

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology  
(SUST)**

**College of Graduate Studies**

**Screening of the Efficacy of  
Some Traditional Herbal Drugs for  
Treatment of *Hymenolepis diminuta*  
Infection in Rats.**

**مسح فاعلية بعض النباتات التقليدية في علاج  
اصابات الهيمنوليس ديمينوتا في الفئران**

**(A thesis Submitted for the Fulfillment of the requirements of M.Sc. degree, in  
Medical Laboratory Sciences, Parasitology and Medical Entomology).**

**By:**

Hussien Sharif Siddig.

B.Sc. (2003) in Medical Laboratory Sciences, Al-neelain  
University.

**Main supervisor:**

Professor Hassan Abdelaziz Musa.

Secretary, Academic Affaires

(The National Ribat University).

**Co-supervisor:**

Professor Ahmed Ali Ismail.

Collage of Vet. Med. & Anim. Prod. (SUST).

(January 2009)



قال تعالى:

وَمَا أُوتِيتُمْ مِّنَ الْعِلْمِ إِلَّا قَلِيلًا ﴿٨٥﴾

﴿٨٥﴾

صدق الله العظيم

الآية 85

سورة الإسراء  
الجزء الخامس عشر  
صفحة 290  
القرآن الكريم

# **Dedication**

To the soul of my mother.

To my father, for his love and  
patience.

To my sisters and brothers for their  
understanding.

To my Teachers in all fields of Science  
throughout my life.

To my Supervisors, for their  
continuous support.

To my Colleagues, for their  
encouragement.

And to all those who helped me in  
one way or another.

I dedicate this work.

# **ACKNOWLEDGEMENT**

Praise Allah, the almighty (Most gracious most Merciful) who gave me the health, the strength, and patience to complete this work.

I would like to express my sincere gratitude and appreciations to my supervisors, Professor Hassan Abdelaziz Musa and Professor Ahmed Ali Ismail for their intensive advises, encouragement and continuous support during this study which might have not been completed without their supervision. My deep appreciation to Dr. Shamsoon khames kafi: The Dean, Faculty of Medical Laboratory Sciences, The National Ribat University, for his keen interest and encouragement. I would also like to thank Dr. Abdalla Mohamed Ibrahim, Faculty of Veterinary Science, University of Bahr-Elgazal (UBG) for his unlimited help and technical advices. My deep appreciation to Professor Ahmed Abdelraheem Gameel, Head Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum for his technical advice and coaching in the histopathology part of this study.

I gratefully appreciate the frequent assistance afforded by the staff members and technicians in the Medicine and Surgery Section, College of Veterinary Medicine and Animal production, Sudan University of Science and Technology (CVMA/SUST). The assistance in the statistical analysis rendered by Dr. Mohamed Tag-Eddin was a vital contribution to the analysis of the results of this study. Deeper appreciation and compassion to my family and friends who accompanied me through the good and bad times, for their patience, compassion and help.

# ABSTRACT

*Hymenolepis nana* (human infecting tapeworm) and *H. diminuta* (rodent infecting tapeworm) are the most common *cestodes* of man. They are prevalent in areas of poor hygiene and sanitation; especially in the warm and arid countries. They cause most of the non-specific bowel disturbances, and severe symptoms due to intestinal autoinfection and cysticercosis.

The present study was designed to study the toxicity and curative efficacy of *Amaranthus viridis*, *Cucurbita maxima*, *Hagenia abyssinica*, and *Balanites aegyptiaca* medicinal plants compared to the curative efficacy of Niclosamide for the treatment of *Hymenolepis* spp infections in experimental rat.

Faecal samples were collected from 250 Albino rats and screened for *Hymenolepis diminuta* natural infection using direct wet mount stool preparation, Formol-Ether concentration technique and Zinc Sulphate flotation technique it was found Formal-Ether concentration technique was the most sensitive technique in the diagnosis of *Hymenolepis spp* infections. The direct and indirect life cycles of *Hymenolepis diminuta* in experimental rats were studied. It was not possible to establish the direct lifecycle. However the Indirect Lifecycle of *Hymenolepis diminuta* was successfully established in the rat, with a prepatent period of 21 days.

There were no signs of toxic effect on the rats due to administration of any of the four tested medicinal plants and there was no significant ( $p > 0.05$ ) difference between the treated and control groups in the body and organ weights, haematological and biochemical parameters. Histopathological examination of the organs did not reveal any abnormalities.

*Hagenia abyssinica* seeds given at a concentration of 25% (w/w) in food or 25% (w/v) in water was very effective in the treatment of *Hymenolepis diminuta* infection in rats. Egg

reduction (100%) was highly significant ( $p < 0.01$ ) as compared to that of the untreated control group of rats (zero%), its deparasitization was

similar to Niclosamide. *Cucurbita maxima* seeds in food or water were active in treatment of *Hymenolepis diminuta* infection in rats. Egg reduction% was 100% in food and water and Deparasitization% was 80% in food and 40% in water both of them showing a significant ( $p < 0.05$ ) difference from those of the untreated control group (zero %).

*Amaranthus viridis* leaves given at a concentration of 25% (w/w) in food and 25% (w/v) in water exhibited a very weak efficacy. It did not reduce eggs in either water or food significantly as compared to control ( $p > 0.05$ ). The deparasitization activity of this plant in food (20%). and water (35%) were not significant.

*Balanites aegyptiaca* seeds (Flesh & kern) given at a concentration of 25% (w/w) in food and 25% (w/v) in water, were not effective in treatment of the infection in rats. Egg counts and deparasitization in food and water, were not significantly ( $p > 0.05$ ) different from those of the untreated control group. Our conclusion was that ***Hagenia abyssinica*** was the most active plant of this group in the treatment of *Hymenolepis diminuta* infection in rats.



# ملخص

أديدان التي تصيب الانسان (هيمنولبس نانا) و التي تصيب الفئران (هيمنولبس ديمينوتا) هي من أكثر الديدان الشريطية إصابة للإنسان , وهي تنتشر في المناطق التي تفتقر لإصحاح البيئة خاصة الاقطار الحارة و القاحلة, كما أنها تسبب اغلب إضطرابات الأمعاء الناتجة عن العدوى الذاتية و تكاثر الطفيل داخل الامعاء و إصابة الأنسجة بالكيسات المذنبة (cysticercoids).

صمم هذا البحث لدراسة الفعالية العلاجية والسمية لأربعة نباتات طبية وهي أمارانسس فردس, كيوكوربيتا ماكسيما, هاجينيا أبيسينيكا وبالانايتس إجييتيكا ومقارنتها بفعالية عقار نيكوساميد, وذلك لعلاج الإصابات بديدان من نوع الهمنولبس في فئران التجارب.

عند جمع عينات من 250 فأر وفحصها باستخدام المسحة السائلة المباشرة لعينة البراز , تفتقن الفورمال-إيثر للتركيز وتفتقن الزنك-سلفات للتركيز بالطفو, وجد ان تفتقن الفورمال-إيثر للتركيز هي أكثر الإختبارات حساسية في الكشف عن إصابة الفئران بهذه الديدان.

أيضاً لقد تمت دراسة دورات الحياة المباشرة وغير المباشرة لديدان هيمنولبس ديمينوتا في فئران التجارب حيث أنه لم يكن ممكناً تثبيت دورة الحياة المباشرة لهذه الديدان في فئران التجارب. ولكن نجد أنه قد تم تثبيت دورة حياتها غير المباشرة في فئران التجارب بنجاح وذلك بعد فترة حضانة دامت 21 يوماً.

لم تكن هناك اي علامات دالة على أثر سمية على الفئران نتيجة لتعاطي أي من النباتات قيد الدراسة بين المجموعات المعالجة ومجموعة الضبط غير المعالجة في ( $p > 0.05$ ) كما أنه لم يكن هناك فرق معتبر أوزان الجسم والأعضاء والمعايير الدموية والبيوكيميائية. وعلى ذات النحو فان إختبارات امراض الانسجة للأعضاء لم تظهر أي اثر غير طبيعي في هذه الانسجة.

بذور نبات هاجينيا أبيسينيكا أعطيت للفئران بتركيز 25% في الطعام أو 25% في الماء, حيث وجد أنها فعالة جداً في علاج إصابات الهمنولبس ديمينوتا في الفئران حيث ان نسبة انخفاض عدد البيض (100%) كانت ذات فرق معتبر ( $p < 0.01$ ) عن مجموعة التحكم (صفر%) وكذلك نسبة الديدان المطرودة كانت مشابهة لفعالية عقار نيكوساميد.

وجد أيضاً أن بذور نبات كيوكوربيتا ماكسيما أعطيت بتركيز 25% في الطعام أو 25% في الماء فعالة في علاج إصابات الهمنولبس ديمينوتا في الفئران حيث أن نسبة انخفاض عدد البيض (في الطعام وفي الماء 100%) وكذلك نسبة الديدان المطرودة (80% في الطعام) (40% في الماء) كانت ذات فرق معتبر ( $p < 0.01$ ) عن مجموعة التحكم (صفر%) غير المعالجة.

أما أوراق نبات أمارانسس فردس عندما أعطيت بتركيز 25% في الطعام و 25% في الماء وجد أنها ذات فعالية ضعيفة جدا في علاج إصابات الهيمنولبس ديمينوتا في الفئران حيث أنها لم تخفض عدد البيض

في الطعام أوفي الماء بصورة جيدة م مقارنة بمجموعة التحكم. وكذلك نسبة الديدان المطرودة (20% في الطعام) ( 35% في الماء) لم تكن بال قدر المطلوب.

أيضا نجد أن غلاف ونواة بذور نباتبالانايتس أجيتيكا عندما أعطيت بتركيز 25% في الطعام أو 25% في الماء وجد أنها غير فعالة في علاج إصابات الهيمنولبس ديمينوتا في الفئران وذلك لأن عدد البيض و نسبة الديدان المطرودة كانتا غير ذوات فرق معتبر ( $p > 0.05$ ) عن مجموعة التحكم عند نهاية التجربة. خلصت هذه الدراسة إلى أن نبات الهيمنولبس ديمينوتا هو النبات الأكثر فعالية في علاج إصابات الهيمنولبس ديمينوتا في فئران التجارب.

## List of contents

<b>Dedication</b>	i
<b>Acknowledgement</b>	ii
<b>Abstract</b>	iii
<b>Arabic abstract</b>	v
<b>List of contents</b>	vii
<b>List of Tables</b>	xii
<b>List of Figures</b>	xiii
<b>List of Plates</b>	xiv
<b>Chapter one: Introduction and objectives</b>	
<b>1.1. Introduction</b>	1
<b>1.2. Rationale</b>	2
<b>1.3. Objectives</b>	3
1.3.1. Over all objective	3
1.3.2. Specific objectives	3
<b>Chapter Two: Literature Review</b>	
<b>2.1. <i>Hymenolepis species</i></b>	4
<b>2.2. Classification.</b>	5
<b>2.3. <i>Hymenolepis nana</i>.</b>	5
2.3.1. Geographical distribution	6
2.3.2. Life cycle and transmission	6
2.3.3. Morphology	7
2.3.4. Clinical disease	7
<b>2.4. <i>Hymenolepis diminuta</i></b>	10
2.4.1. Geographical distribution	10
2.4.2. Beetle Manipulation	10
2.4.3. Life cycle and transmission	10
2.4.4. Morphology	11
2.4.5. Clinical disease	11
<b>2.5. Laboratory diagnosis of <i>Hymenolepis species</i></b>	14
<b>2.6. Treatment of <i>Hymenolepis species</i> infections</b>	14
2.6.1. Using anticestodal medicaments	14
2.6.2. Conventional anticestodal medicaments	15
2.6.2.1. Niclosamide (Yomesan; Bayer, Leverkusen) drug	15
i) Mode of action	15
ii) Dosage in man	15
iii) Side Effects	16
2.6.2.2. Praziquantel	16

i)	Mode of action	16
ii)	Dosage in man	17
iii)	Side Effects	17
2.6.2.3.	Other anticestodal medicaments	18
2.6.3.	Using medicinal plants	18
2.6.3.1.	Anthelmintic activity	19
2.6.3.2.	Anticestodal activity	21
2.6.4.	<i>Amaranthus viridis</i> (Lisan Altair)	22
2.6.4.1.	Physical characteristics	22
2.6.4.2.	Scientific classification	23
2.6.4.3.	Edible uses	23
2.6.4.4.	Medical uses	24
2.6.5.	<i>Balanites aegyptiaca</i> (Higlig, Lalob)	24
2.6.5.1.	Physical characteristics	24
2.6.5.2.	Scientific classification	25
2.6.5.3.	Edible and functional uses	26
2.6.5.4.	Medical uses	26
2.6.6.	<i>Cucurbita maxima</i> (Pumpkins, Garaa)	28
2.6.6.1.	Physical characteristics	28
2.6.6.2.	Scientific classification	29
2.6.6.3.	Edible uses	29
2.6.6.4.	Medical uses	30
2.6.7.	<i>Hagenia abyssinica</i> (Kosso)	31
2.6.7.1.	Physical characteristics	31
2.6.7.2.	Scientific classification	32
2.6.7.3.	Medical uses	32
<b>Chapter Three:</b>	<b>Material and methods</b>	
<b>3.1.</b>	<b>Experimental animals</b>	35
3.1.1.	Rats ( <i>Rattus norvigicus</i> )	35
3.1.2.	Selection of faecal examination technique.	35
3.1.3.	Maintenance of rats	35
3.1.4.	Insects	36
<b>3.2.</b>	<b>Establishment of the life cycle of <i>Hymenolepis</i></b>	37
	<b>species in the rat</b>	
3.2.1.	Preparation of rats for life-cycle establishment	37
3.2.2.	Direct life cycle	38
3.2.2.1.	Source of infective stage ( <i>H. diminuta</i> )	38
3.2.2.2.	Determination of the prepatent period	38
3.2.3.	Indirect life cycle	38
3.2.3.1.	Source of infective stage	38
3.2.3.2.	Source of flour beetles	38
3.2.3.3.	Feeding infective stage to beetles	39
3.2.3.4.	Examining beetles for cysticercoids	39
3.2.3.5.	Feeding infected beetles to rats	39
3.2.3.6.	Determination of the prepatent period	40

<b>3.3.</b>	<b>Herbal treatment of <i>Hymenolepis spp</i> Infection In rats</b>	<b>40</b>
3.3.1.	Source of tapeworms	40
3.3.2.	Medicinal plants	40
3.3.2.1.	<i>Amaranthus viridis</i>	40
3.3.2.2.	<i>Balanites aegyptiaca</i>	41
3.3.2.3.	<i>Cucurbita maxima</i>	41
3.3.2.4.	<i>Hagenia abyssinica</i>	41
3.3.2.5.	Niclosamide	41
3.3.3.	Experimental design	44
3.3.4.	Stool examination	45
3.3.4.1.	Macroscopical examination of stool	45
3.3.4.2.	Microscopical examination of stool	46
3.3.4.2.1.	Direct saline stool preparation	46
3.3.4.2.2.	Zinc sulphate flotation technique	46
3.3.4.2.3.	Formol-Ether Concentration Technique	47
3.3.5.	Examination of treatment groups	48
3.3.5.1.	Stoll's technique for counting eggs	48
3.3.5.2.	Necropsy and worms recovery	49
3.3.6.	Toxicity testing of the crude plants	51
<b>.3.3.6.1</b>	Observations	51
<b>.3.3.6.2</b>	Haematology	51
<b>.3.3.6.3</b>	Clinical Biochemistry	51
<b>.3.3.6.4</b>	Tissue Collection and histology	51
3.3.7.	Chemo therapeutic trials	52
3.3.7.1.	Determination of egg reduction percentage	52
3.3.7.2.	Determination of the treatment efficacy percentage	52
3.3.8.	Statistical analysis	53
<b>Chapter four:</b>	<b>Results</b>	
<b>4.1.</b>	<b>Evaluation of faecal examination techniques</b>	<b>54</b>
<b>4.2.</b>	<b>Establishment of the life-cycle of <i>Hymenolepis diminuta</i> in the rat</b>	<b>55</b>
4.2.1.	Establishment of the direct life-cycle of <i>H diminuta</i>	55
4.2.2.	Establishment of the indirect life-cycle of <i>H diminuta</i>	56
4.2.2.1.	Infection of the intermediate host (Flour beetles <i>Tribolium confusum</i> )	56
4.2.2.2.	Infection of the definitive host (Albino rats)	56
<b>4.3.</b>	<b>Herbal treatment of <i>Hymenolepis spp</i> infection in rats</b>	<b>58</b>
4.3.1.	Toxicity testing of the crude plants	58
<b>.4.3.1.1</b>	General observations	58
<b>.4.3.1.2</b>	Haematology	58
<b>.4.3.1.3</b>	Biochemistry	58

.4.3.1.4	Histological studies	58
4.3.2.	Treatment using crude plants	68
.4.3.2.1	Niclosamide drug	68
.4.3.2.2	<i>Amaranthus viridis</i>	69
.4.3.2.3	<i>Balanites aegyptiaca</i>	70
.4.3.2.4	<i>Cucurbita maxima</i>	72
.4.3.2.5	<i>Hagenia abyssinica</i>	74
Chapter five:	<b>Discussion</b>	82
<b>Conclusion</b>		86
<b>Recommendations</b>		87
<b>References</b>		88

## List of tables

.3.1	.Formulation of Rat's food	36
.4.1	Comparison of the sensitivity of Formol-ether concentration with Zinc sulphate flotation and Wet .preparation techniques	54
.4.2.1	The effects of administration of crude plants, 50% (w/w) with food for 15 days on food consumption (g /group/day) .in rats	59
.4.2.2	The effects of administration of crude plants, 50% (w/w) .with food for 15 days on rat body weight/ g	59
.4.2.3	The effects of administration of crude plants, 50% (w/w) .(with food on organ weight/g in rats (Oneway anova test	60
.4.2.4	The effects of administration of crude plants, 50% (w/w) with food on haematological parameters in rats (Oneway .(anova test	61
.4.2.5	The effects of administration of crude plants, 50% (w/w) with food on differential WBC count in rats (Oneway anova .(test	62
.4.2.6	The effects of administration of crude plants, 50% (w/w) with food on liver function profiles in rats (Oneway anova .(test	63
.4.2.7	The effects of administration of crude plants, 50% (w/w) with food on renal function, minerals, electrolytes profiles .(and glucose level in rats (Oneway anova test	64
.4.2.8	Multiple comparisons of egg counts of <i>H diminuta</i> in rats treated with 4 crude plants, 25% (w/w) in food and 25% .((w/v) in water (Post Hoc Test	77
.4.2.9	Summarizes the results of eggs reduction% 15 days post treatment of <i>H diminuta</i> infections with 4 crude plants, .(25% (w/w) in food and 25%(w/v) in water (Post Hoc Test	78
4.2.10	Summarizes the results of deparasitization% activity 15 days post treatment of <i>H diminuta</i> infections with 4 crude plants, 25% (w/w) in food and 25%(w/v) in water (Post .(Hoc Test	80

## List of figures

.2.1	.Life-cycle and transmission of <i>Hymenolepis nana</i>	9
.2.2	.Life-cycle and transmission of <i>Hymenolepis diminuta</i>	13
.4.1	Histogram representation of the sensitivity of Formol-Ether concentration, Zinc Sulphate flotation and wet preparation techniques	55
.4.2	Increase or decrease in the mean EPG faeces ( $\text{Log}_{10}$ ) in rats infected with <i>H diminuta</i> and treated with crude <i>Amaranthus viridis</i> leafs 25% (w/w) in food or 25% (w/v) in water	70
.4.3	Increase or decrease in the mean EPG faeces ( $\text{Log}_{10}$ ) in rats infected with <i>H diminuta</i> and treated with crude <i>Balanites aegyptiaca</i> seeds flesh 25%(w/w) in food and 25%(w/v) in water	71
.4.4	Increase or decrease in the mean EPG faeces ( $\text{Log}_{10}$ ) in rats infected with <i>H diminuta</i> and treated with crude <i>Balanites aegyptiaca</i> seeds kern, 25% (w/w) in food and 25%(w/v) in water	72
.4.5	Increase or decrease in the mean EPG faeces ( $\text{Log}_{10}$ ) in rats infected with <i>H diminuta</i> and treated with crude <i>Cucurbita maxima</i> seeds 25% (w/w) in food and 25% (w/v) in water	73
.4.6	Increase or decrease in the mean EPG faeces ( $\text{Log}_{10}$ ) in rats infected with <i>H diminuta</i> and treated with crude <i>Hagenia abyssinica</i> seeds 25% (w/w) in food and 25% (w/v) in water	75
.4.7	Histogram summary of the results of Eggs reduction% 15 days post treatment of <i>H diminuta</i> infections with 4 crude plants, 25% (w/w) in food and 25%(w/v) in water	79
.4.8	Histogram summary of the results of deparasitization% activity 15 days post treatment of <i>H diminuta</i> infections with 4 crude plants, 25% (w/w) in food and 25%(w/v) in water	81



## **List of plates**

.a.2.1	( <i>Hymenolepis nana</i> (Embryonated egg	8
.b.2.1	( <i>Hymenolepis nana</i> (Scolex	8
.c.2.1	( <i>Hymenolepis nana</i> (Mature segments	8
.d.2.1	( <i>Hymenolepis nana</i> (Cysticercoid	8
.a.2.2	( <i>Hymenolepis diminuta</i> (Embryonated egg	12
.b.2.2	( <i>Hymenolepis diminuta</i> (Scolex	12
.c.2.2	( <i>Hymenolepis diminuta</i> (Mature segments	12
.d.2.2	( <i>Hymenolepis diminuta</i> (Cysticercoid	12
.2.3	.( <i>Amaranthus viridis</i> (Tree	24
.a.2.4	.( <i>Balanites aegyptiaca</i> (Tree	27
.b.2.4	( <i>Balanites aegyptiaca</i> (Green fruits	27
.c.2.4	( <i>Balanites aegyptiaca</i> (Dry fruits	28
.2.5	.( <i>Cucurbita maxima</i> (Tree	31
.a.2.6	.( <i>Hagenia abyssinica</i> (Tree	34
.b.2.6	.( <i>Hagenia abyssinica</i> (Flowering branch	34
.3.1	Albino rats kept in wide metal cages with saw dust as .bedding	37
.3.2	.Adult flour beetle <i>Tribolium confusum</i>	37
.3.3	:Glass jars containing wheat flour and brewer's yeast	39
.3.4	.( <i>Amaranthus viridis</i> (leafs	42
.a.3.5	.( <i>Balanites aegyptiaca</i> (seeds	42
.b.3.5	.( <i>Balanites aegyptiaca</i> (Kern	43
.c.3.5	( <i>Balanites aegyptiaca</i> (Thick flesh salary	43
.3.6	.( <i>Cucurbita maxima</i> (seeds	43
.3.7	.( <i>Hagenia abyssinica</i> (Seeds	44
.3.8	.Mc Master Slide	49
.a.3.9	The intestine of albino rat was removed	50
.b.3.9	The intestine of albino rat was opened longitudinally in Petri	50