Anti-inflammatory Activity of *Trigonella foenum grecum* and *Ziziphus spina christi* on Rats

By
Marwa Abdalla Ahmed Abdel Kareem
B.V.Sc. University of Khartoum
(2003)

A thesis submitted to University of Khartoum in partial fulfillment of the requirements for the degree of Master of Tropical Animal Health (MTAH)

Department of Preventive Medicine
Faculty of Veterinary Medicine
University of Khartoum

Supervisor
Dr. Samia Mohammed Ali El Badwi

February 2008
First and foremost I am grateful to "God"
For providing strength to conduct the present study

To my family
For all good feeling and unselfish warm love
ACKNOWLEDGEMENTS

First of all my heartfelt thanks to almighty Allah for providing me the strength and patience to complete the work.

I would like to express my warm thanks to my supervisor Dr. Samia Mohamed Ali El Badwi, Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum for her invaluable guidance, encouragement, kindness and the follow up throughout the progress of this research work and preparation of the thesis.

I also wish to extend my gratitude to the academic staff, technician and other members of the Department and my colleagues for their assistance.

I am deeply indebted my father, mother, sisters and my brother Dr. Montasir for their continuous encouragement and support which made me overcome the hardships.
# LIST OF CONTENTS

<table>
<thead>
<tr>
<th>Items</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>ARABIC ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER ONE: LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>1.1 Inflammation</td>
<td></td>
</tr>
<tr>
<td>1.1.1 Etiology of inflammation</td>
<td>3</td>
</tr>
<tr>
<td>1.1.2 Types of inflammation</td>
<td>4</td>
</tr>
<tr>
<td>1.1.3 Acute inflammation</td>
<td>4</td>
</tr>
<tr>
<td>1.1.4 Clinical features of acute inflammation</td>
<td>5</td>
</tr>
<tr>
<td>1.2 Plants with anti-inflammatory activity</td>
<td>5</td>
</tr>
<tr>
<td>1.3 Plants with other medicinal uses</td>
<td>8</td>
</tr>
<tr>
<td>1.4 Plants with toxic effects</td>
<td>11</td>
</tr>
<tr>
<td>1.5 Plants used in the present study</td>
<td>12</td>
</tr>
<tr>
<td>1.5.1 <em>Trigonella foenum grecum</em></td>
<td></td>
</tr>
<tr>
<td>1.5.1.1 Distribution, common name and folkloric uses</td>
<td>12</td>
</tr>
<tr>
<td>1.5.1.2 Chemical constituents</td>
<td>14</td>
</tr>
<tr>
<td>1.5.2 <em>Ziziphus spina christi</em></td>
<td></td>
</tr>
<tr>
<td>1.5.2.1 Distribution in Sudan</td>
<td>16</td>
</tr>
<tr>
<td>1.5.2.2 Description of plant</td>
<td>16</td>
</tr>
<tr>
<td>1.5.2.3 Medicinal uses</td>
<td>16</td>
</tr>
<tr>
<td>1.5.2.4 Chemical composition</td>
<td>17</td>
</tr>
<tr>
<td>CHAPTER TWO: MATERIALS AND METHODS</td>
<td>18</td>
</tr>
<tr>
<td>2.1 Materials and experimental designs</td>
<td>18</td>
</tr>
<tr>
<td>2.1.1 Anti inflammatory activity of <em>Trigonella foenum grecum</em> seeds in carrageenan induced paw edema in rats</td>
<td>18</td>
</tr>
<tr>
<td>2.1.1.1 Animals, housing and management</td>
<td>18</td>
</tr>
<tr>
<td>2.1.1.2 Administration and doses</td>
<td>18</td>
</tr>
<tr>
<td>2.1.1.3 Parameters</td>
<td>19</td>
</tr>
<tr>
<td>2.1.2 Anti inflammatory activity of <em>Ziziphus spina christi</em> in carrageenan induced paw edema in rats</td>
<td>19</td>
</tr>
<tr>
<td>2.1.2.1 Animal housing and management</td>
<td>19</td>
</tr>
<tr>
<td>2.1.2.2 Administration and doses</td>
<td>20</td>
</tr>
<tr>
<td>2.1.2.3 Parameters</td>
<td>20</td>
</tr>
<tr>
<td>2.2 Methods</td>
<td>20</td>
</tr>
<tr>
<td>2.2.1 Preparation of the plants</td>
<td>20</td>
</tr>
<tr>
<td>2.2.2 Haematological methods</td>
<td>21</td>
</tr>
</tbody>
</table>
2.2.1 Haemoglobin (Hb) concentration 21
2.2.2 Packed cell volume (PCV) 21
2.2.3 Red blood cell (RBC) count 22
2.2.4 White blood cell (WBC) count 22
2.2.5 Differential count 22
2.2.3 Histological methods 22
2.2.4 Chemical methods 22
  2.2.4.1 Glutamyl Oxaloacetic Transaminase (Aspartate Amino Transferase, L. Aspartate; 2- oxoglutarateamino-transferase, E.C.6.1.1.; G. O.T, A.S.T) 23
2.2.4.2 Alanine amino transferase (ALT), (Glutamine pyruvic transaminase, L – a spartate, 2 – oxoglutamate, GPT) 24
2.2.4.3 Albumin 24
2.2.4.4 Total protein 25
2.2.5 Statistical methods 25

CHAPTER THREE: RESULTS
3.1 Anti inflammatory activity of methanolic extract of Trigonella foenum grecum on carrageenan induced paw edema in rats 26
  3.1.1 Effects of methanolic extract of Trigonella foenum grecum on edema 26
  3.1.2 Hematological finding 26
  3.1.3 Changes in leukocytes values 30
  3.1.4 Change in serum metabolites 30
  3.1.5 Histopathological findings 33
3.2 Anti-inflammatory effects of Ziziphus spina christi methanolic extract on carrageenan induced paw edema in rats 33
  3.2.1 Effects of methanolic extract of Ziziphus spina christi on edema 33
  3.2.2 Hematological findings 37
  3.2.3 Changes in leukocytes values 37
  3.2.4 Change in serum metabolites 37
  3.2.5 Histopathological findings 41

CHAPTER FOUR: DISCUSSION
CONCLUSIONS AND RECOMMENDATIONS 46
REFERENCES 47
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average (Mean ± SE) values of anti-inflammatory effects of <em>Trigonella foenum grecum</em> methanolic extract on carrageenan-induced paw edema in rats</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>Average (mean±SE) Hematological values of rats treated with <em>Trigonella foenum grecum</em> seeds</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>Average (Mean ± SE) values of leucocytes of rats treated with methanolic extract of <em>Trigonella foenum grecum</em></td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>Average (mean±SE) values of serum metabolites of rats treated with <em>Trigonella foenum grecum</em> methanolic extract.</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>Effect of <em>Ziziphus s spina christi</em> methanolic extract on carrageenan-induced paw edema in rats (mean ± SE)</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Average (Mean ± SE) Heamological values of rats treated with <em>Ziziphus spina christi</em> methanolic extract</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>Average (mean±SE) values of leukocytes values on rats treated with <em>Ziziphus spina christi</em></td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>Average (Mean ± SE) values of rates of serum metabolites of rats treated with <em>Ziziphus spina christi</em> methanolic effect.</td>
<td>40</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trigonella foenum grecum</em> seeds</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td><em>Ziziphus spina christi</em> Natural growth</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Comparison of edema size in rats dosed with <em>Trigonella foenum grecum</em></td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Comparison of inhibition percentage of edema in rats dosed orally with</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td><em>Trigonella foenum grecum</em></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(a) Section of carrageenan group showing heavy infiltration of inflammatory cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Carrageenan + 500 mg/kg <em>Trigonella foenum grecum</em> showing moderate infiltration of inflammatory cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(c) Carrageenan + 1000 mg/kg of <em>Trigonella foenum grecum</em> showing less number of inflammatory cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d) Indomethacine group showing the least number of cellular infiltration</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>Comparison of edema size in rats dosed with <em>Ziziphus spina christi</em></td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>Comparison of inhibition percentage of edema in rats dosed orally with</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><em>Ziziphus spina christi</em> methanolic extract</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>(a) Carrageenan group showing severe infiltration.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Carrageenan + 500 mg/kg <em>showing the least number of inflammatory cells.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(c) Carrageenan 1000 kg/kg showing moderate number of inflammatory cells.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d) Carrageenan + Indomathacine showing number of inflammatory cells.</td>
<td>42</td>
</tr>
</tbody>
</table>
ABSTRACT

This study was designed to investigate the anti-inflammatory activity of methanolic extract of *Trigonella foenum grecum* and *Ziziphus spina christi* at different doses to rats via oral route. The anti-inflammatory potential of extract of both plants was tested in rats by edematous reaction inhibition.

Both methanolic extract of *Trigonella foenum grecum* and *Ziziphus spina christi* were tested, using for each, 24 albino rats arranged in 4 groups (control + 2 test groups + indomethacine group) receiving oral dose rates of 500 and 1000 mg extract/kg body weight/rat, 10 mg indomethacine (reference anti-inflammatory drug)/kg body weight/rat and 1 ml/kg body weight/rat of normal saline (control). All individuals were injected 30 minutes subsequent to extract injection sub-cutaneously with local acute edema inducer (0.1 ml of 10% w/v carrageenan saline suspension) in the sub-planter region of the right hind limb. The diameter of the hind paw was measured for assessment of the edema size at 1, 2, 4, 6 and 24 hours. The methanolic extract of *Trigonella foenum grecum* showed significant anti-inflammatory efficacies against carrageenan induced paw edema in rats at the second, 4th and 24 hours at high dose of the extract with efficacy rates 41.7, 41.17 and 33.33% respectively. The methanolic extract of *Ziziphus spina christi* showed significant (P< 0.05) decrease in edema size at first hr for two doses and at 500 mg/k in 24hr with efficacy rate of 76.36% and a dose 1000 mg/kg at 4th hours with efficacy rate of 18.62% respectively.
Hematological changes showed no significant decrease in RBCs, Hb and PCV in two plants. In WBCs, Neutrophils and Lymphocyte showed significant decrease in two plants. No changes in monocyte and eosinophil. Also sera were analyzed for enzymatic activities of AST and ALT and metabolic indictors, albumin and total protein, showed significant increase in AST in control group of *Z.spina christi* and significant decrease in AST in *T. foenum grecum*, and ALT activity in group (3) and indomethacine group in *Z.spina christi*. No significant changes in Albumin and total protein in two plants. Tissue specimens of hind limb were examined for histopathological lesions.
لا يوجد نص يمكن قراءته بشكل طبيعي من الصورة المقدمة.
INTRODUCTION

Sudan is the largest country in Africa and its climate ranges from completely arid to tropical zones with a wide range of bioclimatic regions. The diversity of the climate of Sudan is responsible for its very rich flora.

Herbal medication has been known since early centuries and continued till now. In parts of the world, plants have been considered a logical source of important alternative drug.

Medicinal plants are now being given serious attention as is evidenced by the recommendation given by the World Health Organization (WHO) (Wondergen et al., 1989).

Traditional medical practitioners constitute the most ready available and in many cases, valuable health resources present in the community, so they better have both botanical and medical training and others suitable qualification. In Sudan there is increasing interest in herbal medicine and many researches work on plants to know their medicinal or toxicological effects.

Also herbal medicine becomes a topic of augmented global importance, having impacted on both world health and in traditional trade (Akerel, 1988).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti inflammatory
agents, should therefore, is viewed as fruitful and logical research strategy in the search for new analgesic and anti inflammatory drugs (Gupta, et al., 2006).

Objectives:

The objectives of this study were:

1. To investigate the anti-inflammatory activity of the methanolic extract of *Trigonella foenum grecum* seeds and *Ziziphus spina christi* leaves in carrageenan induced paw edema in rats.

2. To investigate the safety of the methanolic extract of these two plants at the doses used by measuring some biochemical and haematological parameter.
CHAPTER ONE
LITERATURE REVIEW

1.1 Inflammation:

Inflammation is the body's reaction to invasion by an infectious agent, antigen challenge or even just physical, chemical or traumatic damage. Inflammation is directed at destroying harmful agents and initiating repair.

In the process of inflammation, the reaction is often directed at destroying the inciting agent or, at least, rendering it harmless. Also isolates the agent and prevents spread to other locations. All these activities may cause damage or destruction to normal tissue in the immediate area; the inflammatory process cleans up resulting debris and starts restoring damaged tissues (Cheville, 2006).

1.1.1 Etiology of inflammation:

There are many agents that can provoke an inflammatory response. The painful redness that follows over-exposure to solar radiation (sunlight), a physical agent, is one common example. As but one example of the inflammation-causing effects of some chemicals, turpentine, a common paint solvent, was used by scientists to provoke inflammation in laboratory animals. The pain and redness so commonly associated with rheumatoid arthritis attest to the relationship of inflammation and hypersensitivity. The pain, swelling, and pus associated with a severe bacterial infection is as well an example of inflammatory process (Florey, 1962).
1.1.2 Types of inflammation:

There are two fundamental types of inflammation: acute and chronic. A rapid onset, short duration, and profound signs and symptoms characterize acute inflammation. On the other hand, a slow onset, long duration, and less obvious signs and symptoms characterize chronic inflammation. In addition to the two basic forms (acute and chronic), there are two others that appear less commonly: sub acute and granulomatous chronic inflammation. Sub acute inflammation is an ill-defined form that has some clinical features of acute and some of chronic inflammation. Granulomatous chronic inflammation, as its name signifies, is a special form of chronic inflammation. This type is associated with tuberculosis as well as some other less common diseases (Cheville, 2006).

1.1.3 Acute inflammation:

Acute inflammation is the most studied type of inflammation. It immediately follows injury by physical, chemical, or biologic agents. The events following injury cause blood vessel changes allowing entrance of certain blood cells into the injured area. As these cells grapple with the agent that provoked their appearance, normal surrounding tissue may be damaged or even killed. The sequence of these vascular, cellular, and tissue events have been known for decades and are straightforward. More recently, however, the unfolding molecular basis for them has resulted in a maze of interacting compounds that has complicated the picture considerably (Florey, 1962).
1.1.4 Clinical features of acute inflammation:

Recognizing "cardinal signs" will lead to a diagnosis of acute inflammation. Acute inflammation is easily recognized by its signs and symptoms. The inflamed area is red, warm, swollen, and painful. The part is so sore that the patient protects losing its function. These features are known as the "cardinal signs" of acute inflammation (Kumar, 2005).

1.2 Plants with anti-inflammatory activity:

The crude hydrochloric extract of *Zingiber officinale* which belongs to the family (Zingiberaceae) was able to reduce significantly the serotonin-induced skin edema in the rat at doses of 0.6 and 1.8 mg/site (Penna *et al*., 2003).

Ethanolic extract of *Capparis decidua* (Capparidaceae), known in Sudan as Tundub, showed significant inhibition of carrageenan paw edema in rat (Ageel *et al*., 1985).

Methanolic extract of *Bryophyllum pinnatum* was found to produce significant anti-inflammatory effect against carrageenan-induce inflammation in rat at doses of 100, 200 and 300 mg/kg. The highest dose of 300 mg/kg showed significant inhibition higher than the phenyl butazone (100 mg/kg) (Siddharthapal and Nagchaudhuri, 1990).

Ahmed *et al*. (1993) investigated the anti-inflammatory activity of the ethanolic extract of *Caralluma tuberculata* (Asclepiadaceae) at doses of 125, 250 and 500 mg/kg orally and reported significant inhibition of carrageenan-induced paw edema in rats, also the same doses showed analgesic but not antipyretic effect, was observed.
The aqueous leaf extract of *Persea americana* (Laurceae) at the dose 800 mg/kg produced significant inhibition of the inflammation caused by carrageenan at hour three in rats. This effect was similar to that produced by indomethacine at the same time (*Adeyemi et al.*, 2002).

Ethanolic extract of the park of *Syzygium cumin* (Myrtaceae) was investigated in rats at doses of 100, 300 and 1000 mg/kg. When administered orally, against kaolin-carrageenan and formaldehyde-induced rat paw edema and cotton pellet-induced granuloma it showed significant inhibition of edema (*Muruganandan et al.*, 2001).

The aqueous extract of *Adansonia digitata* (Bombacaceae), commonly known as baobab and locally known as Tabaldy, produced a marked anti-inflammatory effect. It reduced the size of pedal swelling induced by formalin in rat at doses of 400 and 800 mg/kg. Such effect was comparable to that produced by the standard drug pheylbutazone at 15 mg/kg (*Ramadan et al.*, 1994). He mentioned that extract produced analgesic and antipyretic activity in mice.

*Ansari and Ali* (1996) studied the anti-inflammatory effect of six tetracycline triterpenoids isolated from the plant *Pistacia integerrima* (Anacardiaceae) named *pistacig errionnes* A, B, C, D, E and F. Equal doses of 5 mg/kg from the compounds saline suspension (1% w/v) were administered intraperitoneally in rat to induce edema, only *pistacig errimones* C and D gave highly significant inhibition of edema.

*Calotropis procera* belongs to the family (Asclepidaceae), widely growing plant. It’s known in the Sudan as Usher, traditionally the latex and the extract of the plant are used in cheese processing. It has been
reported to posse's medicinal properties. Its latex has been used as emetic, purgative and anthelmintic (Kirtikar and Basu, 1935).

The studies of Kumar and Busu (1994); Dewan et al. (2000 a, b) have demonstrated a potent anti-inflammatory, analgesic, antipyretic and antidiarrhoeal activities of the latex of C. procera.

The Bacoba monneria (Scrophulariaceae) is distributed throughout the Indian subcontinent (Chunekar, 1960; Satyavati et al., 1976). It is used in Ayurvedic medical preparation as a memory enhancer, anti-inflammatory, analgesic, and antipyretic, sedative and also as an anti-epileptic agent (Russo and Borrelli, 2005).

The analgesic and anti-inflammatory properties of Nelsonia conescens of the family (Acanthaceae), was described by Owoyele et al. (2005). They found that the extract of ethanol of the dried leaves of N. canescens significantly inhibited carrageenan-induced paw edema in rat at doses of (50 – 200) mg/kg body weight.

Piper chaba which belongs to the family (Piperaceae) grow in India and Malaya Island (Kirtikar and Bausu, 1980). The evaluation of the analgesic, anti-inflammatory, diuretic and anti-diarrhoeal effect of the stem bark of this plant in rats and mice was reported by Taufig-Ur-Rahman et al., 2005).

Bryophyllum pinnatum (Crassulaceae) has an anti-inflammatory, analgesic and anti pyretic effect. Its anti-inflammatory effect of the methanolic extract of the plant leaves was tested in rats at the doses 50, 100 and 200 mg/kg B.wt (Olajide et al., 1998). The analgesic effect of
the same plant was studied in rats using cotton pellet granuloma. The plant produced a significant inhibition of carrageenan-induced edema as well reduction in cotton pellet granuloma.

1.3 Plants with other medicinal uses:

The ripe fruits of *Tamarindus indica*, (Acaesalpiniaceae), are used to treat malaria, dysentery, rheumatism, wound healing and snake bites. Aqueous extract used of *T. indica* was found to have potent antidiabetogenic activity that reduces blood sugar level in streptozocine s+z-induce diabetic in rats (Maiti, 2004).

*Commiphora myrrha* (Burseraceae) known in Sudan as morr Higazi, is traditionally the plant is used as antiseptic, antispasmodic and in abscess treatment. The ethanolic extract of *C. myrrha* is toxic and lethal to rat by intraperitoneal and intramuscular routes (Omer, 1997).

El-Thahir *et al.* (1999) described the anti-plasmodial activity of *Gardenia lutea*. *Tamarindus indica* (Acaesalpiniaceae) ripe fruit which are used in Sudanese traditional medicine to treat malaria (El Gazali *et al.*, 1994).

*Khaya senegalensis* (Meliaceae) was used in Sudan to treat malaria, chloroform extract of the plant showed high inhibition against *Plasmodium falciparum* while butanolic extract showed a prominent larvicidal activity against *Culex quinquefascitus* (Abadi, 1997).

The aqueous extract from the bark of *Albizia anthelmintica* which is locally known as Grif Eldud (Fabeceae) was found to be highly
effective against experimental *Hymenlepis diminuta* infection in rats (Galal *et al*., 1991).

*Balanites aegyptiaca* (L.) delile (zygophyllaceae) is known in Sudan as Higlig tree and the fruit is lalobe. The fruit is commonly used to remove intestinal parasite and sometimes to treat *Schistosoma japonicum* (Koko *et al*., 2000).

*Piper abyssinica* (Piperaceae) is known in Sudan as shaw makkada and *Jatropha curcas* (Euphorbiaceae) known in Sudan as Habbat El Mulouk. Seed powder and ethanolic extract of both plants showed anthelmintic activity against the poultry cestode *Raillietina tetragona* (Osman, 2001).

Khafagy *et al*. (1971) reported that *Artemisia herba-Alba* (Asteraceae), known in the Sudan as sheeh, leaves are used for their anthelmintic and anti-diabetic properties. The anthelmintic effect of the powdered shoots of the plant was investigated against *Haemonchus contortus* in goats and gave successful results (Idris *et al*., 1982). *Nauclea latifolia* has anthelmintic effect against ovine nematodes in Nigeria (Onyeyiti *et al*., 2001).

The ethanolic extract of *Azadirachta indica* has significant results against the gastro-intestinal nematode *Homonchus contortus* in lambs (Hordegen *et al*., 2003).

When alloxan induced diabetic mice were treated with aqueous plant extract of *Artemisia abyssinica* (Asteraceae) steady decrease in blood glucose level was produced beside acholenergic activity (Mossa, 1985).
Intraperitoneal administration of 50 mg/kg of volatile oil extracted from the spice *Nigella sativa* (Ranunculaceae) produced significant hypoglycemic effect in normal and alloxan induced diabetic in rabbits (Al-Hader *et al*., 1993).

Decoction and ethanolic extract of the plant *Teucrium oliverianum* (Labiatae) lowered blood glucose level in mice with alloxan-induced diabetic (Ajabnoor *et al*., 1986).

In northern Nigeria, the aqueous infusion of *laranthus bengwensis* leaves collected from two host plants lemon and juava supplied for 28 days in drinking water (1.32 kg) to normal and streptozotocin-induced diabetic rats significantly decreased serum glucose level (Obatomi *et al*., 1994).

**Oliver-Bever (1986)** summarized the anti-bacterial and anti-mycotic activity of some medicinal plants such as *Anacardium occidentale* (Anacardiaceae), *Acacia farnesiana*, *Drosera indica* (Droseraceae), *Argemone mexicana*, and *Calotropis procera* (Asclepiadaceae)

*Teucrium polium* that belong to the family (lamiaceae) is known to posses a bactericidal activity against *staphylococcus aureus* and *E. coli* (Al-Yahya *et al*., 1990).

**Govendachari et al. (1999)** reported the anti-fungal activity of *Azadirachta indica*, while (Schneiders, 1986) showed that Neem oil suppressed several species in bacterial activity including *Staphylococcus aureus* and *Salmonella typhi*.
Patel and Trivedi (1962) indicated that neem oil was not effective against *Citrobacter* spp, *E. coli*, *Pseudomonas aeruginosa* and *Streptococcus faecalis*. *Indigofera dendroides* in Nigeria) have antibacterial activity (Esimone *et al.*, 1999).

Petroleum ether extract of *Commiphora mirrha* which belongs to the family (Asteraceae) which known as (Morr Higazi) possesses anti-mycotic activity against *Candida albicans* and anti-bacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Omer, 1997).

1.4 Plants with toxic effects:

As some plants have medicinal uses also many plants have toxic effects. The fruit and park of *Balanites aegyptiaca* (L.) delile (Zygophyllaceae) known in Sudan as Higlig tree and its fruit is lalobe. are used as fish poisons (Watt and Breyer-Brandwijk, 1962).

*Jatropha curcas* (Euphorbiaceae) locally called Habat Elmuluk was reported to be toxic to mice, goats, calves, sheep and chicks (Adam, 1974; Adam and Mazoub, 1975; Ahmed and Adam I, 1979; Ahmed and Adam II, 1979; El Badwi *et al.*, 1990).

*Ambrosia martima* from the family (Asteraceae) and in Sudan Known as Demsisa (Bakhiet and Adam, 1996). They found that feeding of Amaritima shoot in the basic diet to chicks at 2 or 10% for 6 weeks was not lethal, but caused pathological changes.

Khairalla (2002) indicates that the ethanolic extract of the plant *Haplophyllum tuberculatum* (Rutaceae) locally known as Elhaza is toxic
to rabbits at the doses 250, 500 and 1000 mg/kg Bwt. Causing mortalities of 0, 25 and 50% respectively.

Studies of **El Badwi (1997)** showed that *Catropis procera* of the family (Asclepidaceae), which is locally known as usher was found to be toxic to goat at 1 ml/kg daily oral dose. Death occurred between 30 minutes and 4 days at single doses of 0.005 ml/kg/days via the intravenous route.

**Omer et al. (1992)** mentioned that *Abrus precatorius* seed locally known as Habat Elarus was highly toxic and fatal to Lohmann-type chicks when fed at 5, 2 and 0.5% in the basic diet. The majority of chicks on the 5 and 2% *A. precatorius* diets died within 10 days. The main sign of poisoning were dullness, reduction in feed consumption, loss of condition and recumbence.

### 1.5 Plants used in the present study:

#### 1.5.1 *Trigonella foenum grecum*: Figure (1)

*Trigonella* is a member of family Fabaceae (Papilionaceae) is an ancient plant indigenous to Sudan. Recently the crop has attracted much interest as a cheap source of good protein for protein supplement.

#### 1.5.1.1 Distribution, common name and folkloric uses:

In Sudan *T. foenum grecum* is grown in the northern region and known as Helba, and traditional used by lactating women as porridge (with sorghum as millet flour). It’s also boiled with water and taken hot or cold drink to sooth stomach ailment (**Gorafi, 1983**).
Fig. (1): *Trigonella foenum grecum* seeds
It also probably delays subsequent pregnancy due to the presence of diosgenin, the starting material use in the synthesis of sex hormones and oral contraceptives (Marker et al., 1947).

1.5.1.2 Chemical constituents:

Trigonella seeds or fenugreek seeds contents are: moisture (4.3), crude protein (27.3), crude fat (6.7), crude fiber (6.7) and ash (3.8). Constituents were estimated according to the method of the AOAC (1970), and Nitrogen-free extract (NFC) (51.2). The plant contain minerals such as Na (49.3), K (1306), Fe (22.5) Ca (158), P (415), Mn (1550), Zn (9.9), Cu (331) (Studies of Shankarachorya and Natarajan, 1972), and also amino acids, Isoleucine, Lucien, lysine, methonine, phenylalanine, threonine, valien, histidine, tyrosine, alanine, aspartic acid, glutamic acid, glycine, proline, serine, ornithine, phosphor-thanolamine (FAO/WHO, 1973). The Active ingredients of the seed contain alkaloids Trigonelline, choline and contain protein (28%) oil (6%) in addition to saponin, flavonoids, vitamins and volatile oils (ElGhazali et al., 1998).

1.5.2 Ziziphus-spina-christi: Figure (2)

Genus Ziziphus belongs to the buck thorn family (Rhamnaceae). It’s a genus of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world. The common name in Sudan is (Sedir) Nabag are the fruits, in England name is Christ's thorn.
Fig. (2): *Ziziphus spina christi* Natural growth
1.5.2.1 Distribution in Sudan:

Wide spread in the study area and throughout northern and central Sudan.

1.5.2.2 Description of plant:

Spinescent savanna trees up to 10m high with fissured, scaly barks and paired unequal spines. Leaves alternate, petiolate, laminas oblong-elliptic, 2.5-5x 1.5-3 cm, apex obtuss base attenuate to rounded, margin minutely serrate, glabrous on both surfaces, petioles 5-7 mm long strongly 3-nerved from base. Inflorescences cymes up to 1 cm long and 0.2 mm across, pedicels 1-3mm long. Flowers yellow-white. Fruits drupe, ovate-globose, 0.8-2cm across, smooth, reddishbrown, 2-seeded, glabrous (ElGhazali et al., 1998).

1.5.2.3 Medicinal uses:

In Sudan the poultice of the powdered leaves are used to heal swellings, whereas the maceration of the roots are used as anti-purgative. (ElGhazali et al., 1997). Also has anti-diabetic effect (Glombitza et al., 1994).

All parts of the plant are used by the local Arab people to help maintain a healthy lifestyle. The plant has also been used for its soothing properties (Adzu et al., 2001). In Saudi Arabia it’s used for the treatment of ulcers, wounds, eye diseases and bronchitis. The Bedouin use it for the treatment of wounds, skin diseases and as anti-inflammatory. They also use it as a febrifuge and diuretic. In Iran the leaves have been used for washing the hair and body, as antiseptic, antifungal and anti-
inflammatory agent and for healing skin disease such as dermatitis (Amin, 1991).

In China it’s used as a form of birth control, aqueous leaves extract have a calming effect on the central nervous system (Effraim et al., 1998). It has been described as anti-cathartic, astringent, diuretic and tonic (Duke and Ayensu, 1985).

1.5.2.4 Chemical composition:

Studies of Ikram and Tomlinson (1976); Younes et al. (1996) and Mahran et al. (1996) showed the main constituent of essential oil was: Alpha-terpineol (16.4%) and linalool (11.5%). Main neutral hydrocarbons as: n-pentacosane forms (81%). Methyl esters isolated from leaves included methyl palmitate, methyl stearate and methyl myristate, beta-sitosterol, oleanolic acid and maslinic acid were the main aglycones of the glycosides present in leaves.

The plant contains sugars in leaves including lactose, glucose, galactose, arabinose, xylose and rhamnose. The plants also contain four saponin: glycosides (Mahran et al., 1996).

The highest flavonid content was found in the leaves (0.66%). 3-0-rhamnoglycoside. 7-0-rhamnoside and rutin are the main flavonoid compounds present in plant parts (Brantner and Males, 1999). Known alkaloids, zizyphine-F, jubanine-A and Amphibine-H and a new peptide alkaloide spinanine-A has been isolated from stem bark. Spinanine-A is a 14-membered cyclopeptide alkaloide of the amphibine-B type (Abdel-Galil et al., 1991).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials and experimental designs

2.1.1 Anti inflammatory activity of *Trigonella foenum grecum* seeds in carrageenan induced paw edema in rats

2.1.1.1 Animals, housing and management

Twenty four Wistar white Albino rats from both sexes weighing between (60-155g) were obtained from the Medicinal and Aromatic Plants Research Institute (MAPRI), the National Research Center (NRC), Khartoum, Sudan. They were housed in cages and maintained in a room under standard environmental condition and controlled temperature (22 ± 2ºC), relative humidity 60% with free access to water and formula rats feed (2.5 mcal and 20% crude protein).

Animal were apparently healthy and they were identified by color tail marks. Three days were allowed as preliminary adaptive period.

2.1.1.2 Administration and doses:

At the end of adaptive period the animals were weight- distributed and allotted depend on same or similar weight, to four groups, each of six rats. Rats in group 1 were orally dosed with 1 ml/kg body Wt of normal saline and served as untreated control, the methanolic extract of the plant was concentrated by evaporating the solvent and the dried material was redissolved in distilled water and given orally in different doses, at 500 mg/kg body wt/rats to group 2, and at 1000 mg/kg body wt/rats to group 3, while group 4 received indomethacine 10 mg/kg body/wt orally (Hikma
pharmaceutical, Amman, Jordan). After half an hour all groups were injected subcutaneously with 0.1ml of 1% (W/V) carrageenan suspension (Sigma Chemicals Co; St Louis, Mo, USA), in the sub-planter region of the right hind paw as a local acute edema inducer.

2.1.1.3 Parameters:

Paw diameter was measured at 1, 2, 4, 6 and 24 hours post carrageenan injection using Hauptner Tuberculin Caliper (Hauptner, GmbH, Germany) to the nearest millimeter.

Blood samples were obtained from the ocular vein for hematological investigation and serum analysis.

Hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) counts and differential count were estimated.

Sera were analyzed for the activities of AST, ALT and also for concentration of total protein and albumin. Tissue specimens of site of injection in the planter leg of rats were fixed in 10% neutral buffered formalin and processed for histopathology.

2.1.2 Anti-inflammatory activity of *Ziziphus spina christi* in carragenan induced paw edema in rats

2.1.2.1 Animal housing and management

Twenty four Wistar white (Albino) rats of both sex weighing (65-105g) were obtained from the Medicinal and Aromatic Plants Research Institute (MAPRI), The National Research Center (NRC), Khartoum, Sudan, they were housed in cages and maintained in a room under standard
environmental condition, controlled temperature (22 ± 2°C) relative humidity 60% with free access to water and formula rats feed (2.5 mcal and 20% crude protein). Animal were apparently healthy and they were identified by color tail marks, 3 days were allowed as preliminary adaptive period.

2.1.2.2 Administration and doses:

At the end of adaptive period the animals were weight-distributed and allotted depend on same or semi same weight, to four groups, each of six rats. Rats in group 1 orally dosed with 1 ml/kg body Wt of normal saline and served as untreated control, the methanolic extract of the plant was concentrated by evaporating the solvent and the dried material was redissolved in distilled water and given orally in different doses, at 500 mg/kg body wt/rats to group 2, and at 1000 mg/kg body wt/rats to group 3, while group 4 received indomethacine 10 mg/kg body/wt orally (Hikma Pharmaceutical, Amman, Jordan). After half an hour all groups were injected subcutaneously with 0.1ml of 1% (W/V) carrageenan suspension (Sigma Chemicals Co; St Louis, Mo, USA), at the sub-planter region of the right hind paw as a local acute edema inducer.

2.1.2.3 Parameters:

Measurement, blood and sera values were similar to those described in the previous experiment.

2.2 Methods:

2.2.1 Preparation of the plants:

*Trigonella foenum grecum* seeds were obtained from a local Market, Khartoum north, cleaned, shade dried and made into powder. The powder
was extracted with methanol at 40-60° C using soxhelt apparatus, for about six hours. The solvent were evaporated under reduced pressure using Rota vapour apparatus then the extract was allowed to air in Petri dish to be completely dried before being ready for use. *Ziziphus spina Christi* leaves were collected from Khartoum State (ELkadro), shade dried at room temperature and then made into powdered.

The methanolic extract of the plant was prepared as same method of *Trigonella foenum grecum*.

The two plants were identified, classified and authenticated by botanists in Medicinal and Aromatic Plants, Research Institute (MAPRI) The National Research Center (NRC), Khartoum, Sudan.

2.2.2 Haematological methods (Schalm, 1965):

All procedures were carried on blood samples from the ocular veins of rats collected into clean dry bottles containing heparin as an anti-coagulant.

2.2.2.1 Haemoglobin (Hb) concentration:

Hb concentration was determined using the methaemoglobin technique. The method based on the conversion of haemoglobin by Drabkins solution (0.02 g potassium ferricyanide and 1g sodium bicarbonate per liter of distilled water)to cyanmethaemoglobin. The haemoglobin concentration was estimated in g/dl of blood.

2.2.2.2 Packed cell volume (PCV):

Fresh blood samples in capillary tubes were centrifuged for five minute using a micro-hematocrit centrifuge *(Hawksley and Sons Ltd.*,
packed cell volume percent was read off on the scaling instrument provided with the centrifuge.

2.2.2.3 Red blood cell (RBC) count:

Red blood cells were counted using improved Neubauer Haemocytometer (Hawksley and Sons Ltd., England). Formal citrate was used as diluents.

2.2.2.4 White blood cell (WBC) count:

The white blood cells were counted by use of an improved Neubauer Haemocytometer (Hawksley and Son Ltd., England). Turks fluid (1% watery glacial acetic acid, tinged with gentian violet) was used as diluents. A total count of 4 squares was multiplied by 50 and expressed in 1000 cells/mm³.

2.2.2.5 Differential count:

Thin blood films were made, air dried and fixed in methanol for 5 seconds. The films were stained with Giemsa stain (10%) for 30 minutes, then washed in water, air dried and examined. The Battlement method was used; at least 100 cells were identified on each slide and the percentages of each cell types recorded. (Kenyon and Gasmir, 2001)

2.2.3 Histological methods:

The specimens of rat paw, at site of carrageenan injection, were cut immediately after slaughter and fixed in 10% formal saline, embedded in paraffin wax, sectioned at 5um and stained with haemotoxylin and eosin (H&E) using Mayer's haemalum.

2.2.4 Chemical methods:

Determination of serum constituent:
2.2.4.1 Glutamyl Oxaloacetic Transaminase (Aspartate Amino Transferase, L. Aspartate; 2- oxoglutarate amino transferase, E.C.6.1.1.; G. O.T, A.S.T):  

Serum (AST) (GOT) was measured by a commercial kit (Randox Laboratories Ltd, U.K).  

Test principle:  

Aspartate amino transferase catalyses the reversible transfer of an amino group from a spartate to α-ketoglutarate forming glutamate and oxalacetate.

\[
\text{L - spartate} + \alpha\text{-ketoglutarate} \leftrightarrow \text{L - glutamate and oxalacetate} \\
\text{glutamate} + \text{oxalacetate.}
\]

The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH and NADH).

\[
\text{Oxalacetate} + \text{NADH} + \text{H}^+ \leftrightarrow \text{Malate} + \text{NAD}
\]

The rate of decrease in concentration of NADH is proportional to the catalytic concentration of AST present in the serum sample.  

Protocol:  

Non-haemolysed serum was added to a buffered substrate mixture of L-aspartate and α-oxoglutarate. The absorbance at a wave length of 365 nm was read at one minute intervals after mixing the serum with buffered substrate solution. The mean absorbance change per minute (A_{365/ \text{minute}}) was used for calculation of enzyme activity as follows:

\[
\text{IU=}A_{365} \text{nm/ minute} \times 2059.
\]
2.2.4.2 Alanine amino transferase (ALT), (Glutamine pyruvic transaminase, L – a spartate, 2 – oxoglutamate, GPT):

It is an enzymatic method, which measures the glutamic pyruvic transaminase in sera by monitoring the concentration of pyruvate hydrazone formed with 2 – 4 dinitrophenyl hydrazine.

**Principle:**

\[
\text{Oxalglutarate + L- alanine} \leftrightarrow \text{L – glutrate + pyruvate}
\]

The absorbance of samples were read against the reagent blank after five minutes at wave length of 630 nm UV / VIS Spectrophotometer, the ALT was measured in U/ L

2.2.4.3 Albumin:

Albumin was measured by a colorimetric method using a commercial kit (Randox Laboratory Ltd., UK).

**Test principle**

The measurement of serum albumin is based on it is quantitative binding to the indicator 5, 5- di-purple, BCP.

Serum was mixed with a buffered BCP reagent and the mixture was incubated for 2 minutes at room temperature. The absorbance of the sample (A sample) and of standard (A standard) was measured against the reagent blank at wave length of 600 nm and albumin concentration (C) was calculated as follows:

\[
\text{Concentration measure (g / dl)} = \frac{\text{A Sample} \times \text{Concentration of standard}}{\text{A Standard}}
\]
2.2.4.4 **Total protein:**

Total protein was measured by a colorimetric method using a commercial kit (**Randox Laboratory Ltd., UK**).

**Test principle:**

Colorimeter determination of total protein based on the Biuret reaction, serum protein reacts with copper sulphate in the presence of sodium hydroxide.

The Rochelle salt (K- Na- tartarate) contained in the Biuret reagent is utilized to keep the formed cupric hydroxide in solution which gives blue color. The intensity of the color produced is proportional to the amount of protein in the sample. The Absorbencies of the sample (A sample) and of the standard (A standard ) were read against the reagent blank in the Spectrophotometer at a wavelength of 545 nm. The total serum protein concentration (C) was calculated as follows:

\[
C (\text{mg/dl}) = \frac{A_{\text{sample}} \times \text{concentration of the standard}}{A_{\text{standard}}}
\]

2.2.5 **Statistical methods:**

Mean values of data was analyzed by the one way ANOVA. The Efficacies were obtained by calculating the difference between the edema size in the treated and the control and the values were transformed into percentage using mean index using the formula:

\[
\frac{(a-b)}{a} \times 100 = \text{efficacy}
\]

Where:  
- a = Mean of the edema size in the control  
- b = Mean of edema size in the treated rats  

**(Snedecor and Cochran, 1989).**
CHAPTER THREE
RESULTS

3.1 Anti-inflammatory activity of methanolic extract of *Trigonella foenum grecum* on carrageenan induced paw edema in rats

3.1.1 Effects of methanolic extract of *Trigonella foenum grecum* on edema

The anti-inflammatory effect of the methanolic extract of *Trigonella foenum grecum* seeds on rats is shown in Table (1) and the effect, on edema size is shown in Figure (3) and on the inhibition percentage is shown in Figure (4).

Rats of group 2 (500mg/kg) showed decrease on the edema size when compared to the control (carrageenan group) although it was not at the level of significant, and this group showed inhibition percentage of 7.9, 26.05, 26.05, 33.33 and 45.83 at the first, second, fourth, six and twenty fourth hours respectively.

Rats in group 3 (1000mg/kg) showed significant (P<0.05) decrease in edema size in the second, fourth and twenty fourth hours and inhibition percentage at 13.49, 41.17, 41.17, 38.46 and 33.33 at the first, second, fourth, sixth and twenty fourth hours respectively.

Rats in group 4 (indomethacine) showed high decrease (P<0.05) in edema size when compared to the control (untreated group) at the first, second, fourth, sixth and twenty fourth hours and higher inhibition percentage at 54.47, 58.66, 43.69, 50.00 and 50.00 respectively.

3.1.2 Hematological findings:

Table 2 summarizing the hematological changes in blood of rats treated with *Trigonella foenum grecum* methanolic extract
Table (1): Average (Mean ± SE) values of anti-inflammatory effects of *Trigonella foenum grecum* methanolic extract on carrageenan-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>Edema size (mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1.26±0.20(^a)</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>1.16±0.29(^a)</td>
<td>7.90</td>
</tr>
<tr>
<td>G3</td>
<td>1.09±0.13(^ab)</td>
<td>13.49</td>
</tr>
<tr>
<td>G4</td>
<td>0.47±0.18(^b)</td>
<td>54.47</td>
</tr>
<tr>
<td>G1</td>
<td>1.19±0.12(^a)</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>0.88±0.13(^a)</td>
<td>26.05</td>
</tr>
<tr>
<td>G3</td>
<td>0.70±0.13(^b)</td>
<td>41.17</td>
</tr>
<tr>
<td>G4</td>
<td>0.67±0.07(^b)</td>
<td>43.69</td>
</tr>
<tr>
<td>G1</td>
<td>1.19±0.12(^a)</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>0.88±0.13(^a)</td>
<td>26.05</td>
</tr>
<tr>
<td>G3</td>
<td>0.70±0.13(^b)</td>
<td>41.17</td>
</tr>
<tr>
<td>G4</td>
<td>0.67±0.07(^b)</td>
<td>43.69</td>
</tr>
<tr>
<td>G1</td>
<td>0.78±0.19(^a)</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>0.52±0.21(^a)</td>
<td>33.33</td>
</tr>
<tr>
<td>G3</td>
<td>0.48±0.11(^a)</td>
<td>38.46</td>
</tr>
<tr>
<td>G4</td>
<td>0.39±0.08(^b)</td>
<td>50.00</td>
</tr>
<tr>
<td>G1</td>
<td>0.48±0.10(^a)</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>0.40±0.07(^a)</td>
<td>45.83</td>
</tr>
<tr>
<td>G3</td>
<td>0.32±0.07(^b)</td>
<td>33.33</td>
</tr>
<tr>
<td>G4</td>
<td>0.24±0.07(^b)</td>
<td>50.00</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)  
G2 500 mg/kg *Trigonella foenum grecum* + Carrageenan  
G3 1000 mg/kg *Trigonella foenum grecum* + Carrageenan  
G4 10 mg/kg Indomethacine + Carrageenan  
Means in the same column with the same letter are not significantly different (P>0.05).
Fig. (3): Comparison of edema size in rats dosed with *Trigonella foenum grecum* methanolic extract.

Fig. (4): Comparison of inhibition percentage of edema in rats dosed orally with *Trigonella foenum grecum*

- G1 (Control = Carrageenan)
- G2 500 mg/kg *Trigonella foenum grecum* + Carrageenan
- G3 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
- G4 10 mg/kg Indomethacine + Carrageenan
Table (2): Average (mean±SE) Hematological values of rats treated with *Trigonella foenum grecum* seeds.

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>PCV (%)</th>
<th>Hb (g/dl)</th>
<th>RBCs (10⁶/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>37.00±3.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.23±0.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.30±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2</td>
<td>30.00±0.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.67±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.43±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3</td>
<td>32.00±3.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.93±0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.47±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4</td>
<td>36.00±1.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.10±0.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.77±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)
G2 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4 10 mg/kg Indomethacine + Carrageenan

Means in the same column with the same letter are not significantly different (P>0.05).
In all treated groups the values of PCV, RBC and Hb showed no significant difference when compared to the control group

3.1.3 Changes in leukocytes values:

Table (3) summarizes the changes in WBCs count, neutrophil, eosinophil, lymphocyte, and monocyte percentages of rats treated with Trigonella methanolic extract. In group 2 (500mg/kg), neutrophil, monocytes and eosinophil percentage, showed no significant difference when compared to the (carrageenan group), while there were significant (P<0.05) decrease in lymphocyte percentage and total white blood cell count. Group 3 (1000mg/kg) showed no significant differences in monocytes and eosinophil percentage or in white blood cells count, while neutrophil and lymphocytes were significantly decreased (P<0.05). The indo-methacine group, recorded significantly (P<0.05) decreased percentage of neutrophils and total white blood cell count and no significant changes in lymphocytes, monocytes or eosinophil compared to the (carrageenan group) values.

3.1.4 Change in serum metabolites:

Table (4) summarized the change in serum metabolites of rats treated with Trigonella foenum grecum methanolic extract. In all treated groups there were no significant changes in concentration of total protein and albumin and no significant change in the activities of ALT. AST activities significantly increased (P<0.05) in group 3 (1000mg/kg) and in the indomethacine group.
Table (3): Average (Mean ± SE) values of leucocytes count of rats treated with methanolic extract of *Trigonella foenum grecum*.

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>Neutrophils (%)</th>
<th>Lymphocyte (%)</th>
<th>Monocyte (%)</th>
<th>Eosinophil (%)</th>
<th>TWBCs (10^3/mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>80.00±0.57a</td>
<td>27.33±2.33a</td>
<td>1.33±0.33a</td>
<td>1.33±0.33a</td>
<td>5.50±0.76a</td>
</tr>
<tr>
<td>G2</td>
<td>78.67±0.33a</td>
<td>18.00±1.86b</td>
<td>1.33±0.33a</td>
<td>1.00±0.00a</td>
<td>4.27±0.94b</td>
</tr>
<tr>
<td>G3</td>
<td>67.00±0.33b</td>
<td>18.00±1.13b</td>
<td>1.33±0.33a</td>
<td>1.00±0.00a</td>
<td>4.33±0.44a</td>
</tr>
<tr>
<td>G4</td>
<td>67.33±0.33b</td>
<td>22.00±1.73a</td>
<td>1.33±0.33a</td>
<td>1.00±0.00a</td>
<td>3.67±0.29b</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)
G2 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4 10 mg/kg Indomethacine + Carrageenan

Means in the same column with the same letter are not significantly different (P>0.05).
Table (4): Average (Mean ± SE) values of serum metabolites of rats treated with *Trigonella foenum grecum* methanolic extract.

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>AST (i.u/l)</th>
<th>ALT (i.u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>5.70±0.61^a</td>
<td>3.5±0.07^a</td>
<td>113.00±8.15^a</td>
<td>67.00±4.47^a</td>
</tr>
<tr>
<td>G2</td>
<td>5.00±0.25^a</td>
<td>3.5±0.12^a</td>
<td>115.00±5.86^a</td>
<td>60.67±3.18^a</td>
</tr>
<tr>
<td>G3</td>
<td>5.16±0.27^a</td>
<td>3.9±0.23^a</td>
<td>171.667±6.90^b</td>
<td>71.00±8.74^a</td>
</tr>
<tr>
<td>G4</td>
<td>5.33±0.15^a</td>
<td>3.5±0.21^a</td>
<td>121.667±6.44^b</td>
<td>59.67±2.33^a</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)
G2 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4 10 mg/kg Indomethacine + Carrageenan
Means in the same column with the same letter are not significantly different (P>0.05).
3.1.5 Histopathological findings:

Histopathological sections of paw of the rats treated with *Trigonella foenum grecum* were presented in Figure 5 (a – d).

On microscopy, in the test groups, (group 1) the carrageenan group showed severe cellular infiltration. In the treated groups 2 and 3 (500mg/kg and 1000mg/kg, the numbers of cellular infiltration were reducing respectively as compared to the control. The indomethacine treated group showed the lowest number of inflammatory cells.

3.2 Anti-inflammatory effects of *Ziziphus spina christi* methanolic extract on carrageenan induced paw edema in rats

3.2.1 Effects of methanolic extract of *Ziziphus spina Christi* on edema

Table (5) shows the anti-inflammatory effect of *Ziziphus spina Christi* methanolic extract on rats. Figure 6 and 7 showed the effect of the plant on edema size and the effect on inhibition percent respectively.

Treatment effects showed significant (P< 0.05) decrease in edema size in group 2 at the first and twenty fourth hours and showed inhibition percentage of 25.94, 8.26, 13.79, 10.20 and 76.36 at the first, second, fourth, sixth and twenty fourth hours respectively.

Rats in group 3 showed decrease in edema size at the first and fourth hours and inhibition percentage of 36.70, 15.70, 18.62, 6.12 and 9.0 in the first, second, fourth, sixth and twenty fourth hours respectively.

Group 4 (indomethacine) recorded significant decrease in edema size at the first, second, fourth and sixth hours and inhibition a percentage of 36.7, 40.49, 25.15, 41.18 and 40.0 at the first, second, fourth, sixth and twenty fourth hours respectively.
Fig. (5): Paw tissues of rats treated with methanolic extract of *Trigonella foenum grecum* seeds.

(a) Section of carrageenan group showing heavy infiltration of inflammatory cells
(b) Carrageenan + 500 mg/kg *Trigonella foenum grecum* showing moderate infiltration of inflammatory cell
(c) Carrageenan + 1000 mg/kg of *Trigonella foenum grecum* showing less number of inflammatory cells.
(d) Indomethacine group showing the least number of cellular infiltrations
Table (5): Effect of *Ziziphus spina Christi* methanolic extract on carrageenan-induced paw edema in rats (Mean ± SE).

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>Edema size (mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>1.58±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>1.17±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.94</td>
</tr>
<tr>
<td>G3</td>
<td>1.00±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.70</td>
</tr>
<tr>
<td>G4</td>
<td>1.00±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.70</td>
</tr>
<tr>
<td>2 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>1.12±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>1.11±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.26</td>
</tr>
<tr>
<td>G3</td>
<td>1.02±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.70</td>
</tr>
<tr>
<td>G4</td>
<td>0.72±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.49</td>
</tr>
<tr>
<td>4 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>1.45±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>1.25±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.79</td>
</tr>
<tr>
<td>G3</td>
<td>1.18±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.62</td>
</tr>
<tr>
<td>G4</td>
<td>1.08±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.15</td>
</tr>
<tr>
<td>6 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>0.98±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>0.88±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.20</td>
</tr>
<tr>
<td>G3</td>
<td>0.92±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.12</td>
</tr>
<tr>
<td>G4</td>
<td>0.57±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.18</td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>0.55±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>G2</td>
<td>0.13±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.36</td>
</tr>
<tr>
<td>G3</td>
<td>0.50±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.00</td>
</tr>
<tr>
<td>G4</td>
<td>0.33±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.00</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)
G2 500 mg/kg *Ziziphus spina christi* + Carrageenan
G3 1000 mg/kg *Ziziphus spina christi* + Carrageenan
G4 10 mg/kg Indomethacine + Carrageenan

Means in the same column with the same letter are not significantly different (P>0.05).
Fig. (6): Comparison of edema size in rats dosed with *Ziziphus spina christi* methanolic extract.

Fig. (7): Comparison of inhibition percentage of edema in rats dosed orally with *Ziziphus spina christi*

- **G1** (Control = Carrageenan)
- G2 500 mg/kg *Ziziphus spina christi* + Carrageenan
- G3 1000 mg/kg *Ziziphus spina christi* + Carrageenan
- G4 10 mg/kg Indomethacine + Carrageenan
3.2.2 Hematological findings:

Table (6) summarized the hematological changes in blood of rats treated with *Ziziphus spina Christi* methanolic extract.

In groups 2 and 3 there were no significant (P > 0.05) difference in PCV, Hb and red blood cells values. The indomethacine group (group 4) showed significant (P < 0.05) decrease in PCV percentage and significant increase in Hb concentration.

3.2.3 Changes in leukocytes values:

Table (7) is summarizing the changes in white blood cell count, neutrophil, eosinophil, lymphocyte and monocyte percentages of rats treated with *Ziziphus spina christi* methanolic extract.

There were no significant difference in neutrophil, monocyte and eosinophil percentage in group 2 (500mg/kg) but the percentage of lymphocyte and total white blood cells count were significantly (P < 0.05) decreased. In group 3 (1000mg/kg) and group 4 (indomethacine group, the percentage of neutrophil and Lymphocytes were significantly (P<0.05- 0.001) decrease. There were no significant differences in monocytes and eosinophil percentages.

3.2.4 Changes in serum metabolites:

Table (8) is summarizing the changes in serum metabolites of rats treated with *Ziziphus spina christi* methanolic extract.

The activities of AST showed no significant change in group 2, but significant (P < 0.05) in group 3 and 4. ALT activities were significant decrease (P< 0.05) in group 2, 3 and 4. Total protein and albumin showed no significant difference (P > 0.05) in all group when comparing with control group.
Table (6): Average (Mean ± SE) Haemological values of rats treated with *Ziziphus spina christi* methanolic extract

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>PCV %</th>
<th>Hb (g/dl)</th>
<th>RBCs (10⁶/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>39.67±3.28a</td>
<td>13.53±0.43b</td>
<td>8.5±1.420a</td>
</tr>
<tr>
<td>G2</td>
<td>36.00±0.58ab</td>
<td>14.60±0.20b</td>
<td>6.00±0.55a</td>
</tr>
<tr>
<td>G3</td>
<td>32.33±2.85ab</td>
<td>13.57±0.56b</td>
<td>7.00±0.64a</td>
</tr>
<tr>
<td>G4</td>
<td>23.00±8.39b</td>
<td>15.43±0.75a</td>
<td>7.00±1.31a</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)
G2 500 mg/kg *Ziziphus spina christi* + Carrageenan
G3 1000 mg/kg *Ziziphus spina christi* + Carrageenan
G4 10 mg/kg Indomethacin + Carrageenan

Means in the same column with the same letter are not significantly different (P>0.05).
Table (7): Average (mean±SE) values of leukocytes on rats treated with *Ziziphus spina christi*.

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>Neurophils (%)</th>
<th>Lymphocyte (%)</th>
<th>Monocyte (%)</th>
<th>Eosinophil (%)</th>
<th>TWBCs (10³/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>80.00±0.00a</td>
<td>47.20±3.81a</td>
<td>3.00±0.00a</td>
<td>2.00±0.00a</td>
<td>7.40±0.51a</td>
</tr>
<tr>
<td>G2</td>
<td>73.00±3.61a</td>
<td>37.67±1.73b</td>
<td>1.67±0.33a</td>
<td>0.67±0.33a</td>
<td>5.17±0.60b</td>
</tr>
<tr>
<td>G3</td>
<td>40.00±2.89b</td>
<td>24.33±2.76c</td>
<td>2.00±0.58a</td>
<td>1.00±0.58a</td>
<td>5.90±0.33a</td>
</tr>
<tr>
<td>G4</td>
<td>30.33±0.88b</td>
<td>12.17±2.84d</td>
<td>2.00±0.00a</td>
<td>1.33±0.33a</td>
<td>6.5±0.26ab</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)  
G2 500 mg/kg *Ziziphus spina christi* + Carrageenan  
G3 1000 mg/kg *Ziziphus spina christi* + Carrageenan  
G4 10 mg/kg Indomethacine + Carrageenan  
Means in the same column with the same letter are not significantly different (P>0.05).
Table (8): Average (Mean ± SE) values of rates of serum metabolites of rats treated with *Ziziphus spina Christi* methanolic effect.

<table>
<thead>
<tr>
<th>Groups/dose</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>AST (iu/I)</th>
<th>ALT (iu/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>5.37±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.73±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>254.00±7.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.00±9.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2</td>
<td>6.53±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.40±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>169.00±4.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.33±8.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3</td>
<td>6.10±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>147.67±9.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.33±9.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4</td>
<td>4.87±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.00±2.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.33±2.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

G1 (Control = Carrageenan)  
G2 500 mg/kg *Ziziphus spina christi* + Carrageenan  
G3 1000 mg/kg *Ziziphus spina christi* + Carrageenan  
G4 10 mg/kg Indomethacine + Carrageenan  
Means in the same column with the same letter are not significantly different (P>0.05).
3.2.5 Histopathological findings:

Figure 8 (a – d) show the paw section of rat treated with *Ziziphus spina christi* extract on carrageenan induced paw edema.

On microscopy, histopathological study show sever cellular infiltration in group1 (carrageenan group), while group 2(500mg/kg) showed the least number of cellular infiltration even less than indomethacine group.
Fig. (8)  Paw tissues of rats treated with methanolic extract of *Ziziphus spina christi* leaves
(a)  Carrageenan group showing severe infiltration.
(b)  Carrageenan + 500 mg/kg *showing* the least number of inflammatory cells.
(c)  Carrageenan 1000 kg/kg showing moderate number of inflammatory cells.
(d)  Carrageenan + Indomathacine showing number of inflammatory cells.
CHAPTER FOUR
DISCUSSION

Rats have been used as a biological model in this study similar to research on medicinal plants by many workers (Galal, 1991; Omer, 1992; Bakhiet, 1996 and Khairalla, 2002). Considerable variations in the response of animals, rats and chicks to different plant constituent were well documented. Methanol and ethanol were described to be efficient solvent in extracting phytochemical constituents from plants material (Eloff, 1998; Cowan, 1999). In this study methanol was used for extraction of Trigonella foenum grecum and Ziziphus spina christi. Carrageenan induced-edema has been commonly used in experimental animal models for inducing acute inflammation (Hajare et al., (2001). Investigation by Brito and Antonio (1998) and Vinegar et al. (1969)

Inflammation is believed to be biphasic, the early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damage tissue surroundings and the late phase is sustained by prostaglandins release and mediated by bradykinin, leukotriens, polymorphonuclear cells and prostaglandins produced by tissue macrophages.

Also many studies reported that indomethacine is a standard anti-inflammatoty drug in inhibiting response induced by various inflammagens in acute model of inflammation (Levin and Tawio, 1994; Osman, 2005). The reduction in the size of the induced edema indicates the anti-inflammatory activity of the plants, used. Morebise et al. (2001)
reported an inhibition of the size of the induced edema within 3 hr by the plants known as *Chamanthera dependens* and *Liu et al. (2001)*, on the other hand reported the inhibition of the induced edema but after only one hr by *Calligonum comosum*.

The extract was administered 30 minutes before the inflammation inducer this because the inflammatory phase was very short.

The present studies establish the anti-inflammatory activity of the methanolic extract of *Trigonella foenum grecum* seeds and *Ziziphus spina Christi* leaves. Some biochemical assay were recorded including ALT, total protein and albumin and also haematological parameters like Hb, PCV and RBC count reveals no toxicity evidence of the doses used and so indicate effectiveness and safety action for the treatment of conditions associated with inflammation.

The significant increase in AST activity in carrageenan group indicate tissue damage and significant decrease in AST and ALT activities in group 3 (1000 mg/kg) and indomethacine group in *Ziziphus spina Christi* extract can be pointed out as another evidence of anti-inflammatory activity of the plant extract.

The methanolic extract of *Trigonella foenum grecum* seed showed significant inhibition of edema size at the high doses 1000 mg/kg after 2, 4 hr and 24 hr of treatment and showed inhibition percentage of 41.17, 41.17 and 33.33% respectively and this may be due to the insufficient absorption of the extract which was also reported by *Khairalla (2002)* in the study of anti-inflammatory acting of *Haplophyllum tuberclatum*. 
The methanolic extract of *Ziziphus spina christi* leaves (500 mg/kg) showed significant inhibition of edema size at the first and twenty fourth hours the inhibition percentage was 25.94 and 76.36 respectively. It was the same or better than the reference drug (indomethacine group) on first hours, this study agrees with *Liu et al. (2001)* who studies anti-inflammatory activity of *Calligonum comosum*.

In the present study the result obtained in the rats paw edema showed that both extracts of *T .foenum grecum* and *Z .spina Christi* significantly reduced the size of the paw edema, suggesting that the active principles present in the extracts may act as anti-inflammatory. It is well known that some plants constituents can significantly inhibit the biosynthetic pathway of these mediators such as prostaglandins, histamine, serotonin and bradykinin, *(Speroni et al., 2005)*.

In addition, as was mentioned flavonoids and terepenoids had been reported to have anti-inflammatory effects. A number of pervious studies suggested that flavonoids may interact directly with the prostaglandins system in the same way as non-steroidal anti-inflammatory drugs *(Panthong et al., 1989; Recio et al., 1995)*. Among the above facts flavonoids can be responsible for the anti-inflammatory effects of both extracts in this study.

Neutrophils participate in the endogenous control of the inflammatory pain in rats, *(Giorgi et al., 1998)*. In both plants significant decrease in white blood cells, lymphocyte and neutrophil percentage may be due to anti-inflammatory activity of extract for both plants. Where as the carrageeanan group recorded the higher percentage of neturopihils and
lymphocytes and higher count of white blood cells. This was explained in the study of USEPA (1991) that the animal body may point out some chemical and their metabolite as invading hemotoxicants. No change in monocytes and eosinophil count (eosinophil share in parasitic inflammation and monocytes share in viral inflammation).

**Conclusions:**

1. Both plants extract (*Trigonella foenum grecum* and *Ziziphus spina christi*) possess anti-inflammatory activity.

2. Both plants extracts were safe at the doses used.

3. *Ziziphus spina christi* possesses a better anti-inflammatory activity than *Trigonella foenum grecum* and even somewhat better than indomethacine.

**Recommendations:**

1. Investigation of anti-inflammatory activity of both plants by using higher doses may record higher activity.

2. Investigation of both plants in order to establish their range of safety when used topically on skin.

3. Investigation of other parameters and other phytochemical by different doses and long period of time needed to be done.
REFERENCES


51


