريت دراسة التأثير الحاد لحقن الفئران تحت الجلد بمستخلص التمر هندي.

وقد تمت دراسة تثبيط فتك سمية ثلاثة أفاعي بحقن مستخلص التمر هندي تحت جلد فئران التجارب.. وقد ثبتت جرعات سموم الأفاعي وكذلك أماكن حقنها؛ مع تغيير جرعات، ووقت وأماكن حقن مستخلص التمر هندي. وأظهرت نتائج تثبيط فاعلية انزيمات سموم الأفاعي على مقدرة مستخلص التمر هندي على اضعاف تأثير سُمية انزيمات الأفاعي الثلاثة (انزيمات الفوسفوليبيد 2، بروتينوليتك، والفوسفومونواستيريز). وقد أظهرت تجارب فصل الرحلان الكهربائي لهلام الأكريلاميد عطل رحلان سموم الأفاعي عند استعمال مستخلص التمر هندي. ولمدة 4 أسابيع لم يُلاحظ ظهور أعراض لتسمم الفئران المحقونة بمستخلص التمر هندي بجرعة 60 ملج/20 ملج باستثناء التقرحات الجلدية. ولم يُظهر الفحص النسيجي للكبد والكليتين ومواقع الحقن تحت الجلد أي تغييرات بالمقارنة بفئران مجموعة التحكم والتي حقنت بالماء المقطر. وقد رفع مستخلص التمر هندي معدل بقاء الفئران على قيد الحياة عند تعرضها لسُموم كل من الثلاثة أفاعي بغض النظر عن موقع الحقن تحت الجلد. ارتفع معدل بقاء الفئران على قيد الحياة لمدة 24 ساعة التي حُقنت بسموم أفعى الأميوفيجس هانا وذلك عند التعجيل بحقنها بجرعة عالية من مستخلص التمر هندي. أما الفئران التي حُقنت بسموم أفعى الناجاكوثيا والدايبا روسلي فقد زاد معدل بقائها على قيد الحياة لمدة 24 ساعة عند حقنها بمستخلص التمر هندي كل 15 دقيقة. واستنتجت الدراسة أن لدى مستخلص التمر هندي قدرة على تأخير آثار سموم أفاعي الأميوفيجس هانا، والناجاكوثيا والدايبا روسلي عند حقنها تحت الجلد بمستخلص التمر هندي بجرعات تتراوح بين 60 ملج الى 20 ملج.

APPROVAL PAGE

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DECLARATION PAGE

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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This thesis is dedicated to my loving family;

To my respectful and loving parents; Haji Ismail Bazain and Allahyarharmah Hajjah Khadijah Haji Mohd Said, thank you very much for allowing me to pursue my dreams and supporting me all the way. Without your blessing I will never be where I am today. Thank you for believing in me.

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LIST OF ABBREVIATIONS

AMSEM	Advanced workshop on marine and snake envenomation management
H & E	Haematoxylin - Eosin
HPLC	High performance liquid chromatography
IM	Intramuscular
IV	Intravascular
LD ₅₀	50% of lethal dose
LD ₁₀₀	100% of lethal dose
MHD	Minimum haemorrhagic dose
MPT	<i>Mimosa pudica</i> extract (<i>Mimosa pudica</i> tannin)
OECD	Organization for Economic Co-operation and Development
PLA ₂	Phospholipase A ₂
PME	Phosphomonoesterase
rpm	Revolution per minute
SC	Subcutaneous
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SMVPs	Snake venom metalloproteinases
TSE	Tamarind seed extract

LIST OF DEFINITIONS

Antivenom

A purified fraction of immunoglobulins or its fragments fractionated from the plasma of animals that have been immunized against venom from one or several species of snakes

Venom

The toxic secretion of a specialized venom gland which in the case of the snake, it is delivered through the fangs and provokes deleterious effects. Venoms usually comprise many different protein components of variable structure and toxicity. Venom enters the victims via 'injection'.

Toxin

A toxic substance, especially a protein, which is produced by living cells or organism and is capable of causing disease when introduced into the body tissues. It is also often capable of introducing neutralizing antibodies or antitoxins. Toxin enters the victims via ingestion.

Calloselasma rhodostoma

Synonym to i) *Agkistrodon rhodostoma*

Common name: Malayan pit-viper

Daboia russelli

Synonym to: *Vipera russelli*

Common name: Russell's viper, Chain viper

Daboia russelli siamensis

Synonym to: *Vipera russelli*

Common name: Eastern Russell's viper, Siamese Russell's viper

Naja kaouthia

Synonym to: *Naja naja kaouthia*, *Naja-naja*

Common name: Monocled cobra

It was formerly treated as a subspecies of Indian cobra (*Naja naja*)

Naja sumatrana

Synonym to: *Naja naja sputatrix*

Common name: Spitting cobra

Ophiophagus hannah

Synonym to: Hamadryad

Common name: King cobra

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Snakes have been the subject of fascination, fear and myths all through history. In ancient Egyptian times, the cobra was worshipped and its replica was used to decorate the crowns of the Pharaohs (Belluccio & Johnson, 1992). In ancient Greek too, Asclepius the demi-god of medicine carried a pole with two snakes entwined on it (Wilcox & Whitham, 2003). The Greek mythology associates snake with being deadly and dangerous. The infamous Medusa had snakes in place of hair (Graves, 1990). In Hindu mythology, the cobra occupies the revered position i.e around Lord Shiva's neck and is protected from enemy, eagle or garuda (Lochtefeld, 2002). In ancient Chinese astrology, the snake represents a month in the Chinese zodiac calendar (Eberhard, 2006).

The scientific classification of snake is shown below in Figure 1.1. The phylogeny of snakes have gone through many updates through the years. The latest update uses snake classifications by Pyron et al (2011). This classification is available in Appendix A.

Globally, over 3400 species of snakes have been documented (Uetz & Hosek, 2015a). From that, peninsular Malaysia has over 141 known species of land and sea snakes. Of these, 26 species of land snakes (Das, Ahmed, & Liat, 2015) and 21 species of sea snakes are venomous (Lim, 1982). The medically significant venomous snakes