



**SUDAN UNIVERSITY OF SCIENCE & TECHNOLOGY  
COLLEGE OF GRADUATE STUDIES**

**ASSESSMENT OF THE POTENTIAL EFFICACY OF  
BEE VENOM AS AN ANTILEISHMANIAL  
CHEMOTHERAPEUTIC (*IN VITRO*)**

**BY:**

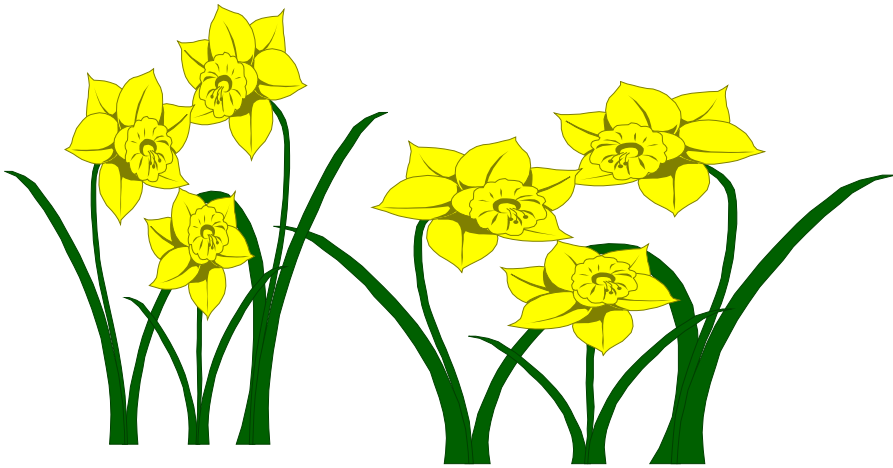
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*A thesis submitted in partial fulfillment of  
MSc. Degree in Clinical Biochemistry*

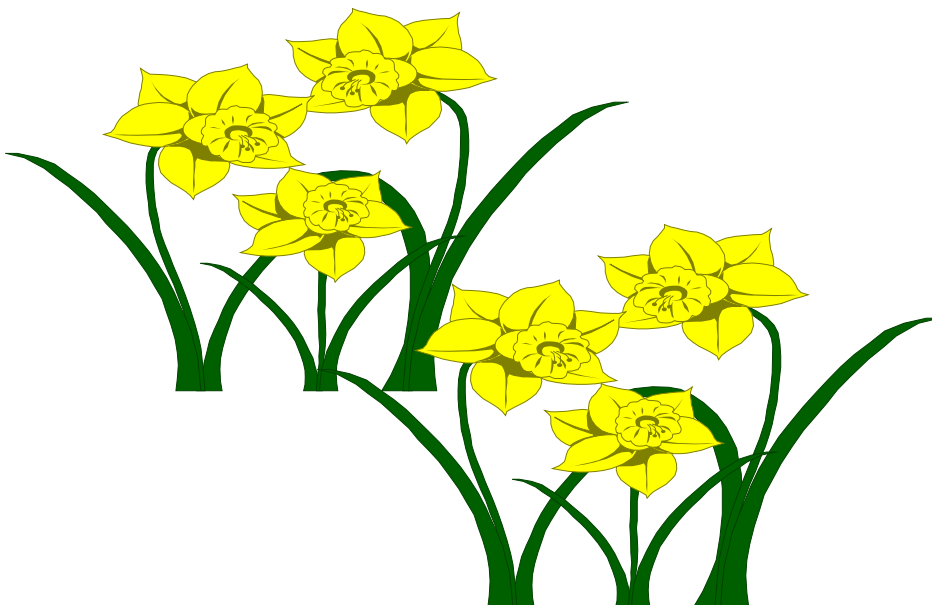
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**2005**



# Dedication

To my dear parents, sisters  
and brothers.  
With my best regards, respect  
and love



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال الله تعالى

وَأَوْحَىٰ رَبُّكَ إِلَى النَّحْلِ أَنِ اتَّخِذِي مِنَ الْجِبَالِ بُيُوتًا وَمِنَ الشَّجَرِ وَمِمَّا يَعْرِشُونَ (68) ثُمَّ كُلِي مِن كُلِّ الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلًّا يَخْرُجُ مِنْ بُطُونِهَا شَرَابٌ مُّخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاءٌ لِلنَّاسِ إِنَّ فِي ذَلِكَ لَآيَةً لِّقَوْمٍ يَتَفَكَّرُونَ (69) وَاللَّهُ خَلَقَكُمْ ثُمَّ يَتَوَفَّاكُمْ وَمِنكُم مَّن يَردُّ إِلَىٰ أَرْدَلِ الْعُمْرِ لِكَيْ لَا يَعْلَمَ بَعْدَ عِلْمٍ شَيْنًا إِنَّ اللَّهَ عَلِيمٌ قَدِيرٌ (70)

صدق الله العظيم

سورة النحل الآيات 68-70

## DECLARATION

The work prescribed in this thesis has been conducted by the undersigned in the Department of Clinical Biochemistry at the College of Medical Laboratory Science, Sudan University for Science & Technology.

It has not been previously accepted in substances and is not being concurrently submitted in candidature for any other degree.

**Signature**                      **(Candidate)** .....

**Signature**                      **(Supervisor)** .....

Date June 2005

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## ملخص الدراسة

اجريت هذه الدراسة على سم النحل بغرض قياس مدى كفاءته كمضاد لطفيل الليشمانيا دونافانى الذى يصيب الانسان . كما ان كثير من الدراسات اثبتت مؤخرامقاومة الطفيل لعدد من انواع الادويه العلاجيه المستخدمه . اجريت التجارب باستخدام الطفيل *L. donovani archabadi* Mon 82 الذى تم تربيته فى نوعين من اللاوساط الغذائية وهما Hamin agar و NNN فى درجة حراره 25م لمدة اسبوعين . وجد ان معدل نمو الطفيل فى الوسط الغذائى Haemin agar اسرع من نموه فى الوسط الغذائى NNN . وقد اجريت تجربه على طفيل الليشمانيا الذى تم اخذ 50 ميكروليتر منه بعد ضبط تركيزه الى  $(3 \times 10^6 \text{ X})$  خليه, و اضافته الى تراكيز مخففه من سم النحل بمعدل التخفيف 10 وتتراوح ما بين  $175 \times 10^{-3}$  الى  $0.0017 \times 10^{-3}$  ميكروجرام /مليتر فى درجة حراره 25 لمدة 24 ساعه . وقد لوحظ ان اعلى معدل تثبيط الطفيل <70% عند تركيز اعلى من  $110^{-3} \text{ X}$  ميكروجرام /مليتر, وعند القيام باعادة تجربه بمعدل تخفيف يساوى 3 وجد ان اعلى معدل تثبيط للطفيل عند تركيز  $19 \times 10^{-3}$  ميكروجرام /مليتر. وعند استخدام التحليل الاحصائى Probit regression analysis لحساب التركيز ذو الفعالية المتوسطة ( $IC_{50}$ ) وجد انه يعادل  $3 \times 10^{-3}$  ميكروجرام /مليتر ومقارنة مع دواء البنتوستام الذى تبلغ  $41.7$   $IC_{50}$  ميكروجرام /مليتر . مما يثبت درجة فعالية سم النحل . كما قامت الدراسة ايضا بقياس مدى سمية سم النحل مقارنة مع البنتوستام على على الخلايا الليمفاوية المستخلصة من دم الإنسان تحت نفس الظروف بمعدل التخفيف 10 تحت درجة 37 لمدة 48 ساعه . وقد لوحظ ان هنالك درجة سميته تبلغ 40% عند تركيز اعلى من  $1 \times 10^{-3}$  ميكروجرام /مليتر مقارنة مع البنتوستام الذى ليس له درجة سميته حتى عند تركيز 500 ميكروجرام /مليتر بينما اظهرت الدراسة ان هنالك زيادة فعالية ( $P < 0.05$ ) فى معدل انقسام الخلايا الليمفاويه يتراوح ما بين (50%-100%) عند تركيزات تتراوح ما بين (0.1-0.001) ميكروجرام /مليتر.

## ABSTRACT

This study essentially evaluated the antileishmanial activity and toxicity of the bee venom using *in vitro* techniques. The study also compared *in vitro* the maintenance of *L. donovani* promastigotes in 2 types of culture media, 3N and Hamin- agar media .The preliminary *in vitro* examination of the potential antileishmanial effect of bee venom upon *L. donovani* promastigotes when treated with 10-fold dilutions of this chemotherapeutic agent ( $175 \times 10^{-3}$  -  $0.0017 \times 10^{-3} \mu\text{g/ml}$ ) for 24h at  $25\text{C}^\circ$ . The highest response of growth inhibition ( $>70\%$ ) was obtained when the parasite subjected to bee venom concentrations  $>1.0 \times 10^{-3} \mu\text{g/ml}$  but a total inhibition of parasite population growth was only recorded at concentration  $175 \times 10^{-3} \mu\text{g/ml}$ .  $\text{IC}_{50}$  was calculated as  $3.0 \mu\text{g/ml}$ .

The lymphocyte proliferation assay for toxicity was used to compare the bee venom toxicity with Pentostam (Wellcome, UK), a standard antileishmanial drug. The cytotoxic effect of bee venom has been expressed as the mean percent inhibition of cell proliferation after 48h treatment with bee venom. About 40% inhibition of lymphocyte proliferation was observed when the lymphocytes were subjected *in vitro* to the highest concentration of this therapeutic agent ( $175 \times 10^{-3} \mu\text{g/ml}$ ) , Pentostam had no effect even at maximum tested concentration  $500 \mu\text{g/ml}$  whereas bee venom appeared to have some toxicity at concentrations  $<0.1 \times 10^{-3} \mu\text{g/ml}$  only. On the other hand treatment of PMBC with low concentrations ( $0.001$ - $0.1 \times 10^{-3} \mu\text{g/ml}$ ) of bee venom significantly stimulated PMBC proliferation ( $P < 0.05$ ).



## ACKNOWLEDGMENT

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