# Sudan University of Science and Technology

### College of Graduate studies

Anti-inflammatory Activity with Pharmacological and Sub-chronic Toxicity
Studies of some Medicinal Plants Extracts

دراسة الخواص المضادة للإلتهاب ، الدراسات الدوائية و السمّية لمستخلصات بعض النباتات الطبية

By

Asia Abdallah Elsaeed Amir

(B.V.Sc., M.V.Sc., U of K)

A Thesis Submitted in Fulfillment of the Requirements for The Degree of Philosophy of Doctorate (Ph.D.) in Veterinary Medicine

( Pharmacology )

Supervisor:

Professor . Dr : Osman Saad Ali Mohammed

Department of Pharmacology and Toxicology , College of Veterinary Medicine Sudan university of science and Technology

Co-supervisor:

Dr. Reem Hassan Ahmed

Department of Pharmacology and Toxicology , Medicinal and Aromatic Plant Research Institute ( MAPRI )

March 2015

#### Acknowledgments

I would like to express my sincere appreciation to my supervisor, Professor Osman Saad and co-supervisor Dr. Reem Hassan Ahmed, for their valuable guidance encouragement and continuous advice throughout the course of this study.

Iam also greatly indepted to Professor Abd ElWahab Hassan Mohamed for his valuable assistance and advice and Professor AbdElrahim Idris for his invaluable efforts.

I would particularly like to express my thanks to Professor Ahmed Mohammed Gameel, Department of Pathology Faculty of Veterinary Medicine university of Khartoum who generously contribute his time and efforts to help me.

Special thanks go to the academic and technical staff of Pharmacology and Toxicology laboratory, Medicinal and Aromatic Plant Research Institute for their unlimited help, advice especially with the practical aspects of the study and the pharmacological techniques.

The assistance of the Herbarium Academic and technical staff, Medicinal and Aromatic Plant Research Institute, for the collection of plants and classification of the plants by Dr. Haidar Abd ELGadir is deeply appreciated.

My thanks are also to the academic and technical staff of Plant Chemistry Department , Medicinal and Aromatic Plant Research Institute , for the help , advice and extraction of the plants .

My thanks to Mohammed ELdaw for his rearing laboratory animals.

Warm appreciations go to my husband and my daughters and sons for the constant support and the encouragement and for making everything wonderful, specially my lovely daughter Dr.Mai Alauddin.

# **Table of Contents**

List of Tables

List of Figures

List of Abbreviations

Abstract

Arabic Abstract

# LIST OF TABLES

	Page	e no.
Table 1.1: Wild medicinal and aromatic plants of the Sudan		5
Table 1.2 : Cultivated medicinal and aromatic plants of the Sudan		8
Table 2.1: Percentage yield of plants extracts		36
<b>Table 3.1</b> : Anti-inflammatory effect of crude ethanolic extract of <i>Aerva javanica</i> on the rat paw oedema at doses of 250 and 500 mg/kg	а	53
Table 3.2 : Effect of treatment with crude ethanolic extract of	56	
Aerva javanica on body weight and body weight gain at 250		
and 500 mg/kg/day doses for 2 and 4 weeks		
Table 3.3 : Effect of treatment with crude ethanolic extract	57	
of Aerva javanica on haematological parameters at 250		
and 500 mg/kg/day doses for 2 and 4 weeks		
Table 3.4 : Effect of treatment with crude ethanolic extract	58	
of Aerva javanica on plasma biochemical parameters at 250		
and 500 mg/kg/day doses for 2 and 4 weeks		
Table 3.5 : Anti-inflammatory effect of crude ethanolic extract		60
of Amaranthus viridis on the rat paw oedema at doses of 250		
and 500 mg/kg		
Table 3.6 : Effect of treatment with crude ethanolic extract	65	
of Amaranthus viridis on body weight and body weight gain		
at 250 and 500 mg/kg/day doses for 2 and 4 weeks		
Table 3.7 : Effect of treatment with crude ethanolic extract	66	
of Amaranthus viridis on haematological parameters at 250		
and 500 mg/kg/day doses for 2 and 4 weeks		

Table 3.8 : Effect of treatment with crude ethanolic extract	67
of Amaranthus viridis on plasma biochemical parameters	
at 250 and 500 mg/kg/day doses for 2 and 4 weeks	
Table 3.9 : Anti-inflammatory effect of crude ethanolic extract	69
of Lepidium sativum on the rat paw oedema at doses of 250	
and 500 mg/kg	
Table 3.10 : Effect of treatment with crude ethanolic extract	73
of Lepidium sativum on body weight and body weight gain at	
250 and 500 mg/kg/day doses for 2 and 4 weeks	
Table 3.11 : Effect of treatment with crude ethanolic extract	74
of Lepidium sativum on haematological parameters at 250	
and 500 mg/kg/day doses for 2 and 4 weeks	
Table 3.12 : Effect of treatment with crude ethanolic extract	75
of Lepidium sativum on plasma biochemical parameters at	
250 and 500 mg/kg/day doses for 2 and 4 weeks	

# LIST OF FIGURES

Pag	e no
Fig 3.1 : Contraction effect of ethanolic extract of Aerva javanica at	54
different doses ( $1$ , $2$ , $4$ and $8$ mg/ml ) on isolated rabbit jejunum strip	
Fig 3.2 : Contraction effect of ethanolic extract of Aerva javanica at	55
different doses (1, 2, 4 and 8 mg/ml) on isolated rabbit jejunum strip	
; that blocked by atropine ( $5 \mu g/ml$ )	
Fig 3.3: Contraction effect of ethanolic extract of Amaranthus viridis at	62
different doses (1, 2 and 4 mg/ml) on isolated rabbit jejunum strip	
<b>Fig 3.4</b> : Contraction effect of ethanolic extract of <i>Amaranthus viridis</i> at different doses (1, 2 and 4 mg/ml) on isolated rabbit jejunum strip; that blocked partially by atropine (5 $\mu$ g/ml)	63
<b>Fig 3.5</b> : Contraction effect of ethanolic extract of <i>Amaranthus viridis</i> at different doses ( $1$ , $2$ and $4$ mg/ml $)$ on isolated rabbit jejunum strip ; that blocked by cyproheptidine ( $40~\mu g/ml$ )	64
Fig 3.6: Contraction effect of ethanolic extract of Lepidium sativum at	70
different doses (1, 2 and 4 mg/ml) on isolated rabbit jejunum strip	
Fig 3.7: Contraction effect of ethanolic extract of Lepidium sativum at	71
different doses ( $1$ , $2$ and $4~mg/ml~)~$ on isolated rabbit jejunum strip ; that blocked partially by atropine ( $5~\mu g/ml~)$	
Fig 3.8: Contraction effect of ethanolic extract of Lepidium sativum at	72
different doses ( $1$ , $2$ and $4$ mg/ml $)$ on isolated rabbit jejunum strip ; that blocked by cyproheptidine ( $40~\mu g/ml$ )	

#### **List of Abbreviations:**

ALP Alkaline phosphatase

ALT Alanine transaminase

AST Aspartate transaminase

CEE Crude ethanolic extract

EVM Ethnoveterinary medicine

GIT Gastrointestinal tract

Hb Haemoglobin

Hrs Hours

INF-Y Interferon - Y

IP Intraperitoneal

K Potassium

Kg Kilograms

MAC Membrane attack complex

MCH Mean corpuscular haemoglobin

MCHC Mean corpuscular haemoglobin concentration

MCV Mean corpuscular volume

mg milligrams

Na Sodium

PAMPs Pathogen associated molecular patterns

PCV Packed red cell volume

PRRs Pattern recognition receptors

R.B.C Red blood cells

S.E.D Standard error of deviation

TNF- $\alpha$  Tumour necrosis factor - $\alpha$ 

W.B.C White blood cells

#### Abstract

In the current study experiments were conducted to evaluate the possible antiinflammatory effects of Crude Ethanolic Extracts CEE of *Aerva javanica* whole plant , *Amaranthus viridis* whole plant and *Lepidium sativum* seeds in rats injected with Carrageenan so as scientifically appraise its ethnomedicinal use and to detect any side effects that may be associated with their use . The ethanolic extract of the three plants were studied on selected isolated tissue preparations and their sub-chronic toxicity studies were evaluated .

In the study , using a dose of 250 mg/kg of ethanolic extracts of the three plants was found to produce reduction or inhibition of the inflammation as 48.3% at 3 hrs for CEE of *Aerva javanica*. The CEE of *Amaranthus viridis* produced inhibition of inflammation as 40.3% at 3 hrs and the CEE of *Lepidium sativum* produced inhibition of inflammation as 38.6% at 3 hrs respectively .

The anti-inflammatory activity of the three plants was also confirmed by using the dose of  $500 \, \mathrm{mg} \, / \, \mathrm{kg}$  and the inhibition of inflammation was found as  $85.2 \, \%$  for the CEE of *Aerva javanica* at 3 hrs . The CEE of *Amaranthus viridis* produced inhibition of inflammation as  $70.4 \, \%$  at 3 hrs and the CEE of *Lepidium sativum* produced inhibition of inflammation as  $46.0 \, \%$  at 3 hrs .

The effect of CEE of the three plants on smooth muscle of rabbit jejunum were tested . The CEE of *Aerva javanica* whole plant showed contraction of the smooth muscle of rabbit jejunum . The contraction was blocked by atropine (  $5~\mu g$  / ml ) , antagonistic property . The CEE of *Amaranthus viridis* whole plant and the CEE of *Lepidium sativum* seeds showed a dose dependent ( 1,2,4~mg/ml ) stimulation of the isolated rabbit jejunum preparation , this stimulation was partially blocked by atropine ( $5~\mu g$  / ml ) , but was blocked by cyproheptadine ( $40~\mu g$  / ml ) , (a non-selective 5-HT blocker ) .

In the sub-chronic toxicity study , the result of haematological studies of the three plants , concerning haemoglobin ( Hb ) , packed cell volume ( PCV ) , mean corpuscular volume ( MCV ) , mean corpuscular haemoglobin ( MCH ) , mean corpuscular haemoglobin concentration ( MCHC ) , red blood cell count ( RBCs ) and white blood cell count ( WBCs ) showed no significant change from the control group for the two different doses at the two different periods of time

•

The result of the biochemical assays of the three plants, concerning liver function and the kidney function tests, the ethanolic extracts of the three plants showed no significant change from the control group for the two different doses at the two different periods of time.

Histopathological changes of the tissues of liver, kidneys, intestines, spleen and heart showed no histopathological changes were observed in the spleen and heart tissue, but there were congested vacuolated hepatocytes with dilated hepatic blood vessels and dilated liver sinusoids with perilobular and centrilobular hepatocytic necrosis.

The renal tubules were congested and dilated with variations in renal glomeruli size and many lobulated glomeruli tufts with dilated bowman space and focus of macrophage collection.

The intestinal villi and the intestinal mucosal epithelium were desquamated and the lumen contain RBCs and shreads of epithelial cells and WBCs.

#### ملخص الاطروحه

إن الادويه التقليدية المستعملة لعلاج الالتهاب و الاعراض المصاحبة ، اما ان تكون غالية الثمن أو سامة أو غير متوفرة مما نتج عنه الالتفات لاستعمال النباتات كبديل للأدوية المستعملة لمعاجة الأمراض . و عليه قد تم في هذا البحث إجراء تجارب عملية لاختبار فعالية المستخلصات الكحولية لنباتات راس الشايب ، لسان الطير كبير و بذور نبات حب الرشاد ضد الالتهاب في المختبر و على فئران التجارب التي تم حقنها بمادة الكاراجينان المحفزة للالتهاب ، و لمعرفة الاثار الجانبية المصاحبة لاستعمال هذه المستخلصات .

في هذه الدراسة تم اختبار المستخلص الكحولي لنباتات راس الشايب ، لسان الطير كبير و بذور حب الرشاد على أنسجه معزولة من أمعاء الارنب كما تمت دراسة و تقييم السمية لهذه المستخلصات .

و قد أوضحت التجارب المختبرية بأن استعمال جرعة 250 ملجرام / مل من المستخلصات الكحولية لنباتات راس الشايب ، لسان الطير كبير و بذور حب الرشاد نتج عنها نزول في مقياس درجة الالتهاب بعد مرور تلاتة ساعات من المعالجة بالمستخلص الكحولي لنبات راس الشايب 48.3 % و بالمستخلص الكحولي لنبات لسان الطير كبير 40.3 % و بالمستخلص الكحولي لبذور حب الرشاد 38.6 % على التوالى .

و تم تأكيد فعالية مستخلصات النباتات الثلاث في معالجة الالتهاب باستعمال جرعة 500 ملجرام / مل من المستخلصات الكحولية لنباتات راس الشايب / لسان الطير كبير و بذور حب الرشاد نتج عنها نزول في مقياس درجة الالتهاب بعد مرور ثلاثة ساعات من المعالجة بالمستخلص الكحولي لنبات راس الشايب 85.2% و بالمستخلص الكحولي لنبات لسان الطير كبير 70.4% و بالمستخلص الكحولي لبذور حب الرشاد 46.0% .

في هذه الدراسة تم اختبار المستخلص الكحولي لنباتات راس الشايب ، لسان الطير كبير و بنور حب الرشاد على أنسجه معزولة من أمعاء الارنب . لقد احدث مستخلص نبات راس الشايب انقباضا لامعاء الارنب و هذا الانقباض كان لمادة الاتروبين اثرا انعكاسيا عليه . أما المستخلص الكحولي لنبات لسان الطير كبير و بذور حب الرشاد اظهر تنشيطا متناسبا طرديا مع الجرعة على أمعاء الارنب المعزولة ، هذا الاثر الذي لم يستجب للاتروبين (استجابة جزئية) و لكن تم قفله نهائيا بواسطة مضاد السرتونين السبرو هيبتادين .

نتائج قياسات السمية للمستخلصات الكحولية لنباتات راس الشايب ، لسان الطير كبير و بذور حب الرشاد لم تظهر اي تغيير ملحوظ لقياس مكونات الدم المختلفة ، القياسات الكيميائية و السمية لاختبارات وظائف الكيد و الكلى .

في هذه الدراسة تمت معرفة تأثير المستخلصات الكحولية لنباتات راس الشايب ، لسان الطير كبير و بذور حب الرشاد على إمراضية انسحة الكبد ، الكلى ، الامعاء ، الطحال و القلب و قد اظهرت النتائج عدم حدوث أي تغييرات على أنسجة الطحال و القلب إلا أن هناك فجوات و تورم بالخلايا الكبدية مع توسع و تضخم الاوعية الدموية بها كما لوحظ توسع في جيبانيات الكبد و تغييرات نخرية حول و داخل نسيج الكبد . هناك احتقان و توسع النبيبات الكلوية مع تورم احواض النبيبات كما لوحظ , اختلافات في حجم الكبيبات الكلوية و الكثير من لمات الكبيبات المفصصة مع توسع في فراغات بومان و وجود بؤرة

لتجمع البلاعم . أما بالنسبة لنسيج الامعاء فقد لوحظ توسف الزغابات المعوية و الغشاء المخاطي المعوي , كما لوحظ وجود انحلال دموي به .

## **CHAPTER ONE**

## LITERATURE REVIEW

		Page No
1.1	Ethnoveterinary medicine	3
1.2	Uses of some wild medicinal and aromatic plants in Sudan	3
1.3	Uses of some cultivate medicinal and aromatic plants in Sudan	5
1.4	Some medicinal plants as potential source of anti-microbial agent	
1.5	Inflammation and anti-inflammatories	9
1.5.1	Causes of inflammation	9
1.5.2	Types of inflammation	11
1.5.3	Cell repair and wound healing	13
1.5.4	Anti-inflammatories	13
1.5.4.	1 Types of anti-inflammatories	14
1.6 A	nti-inflammatory plants	18
1.6.1	Family Amaranthaceae	19
1.6.1.	1 Aerva javanica	20
1.6.1.	1.1 Classification	20
1.6.1.	1.2 Distribution	20
1.6.1.	1.3 Botanical description	21
1.6.1.	1.4 Active constituents	21
1.6.1.	1.5 Medicinal folk-uses	22
1.6.1.	1.6 Pharmacological studies	23
1.6.1.	2 Amaranthus viridis	23
1.6.1.	2.1 Classification	24
1.6.1.	2.2 Distribution	24
1.6.1.	2.3 Botanical description	24

1.6.1.2.4 Active constituents	25
1.6.1.2.5 Medicinal folk-uses	25
1.6.1.2.6 Pharmacological studies	25
1.6.2 Family <i>Brassicaceae</i>	26
1.6.2.1 Lepidium sativum	27
1.6.2.1.1 Classification	28
1.6.2.1.2 Distribution	28
1.6.2.1.3 Botanical description	28
1.6.2.1.4 Active constituents	29
1.6.2.1.5 Medicinal folk-uses	29
1.6.2.1.6 Pharmacological studies	30
1.7 Neurotransmitters , receptors , stimulants , blockers and action	30

# **CHAPTER TWO**

# MATERIALS AND METHODS

	Page No
2.1 materials	34
2.1.1 plants	34
2.1.2 Samples collection	34
2.1.2.1 plants collection	34
2.1.3 Chemicals	34
2.1.3.1 Physiological salts	34
2.1.3.2 Solvents	35
2.1.3.3 Standard drugs	35
2.1.4 Apparatus	35
2.1.5 Laboratory animals	35
2.2 Methods	36
2.2.1 Method of extraction	36
2.2.1.1 Preparation of the ethanolic extract	36
2.3 Experiments	37
2.3.1 Anti-inflammatory activity of ethanolic extract	38
of Aerva javanica in Albino rats using rat paw oedema model	
2.3.2 Anti-inflammatory activity of ethanolic extract of	38
Amaranthus viridis in Albino rats using rat paw oedema model	
2.3.3 Anti-inflammatory activity of ethanolic extract of	39
Lepidium sativum in Albino rats using rat paw oedema model	

2.3.4 The effect of <i>Aerva javanica</i> plant extract on the	40
isolated rabbit jejunum motility	
2.3.5 The effect of Amaranthus viridis plant extract	40
on the isolated rabbit jejunum motility	
2.3.6 The effect of <i>Lepidium sativum</i> plant extract on the	41
isolated rabbit jejunum motility	
2.3.7 The toxic effects of ethanolic extract of Aerva javanica	42
in Albino rats	
2.3.8 The toxic effects of ethanolic extract of Amaranthus viridis	43
in Albino rats	
2.3.9 The toxic effects of ethanolic extract of <i>Lepidium sativum</i>	43
in Albino rats	
2.3.10 Blood samples	44
2.3.11 Preparation of the plasma	44
2.3.12 Blood and biochemical analysis	45
2.3.12.1 Glutamate Oxalate Transaminase (GOT) Aspartate	46
Transaminase ( AST )	
2.3.12.2 Glutamate Pyruvate Transaminase (GPT) Alanine	46
Transaminase ( ALT)	
2.3.12.3 Alkaline Phosphatase (ALP)	47
2.3.12.4 Total Protein	47
2.3.12.5 Albumin	48
2.3.12.6 Globulin	48
2.3.12.7 Bilirubin	48

2.3.12.8 Urea	48
2.3.12.9 Cholestrol	49
2.3.12.10 Sodium Determination	49
2.3.12.11 Potassium Determination	50
2.3.13 Histopathology examination	50
2.3.14 Statistical analysis	50

## **CHAPTER THREE**

## **RESULTS**

	Page No
Aerva javanica	
3.1.1 : Anti-inflammatory experiment	52
3.1.1.1 : Anti-inflammatory activity in a dose of 250 and	52
500 mg/kg of plant extract	
3.1.2 : Pharmacological studies	53
3.1.2.1 : Effect of <i>Aerva javanica</i> extract on isolated rabbit	53
jejunum strip	
3.1.3 : Sub-chronic toxicity study	55
3.1.3.1 : Effect of <i>Aerva javanica</i> whole plant ethanolic extract	55
on clinical signs and body weight findings at 250 and 500 mg/kg/day	
doses for 2 and 4 weeks	
3.1.3.2 : Haematological findings	56
3.1.3.3 : Plasma biochemical findings	57
3.1.3.4 : Histopathological findings	58
Amaranthus viridis	
3.2.1 : Anti-inflammatory experiment	59
3.2.1.1 : Anti-inflammatory activity in a dose of 250	59
and 500 mg/kg of plant extract	
3.2.2 : Pharmacological studies	61
3.2.2.1 : Effect of <i>Amaranthus viridis</i> extract on isolated rabbit	61
jejunum strip	
3.2.3 : Sub-chronic toxicity study	65

3.2.3.1: Effect of <i>Amaranthus viridis</i> whole plant ethanolic	65
extract on clinical signs and body weight findings at 250	
and 500 mg/kg/day doses for 2 and 4 weeks	
3.2.3.2 : Haematological findings	65
3.2.3.3 : Plasma biochemical findings	66
3.2.3.4 : Histopathological findings	68
lepidium sativum	
3.3.1: Anti-inflammatory experiment	68
3.3.1.1 : Anti-inflammatory activity in a dose of 250	68
and 500 mg/kg of plant extract	
3.3.2 : Pharmacological studies	69
3.3.2.1 : Effect of <i>lepidium sativum</i> extract on isolated	69
rabbit jejunum strip	
3.3.3 : Sub-chronic toxicity study	73
3.3.3.1 : Effect of <i>lepidium sativum</i> seeds ethanolic extract on	73
clinical signs and body weight findings at 250 and 500 mg/kg/day	
doses for 2 and 4 weeks	
3.3.3.2 : Hamatological findings	73
3.3.3.3 : Plasma biochemical findings	74
3.3.3.4 : Histopathological findings	75

# **CHAPTER FOUR**

	Page No
Discussion	77
Conclusion	85
Recommendatios	85
References	86