Effect of *Leptadenia arborea* and *Syzygium aromaticum* on Albino Rats

By
TWADU ALI SAEED
BVSc. (1998), Khartoum.

A Thesis Submitted in accordance with the requirements of the University of Khartoum for the Degree of Master in veterinary science (Veterinary Toxicology)

Supervisor
Dr. Samia Mohammed Ali El Badwi
Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum.

Co-Supervisor
Professor Mohammed Galal Mohammed
Medicinal and Aromatic Plants Institute, National Research Center
Ministry of Science and Technology, Khartoum.

Faculty of Veterinary Medicine
University of Khartoum
April, 2006
To my Husband Dr Osman and to my twin who are the real skeleton of my all success
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>Vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>Ix</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td></td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xii</td>
</tr>
<tr>
<td>Abstract</td>
<td>Xiii</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>CHAPTER ONE</strong></td>
<td></td>
</tr>
<tr>
<td><strong>LITERATURE REVIEW</strong></td>
<td></td>
</tr>
<tr>
<td>1.1 Toxic plants</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Medicinal uses of plants</td>
<td>4</td>
</tr>
<tr>
<td>1.2.1 Anti-microbial effects</td>
<td>4</td>
</tr>
<tr>
<td>1.2.2 Anti-malarial effect</td>
<td>5</td>
</tr>
<tr>
<td>1.2.3 Anti-diabetic effects</td>
<td>5</td>
</tr>
<tr>
<td>1.2.4 Anthelmentic effects</td>
<td>6</td>
</tr>
<tr>
<td>1.2.5 Anti-inflammatory effects</td>
<td>6</td>
</tr>
<tr>
<td>1.3 Plants used in the present study</td>
<td>9</td>
</tr>
<tr>
<td>1.3.1 <em>Leptadenia arborea</em></td>
<td>9</td>
</tr>
<tr>
<td>1.3.1.1 Distribution</td>
<td>9</td>
</tr>
<tr>
<td>1.3.1.2 Traditional uses</td>
<td>9</td>
</tr>
<tr>
<td>1.3.1.3 Constituents</td>
<td>9</td>
</tr>
<tr>
<td>1.3.2 <em>Syzygium aromaticum</em></td>
<td>10</td>
</tr>
<tr>
<td>1.3.2.1 Distribution</td>
<td>10</td>
</tr>
<tr>
<td>1.3.2.3 Traditional uses</td>
<td>10</td>
</tr>
</tbody>
</table>
## 1.3.2.4 Constituents

10

## 1.3.2.5 Medicinal uses

10

### CHAPTER TWO

#### Materials and Methods

2.1 Materials and experimental designs 13

2.1.1 Toxicity to Albino rats of *Leptadenia arborea* ethanolic extract 13

2.1.1.1 Animals, housing and management 13

2.1.1.2 Administration and dose rates 13

2.1.1.3 Parameters 13

2.1.2 Toxicity to Albino rats of *Syzygium aromaticum* ethanolic extract 14

2.1.2.1 Animals, housing and management 14

2.1.2.2 Administration and dose rates 15

2.1.2.3 Parameters 15

2.1.3 Anti-inflammatory activity of *Leptadenia arborea* ethanolic extract. 15

2.1.3.1 Animals, housing and management 15

2.1.3.2 Administration and doses 15

2.1.3.3 Parameters 15

2.1.4 Anti-inflammatory activity of *Syzygium aromaticum* ethanolic extract 16

2.1.4.1 Animals, housing and management 16

2.1.4.2 Administration and dose levels 16

### 2.14.3 Parameters

16

#### 2.2 Methods

17

2.2.1 Preparation of the plant extracts 17

2.2.2 Haematological methods 17

2.2.2.1 Haemoglobin (Hb) concentration 17

2.2.2.2 Packed Cell Volume (PCV) 18

2.2.2.3 Red Blood Cell (RBC) count 18

2.2.2.4 White Blood Cell (WBC) count 18

2.2.3 Histological methods 18

2.2.4 Chemical methods 18

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) 19

2.2.4.2 Alanine amino transferase, ALT(Glutamic pyruvic transaminase, L- 20
aspartate, 2-oxoglutarate, GPT)

<table>
<thead>
<tr>
<th>2.2.4.3 Albumin</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.4.4 Total protein</td>
<td>20</td>
</tr>
<tr>
<td>2.2.4.5 Creatinine</td>
<td>21</td>
</tr>
<tr>
<td>2.2.4.6 Total bilirubin</td>
<td>21</td>
</tr>
<tr>
<td>2.2.4.7 Urea</td>
<td>22</td>
</tr>
<tr>
<td>2.2.5 Statistical methods</td>
<td>23</td>
</tr>
</tbody>
</table>

CHAPTER THREE
RESULTS

3.1 Response of Albino rats to the administration of *Leptadenia arborea* ethanolic extract

<table>
<thead>
<tr>
<th>3.1.1 Clinical Changes</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.2 Pathological Changes</td>
<td>24</td>
</tr>
<tr>
<td>3.1.3 Histopathological changes</td>
<td>24</td>
</tr>
<tr>
<td>3.1.5 Changes in serum metabolites</td>
<td>26</td>
</tr>
<tr>
<td>3.4.6 Hematological findings</td>
<td>26</td>
</tr>
</tbody>
</table>

3.2. Response of Albino rats to the *Syzygium aromaticum* ethanolic extract

<table>
<thead>
<tr>
<th>3.2.1 Clinical signs</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.2 Pathological Changes</td>
<td>27</td>
</tr>
<tr>
<td>3.2.3 Histopathological changes</td>
<td>27</td>
</tr>
<tr>
<td>3.2.4 Changes in serum metabolites</td>
<td>29</td>
</tr>
<tr>
<td>3.2.5 Hematological findings</td>
<td>29</td>
</tr>
</tbody>
</table>

3.3. Anti-inflammatory effect of *Leptadenia arborea* ethanolic extract on rats.

| 3.4. Anti-inflammatory effect of *Syzygium aromaticum* ethanolic extract on rats. | 31 |

CHAPTER FOUR
DISCUSSION

Conclusion

Suggestions for future work

REFERENCES

v
<table>
<thead>
<tr>
<th>Table No.</th>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average (mean ± S.D.) values of serum metabolites treated rats with <em>Leptadenia arborea</em> ethanolic extract for 3 weeks</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>Average (mean ± S.D.) haematological values of rats treated with <em>Leptadenia arborea</em> ethanolic extract for 3 weeks.</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Average (mean ± S.D.) values of serum metabolites of rats treated with <em>Syzygium aromaticum</em> ethanolic extract for 3 weeks</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>Average (mean ± S.D.) haematological values of rats treated with <em>Leptadenia arborea</em> ethanolic extract for 3 weeks.</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Average (mean ± S.D.) values of anti-inflammatory effect of <em>Leptadenia arborea</em> on carrageenan -induced paw oedema on rats.</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Average (mean ± S.D.) values of anti-inflammatory effect of <em>Syzygium aromaticum</em> ethanolic extract on carrageenan -induced paw oedema on rats.</td>
<td>32</td>
</tr>
</tbody>
</table>
### List of Figures

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dilatation of kidney tubules and shrinkage of glomeruli in kidneys of a rat dosed with ethanolic extract of <em>Leptadenia arborea</em> at 1000mg/kg at the end of week 2. <em>H&amp;E x 10.</em></td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Generalized fatty changes in the liver of a rat treated with ethanolic extract of <em>Leptadenia arborea</em> at 500mg/kg at the end of week 3. <em>H&amp;E x 10.</em></td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Severe fatty changes and area of necrosis of hepatocytes in liver of a rat treated with ethanolic extract <em>Leptadenia arborea</em> at 1000mg/kg at the end of week 3. <em>H&amp;E x 10.</em></td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>Congestion in renal blood vessels and dilatation in kidney tubules of a rat treated with <em>Syzygium aromaticum</em> ethanolic extract at 1000 mg/kg at the end of week 2. <em>H&amp;E x 10.</em></td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Necrosis in the cortex tubules and shrinkage of glomeruli in kidneys of a rat treated with <em>Syzygium aromaticum</em> ethanolic extract at 1000 mg/kg at the end of week 2. <em>H&amp;E x 10.</em></td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>Congestion in the portal tract with necrosis of hepatocytes in liver of a rat treated with <em>Syzygium aromaticum</em> ethanolic extract at 1000 mg/kg at the end of week 3. <em>H&amp;E x 10.</em></td>
<td>28</td>
</tr>
</tbody>
</table>
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>Aspartate amino-transferase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine amino transferase</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>i.m</td>
<td>Inta muscular</td>
</tr>
<tr>
<td>i.p</td>
<td>interaperitoneally</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum Bactericidal Concentration</td>
</tr>
<tr>
<td>MI C</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>NS</td>
<td>Not significant</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>p.o</td>
<td>per os</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed cell volume</td>
</tr>
<tr>
<td>PDH</td>
<td>Phosphate dehydrogenase</td>
</tr>
<tr>
<td>PM</td>
<td>Post mortem</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>ppm</td>
<td>Part per million</td>
</tr>
<tr>
<td>PV</td>
<td>Para Vilci</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>SAFB</td>
<td><em>Syzygium aromaticum</em> flower bud</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SSMO</td>
<td>Sudan Standards and Meterology Organization</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozocine</td>
</tr>
<tr>
<td>WBCs</td>
<td>White blood cells</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I wish to express my thanks and deep gratitude to my supervisors. Dr. Samia Mohamed Ali EL Badwi, Faculty of Veterinary Medicine, Department of Medicine, Pharmacology and Toxicology, University of Khartoum, for her guidance and encouragement throughout this work.

I am grateful to my co-supervisor professor Mohamed Galal, Medicinal and Aromatic Plant Institute, National Centre for Research, Khartoum, Sudan for his assistance throughout this work.

My lovely attitude and my deep feeling are directed to my family and to my husband Dr. Osman. A. Osman Sudanese Standard and Metrology Organization,(SSMO), Technical Department for his encouragement and utmost care and to my twins Ahmed and Alla for their patience throughout this work and for the long time I have been away from them.

Last but not least, my unlimited thanks to those not mentioned here by names and helped me throughout this work in one way or another.

TWADU
ABSTRACT

In this study the plants used in traditional and modern medicine are reviewed beside the toxic plants with focusing on *Leptadenia arborea* and *Syzygium aromaticum* covering their active ingredients and uses. This study was designed to investigate the toxicity of the ethanolic extracts of *Leptadenia arborea* and *Syzygium aromaticum* at different doses to rats via oral route. The anti-inflammatory potential of extracts of both plants was tested in rats by oedematous reaction inhibition.

Both ethanolic *Leptadenia arborea* and *Syzygium aromaticum* extracts were tested, using for each, 24 albino rats arranged in 4 groups (Control + 3 test groups). Three test groups were used for oral dosing at 250, 500 and 1000 mg/kg body wt./rat/day, continued for three weeks. Half survival animals were slaughtered at the end of week 2 and the rest at the end of week 3.

The anti-inflammatory activity of the two extracts was also recorded. Forty eight Albino rats were used, 24 rats for each extract were used in 4 groups receiving oral dose rates of 250 and 500 mg extract/kg body weight/rat, 10 mg indomethacine (reference anti-inflammatory)/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 subcutaneously with a local acute oedema inducer (0.1 ml of a 10% w/v carragenan saline suspension) in the sub-planter region of the left hind limb. Oedema size was monitored at 1, 2, 4, 6 and 24 hours.

Clinical signs were observed regularly. Sera were analyzed for enzymatic activities of AST and ALT and metabolic indicators albumin, total protein, bilirubin, urea, and creatinine. Also hematological changes in Hb, PCV, RBCs and WBCs were recorded. Tissue specimens of liver, kidneys, heart, intestines and spleen were examined for histopathology. The diameter of the hind paw was measured for assessment of the edema size.
For both experiments clinically no signs of toxicity were observed nor death or gross changes in the examined vital organs. Toxicity of *Leptadenia arborea* extract was characterized by dilatation of kidney tubules, generalized fatty changes in the liver and severe fatty changes and area of necrosis of hepatocytes in liver while *Syzygium aromaticum* treated rats showed congestion in renal blood vessels and dilatation in kidney tubules. Necrosis in the cortical tubules and shrinkage of glomeruli in kidneys and congestion in the portal tract with necrosis of hepatocytes. There are no significant changes in the serum (AST) and (ALT) activities and in the serum metabolites bilirubin, albumin, total protein, bilirubin, urea, and creatinine. On the other hand there were no significant changes in the values of RBCs, WBCs, Hb and PCV.

The ethanolic extract of *Leptadenia arborea* showed weak anti-inflammatory efficacies against carrageenan-induced paw edema in rats at the 4th hour. The ethanolic extract of *Syzygium aromaticum* highly (P<0.001) decreased the edema size at efficacy rates 79.41, 82.39 and 63.92 % for the dose, 500 mg/kg body weight at second, fourth and six hour respectively. The results were discussed with suggestions for future studies.
INTRODUCTION

Medicinal plants are most important to daily health and practices particularly in developing countries. Also herbal medicine became a topic of augmented global importance, having impacted on both world health and international trade (Akerele, 1988). Also recognition and development of medical and economic benefits of traditional medicinal plants is on increase in both developing and industrialized countries (WHO, 1998). Many sources from plants materials such as artemisinin and its derivatives, used for treatment of cerebral malaria, has turned attention to plants as potential sources of anti-malarial drugs (Wright and Philipson, 1990). In the Sudan many plants were used traditionally for treating different disorders. The traditional uses of plants need to validate their safety; therefore, there has been great importance in the study of herbal toxicities. Many studies were carried out on the medicinal uses of Leptadenia arborea and Syzygium aromaticum but the open literature mentioned no information on their toxicities.

This study was designed to examine the toxicity of Leptadenia arborea and Syzygium aromaticum ethanolic extracts to rats and also testing the anti-inflammatory activity of the extracts. Experimental design, materials used and methods adopted were described in Chapter Two, Results were evaluated and discussed in the subsequent Chapters.
CHAPTER ONE
LITERATURE REVIEW

1.1 Toxic plants

Medicinal plants were known to man from prehistoric times. Sudan has very rich and varied vegetation because of the large lands and various climates in different States. Many plants are used in the treatments of various disorders, and this encourage students and researchers to investigate the effects and the activities of plants.

*Caltropis procera* belongs to the family Asclepiaceae, which is locally known as Ushar, was found to be toxic to goats at 1 ml/kg/daily the oral dose and death occur between 30 minutes and 4 days at single doses of 0.005 ml/kg/day via the intravenous route (Elbadwi 1997).

Studies of Elsayed *et al.* (1983); Galal *et al.* (1985) and Bakhiet and Adam (1996) showed that *Cassia senna* and *C. italica* (Fabaceae/Caesalpinaceae) is known in the Sudan as Sanamakka, are toxic to ruminants and chicks.

*Albizia anthelmintica* (Fabaceae/Mimosaceae) is known in the Sudan as Girf el-dud. The butanolic fraction of the plant bark was highly toxic to rats at dose rates of 25 – 150 g/kg body weight (Galal *et al.*, 1991).

According to Watt and Breyer-Brandwijk, (1962) *Balanites aegyptiaca* (L.) Delile(Zygophyllaceae) which known in the Sudan as Higlig tree and its fruit is Lalobe, the fruit and the bark are a fish poisons.

*Arestolochia bracteolata* (Aristolochiaceae) of the common name Um Galagel, in the Sudan and folkolorically used as an antidote against scorpion;
stings and snake bites (El Diridiri et al., 1987). The acute toxicity of *A. bracteolata* to rats showed hepatonephropathies (Hashimoto et al., 1999), while Khairalla (2002) found that the ethanolic extract of the plant showed high toxicity to rabbits causing 100% mortality rates at 250, 500, and 1000 mg/kg body weight within 4-7 days.

The Euphorbiaceous *Jatropha curcas* is known in the Sudan as Habbat EL Mulouk. It was proved to be toxic to mice, goats, calves, sheep and chicks (Adam, 1974; Adam and Magzoub, 1975; Ahmed and Adam, 1979* and EL Badawi, 1990).

Ali and Adam (1978) described the toxicity of the plant *Acanthospernum hispidium* (Compositeae) to mice and found that shoots of *A. hispidium* fed to mice at 1, 5, 10, 30 and 50% of the diet were toxic.

*Ambrosia maritima* belongs to the family Asteraceae and in the Sudan is known as Demsisa (Bakhiet and Adam, 1996). They found that feeding of *A. maritima* shoots in the basic diet to chicks at 2 or 10% for 6 weeks was not lethal, but caused pathological changes.

Administration of aqueous suspension of the green or dried leaves of *Azadarichia indica* to goats and guinea pigs at doses of 50 or 200 mg/kg orally for eight weeks produced toxicity signs characterized by decrease in body weight, weakness, in-appetence and loss of condition (Ali, 1987). Also Ibrahim (1990), found that the concentration of 2% and 5% of *A. indica* leaves, ripe and unripe fruits in the basic diet are toxic to Brown Hisex chicks and that toxicities are mainly associated with hepatonephropathies and reduced growth rate. Osman (2005) in his investigations reported that *A. indica* seed and seed kernel methanolic extract caused histopathological and biochemical changes when given to rats and chicks by different routes.
Khairalla (2002), indicates that the ethanolic extract of the plant *haplophyllum tuberculatum* (Rutaceae) locally known as Um Museik, is toxic to rabbits at the doses 250,500 and 1000mg /kg body weight causing mortalities of 0%, 25% and 50% respectively.

Omer *et al.*, (1992) mentioned that *Abrus precatorius* seed locally know as Habet Elarus was highly toxic and fetal 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 signs of poisoning were dullness, reduction in feed consumption, loss of condition and recumbencey.

The seeds of *Piper abyssinca* (Piperaceae), locally known as Shaw Makkada, was found to be toxic for Nubian goats and chickens and the toxicity was manifested by diarrh0ea, bloat, dysopnoe , conjunctivitis, in appetence and recumbencey (Ali ,1995).

1.2. Medicinal uses of plants

1.2. 1 Anti-microbial effects

Schneider (1986) found that Neem oil has suppressed several species of bacteria including *Staphylococcus aureus* and *Salmonella typhosa* while studies of Patel and Trivdi (1962), indicate that Neem oil is not effective against *Citrobacter*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptcoccus faecalis*.

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

*Teucrium polium* that belongs to the family Lamiaceae is known to posses a bactericidal activity against *Staphylococcus aureus* and *E. coli* (Al-yahya *et al.*, 1990).
The latex of *Ipomea fistalosa* (Convolvulaceae) is used in folk medicine as antiseptic and the chloroform extracts of the stem and flower of the plant have been investigated for their activity against 7 Gram positive bacteria, 13-Gram negative bacteria and 8 fungi using the disc diffusion method and exhibited potent antibacterial activity against *Shigella* and *Bacillus* species. For example, oils from Basil, Bay, Clove, Thyme and Rosemary had anti-bacterial activities against *L. monocytogenes* and other pathogens (O'gara et al., 2000).

*Commiphora mirrha* (Morr Higazi) petroleum ether extract possesses anti bacterial activity against *Bacillus subtilis* and *Staphylococcus areus* and high antimycotic activity against *Candida albicans* (Omer, 1997).

1.2.2 Anti-malarial effect

*Tamarindus indica* (Caesalpiniaaceae) ripe fruits are, used in Sudanese traditional medicine to treat malaria, dysentery, rheumatism, wound healing and snake bites (ElGazali et al., 1994).

*Khaya senegalensis* (Meliaceae) was used in the Sudan to treat malaria. The plant activity in vitro investigations against *Plasmodium falciparum* chloroform extract showed high inhibition while the butanolic extract showed a prominent larvicidal activity against *Culex quinquefascitutus* (Abadi, 1997). The study of El Tahir et al. (1999) described the antiplasmoidal activity of selected Sudanese medicinal plants.

1.2.3 Anti-diabetic effects

An aqueous extract of seed of *Tamarindus indica* was found to have potent antidiabetogenic activity that reduces blood sugar level in streptozocine (STZ-induced) diabetic in rats (Maiti et al., 2004). The anti-hyper/hypoglycemic activities of Neem parts was reported (Mutry and Rao,
1978; Dixit et al., 1986; Chattopadhyay et al., 1986; 1987a,b; Chattopadhyay, 1999; Osadebe et al., 2004 and Gupta et al., 2004).

1.2.4 Anthelminthic effects

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).

The ethanolic extract of Azadirachta indica gave significant results against the gastrointestinal nematode H. contortus in lambs (Hordegen et al., 2003).

Nauclea latifolia has anthelmintic effect against ovine nematodes in Nigeria (Onyeyiti et al., 2001). Boscia angustifolia belongs to the family Capparidaceae and showed purgative effects (Elegami et al., 2001).

The aqueous extract from the bark of Albizia anthelmintica which is known locally as Girf Eldud (Fabaceae) was found to be highly effective against experimental Hymenlepis diminuta infection in rats (Galal, et al., 1991). Piper abyssinica belongs to the family Piperaceae, locally known as Shaw Makkada, of which anthelmintic activity against experimental infection of the tape worm Raillietina tetragona in chicks was evaluated by Osman, (2001).

1.2.5 Anti-inflammatory effects

Trigonella foenum-graecum (TFG; Fenugreek) belongs to the family Fabaceae known in the Sudan as Hilba. The anti-inflammatory and antipyretic effects of the plant seeds were described by Ahmadani et al. (2001).

The crude hydrochloric extract of Zingiber officinale which belong 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). The anti-inflammatory activity of the ethanolic extract of Capparis decidua (Capparidaceae), known in the Sudan as Tundub, showed significant inhibition of carrageenan paw edema in rats (Ageel et al., 1985).

The studies of Kumar and Busu (1994), Dewan et al. (2000 a,b) and Kumar et al. (2001) 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 and 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 antiepileptic agent (Russo and Borrelli, 2005).

The methanolic extract of Bryophyllum pinnatum was found to produce significant anti-inflammatory effect against carrageenan-induced inflammation in rats at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg. The highest dose of 300mg showed significant inhibition higher than the phenylbutazone at (100 mg/kg) Siddharthapal (1990).

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

Piper chaba which belongs to the family Piperaceae grow India and Malaya Island (Kirtikar and Bausu, 1980). The evaluation of the analgesic, anti-inflammatory, diuretic and anti-diarrhoeal effects of the stem bark of this plant in rats and mice was reported by Taufiq-Ur-Rahman et al. (2005).
The Anti-inflammatory effect of *Azadirchta indica* was studied by Raji *et al.* (2004), Chattopadhyay *et al.* (1992), Chattopadhyay (19980 and Osman (2005).

Ahmed *et al.* (1993) investigated the anti-inflammatory activity of the ethanolic extract of *Caralluma tuberculate* (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 no antipyretic effect was observed.

The aqueous leaf extract of *Persea americana* (laurceae) produced significant inhibition at the dose 800 mg/kg of the inflammation caused by carrageenan at hour therein rats. This effect was similar to that produced by indomethacine at the same time (Adeyemi *et al.*, 2002).

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

*Sysygium cumin* (Myrtaceae) ethanolic extract of the bark was investigated in rats at doses of 100,300 and 1000 mg/kg, administered orally, against kaolin –carrageenaan and formaldehyde – induced rat paw edema and cotton pellet-induced granuloma where the plant showed significant inhibition (Muruganandan and *tal.* 2001).
1.3. Plants used in the present study

1.3.1 Leptadenia arborea

*Leptadenia arborea* is a member of family Asclepiadaceae.

1.3.1.1 Distribution and vernacular name

Widespread in the Sudan and known as Shaaloub

1.3.1.2 Traditional uses

Traditionally the plant is used in India as galactagogue for both women and cows (*Narasimhamurthy, 1969*). In the Sudan the powdered stem is used for nose and tooth swelling.

1.3.1.3 Constituents

Different compounds were isolated from *Leptadenia* spp such as amyrins, diosmetin, luteolin, sterol and leanolic acid (*Andrews, 1952*). The following compounds were isolated from the leaves, stem and fruit of *Leptadenia hetrophylla* (syn); a hydrocarbon (C17H36), beta-amyrin, alpha-amyrin, a mixture of campesterol and stigmasterol, a triterpene alcohol and the flavonoids, chrysoeriol, apigenin, kaempferol, luteolin, astragalin, cosmosiin, isoquercitrin, luteolin-7-0-beta-D-glucopyranoside and paonoside (*EL-Moghazy et al., 1980*). The aerial part of *Leptadenia arborea* has been shown to contain pinoresinol, syringaresinol, leucanthemitol and E-ferulaldehyde. These known compounds are being reported for the first time from this plant. Among them, syringaresinol has shown an inhibitory effect against acetylcholinesterase (*EL-Hassan et al., 2003*).
1.3.2 *Syzygium aromaticum*

*Syzygium aromaticum* belongs to family Myrtaceae.

1.3.2.1 Distribution and vernacular names

It is indigenous to Moluccas and Southern Philippines, India, Zanzibar, Mauritius and Sri Lanka. It is also cultivated in Africa including Madagascar and United Republic of Tanzania. It is known as Clove, Cloves and Koronfoul (WHO, 1998).

1.3.2.2 Description

It is a small evergreen tree, 10-20 m high. Leaves are pinkish to dark green. Fruits are dark red. Buds readily exude oil when pressed (WHO, 1998).

1.3.2.3 Traditional uses

The dried flower bud (Clove) of the plant was reported as aphrodisiac, stomachic, carminative, antispasmodic and useful in cataract. It has also been used for nausea and vomiting, while in tropical Asia, it has been given to treat such diverse infections as malaria, cholera and tuberculosis (Chevaillier, 2001).

1.3.2.4 Constituents

Two new apigenin triglycosides, apigenin 6-C-[β-d-xylopyranosyl-(1″→2″)-β-d-galactopyranoside]-7-O-β-d-glucopyranoside and apigenin 6-C-[β-d-xylopyranosyl-(1″→2″)-β-d-galactopyranoside]-7-O-β-d-(6″-O-p-coumarylglucopyranoside) were isolated from the ethanol extract of the seeds of *Syzygium aromaticum* (Nassar, 2006).

1.3.2.5 Medicinal uses

Yukawa et al., (1996) in his investigation found that hot water extracts of four traditional herbs, *Geum japonicum*, *Syzygium aromaticum*, *Terminalia chebula* and *Rhus javanica* have anti-herpes simplex virus (HSV) activity in vivo. The
molluscidal activity of dried root latex powder of *Ferula asafoetida*, flower-bud powder of *Syzygium aromaticum* and seed powder of *Carum carvi* against the snail *Lymnaea acuminata* was studied. The molluscicidal activity of all the three plant products was found to be both time and concentration dependent. The toxicity of *Syzygium aromaticum* flower-bud powder (96 h LC50:51.98 mg/l) was more pronounced than that of root latex powder of *F. asafoetida* (96 h LC50:82.71 mg/l) and seed powder of *C. carvi* (96 h LC50:140.58 mg/l) (Kumar and Singh, 2005).

Ghelardini et al., 2001) studied the local anesthetic activity of β-caryophyllene, one of the main components of clove oil obtained from the dried flower-buds of *Syzygium aromaticum*. Anaesthetic activity was evaluated *in vivo* in the rabbit conjunctival reflex test and *in vitro* in a rat phrenic nerve-hemidiaphragm preparation.

According to (Dung et al., 2005) (Clove: *Syzygium aromaticum* L.) Which is used as supplementary ingredients by some local starter producers, was observed to stimulate the mould and yeast growth? Based on traditional starter manufacturing methods and modified on the basis of optimization experiments, a laboratory-scale manufacturing process for defined mixed-culture starter granules was established. In accordance with the national standard method, the wine produced with new experimental starter granules was found to have superior flavor and overall acceptability, compared with local commercial rice wines.

The effect of aqueous extract of *Syzygium aromatic* (L.) Merr. et Perry (Myrtaceae) flower bud (SAFB) was used immediate hypersensitivity induced by compound 48/80 systemic anaphylaxis in rats (LD50=31.25 mg/kg, i.p.).
The results indicate that SAFB inhibits immediate hypersensitivity by inhibition of histamine release from mast cells in vivo (Kim et al., 1998).

The antimicrobial activity of the essential oils of Eucalyptus (Eucalyptus globules), Ttea tree (Melaleuca alternifolia), Rosemary (Rosmarinus officinalis), Mint (Mentha piperita), Rosa moschata (Rosa moschata), Clove (Syzygium aromaticum), Llemon (Citrus limonum), Oregano (Origanum vulgare), Pine (Pinus silvestry) and sweet Basil (Ocimum basilicum) on survival and growth of different strains of E. coli O157:H7. The strains of E. coli exhibited similar susceptibilities to the action of the essential oils assayed. The essential oil with the lowest MIC and MBC (Minimum Inhibitory and Bactericidal Concentration, respectively) was Clove (0.25 ml and 0.3 ml/100 ml, respectively) and the results demonstrated that Clove exerted a significant bactericidal and bacteriostatic action (Moreira et al., 2005).

Ten herbal extracts with therapeutic antiherpes simplex virus type 1 (HSV-1) activity. Among these, Geum japonicum Thunb., Rhus javanica L., Syzygium aromaticum (L.) Merr. et Perry, or Terminalia chebula Retzus showed a stronger anti-HSV-1 activity in combination with acyclovir than the other herbal extracts in vitro. When acyclovir and/or a herbal extract were orally administered at doses corresponding to human use, each of the 4 combinations significantly limited the development of skin lesions (Kurokawa et al., 1995).
2.1 Materials and experimental designs

2.1.1 Toxicity to Albino rats of *Leptadenia arborea* ethanolic extract

2.1.1.1 Animals, housing and management

Twenty four male Wister white (Albino) rats weighing 100-150gm were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartom, Sudan, where they were housed in cages (each of dimensions 12x12x12cm, accommodating one dose-group) and maintained in a room under standard environmental condition, controlled temperature (22±2°C), relative humidity (60%) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by color tail marks. One week was allowed as a preliminary adaptive period.

2.1.1.2 Administration and dose rates

At the end of the adaptation period, the animals were weight-distributed and allotted randomly to four groups, each of six rats. Rats in Group 1 were the undosed control. *Leptadenia arborea* ethanolic extract was concentrated by evaporating the solvent and the dried material was redissolved in distilled water and given orally in daily doses at 250 mg/kg body wt./rat to Group 2. Rats in Group 3 received 500 mg/kg body wt./rat/day, while rats in Group 4 received 1000 mg/kg body wt./rat/day. Dosing continued for three weeks.

2.1.1.3 Parameters

Clinical signs and mortality rates were recorded. Blood samples were obtained from the ocular vein before the start of the experimental dosing and thereafter fortnightly for haematological investigations and serum analysis. Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC) and White Blood Cell (WBC) counts were estimated.
Sera were analyzed for the activities of AST, ALT and also for the concentrations of metabolic indicators creatinine, bilirubin, urea, albumin and total protein; Lots of three rats from each group were anaesthetized with diethyl ether and sacrificed mid experiment and at week 3. Tissue specimens of liver, kidneys, heart, intestines and spleen were fixed in 10% neutral buffered formalin and processed for histopathology.

2.1.2 Toxicity to Albino rats of *Syzygium aromaticum* ethanolic extract

2.1.2.1 Animals, housing and management

Twenty four male Wister white (Albino) rats weighing 100-150gm were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research Khartom, Sudan, where they were housed in cages (each of dimensions 12x12x12cm. accommodating one dose-group) and maintained in a room under standard enviromental condition, controlled temperatur (22±2°C), relative humidity (60 %) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by tail marks. Experimental animals were allowed one week as a preliminary adaptive period.

2.1.2.2 Administration and dose rates

At the end of the adaptation period, the animals were weighed-distributed and allotted randomly to four groups, each of six rats. Rats in Group 1 were the undosed control. *Syzygium aromaticum* ethanolic extract was concentrated by evaporating the solvent and the dried material was redissolved in distilled water and given orally in daily doses at 250 mg/kg body wt./rat to Group2. Rats in Group3 received 500 mg/kg body wt./rat/day, while rats in Group4 received 1000 mg/kg body wt./rat/day. Dosing continued for three weeks.

2.1.2.3 Parameters
Parameters adopted in clinical signs, blood and sera values were similar to those described for rats challenged with the *Leptadenia arborea* ethanolic extract.

### 2.1.3 Anti-inflammatory activity of *Leptadenia arborea* ethanolic extract.

#### 2.1.3.1 Animals, housing and management

Twenty four male Wister white (Albino) rats weighing 100-130gm were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research Khartom, Sudan, where they were housed in cages (each of dimensions 12x12x12cm, accommodating one dose-group) and maintained in a room under standard environmental condition, controlled temperature (22±2°C), relative humidity (60 %) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by tail color marks. One week was allowed as a preliminary adaptive period.

#### 2.1.3.2 Administration and doses

Post adaptive period, rats were weighed-distributed and divided randomly to 4 groups each of 6 rats. All groups' individuals were injected subcutaneously with 0.1 ml of a 10% w/v carragenan (Sigma Chemical Co; St Louis, Mo, USA) suspension (0.1ml of a 1% suspension in 10% saline in the sub-planter region of the left hind limb as a local acute oedema inducer 30 minutes subsequent to extract injection. *Leptadenia arborea* ethanolic extract was redissolved in distilled water and given orally to rats of **Group 1** at 500 mg/kg body wt. and 1000 mg/kg body wt. to rats of **Group 2**. Rats in **Group 3** were treated po with indomethacine (Hikma Pharmaceutical, Amman, Jordan) 10 mg/kg body wt. as a reference compound. **Group 4** rats were the un-treated control and received po 1ml /kg body wt. of normal saline.

#### 2.1.3.3 Parameters
Paw diameter was measured initially (zero hr.) and successively at 1, 2, 4, 6 and 24 hours post carregeenan injection using Hauptner tuberculin caliper (Hauptner, GmbH, Germany) to the nearest millimeter.

2.1.4 Anti-inflammatory activity of *Syzygium aromaticum* ethanolic extract

2.1.4.1 Animals, housing and management

Twenty four male Wister white (Albino) rats weighing 100-130gm were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research Khartom, Sudan, where they were housed in cages (each of dimensions 12x12x12cm, accommodating one dose-group) and maintained in a room under standard environmental condition, controlled temperature (22±2°C), relative humidity (60 %) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by tail color marks. One week was allowed as a preliminary adaptive period.

2.1.4.2 Administration and dose levels

Post adaptive period, rats were weighed-distributed and divided randomly to 4 groups each of 6 rats. *Syzygium aromaticum* ethanolic extract preparation and injection at different dose levels to test groups, concentration and subcutaneous injection of carregeenan and indomethacine were all done similar to that with *Leptadenia arborea* ethanolic extract. **Group 4** rats were the untreated control and received orally 1ml /kg body wt. of normal saline.

2.1.4.3 Parameters

The diameter of the hind paw was measured as described in the previous experiment.

2.2 Methods
2.2.1 Preparation of the plant extracts

*Syzygium aromaticum* fruits 200 gm were obtained from a local market Khartoum North, cleaned, dried and finely ground by an electric mill. The powder obtained was successively extracted with ethanol for 14 hrs. using soxhelt apparatus. The ethanolic extract was occasionally shaken during the first four hours and was then filtrated. The filtrate was evaporated under vacuum, and the residue is brownish in color and weighed 23gm (11.50% yield).

A total of 470 gm *Leptadenia arboresa* whole plant was collected from Khartoum North area, identified at the Medicinal and Aromatic Plants Institute. The ethanolic extract of the plant was prepared following the same method of *Syzygium aromaticum* ethanolic extract the residue is greenish in color and weighed 65.50gm (13.93% yield).

2.2.2 Haematological methods

These were described by Schalm (1965). Blood samples from rats were collected into clean dry bottles containing the anti-coagulant heparin from the ocular veins.

2.2.2.1 Haemoglobin (Hb) concentration

The concentration of haemoglobin was determined by the methaemoglobin technique. The method is based on the conversion of haemoglobin by Drabkins solution (0.2 g potassium cyanide, 0.2 g potassium ferricyanide and 1 g 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 were centrifuged in a micro haematocrit centrifuge (Hawksley and Sons Ltd. England) for 5 minutes. 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 with an improved Neubauer haemocytometer (Hawksley and Sons Ltd., England). Formal citrate was used as a diluent.

2.2.2.4 White Blood Cell (WBC) count

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

2.2.3 Histological methods

The specimens were collected immediately after slaughter and fixed in 10% formal saline, embedded in paraffin wax, sectioned at 5 µm and stained with haemotoxylin and eosin (H & E) using Mayer's haemalum.

2.2.4 Chemical methods

Blood samples obtained, initially and every two weeks thereafter, from the ocular vein of rats on dosing with the test extracts were used to prepare sera for the chemical methods. Venous blood samples were allowed to clot. Serum was separated by centrifugation at 3000 r.p.m. for 5 minutes and stored at -20°C until analyzed. Spectrophotometer, Merck Mega, Version 0.6, 1995 (E. Merck, Darmstadt, Germany) was used to analyze serum activities of enzymes AST and ALT and serum metabolites albumin, total protein, creatinine, total bilirubin and urea.
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 activity was measured by a commercial kit (Randox Laboratories Ltd, U.K.)

Test principal: Aspartate aminotransferase catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxalacetate:

\[
\text{L-aspartate} + \alpha\text{-ketoglutarate} \stackrel{\text{GOT}}{\leftrightarrow} \text{L-glutamate} + \text{oxalacetate.}
\]

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

\[
\text{Oxalacetate} + \text{NADH} + \text{H}^+ \stackrel{\text{MDH}}{\rightarrow} \text{Malate} + \text{NAD}
\]

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 wavelength of 365 nm was read at one minute intervals after mixing the serum with the buffered substrate solution. The mean absorbance change per minute (\(A_{365}/\text{minute}\)) was used for calculation of enzyme activity as follows:

\[
1\text{ U} = A_{365} \text{ nm/minute} \times 2059
\]
2.2.4.2 Alanine amino transferase, ALT (Glutamic pyruvic transaminase, L-aspartate, 2-oxoglutarate, GPT)

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

Test principal:

\[
\text{Oxoglutarate} + \text{L-alanine} \overset{\text{GPT}}{\longrightarrow} \text{L-glutarate} + \text{pyruvate}
\]

The absorbance of samples were read against the reagent blank after 5 minutes at wavelength of 630 nm UV/VIS Spectrophotometer. The GPT was measured in U/L.

2.2.4.3 Albumin

Serum albumin was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.)

Test principle

The measurement of serum albumin is based on its 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 the standard (A standard) was measured against the reagent blank at a wavelength of 600 nm and albumin concentration (C) was calculated as follows:

\[
C \text{ (g/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times \text{concentration of standard}
\]

2.2.4.4 Total protein
Total serum protein was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.)

**Test principle**

Colorimetric determination of total protein in serum is based on the Biuret reaction. The 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 the blue colour. The intensity of the colour 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:

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

**2.2.4.5 Creatinine**

Serum creatinine concentration was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

**Test principle**

Creatinine forms a coloured complex with picric acid in an alkaline medium. The colour intensity is proportional to the concentration of the reaction mixture. Serum was mixed with a mixture of picric acid and sodium hydroxide. Absorbance change/minute (A_{492}/min.) of the sample and standard was recorded at a wavelength of 492 nm and the change in absorbance was used in calculations as follows:

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

**2.2.4.6 Total bilirubin**
Serum total bilirubin concentration was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.)

**Test principle**

Albumin-bound bilirubin is released by a detergent and the total bilirubin reacts with 2, 4-di-chloroalanine to form a coloured azobilirubin which is measured in the Spectrophotometer at a wavelength of 456 nm.

Serum was mixed with the working reagent solution and the mixture was allowed to stand for 10 minutes protected from light. Absorbance of the sample (A- sample) was measured against the sample blank and concentration (C) of bilirubin was estimated as follows:

\[ C \text{ (mg/dl)} = A_{\text{sample}} \times 12.5 \]

2.2.4.7 Urea

Serum urea concentration was estimated by an enzymatic colorimetric method using a kit (Randox Laboratories Ltd., U.K.).

**Test principle**

Ammonia and carbon dioxide are produced when urea is hydrolysed by urease

\[ \text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} \text{Ammonium carbonate} + \text{CO}_2 \]

Ammonium ions react with phenol and hydrochlorite to give a coloured complex.

**Protocol**

The serum was mixed with a buffered urease solution and the mixture was incubated for 10 minutes at room temperature. The hydrochloride solution was added and mixed and the contents were incubated at room temperature for 15 minutes. The absorbency of the sample (A sample) and of the standard (A standard) were read against the blank at a wavelength of 546 nm and the concentration (C) of urea was calculated as follows:
C (mg/dl) = \frac{A \text{ sample}_X \text{ concentration of the standard}}{A \text{ standard}}

2.2.5 Statistical methods

The difference between mean values of data was analyzed by the un-paired students- t-test. The efficacies were obtained by calculating the differences 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 \(a\) is mean of the edema size in the control and \(b\) the edema size in the treated rats. (Snedecor and Cochran, 1989).
CHAPTER THREE
RESULTS

3.1 Response of Albino rats to the administration of *Leptadenia arborea* ethanolic extract.

3.1.1 Clinical Changes

Rats in groups 2 (250mg/kg), 3 (500mg/kg) and 4 (1000mg/kg) showed no clinical signs during the experimental period (3 weeks) and no mortalities were recorded. In the un-dosed control rats (group1) there were no abnormalities seen.

3.1.2 Pathological Changes

For all test groups 2, 3 and 4, no gross changes were observed at postmorteun 10days post dosing or at the end of the experiment (3 weeks). No lesions were observed in the control group (group1).

3.1.3 Histopathological changes

The changes in the organs section were characterized by dilatation of kidney tubules and hrinkage of the glomeruli in group3(1000mg/kg)(Fig.1).There was generalized fatty changes in the liver of rat treated with 500mg/kg (Fig. (2) and sever fatty changes and area of necrosis of hepatocytes in liver of rat treated with 1000mg/kg .(Fig. 3).
Fig. (1) Dilatation of kidney tubules and Shrinkage glomeruli in kidneys of a rat treated with ethanolic extract of *Leptadenia arborea* at 1000mg/kg at the end of week 2. *H&E x 10.*

Fig. (2) Generalized fatty changes in the liver of a rat treated with ethanolic extract of *Leptadenia arborea* at 500mg/kg at the end of week 3. *H&E x 10.*

Fig. (3) Severe fatty changes and area of necrosis of hepatocytes in liver of a rat treated with ethanolic extract of *Leptadenia arborea* at 1000mg/kg at the end of week 3. *H&E x 10.*
3.1.4 Changes in serum metabolites

Table 1 shows the changes in serum metabolites of rats treated with *Leptadenia arborea* ethanolic extract for 3 weeks.

Table 1. Average (mean ± s.d.) values of serum metabolites of treated rats with *Leptadenia arborea* ethanolic extract for 3 weeks.

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>AST (i.u/l)</th>
<th>ALT (i.u/l)</th>
<th>T.protien (g/dL)</th>
<th>Albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (un-dosed control)</td>
<td>07.00 ± 00.71</td>
<td>08.00 ± 01.41</td>
<td>06.95 ± 00.35</td>
<td>04.80 ± 00.14</td>
</tr>
<tr>
<td>G2 (250 mg/kg/day)</td>
<td>10.33 ± 01.53</td>
<td>09.00 ± 1.00</td>
<td>07.20 ± 00.20</td>
<td>04.10 ± 00.20</td>
</tr>
<tr>
<td>G3 (5000 mg/kg/day)</td>
<td>10.33 ± 01.53</td>
<td>10.67 ± 1.15</td>
<td>07.00 ± 00.10</td>
<td>04.20 ± 00.30</td>
</tr>
<tr>
<td>G4 (1000 mg/kg/day)</td>
<td>09.33 ± 01.15</td>
<td>08.67 ± 1.15</td>
<td>07.20 ± 00.17</td>
<td>04.10 ± 00.17</td>
</tr>
</tbody>
</table>

Table 1 continued

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>Billirubin (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (un-dosed control)</td>
<td>00.65 ± 00.07</td>
<td>24.50 ± 00.71</td>
<td>00.75 ± 00.07</td>
</tr>
<tr>
<td>G2 (250 mg/kg/day)</td>
<td>00.87 ± 00.12</td>
<td>26.00 ± 02.00</td>
<td>00.87 ± 00.12</td>
</tr>
<tr>
<td>G3 (5000 mg/kg/day)</td>
<td>00.73 ± 00.10</td>
<td>25.67 ± 00.06</td>
<td>00.73 ± 00.10</td>
</tr>
<tr>
<td>G4 (1000 mg/kg/day)</td>
<td>00.77 ± 00.06</td>
<td>26.00 ± 01.00</td>
<td>00.77 ± 00.06</td>
</tr>
</tbody>
</table>

All values of the test groups are similar (p<0.05) to the control.

The activities of AST and ALT showed no significant changes (P>0.05) for all groups. Normal values were recorded in the control group. While all groups showed no significant changes (P>0.05) in the concentration of urea, albumin, total protein, billirubin creatinine. No abnormalities were recorded in the control group.

3.1.5 Haematological findings

Table 2 is summarizing the hematological changes in blood of rats treated with *Leptadenia arborea* ethanolic extract.
Table 2 Average (mean ± s.d.) heamatological values of rats treated with *Leptadenia arborea* ethanolic extract for 3 weeks.

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>RBCs (10⁶/mm³)</th>
<th>WBCs (10⁴/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁ (un-dosed control)</td>
<td>10.50 ± 0.57</td>
<td>32.50 ± 3.54</td>
<td>01.31 ± 0.08</td>
<td>00.58 ± 0.02</td>
</tr>
<tr>
<td>G₂ (250mg/kg/day)</td>
<td>11.23 ± 0.10</td>
<td>29.33 ± 0.16</td>
<td>02.61 ± 0.14</td>
<td>00.77 ± 0.21</td>
</tr>
<tr>
<td>G₃ (500mg/kg/day)</td>
<td>12.07 ± 0.00</td>
<td>33.33 ± 0.79</td>
<td>01.59 ± 0.07</td>
<td>00.81 ± 0.14</td>
</tr>
<tr>
<td>G₄ (1000mg/kg/day)</td>
<td>11.00 ± 0.07</td>
<td>31.67 ± 0.53</td>
<td>01.56 ± 0.07</td>
<td>00.89 ± 0.06**</td>
</tr>
</tbody>
</table>

All values of the test groups are similar (p<0.05) to the control ** denotes p<0.001

For all groups no significant (P>0.05) effects in the number of RBCs while the WBCs number is significant (P<0.05). The concentration of Hb and PCV all groups showed no significant (P>0.05) changes .The control group showed normal values.

3.2. Response of Albino rats to the *Syzygium aromaticum* ethanolic extract

3.2.1 Clinical sings

Rats in groups 2, 3 and 4 showed no clinical signs during the experimental period (3 weeks). No mortalities were recorded. No abnormal behaviors were recorded in the un-dosed control rats (group1) and also no mortalities recorded.

3.2.2 Pathological Changes

For all test groups 2, 3 and 4, no gross changes were observed at post-mortem two weeks post dosing or at the end of the experiment (3 weeks). No abnormalities were observed in all experimental groups.

3.2.3 Histopathological changes

Histopathological changes in rats treated with 1000mg/kg (group 3) are mainly congestion in renal blood vessels and dilatation in kidney tubules (Fig. 4), necrosis in the cortical tubules and shrinkage of glomeruli in
kidneys (Fig. 5) and congestion in the portal tract with necrosis of hepatocytes in liver (Fig. 6).
Fig. (6) Congestion in the portal tract with slight necrosis of hepatocytes in liver of a rat treated with Syzygium aromaticum ethanolic extract at 1000 mg/kg at the end of week 3. H&E x 10.

3.2.4 Changes in serum metabolites

Table 3 is summarizing the changes in serum metabolites of rats treated with Syzygium aromaticum ethanolic extract.

The activities of AST and ALT showed no significant changes (P>0.05) for all groups. Normal values were recorded in the control group. Also all groups showed no significant (P>0.05) changes in the concentration of urea, albumin, total protein, billirubin and creatinine. No abnormal values were recorded in the control group.

Table 3 Average (mean ± s.d.) values of serum metabolites of rats treated with Syzygium aromaticum ethanolic extract for 3 weeks

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>AST (i.u/L)</th>
<th>ALT (i.u/L)</th>
<th>T.protein (g/dL)</th>
<th>Albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (un-dosed control)</td>
<td>7.00 ± 00.71</td>
<td>8.00 ± 01.41</td>
<td>06.95 ± 00.35</td>
<td>04.80 ± 00.14</td>
</tr>
<tr>
<td>G2 (250mg/kg/day)</td>
<td>6.67 ± 01.15</td>
<td>11.67 ± 4.93</td>
<td>07.03 ± 00.21</td>
<td>03.77 ± 00.32</td>
</tr>
<tr>
<td>G3 (5000mg/kg/day)</td>
<td>6.00 ± 02.00</td>
<td>9.67 ± 03.79</td>
<td>07.20 ± 00.26</td>
<td>04.17 ± 00.74</td>
</tr>
<tr>
<td>G4 (1000 mg/kg/day)</td>
<td>9.00 ± 02.56</td>
<td>8.67 ± 05.51</td>
<td>07.13 ± 00.35</td>
<td>03.67 ± 00.21</td>
</tr>
</tbody>
</table>

Table 3Continued

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>Billirubin (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (un-dosed control)</td>
<td>00.65 ± 00.07</td>
<td>24.50 ± 00.71</td>
<td>00.75 ± 00.07</td>
</tr>
<tr>
<td>G2 (250mg/kg/day)</td>
<td>00.53 ± 00.15</td>
<td>25.33 ± 02.52</td>
<td>00.77 ± 00.15</td>
</tr>
<tr>
<td>G3 (5000mg/kg/day)</td>
<td>00.10 ± 02.65</td>
<td>24.67 ± 02.52</td>
<td>00.73 ± 00.15</td>
</tr>
<tr>
<td>G4 (1000 mg/kg/day)</td>
<td>00.70 ± 04.58</td>
<td>21.67 ± 02.08</td>
<td>00.77 ± 00.15</td>
</tr>
</tbody>
</table>

All values of the test groups are similar (p<0.05) to the control.
3.2.5 Hematological findings

Table 4 summarized the hematological changes in blood of rats treated with *Syzygium aromaticum* ethanolic extract.

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>RBCs (10^6/mm^3)</th>
<th>WBCs (10^4/mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0 (un-dosed control)</td>
<td>10.50 ± 00.57</td>
<td>32.50 ± 03.54</td>
<td>01.31 ± 00.08</td>
<td>00.58 ± 00.02</td>
</tr>
<tr>
<td>G1 (250mg/kg/day)</td>
<td>12.70 ± 02.20</td>
<td>31.30 ± 01.53</td>
<td>02.18 ± 00.18</td>
<td>00.56 ± 00.06</td>
</tr>
<tr>
<td>G2 (500mg/kg/day)</td>
<td>15.46 ± 02.17</td>
<td>29.00 ± 15.59</td>
<td>02.49 ± 00.45</td>
<td>00.50 ± 00.02</td>
</tr>
<tr>
<td>G3 (1000mg/kg/day)</td>
<td>12.26 ± 01.40</td>
<td>36.66 ± 06.35</td>
<td>02.00 ± 00.12</td>
<td>00.54 ± 00.11</td>
</tr>
</tbody>
</table>

Table 4 Average (mean ± s.d.) heamatological values of rats treated with *Syzygium aromaticum* ethanolic extract for 3 weeks.

All values of the test groups are similar (p<0.05) to the control.

In all groups there were no significant (P>0.05) changes in the number of WBCs and RBCs and in the values of Hb and PCV. The control group showed no abnormalities.

3.3. Anti-inflammatory effect of *Leptadenia arborea* ethanolic extract on rats.

Table 5 shows the anti-inflammatory effect of *Leptadenia arborea* ethanolic extract on rats.

Rats of group 1 showed no significant (P>0.05) decrease in edema size in the first, second, fourth and 24th hours as compared to the control group, while in hour six the reduction of the oedema size is significant (P< 0.01). Efficacy rates of 37.06, 11.87, 28.89, 72.31 and 53.55% were at hrs 1,2,4,6 and 24 respectively.
Rats of group 2 showed no significant (P>0.05) decrease in edema size in the first, second and 4th fourth with efficacies of 30.00, 31.03 and 47.56 respectively, while it showed significant (P<0.05-0.01) effect in the sex and 24th hours as compared to the control group providing efficacies of 61.17 and 81.54 respectively.

Rats in group 3 (indomethacine) showed significant (P<0.05) difference in the edema size when compared to the control group only in the sixth hour with an efficacy of 65.57, but inhibition percentages of indomethacine during all hrs is better than the two doses of the plant extract.

Table 5. Average (mean ± s.d.) values of anti-inflammatory effect of *Leptadenia arborea* ethanolic extract on carrageenan-induced paw oedema in rats.

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>1hr Edema size</th>
<th>Inhibition %</th>
<th>2hr Edema size</th>
<th>Inhibition %</th>
<th>4hr Edema size</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (250mg/kg)</td>
<td>0.087± 0.044</td>
<td>37.06</td>
<td>0.027±0.039</td>
<td>11.87</td>
<td>0.016± 0.070</td>
<td>28.89</td>
</tr>
<tr>
<td>G2 (500mg/kg)</td>
<td>0.098 ± 0.042</td>
<td>30.00</td>
<td>0.014±0.042</td>
<td>31.03</td>
<td>0.018± 0.021</td>
<td>47.56</td>
</tr>
<tr>
<td>G3 10mg/kg (indomethacine)</td>
<td>0.073 ± 0.019</td>
<td>47.06</td>
<td>0.010±0.048</td>
<td>45.81</td>
<td>0.067± 0.012</td>
<td>70.22</td>
</tr>
<tr>
<td>G4 (un-dosed control)</td>
<td>0.140 ± 0.036</td>
<td>00.00</td>
<td>0.023 00.32</td>
<td>00.00</td>
<td>0.025 ± 0.021</td>
<td>00.00</td>
</tr>
</tbody>
</table>

Table 5. Continued
3.4. Anti-inflammatory effect of *Syzygium aromaticum* ethanolic extract on rats.

The anti-inflammatory effects of *Syzygium aromaticum* ethanolic extract on rats are shown in Table 6.

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>6 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Edema size</td>
<td>Inhibition %</td>
</tr>
<tr>
<td><strong>G1 (250mg/kg)</strong></td>
<td>0.85 ± 0.33**</td>
<td>53.55</td>
</tr>
<tr>
<td><strong>G2 (500mg/kg)</strong></td>
<td>0.70 ± 0.19**</td>
<td>61.17</td>
</tr>
<tr>
<td><strong>G3 10mg/kg</strong></td>
<td>0.63 ± 0.08**</td>
<td>65.57</td>
</tr>
<tr>
<td>(indomethacine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G4 (un-dosed control)</strong></td>
<td>0.83 ± 0.27</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 6. Average (mean ± s.d.) values of anti-inflammatory effect of *Syzygium aromaticum* ethanolic extract on carrageenan-induced paw oedema in rats.
Table 6. Continued

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>Edema size 6 hr</th>
<th>reduction %</th>
<th>Edema size 24 hr</th>
<th>reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.63 ± 0.21 NS</td>
<td>35.05</td>
<td>0.18 ± 0.12 NS</td>
<td>72.31</td>
</tr>
<tr>
<td>G₂ 500mg/kg</td>
<td>0.35 ± 0.22*</td>
<td>63.92</td>
<td>0.20 ± 0.90*</td>
<td>69.23</td>
</tr>
<tr>
<td>G₃ 10mg/kg (indomethacin)</td>
<td>0.63 ± 0.08 **</td>
<td>35.05</td>
<td>0.2 ± 0.23 NS</td>
<td>61.23</td>
</tr>
<tr>
<td>G₄ (un-dosed control)</td>
<td>0.97 ± 0.1</td>
<td>0.00</td>
<td>0.65 ± 0.19</td>
<td>0.00</td>
</tr>
</tbody>
</table>

NS = Not significant  
* denotes P<0.05  
*** denotes P<0.001

Group1 showed no significant (P>0.05) difference when compared to the control group in the first, six th and 24th hours and providing efficacies of 47.00, 35.05, 72.31 respectively. While at the second and the fourth hours showed significant (P<0.01) reduction in the edema size with efficacies of 54.12 and 54.23 respectively. Rats in group2 demonstrate high (P<0.001) reduction in the edema size in comparison to the control group in the second, fourth and six th hours providing efficacy rates of 79.41, 82.93 and 63.92 % respectively. Values of group 3 (indomethacin) indicate significant (P< 0.05-0.01) difference in the oedema size when compared to the control group only in the fourth and six th hours and efficacies were52.82 and 35.05 respectively. The inhibition percentages of the higher dose of the extract (500mg/kg).Throughout the period of experiment is better than the lower dose of the extract and even better than indomethacin.
Chapter Four

DISCUSSION

Methanol and ethanol were described to be efficient solvents in extracting phytochemicals from plant material (Eloff, 1998) and (Cowan, 1999). In this study ethanol was used for extracting Leptadenia arborea and Syzygium aromaticum.

The daily oral doses of both ethanolic extracts of Leptadenia arborea and Syzygium aromaticum to rats caused neither death nor any observable clinical toxicity signs for both extracts during the experimental period (3 weeks) at the doses used and these observations were reported also by Osman, (2005). This may be related to the dose levels administered and the duration of treatment.

Our findings in this study showed that the daily oral administration of the extracts caused changes in the vital organs and tissues of the treated rats with Leptadenia arborea. The changes in the organs section were characterized by dilatation of kidney tubules, generalized fatty changes in the liver and severe fatty changes and area of necrosis of hepatocytes in liver. While Syzygium aromaticum treated rats showed histopathological changes mainly, congestion in renal blood vessels and dilatation in kidney tubules, necrosis in the cortical tubules and shrinkage of glomeruli in kidneys beside congestion in the portal tract with necrosis of hepatocytes in the liver.

The assessment of the activities of enzymes AST and ALT in the serum and the concentration of urea and total protein used routinely for evaluating the functional status of the liver and of the renal toxicity in both clinical and experimental settings (Ahmed and Adam, 1979**, Yanpellewar et al., 2002 and Medani, 2003). In this study no significant changes were recorded in the biochemical constituents although there are histopathological
changes include congestion in renal blood vessels, dilatation in kidney tubules, necrosis in the cortical tubules, shrinkage of glomeruli in kidneys and congestion in the portal tract with necrosis of hepatocytes in liver. This is not surprising because many studies have described the presence of lesion in hepatic centriloblar zone without marked interference with excretory ability of the liver of ruminant such as damages produced by *Chrozophora plicata* (Galal and Adam, 1988). *Crotolaria saltina* (Barri and Adam, 1981) and *Jatropha curcas* (Ahmed and Adam, 1979)**.

Idris, et al. (1982) and Marrid et al. 1995) found that there were no changes in the concentration of creatinine and activity of AST in goats treated with *Artemisia herba alba*. And the aqueous extract of *Artemisia herba alba*. Histopathologically, he was mild hydropic degeneration in hepatocytes and proximal convullated tubules.

Carter (1990) recorded normal serum AST activity in Angus cows poisoned by *Cucumis myriocarpus*. On the other hand Dalvi (1985) reported that in rats received 250 mg/kg oral dose of Solanine, there were failure to monitor statistically significant increase in serum AST and ALT activities. This may be attributed to solanine slower absorption and faster degradation in the gastrointestinal tract compared to other routes. No changes in activities serum AST and ALT were detected by Seawright (1963) in *Lantana camara* poisoning despite the liver damage.

In rats treated with *L.arborea* and *S.aromaticum* ethanolic extract there was negligible effect on the haematology parameters tested. Rogers et al. (1979) noticed that in cattle poisoned by *Cassia occidentalis* there was no marked change in haematological parameters.

So we can conclude that *L.arborea* and *S.aromaticum* normal activities of serum AST and ALT with slight histopathological change in experiments with had no effect on liver, heart or kidneys of rats at the doses used.
The normal concentration of creatinine or urea together with mild effect of gross and microscopic renal changes indicated that *L. arborea* and *S. aromaticum* ethanolic extract had no effect on the kidney. 

*(DiRosa et al., 1971; Goetzl, 1980)* in their investigation reported that carrageenan-induced rat paw edema is associated with three distinct phases. The early first phase (1st hour) is mediated by mast cell degranulation, histamine and serotonin release. The second phase (2nd hour) is characterized by bradykinin release and pain, and in the last phase (3rd - 4th hour) eicosanoid production. In this study carrageenan was used as an acute form inducer of inflammation. The extract administered 30 minutes before the inflammation inducer and this because the inflammation phases were very short. Also many studies reported indomethacine, the non-steroidal anti-inflammatory drugs (NSAIDs), as a reference compound and it was used also in our study *(Levin and Taiwo, 1994 and Osman, 2005)*

The ethanolic extract of *Leptadenia arborea* demonstrates weaker inhibition effects in the edema size and this may be due to the insufficient absorption of the extract. The effects that produced by the reference compound indomethacine, observed during the phases was stronger than the extract of *Leptadenia arborea*.

The ethanolic extract of *Syzygium aromaticum* showed high inhibition effect at the dose 500 mg/kg. This dose showed neither death nor any untoward clinical signs. This indicates that the extract has a wide range of safety and its administration may not cause immediate toxic effect at least at the doses used in this study. These results agree with the earlier studies of the anti-inflammatory activities of some medicinal plants tested against rat paw edema *(Maulik et al., 1997; Chattopadhayay, 1998; Khairalla, 2002; Yanpallewar et al., 2002; Penna et al., 2003; 2005; Speroni et al., 2005 and Osman, 2005)*. These findings obtained in the rats paw oedema indicate the anti-
inflammatory potential of these plants is stronger even than the reference drug indomethacine.

Conclusion

- *L.arborea* ethanolic extract showed no signs of toxicity to rats at the doses used and showed weaker anti-inflammatory activities against rat paw edema.
- *S.aromaticum* ethanolic extract showed no signs of toxicity to rats at the doses used and showed potent anti-inflammatory effect against rat paw edema.
- Both plants extract posses marked anti-inflammatory activities verified by the high percentage inhibitory effect of the odema size, and the anti-inflammatory activity of *S.aromaticum* ethanolic extract is higher than that of *L.arborea* ethanolic extract

Suggestions for future work

- This study has demonstrated details of the effect of *L.arborea* and *S.aromaticum* ethanolic extracts for a period of three weeks. Studies are needed to investigate these extracts in higher doses and for a longer period with different routes of administration.
- Further investigation of the phytochemical constituents present in these extracts is needed to determine the mode of action of these plants.
- Investigations on other parameters such as glucose, liver specific enzymes and trace element needed to be done.
REFERENCES


foenicum – graecum leave in the rat. J. Ethnopharmacol, 75: 283-286.


Kurokawa, M; Nagasaka, K; Hirabayashi, T; Uyama, S; Sato, H; Kageyama, T; Kadota, S; Ohyama, H; Hozumi, T; Namba, T and Shiraki, K. (1995). Efficacy of traditional herbal medicines in combination with acyclovir against herpes simplex virus type 1


