University of Khartoum
College of Graduate Studies

Anthelmintic Activity of Extracts from Three Putative Medicinal Plants Against Caprine Haemonchosis

By

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A Thesis Submitted in Accordance with The Requirements of The University of Khartoum for The Degree of doctor of Philosophy (Ph.D.)

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Dedication
To my affectionate father, mother, brothers and sisters with my love.
CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction  1
1.2 Ethno veterinary medicine  4
1.3 Previous research and shortcomings 10
1.4 Haemonchosis 17
  1.4.1 *Haemonchus contortus* 17
1.4.2 Symptoms-pathogenicity 19
1.4.3 Epidemiology 19
1.4.4 Treatment 20
1.4.5 Control 20
1.5 Anthelmintic plants 23
  1.5.1 *Balanites aegyptiaca* 23
    1.5.1.1 Classification 23
    1.5.1.2 Distribution 24
    1.5.1.3 Botanical description 24
    1.5.1.4 Active constituent 24
    1.5.1.5 Medicinal Folk-uses 25
    1.5.1.6 Pharmacological studies 26
  1.5.2 *Peganum harmala* 27
    1.5.2.1 Classification 27
    1.5.2.2 Distribution 27
    1.5.2.3 Botanical description 28
    1.5.2.4 Active constituent 28
    1.5.2.5 Pharmacological studies 28
List of contents

1.5.2.6 Animal toxicity 31
1.5.3 *Zizyphus spina-christi* 32
   1.5.3.1 Classification 32
   1.5.3.2 Distribution 33
   1.5.3.3 Botanical description 33
   1.5.3.4 Active constituent 33
   1.5.3.5 Medicinal Folk-uses 34
   1.5.3.6 Pharmacological studies 34

CHAPTER TWO: MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Section</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Plants 36</td>
</tr>
<tr>
<td>2.2</td>
<td>Plant extracts 36</td>
</tr>
<tr>
<td>2.3</td>
<td>Animals 36</td>
</tr>
<tr>
<td>2.4</td>
<td>Parasites 37</td>
</tr>
<tr>
<td>2.5</td>
<td>Samples collection 37</td>
</tr>
<tr>
<td>2.6</td>
<td>Preparation of the aqueous extract of the plants 37</td>
</tr>
<tr>
<td>2.7</td>
<td>Preparation of methanolic extract of the plants 37</td>
</tr>
<tr>
<td>2.8</td>
<td><em>In vitro</em> anthelmintic activity 38</td>
</tr>
<tr>
<td>2.9</td>
<td><em>In vivo</em> anthelmintic activity 38</td>
</tr>
<tr>
<td>2.10</td>
<td>Blood and biochemical analysis 42</td>
</tr>
<tr>
<td>2.11</td>
<td>Pharmacological experiments 47</td>
</tr>
<tr>
<td>2.12</td>
<td>Statistical Analysis 49</td>
</tr>
</tbody>
</table>

CHAPTER THREE: RESULTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td><em>Balanites aegyptiaca</em> 50</td>
</tr>
<tr>
<td>3.1.1</td>
<td><em>In vitro</em> anthelmintic activity 50</td>
</tr>
<tr>
<td>3.1.2</td>
<td><em>In vivo</em> anthelmintic activity 50</td>
</tr>
<tr>
<td>3.1.3</td>
<td>Haematological findings 55</td>
</tr>
<tr>
<td>3.1.4</td>
<td>Biochemical finding 55</td>
</tr>
<tr>
<td>3.1.5</td>
<td>Pharmacological studies 62</td>
</tr>
<tr>
<td>3.2</td>
<td><em>Peganum harmala</em>: 67</td>
</tr>
<tr>
<td>3.2.1</td>
<td><em>In vitro</em> anthelmintic activity 67</td>
</tr>
<tr>
<td>3.2.2</td>
<td><em>In vivo</em> anthelmintic activity 67</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Haematological findings 73</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Biochemical finding 73</td>
</tr>
<tr>
<td>3.2.5</td>
<td>Pharmacological studies 79</td>
</tr>
<tr>
<td>3.3</td>
<td><em>Zizyphus spina-christi</em> 85</td>
</tr>
<tr>
<td>3.3.1</td>
<td><em>In vitro</em> anthelmintic Activity 85</td>
</tr>
</tbody>
</table>
List of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.2</td>
<td><em>In vivo</em> anthelmintic activity</td>
<td>85</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Haematological findings</td>
<td>91</td>
</tr>
<tr>
<td>3.3.4</td>
<td>Biochemical finding</td>
<td>91</td>
</tr>
<tr>
<td>3.3.5</td>
<td>Pharmacological studies</td>
<td>97</td>
</tr>
<tr>
<td>3.4</td>
<td>Ivermectin (ivomec)</td>
<td>103</td>
</tr>
<tr>
<td>3.4.1</td>
<td><em>In vitro</em> anthelmintic Activity</td>
<td>103</td>
</tr>
<tr>
<td>3.4.2</td>
<td><em>In vivo</em> anthelmintic activity</td>
<td>105</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Haematological findings</td>
<td>107</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Biochemical finding</td>
<td>107</td>
</tr>
</tbody>
</table>

CHAPTER FOUR

Discussion  
Conclusion  
Recommendation  
References
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td><em>In vitro</em> assay of some medicinal plant preparations evaluated against the nematode <em>Haemonchus contortus</em></td>
<td>7</td>
</tr>
<tr>
<td>1-2</td>
<td><em>In vivo</em> evaluation of some medicinal plants against <em>Haemonchus contortus</em> infection in ruminants.</td>
<td>8</td>
</tr>
<tr>
<td>1-3</td>
<td><em>In vivo</em> evaluation of some plants preparations against mixed GI nematode infections in ruminant hosts.</td>
<td>9</td>
</tr>
<tr>
<td>3-1</td>
<td>Effect of different doses of aqueous extract of <em>Balanites aegyptiaca</em> on faecal egg counts and total worms recovered at necropsy in goats infected with <em>Haemonchus contortus</em>.</td>
<td>53</td>
</tr>
<tr>
<td>3-2</td>
<td>Effect of different doses of methanolic extract of <em>Balanites aegyptiaca</em> on faecal egg counts and total worms recovered at necropsy in goats infected with <em>Haemonchus contortus</em>.</td>
<td>54</td>
</tr>
<tr>
<td>3-3</td>
<td>Plasma constituents in goats infected with <em>Haemonchus Contorts</em></td>
<td>59</td>
</tr>
<tr>
<td>3-4</td>
<td>Effect of treatment with 400 mg/kg of aqueous extract of <em>Balanites aegyptiaca</em> on plasma constituents in infected goats with <em>Haemonchus contortus</em></td>
<td>59</td>
</tr>
<tr>
<td>3-5</td>
<td>Effect of treatment with 400 mg/kg of methanolic extract of <em>Balanites aegyptiaca</em> on plasma constituents in infected goats with <em>Haemonchus contortus</em></td>
<td>60</td>
</tr>
<tr>
<td>3-6</td>
<td>Effect of treatment with 800 mg/kg of methanolic extract of <em>Balanites aegyptiaca</em> on serum constituents in infected goats with <em>Haemonchus contortus</em></td>
<td>60</td>
</tr>
<tr>
<td>3-7</td>
<td>Effect of different doses of aqueous extract of <em>Peganum harmala</em> on faecal egg counts and total worms recovered at necropsy in goats infected with <em>Haemonchus contortus</em></td>
<td>71</td>
</tr>
<tr>
<td>3-8</td>
<td>Effect of different doses of methanolic extract of <em>Peganum harmala</em> on faecal egg counts and total worms recovered at necropsy in goats infected with <em>Haemonchus contortus</em></td>
<td>72</td>
</tr>
</tbody>
</table>
Table 3-9  Plasma constituents in goats infected with *Haemonchus contortus*

Table3-10  Effect of treatment with 200mg/kg aqueous extract of *Peganum harmala* on plasma constituents in goats infected with *Haemonchus contortus*

Table3-11  Effect of treatment with 200mg/kg methanolic extract of *Peganum harmala* on plasma constituents in goats infected with *Haemonchus contortus*

Table3-12  Effect of different doses of aqueous extract of *Zizyphus spina-christi* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemonchus contortus*

Table3-13  Effect of different doses of methanolic extract of *Zizyphus spina-christi* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemonchus contortus*.

Table3-14  Plasma constituents of goats infected with *Haemonchus contortus*

Table 3-15  Effect of treatment with 400 mg/kg of aqueous extract of *Zizyphus spina-christi* on plasma constituents in goats infected with *Haemonchus contortus*

Table3-16  Effect of treatment with 400 mg/kg of methanolic extract of *Zizyphus spina-christi* on plasma constituents in goats infected with *Haemonchus contortus*

Table3-17  Effect of treatment with 800 mg/kg of methanolic extract of *Zizyphus spina-christi* on plasma constituents in goats infected with *Haemonchus contortus*.

Table3-18  In vitro effect of aqueous and methanolic extracts of *Balanites aegyptiaca*, *Peganum harmala* and *Zizyphus spina-christi* on *Haemonchus contortus* in comparison with ivermectin.
Table 3-19  Effect of ivermectin (1ml/50kg) on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemonchus contortus*

Table 3-20  Effect of ivermectin on plasma constituent of goats infected by *Haemonchus contortus*
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Title</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig 1 Comparison of mean percentage of survival adult <em>H. contortus</em> for 24 hours exposure to methanolic and aqueous extracts of <em>Balanites aegyptiaca</em> with control (phosphate buffer saline)</td>
<td>52</td>
</tr>
<tr>
<td>Fig. 2 Effect of AE &amp; ME of <em>Balanites aegyptiaca</em> on PCV% in goats infected with <em>H.contortus</em></td>
<td>57</td>
</tr>
<tr>
<td>Fig. 3 Effect of treatment with AE&amp;ME of <em>Balanites aegyptiaca</em> on Hb concentration in goats infected with <em>H.contortus</em></td>
<td>58</td>
</tr>
<tr>
<td>Fig. 4 Effect of different doses of methanolic extract of <em>Balanites aegyptiaca</em> on plasma total protein concentration in goats infected with <em>H.contortus</em></td>
<td>61</td>
</tr>
<tr>
<td>Fig. 5 Contraction effect of methanolic extract of <em>Balanites aegyptiaca</em> at different doses of on isolated rabbit jejunum</td>
<td>63</td>
</tr>
<tr>
<td>Fig. 6 Contraction effect of aqueous extract of <em>Balanites aegyptiaca</em> at different doses on isolated rabbit jejunum.</td>
<td>64</td>
</tr>
<tr>
<td>Fig. 7 Contraction effect of methanolic extract of <em>Balanites aegyptiaca</em> at different doses on isolated rabbit jejunum; that blocked by atropine</td>
<td>65</td>
</tr>
<tr>
<td>Fig. 8 Contraction effect of aqueous extract of <em>Balanites aegyptiaca</em> at a dose of 5 mg/ml on isolated rabbit jejunum, the contraction blocked by atropine</td>
<td>66</td>
</tr>
<tr>
<td>Fig. 9: Comparison means percentage of survival adult <em>H. contortus</em> for 24 hours exposure to methanolic and aqueous extracts of <em>Peganum harmala</em> comparison with control (phosphate buffer saline)</td>
<td>70</td>
</tr>
<tr>
<td>Fig. 10 Effect of treatment with AE &amp; ME of <em>Peganum harmala</em> on PCV% in goats infected with <em>H.contortus</em>.</td>
<td>74</td>
</tr>
</tbody>
</table>
Fig. 11: Effect of treatment with AE & ME of *Peganum harmala* on Hb concentration of *H. contortus* infected goats

Fig. 12: Effect of aqueous and methanolic extracts of *Peganum harmala* on plasma urea concentration in goats infected with *H. contortus*

Fig. 13: Relaxation effect of methanolic extract of *Peganum harmala* seed at different dose on isolated rabbit jejunum.

Fig. 14: Relaxation effect of aqueous extract of *Peganum harmala* seed at different doses on isolated rabbit jejunum.

Fig. 15: Relaxation effect of methanolic extract of *Peganum harmala* seed at different doses (5, 7.5 mg/ml) on isolated rabbit jejunum; the relaxation was not blocked by a dose of 0.5 mg/ml of propranolol.

Fig. 16: Relaxation effect of methanolic extract of *Peganum harmala* seed at different doses on isolated rabbit jejunum, that blocked by doses of 0.5, 1 mg/ml of tolazoline.

Fig 17: Comparison of mean percentage of survival adult *H. contortus* for 24 hours exposure to methanolic and aqueous extracts of *Zizyphus spina-christi* with control (phosphate buffer saline)

Fig. 18: Effect of AE&ME of *Zizyphus spina-christi* on PCV% in goats infected with *H. contortus*

Fig 19: Effect of AE&ME of *Zizyphus spina-christi* on Hb concentration in goats infected with *H. contortus*

Fig 20: Effect of aqueous and methanolic extracts of *Zizyphus spina-christi* on plasma sodium concentration in goats infected with *H. contortus*

Fig 21: Relaxation effect of methanolic extract of *Zizyphus spina-christi* leaf at different doses on isolated rabbit jejunum.
Fig. 22: Relaxation effect of aqueous extract of *Zizyphus spina-christi* leaf at different doses on isolated rabbit jejunum.

Fig. 23: Relaxation effect of methanolic extract of *Zizyphus spina-christi* leaf at different doses on isolated rabbit jejunum; that not blocked by a dose of 0.5mg/ml of propranolol.

Fig. 24: Relaxation Effect of methanolic extract of *Zizyphus spina-christi* leaf in different doses on isolated rabbit jejunum, that not blocked by a dose of 0.5mg/ml of tolazoline.

Fig. 25: Effect of methanolic extract of *Zizyphus spina-christi* leaf at dose (5 mg/ml) on isolated rabbit jejunum; the extract diminished the effect of acetylcholine (5 µg/ml).

Fig. 26: Effect of ivermectin on PCV percentage in goats infected with *H.contortus*.

Fig. 27: Effect of ivermectin on Hb concentration in goats infected with *H.contortus*. 
**Abbreviations**

BZs  Benzimidazole/ probenzimidazoles anthelmintic products
AE  Aqueous extract
ME  Methanolic extract
CP  Powder of crude plant
EPG  Egg per gram of faeces
EVM  Ethnoveterinary medicine
ECR  Egg count reduction
G  Gram
GI  Gastrointestinal
GOT  Glutamate oxalate transaminase
GPT  Glutamate pyruvate transaminase
Hb  Haemoglobin
Hrs  Hours
IV  Intravenous
L3  Infective larvae
LD50  leathal dose50
Kg  kilogram
mg  Milligram
ml  Milliliters
PBS  Phosphate buffer saline
PCV  Packed cell volume
SC  Subcutaneous
S.D  Stander error of deviation
Abstract

Objectives: This research aimed to study anthelmintic effects of aqueous extracts (AE) and methanolic extracts (ME) of *Balanites aegyptiaca* kernel, *Peganum harmala* seed and *Zizyphus spina-christi* leaves in Nubian goats infected with *Haemoncus contortus*.

Methodology: The study was carried out in two phases *in vivo* and *in vitro*. In the *in vitro* study, a dose of 25mg/ml of the three plant extracts was used against adult *Haemoncus contortus* worms. *In vivo* study, the anthelmintic efficacy of the plant extracts was investigated through faecal egg count reduction at regular intervals for a period of three weeks post treatment by different doses in goats artificially infected with *Haemoncus contortus*. Biochemical tests were carried out which included liver and kidney function tests. LD$_{50}$ of methanolic extract of the plants was calculated using Wister rats. The effect of the plants extracts on the smooth muscles of rabbit intestine was tested in *vitro*.

Results: The result of the *in vitro* study revealed that the aqueous and methanolic extracts of the three plants against adult *Haemoncus contortus* worms produced mortality rate of 90% for AE and 25% for ME of *Balanites aegyptiaca* at 12 hrs. The AE and ME of *Peganum harmala* produced mortality rate of 95% and 75% at 12 hrs, while the AE and ME of *Z.spina-christi* produced mortality rate of 40% and 100% at 12 hrs respectively. The anthelmintic activity of the three plants was also confirmed by graded dose response in reduction of egg count per gram (EPG) of faeces and reduction in the number of adult *Haemoncus contortus* found in abomasa of
goats treated with AE and ME of the plants. The AE of *B. aegyptiaca* at the doses of 100mg/kg and 400mg/kg showed 9.3% and 61.2% reduction in EPG of faeces on day 21 post treatment respectively. The ME at the doses of 100mg/kg, 400mg/kg and 800mg/kg showed maximum reduction of 20%, 74.7% and 79.2% reduction in EPG of faeces at day 21 post treatment. The AE of *Peganum harmala* at dose of 100mg/kg and 200mg/kg showed 85.4% and 86.6% reduction in EPG of faeces at day 21 post treatments, while the ME at dose of 100mg/kg and 200mg/kg resulted in a maximum reduction of 23.9% and 70.3% in EPG at day 21 post treatments respectively.

Regarding the AE of *Zizyphus spina-Christi*, the doses of 100mg/kg and 400mg/kg showed 61.5% and 78.7% reduction in EPG of faeces on day 21 post treatment. While ME at the doses of 100mg/kg, 400mg/kg and 800mg/kg showed maximum reduction of 24.4%, 73.1% and 85.1% reduction in EPG of faeces at day 21 post treatment, respectively.

The result of the biochemical assays of the effect of the extracts of *Balanites aegyptiaca* and *Zizyphus spina-Christi* on liver and kidney function tests showed no change. For the extracts of *Peganum harmala*, the liver function tests revealed no change, but kidney function tests showed slight decrease in urea concentration.

In Wister rats, the oral LD$_{50}$ of methanloic extracts for *Balanites aegypticea* kernel was more than 25g/kg, for *Peganum harmala* seed was 7g/kg and for *Zizyphus spina-christi* was more than 8g/kg.

The AE and ME of *Balanites aegyptiaca* showed contraction of the smooth muscle of rabbit jejunum. The contraction was blocked by atropine. The AE and ME of *Peganum harmala* inhibited the spontaneous movements of the rabbit jejunum. These inhibitions were refractory to the adrenergic
blockers (propranolol and tolazoline). The AE and ME of *Zizyphus spina-christi* showed relaxation of the rabbit jejunum. These relaxations were blocked by the adrenergic blocker tolazoline.

**CONCLUSION:** It is concluded that the aqueous and methanolic extracts of *Balanites aegyptiaca*, *Peganum harmala* and *Zizyphus spina-christi* possess appreciable anthelminthic effects against *Haemoncus contortus* in goats.
ผลกระทบات ثلاثية نباتية طبية على ديدان الهمونكس في الماعز

الأهداف: لقد هدف هذا البحث لدراسة فعاليّة المستخلصات المائية والكحوليّة لأوراق السدر وبذور الحرم ونواة بذور اللالوب ضد ديدان الهمونكس في المختبر وعلى الماعز المصاب بهذه الديدان.

منهجية البحث: قد تم إجراء تجارب مختبرية باستعمال جرعة 25ملجم/مل من المستخلصات المائية والكحوليّة لأوراق السدر وبذور الحرم ونواة بذور اللالوب ضد ديدان الهمونكس البالغة. أيضاً تم اختبار فعالية هذه المستخلصات على الماعز المصابة بهذه الديدان وذلك بقياس الانخفاض في عدد بيوس الديدان لكل جرام من الروث خلال الاسبوع الثلاثي التالي لاعطاء مستخلصات النباتات الثلاث. اضافةً تم استكشاف أثر السموم في هذه النباتات عن طريق قياس أثرها على وظائف الكبد والكلى. في هذه الدراسة أيضاً تم اختيار الجرعة الوسطية المماثلة للمستخلص الميثولوجي للنباتات على الفئران. كما تم اختبار المستخلصات المائية والكحوليّة لهذه النباتات على انسجة معزولة من اعماق الإربيب.

النتائج: قد أوضحت التجارب المختبرية بأن استعمال جرعة 25ملجم/مل من المستخلصات المائية والكحوليّة لأوراق السدر وبذور الحرم ونواة بذور اللالوب ذات فعالية ضد ديدان الهمونكس. بلغ معدل الموت في ديدان الهمونكس بعد مرور 12 ساعة من المعالجة بالمستخلص المائي نواة بذور اللالوب 90% وبالمستخلص الكحولي لنياحة بذور اللالوب 25% والمستخلص المائي لبذور الحرم 95% وبالمستخلص الكحولي لبذور الحرم 75% والمستخلص المائي لأوراق السدر 40% بالمستخلص الكحولي لأوراق السدر 100%. أما التجارب على الحيوانات المصابية بديدان الهمونكس فقد أظهرت أن المستخلصات المائية والكحوليّة للنباتات الثلاث تثير مضاد للديدان يعتمد على الجرعة والفترة الزمنية بعد العلاج. لقد اثبتت التجارب أن المستخلص المائي لناة بذور اللالوب بجرعة 100ملجم/كيلوغرام ليس له تأثيراً معنوباً على خفض عدد بيوس الديدان، و بجرعة 400 ملجم/كيلوغرام ادى إلى خفض عدد بيوس الديدان لكل جرام من الروث بنسبة 60.2% بعد مرور 21 يوم من إعطاء الجرعة للحيوانات. أما المستخلص الكحولي لبذرة اللالوب بجرعة 100, 400 و800ملجم/كيلو جرام فقد خفض في عدد بيوس الديدان لكل جرام من الروث بنسبة 20%, 74.7% و79.2% بعد مرور 21 يوم من إعطاء الجرعة على...
الخلاصة: استخلص من هذه الدراسة أن المستخلصات المانية والكحولية لأوراق السدر وبذور الحرم بمعدل 100 و200 ملجرام/كيلو جرام فقد ادى إلى خفض عدد بيوض الديدان لكل جرام من الروث بنسبة 85.4% و 86.8% بعد مرور 21 يوم من إعطاء الجرعة. وادى المستخلص الكحولي لبذور الحرم بجرعة 100 و200 ملجرام/كيلو فقد خفض عدد بيوض الديدان لكل جرام من الروث بنسبة 7.3% و 12.5% بعد مرور 21 يوم من إعطاء الجرعة. هذا وظائف الكبد والكلي. أما المستخلصات المانية والكحولية لهذا الورق فقد تظهرت تغيرات باستمرار الاختبارات ووظائف الكبد والكلي. أوضح التجربة المختبرية أن الجرعة الوسطية الممتزة للمستخلص الميثيلي لأوراق السدر أكثر من 8 جرام/كيلو جرام وبذور الحرم 7 جرام/كيلو جرام ولوادة بذور اللالوب أكثر من 25 جرام/كيلو جرام. أحدث المستخلص اللالوب انقباضا لامعة الأرنب، هذا الانقباض تعاكس بالاتروبين. أما المستخلص المانى والميثيلي لبذور الحرم فقد احدث انقباضا لامعة الأرنب. هذا الانقباض تعاكس بغالبية مستقبلات الألفا الودية (تولازولون). أما المستخلص المانى والميثيلي لأوراق السدر فقد احدث أيضا انقباضا لامعة الأرنب لم يستجب للغلفات الودية (البروبانولول والتولازولون).
CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1. 1: Introduction:
Livestock are an important and integrated component of agricultural production system in developing countries. They are reared under a wide variety of production systems ranging from traditional smallholder and village production system to large-scale intensive commercial farms. Like in many developing countries, the majority of the farmers in Sudan raise their livestock and traditional production as a sideline to the main agricultural activities. However, livestock production plays a significant role in supporting farmers’ income.

Most developing countries of the world lie in the tropical and subtropical region. The warm and humid climatic conditions provide favorable environment for development of worm egg to infective larvae. Thus, helminth parasites problem is unquestionably being major limiting in the improvement of livestock production. Helminthiasis is one of the most important animal heath problems, which inflict heavy production losses in grazing animals. The disease is highly prevalent particularly in developing countries (Dhar, et al., 1982). In Sudanese livestock, gastrointestinal parasites are major problems in ruminants, causing varying degree of morbidity and mortality and lead to decrease in production (Eisa, et al., 1979).

Control of gastrointestinal helminth infection in the livestock relies mainly on the use of commercial anthelmintics in combination with farm management, although synthetic anthelmintics are currently viewed as the most effective means of helminth control (Hammond, et al., 1997). Their use is faced with numerous constraints. These include lack of foreign exchange to import them, farmers lacking capital to purchase them, anthelmintic resistance, their unavailability
especially in rural areas, lack of know-how on the proper use of them, environmental pollution and unreliable manufactures who at times unscrupulously produce anthelmintics with little or no efficacy at all (Kinoti, et al., 1994). For a diversity of reasons, interest in the screening of putative medicinal plants for their anthelmintic activities attracts scientific interest despite the extensive use of synthetic chemicals in modern clinical practices all over the word.

The plant kingdom is known to provide a rich source of botanical anthelmintics (Satyavati, et al., 1976). A number of medicinal plants have been used to treat parasitic infection in man and animals (Nadkarni, 1954, Chopra, et al., 1956, Said, 1969). The use of medicinal plants may present a cheaper, sustainable and an alternative if the compounds were demonstrated to work. Such herbal preparations have been used over time by pastoralists and smallholder farmers for treatment of their livestock against helminth parasites. The present work is an attempt to carry out evaluation of anthelmintic efficacy of three medicinal plants preparations (Balanites aegyptiaca, Peganum harmala, Zizyphus spina-christi) used by pastoralists and small holder farmers in Sudan against helminth parasites. Evaluation was carried out in vitro using mature Haemoncus contortus worms and in vivo in ruminant host parasite model using goats artificially infected with these worms.
OBJECTIVE:

1. To determine the anthelmintic activity of aqueous and methanolic extracts of *Balanites aegyptiaca, Peganum harmalal, Zizyphus spina-christi in vitro* using live *Haemoncus contortus* and *in vivo* using experimentally induced *Haemoncus contortus* infection in Nubian goats.

2. To assess primary pharmacological screening of the plant extracts on isolated tissue.

3. To study toxicity and mean lethal dose (LD 50) for each of the three plants.
1.2: Ethno veterinary medicine (EVM):

There is increased awareness among medical and scientific communities that the importance of medicinal plant studies goes beyond anthropological curiosity. Plant anthelmintics have been in the forefront of this growing awareness (Hammond, et al., 1997). A reason for this could be that they fall into the category of readily applicable elements of ethno veterinary medicine in livestock development (McCorkle and Mathias-Mandy, 1992). Studying herbal medicine can serve to validate and enhance existing local uses and can give clues to remedies with further potential.

Ethno veterinary medicine was defined by McCorkle, 1995 as "The wholistic, interdisciplinary study of local knowledge and its associated skill, practices, beliefs, practitioners, and social structures pertaining to the health care and healthful husbandry of food, work, and other income-producing animals, always with an eye to practical development application within livestock production and livelihood systems, and with the ultimate goal of increasing human well-being via increased benefits from stock raising"

According to Tabuti et al., (2003) and other systematic studies on ethno veterinary medicine the venture can be justified for three main reasons:-

1. They can generate useful information needed to develop livestock healing practices and methods that are suited to the local environment.
2. Ethno veterinary medicine could be a key for veterinary resources and could add useful new drugs to the pharmacopoeia.
3. Ethno veterinary medicine can contribute to biodiversity conservation.

Several books have been written on ethno veterinary medicine (Mathias-Mundy & Mc Corkle, 1989, Anonymous, 1994, 1996, Bizimana, 1994; McCorkle et al., 1996 Köhler-Rollefson et al., 2001, Martin, et al., 2001) and a few databases and web sites on the subject exist (NUFFIC, 2001; Ethnovet web, 2003; PRELUDE, 2003;
SPIRAL, 2006). However, in most of these sources, there is only brief description of the plants used and proposed conditions that they treat. Most of the research on testing of EVM preparations has so far been carried out in Asia (Akhtar, et al., 2000). Conversely, a number of publications have been produced that relate to diseases that affect humans and livestock in Africa (Gachathi, 1993, kokwaro, 1993; Bizimana, 1994).

In Africa, Ibrahim, et al., (1984) screened 18 species of plants used in West Africa for their anthelmintic activity, while, Kasonia, et al., (1991) reported 11 plants used for the same purpose in Zaire. In Nigeria, 92 species of plants were identified to be in traditional veterinary practice, with 15 reported to be used against general worm infestation and three against fasciolosis in cattle (Nwuhe & Ibrahim, 1980). A study in Madikwe area of South Africa identified 8 plants that were used as dewormers of cattle (VanderMerwe, 2000). In the Sanaag area in Somalia, six plants were reported to be used for treatment of helminthes in livestock (Catley & Mohammed, 1996).

Tagboto and Townson (2001) in their review listed 39 plants used against cestodes, 16 against trematodes and 45 against nematodes in human wide world. There are also recent reports in Africa of plants that had anthemintic activity when fed to livestock due to their condensed tannins (Kabasa, et al., 2000, Kahiya, et al., 2003).

In Sudan, the anthemintic activity of 14 plants species that represent seven families of the Sudanese flora was examined using the free-living nematode (Ibrahim 1992).

In many instances, EVM remedies have been identified for treatment of different conditions. However, some evaluations have been carried out to validate putative anthemintic properties. Some evaluations of plant preparations have been tried against nematode parasites *Haemoncus contortus in vitro* as summarized in table
A number of parasitic nematodes have been used in many in vitro assays for determination of anthelmintic efficacy. For example, in several trials, in vitro effects of plant extracts on the free-living nematode Caenorhabditis elegans and Rhabditis pseudoengata (Okpekon, et al., 2004) were evaluated. An investigation in south Africa tested the in vitro activity of 72 species of plants from 18 families, against C. elegans (McGaw et al., 2000) while in the Ivory Coast 17 species from 13 families were evaluated against R. pseudoengata (Okpekon, et al., 2004).

Previously, several investigations had assessed plant efficacy against different helminth parasites in animal and human hosts. In a few studies, the anthelmintic properties of plants in naturally or artificially infected sheep, goats and cattle, have been determined against nematode Haemoncus contortus as summarized in table (1-2) as well as against mixed Trichostrongydae nematode infection in ruminants (Table 1-3). Similarly in vivo efficacy of plant preparation was investigated against cestode infected hosts such as in human (Desta, 1995) rodents (Galal, et al., 1991, Vishnyanskas, et al., 1993; Ghosh, et al., 1996; De Amorin, et al., 1999; Molgaard, et al., 2001) and ruminants (Aktar & Riffat, 1986).
Table 1: *In vitro* assay of some medicinal plant preparations evaluated against the nematode *Haemonchus contortus* (adult parasite, eggs or infective larva).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part used</th>
<th>Target</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Adhatoda vesica</em></td>
<td>Roots</td>
<td>Adult parasite</td>
<td>Lateef <em>et al</em>., 2003</td>
</tr>
<tr>
<td><em>Annona senegalensis</em></td>
<td>Bark</td>
<td>Eggs</td>
<td>Alawa <em>et al</em>., 2003</td>
</tr>
<tr>
<td><em>Piliostigma thonningii</em></td>
<td>Bark</td>
<td>infective larvae</td>
<td>Asuzu, Gray &amp; Waterman, 1999; Fakae <em>et al</em>., 2000</td>
</tr>
<tr>
<td><em>Chenopodium ambrosioides</em></td>
<td>Oil, Leaves</td>
<td>Eggs</td>
<td>Ketzis <em>et al</em>., 2002</td>
</tr>
<tr>
<td><em>Vernonia amygdalina</em></td>
<td>Leaves</td>
<td>Eggs</td>
<td>Alawa <em>et al</em>., 2003</td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td>Bulbs</td>
<td>Adult parasite</td>
<td>Igbal <em>et al</em>., 2001</td>
</tr>
<tr>
<td><em>Spigelia anthelmia</em></td>
<td>Shoots</td>
<td>E, infective larvae</td>
<td>Assis <em>et al</em>., 2003</td>
</tr>
<tr>
<td><em>Nauclea latifolia</em></td>
<td>Leaves</td>
<td>infective larvae</td>
<td>Fakae <em>et al</em>., 2000</td>
</tr>
<tr>
<td><em>Ficus</em></td>
<td>Bark</td>
<td>A Adult parasite</td>
<td>Igbal <em>et al</em>., 2001</td>
</tr>
<tr>
<td><em>Zingiber officianale</em></td>
<td>Rhizomes</td>
<td>A Adult parasite</td>
<td>Igbal <em>et al</em>., 2001</td>
</tr>
<tr>
<td><em>Ocimum giatissimun linn(labideae)</em></td>
<td>Oil</td>
<td>Eggs</td>
<td>Pessoa <em>et al</em> 2002</td>
</tr>
<tr>
<td><em>Croton macrostchyus</em></td>
<td>Seed</td>
<td>Eggs, A Adult parasite</td>
<td>Eguale <em>et al</em> 2006</td>
</tr>
<tr>
<td><em>Ekebergia capensis</em></td>
<td>Seed</td>
<td>Eggs, A Adult parasite</td>
<td>Eguale <em>et al</em> 2006</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>Seed</td>
<td>Eggs, A Adult parasite</td>
<td>Eguale <em>et al</em> 2006</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>Seed</td>
<td>infective larvae</td>
<td>Hordeyen <em>et al</em> 2006</td>
</tr>
<tr>
<td><em>Artemisia brevifolia</em></td>
<td>Shoots</td>
<td>Adult parasite</td>
<td>Igbal <em>et al</em> 2004</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>Leaves</td>
<td>Adult parasite</td>
<td>Igbal 2008</td>
</tr>
<tr>
<td><em>Calotropsis procera</em></td>
<td>Friuts</td>
<td>Adult parasite</td>
<td>Igbal <em>et al</em> 2005</td>
</tr>
<tr>
<td><em>Hedera helix corrandrum</em></td>
<td>Friuts</td>
<td>Adult parasite</td>
<td>Eguale <em>et al</em> 2007</td>
</tr>
<tr>
<td><em>Sativum</em></td>
<td>Seed</td>
<td>Adult parasite</td>
<td>Eguale <em>et al</em> 2006</td>
</tr>
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</table>
Table 1-2: *In vivo* evaluation of some medicinal plants against *Haemoncus contortus* infection in ruminants.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part used</th>
<th>Host</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica</td>
<td>Leaves</td>
<td>Goat</td>
<td>Kahiya <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Allium stavium</td>
<td>Bulbs</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Ananas comosus</td>
<td>Leaves</td>
<td>Sheep, Bovine</td>
<td>Jovellanos, 1997; Baldo, 2001; Hördegen <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Annona squamosa</td>
<td>Leaves</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Artemisia herba-alba</td>
<td>Shoots</td>
<td>Goat</td>
<td>Idris <em>et al.</em>, 1982</td>
</tr>
<tr>
<td>Chenopodium ambrosioides</td>
<td>Leaves</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Calatropis procera</td>
<td>Leaves</td>
<td>Sheep</td>
<td>Al-Qarawi <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Canavalia brasiliensis</td>
<td>Seeds</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>Seeds</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Chrysophyllum cainito</td>
<td>Stem</td>
<td>Bovine</td>
<td>Fernandez, 1991</td>
</tr>
<tr>
<td>Momordica Charantia</td>
<td>Stem</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Musa acuminate</td>
<td>Leaves</td>
<td>Goat</td>
<td>Vieira <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Tinospora rumphii</td>
<td>Stem</td>
<td>Goat</td>
<td>Fernandez, 1991</td>
</tr>
</tbody>
</table>
Table 1-3: *In vivo* evaluation of some plants preparations against mixed gastrointestinal nematode infections in ruminant hosts.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part used</th>
<th>Host</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Adhatoda vesica</em></td>
<td>Roots</td>
<td>Sheep</td>
<td>Lateef <em>et al.</em>, 2003</td>
</tr>
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<td><em>Albizia anthelmintica</em></td>
<td>Bulbs, Root bark</td>
<td>Sheep</td>
<td>Grage &amp; Longok, 2000; Gakuya, 2001; Gathuma <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><em>Ananas comosus</em></td>
<td>Leaves</td>
<td>Sheep, Bovine</td>
<td>Jovellanos, 1997; Baldo, 2001; Hördegen <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><em>Annona squamosa</em></td>
<td>Leaves</td>
<td>Goat, Bovine</td>
<td>Jovellanos, 1997; Vieira <em>et al.</em>, 1999</td>
</tr>
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<td><em>Chenopodium ambrosioides</em></td>
<td>Leaves, Seeds Oil</td>
<td>Sheep</td>
<td>Ketzis <em>et al.</em>, 2002</td>
</tr>
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<td><em>Chrysanthemum cinerariaefolium</em></td>
<td>Flowers</td>
<td>Sheep</td>
<td>Mbaria <em>et al.</em>, 1998</td>
</tr>
<tr>
<td><em>Caesalpinia crista</em></td>
<td>Seeds</td>
<td>Sheep</td>
<td>Hördegen <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><em>Embelia ribes</em></td>
<td>Fruits</td>
<td>Sheep</td>
<td>Hördegen <em>et al.</em>, 2003</td>
</tr>
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<td><em>Fumaria parviflora</em></td>
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<td>Sheep</td>
<td>Hördegen <em>et al.</em>, 2003</td>
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<td><em>Hagenia abyssinica</em></td>
<td>Fruits</td>
<td>Goat</td>
<td>Abebe <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><em>Hildebrandtia</em></td>
<td>Root bark</td>
<td>Sheep</td>
<td>Gathuma <em>et al.</em>, 2004</td>
</tr>
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<td><em>Khaya anthotheca</em></td>
<td>Bark</td>
<td>Bovine</td>
<td>Nfi <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Maerua</em></td>
<td>Tuber</td>
<td>Sheep</td>
<td>Gakuya, 2001</td>
</tr>
<tr>
<td><em>Myrsine Africana</em></td>
<td>Fruits</td>
<td>Sheep</td>
<td>Gathuma <em>et al.</em>, 2004</td>
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<td><em>Nauclea latifolia</em></td>
<td>Bark</td>
<td>Sheep</td>
<td>Onyeiyili <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><em>Solanum aculeastrum</em></td>
<td>Roots</td>
<td>Bovine</td>
<td>Nfi <em>et al.</em>, 1999</td>
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<td><em>Terminalia glaucescens</em></td>
<td>Bark</td>
<td>Bovine</td>
<td>Nfi <em>et al.</em>, 1999</td>
</tr>
</tbody>
</table>
1.3: Previous research and shortcomings:

1.3.1: *In vitro* research:


Many of the *in vitro* investigations on anthelmintic activity of plants, their oils or extracts have been based on their effect on *Haemonchus contortus* eggs, larvae and adult worms. Pessoa, *et al.*, (2002) tested the ovicidal activity of the essential oil of Ocimum gratissium linn and its main component eugenol in *Haemonchus contortus* of small ruminants. They revealed at 0.5% concentration of the essential oil and ergenol showed a maximum eclodibility inhibition. The alcoholic extract of Punica granatum showed anthelmintic activity as revealed by a dose dependant inhibition of transformation of eggs to larva of *H. contortus* (Prakash, *et al.*, 1980).


Bromelain, the enzyme complex of the stem of Ananas, the ethanolic extracts of seeds of Azardirracthy indica, Caesalpinia and Vernonia anthemintica, and the ethanolic extract of the whole plant of Fumaria pravilora and of the fruit of Embelia ribs were tested against larvae of *H. contotus* they showed efficacy of up to 93% (Hödregen, *et al.*,2006). Spigelia anthelmic extracts obtained with hexane,
chloroform, ethyl acetate and methanol were tested on *H. contortus* eggs and larvae via egg hatch and larval development test. The ethyl acetate extract inhibited 100% of the egg hatching and 81.2% of the larval development. The methanolic extract inhibited 97.4% of the egg hatching and 84.4% of larval development (Assis, *et al*., 2003). *In vitro* anthelmintic of crude aqueous and hydro-alcoholic extract of the seeds of *Coriandrum Staium* were investigated on egg and adult nematode parasite *H. contortus*. Both extract types of *Coriandum sativum* inhibited hatching of eggs completely at concentration less than 0.5mg/ml. The hydro-alcoholic extract showed better in vitro activity against adult parasites than the aqueous one (Eguale *et al*., 2007). Crude extract of the ripe and fruits of *Hedera helix* investigated on egg and adult *H. contortus*. The extracts revealed ED50 for egg hatch inhibition at adose of 0.12 and 0.17 mg/ml for aqueous and hydro-alcoholic extracts respectively. There was no statistically significant difference in the activity of the two extract types. Hydro-alcoholic extract showed better in vitro activity against adult parasites compared to the aqueous extract. (Eguale *et al*., 2007)

Anthelmintic effect of crude aqueous and hydroalcoholic extract of the seeds of *Croton macrostachyus*, *Ekebergia capensis*, *Acacia nilotica* and *Terminalia schimperrania* on eggs and adult *H. contortus* were investigated. Both extracts of *C. macrostachus* and *E. capensis* as well as aqueous extract of *A. nilotica* induced complete egg hatch inhibition at concentration less than or equal to 2mg/ml. Hydro-alcoholic extracts of *C. macrostachus* and *E. capensis* produced significantly mortality of 90 and 60% of adult *H. contortus* at concentration of 8mg/ml. While aqueous extract of these plants produced only 36.67 and 43.33% mortality respectively at the same concentration (Eguale, *et al*., 2006).

The anthelmintic studies of the essential oils of *Cymbopogon nardus*, *C. itrcates* and *Zanthoxylum alatum* have revealed that oil of *C. Nadus* has very good effect against earthworms while the oils of *C. citrates* and *Z. alatum* have moderate
activity (Kokate and Varma, 1971). A steam volatile oil from petroleum ether of *Withan caoagulans* has been found to possess lethal effect on earthworms. Essential oils of *Boswellia serrata* and *Cinnamomum tamala* had better in vitro activity than piperazine citrate against earthworms, (Girgune, *et al.*, 1978). Likewise, *zanthoxylum alatum* has been found better than piperazine phosphate against earthworms (Methta, *et al.*, 1981) Alkaloid hydrochlorides extracted from seed of *Butea fundosa* (in dose 0.1-20.0mg/ml) proved 100% lethal to earthworms within 24 h (Kalesaraj and Kurup, 1962). The essential oil of *Piper betle* has revealed anthelmintic effect on earthworms (Ali and Mehta, 1970).

**1.3.2: In vivo research**

Several investigations had assessed *in vivo* plant efficacy against *H. contortus* in sheep and goats. Frenandez, (1999) studied the potential of *Tinspora rumphic* as anthelmintic against *H. contortus* in goats. The study showed the crude extract of *T. rumphic* given at a dose of 40mg/kg body weight significantly reduced the number of worm eggs in faces of naturally infected goats. The anthelmintic activity of *Calotropics procera* latex was investigated in sheep infected with *H. contortus* by Al-Qurawi, *et al.*, (2001). In this study, the egg production was significantly reduced, but not completely suppressed and fewer adult *Haemonchus* worms were found in the abomasa. Aqueous extract of *Hedera helix* was evaluated for in vivo anthelmintic activity at dose of 1.13 and 2.25g/kg in sheep artificially infected with *H. contortus*. Significant faecal egg counts reduction was detected in the groups treated with both doses of *H. helix* and significant, dose dependent reduction in total worm count was observed (Equale, *et al.*, 2007). Githiori (2004), tested seven plant parations of *Hagenia abyssinica*, *Olea europaea var.africana*, *Annona saquamosa*, *Ananas comosus*, *Dodonea angustifolia*, *Hildebrandtia sepalosa* and *Azadirachta indica* in 151 lambs infected with 5000 or 3000 *L*₃ *Heamonchus contortus* in three experiments and all were found to be ineffective. The anthelmintic efficacy of
Artemisia herba-alba was investigated in experimental haemonchosis in Nubian goats. The results obtained that ahervba-alba had overall efficacy of 66.6% (Idris, et al., 1982). Githiori, et al., (2003) investigated the anthelmintic efficacy of Albizia anthelmintica against Haemonchus contortus of sheep. The study revealed significant reductions in faecal egg counts, but the efficacy levels achieved were well below the 70% reduction required.

In vivo efficacy of various plant preparations were investigated against different helminth parasites in animal host. Garg and Atal, (1963) reported remarkable vermicidal activity of Calotropain (proteolytic enzyme isolated from latex of Calotropis procera and Bromelain (an enzyme obtained as byproduct from pineapple industry) against oesphagostumum colombianum and Bunostomun trigoncephalum of sheep origin compared to phenothiazine. Gauthuma, et al., (2004) tested the efficacy of Myrsine africana, Albiziz_anthelmintica and Hildebrandtia epsalosa against mixed natural helminthosis in sheep. Albizia anthelmintica and hilderbrantia spalosa treatment showed significant improvement over controls from day 4 after treatment to day 12. On day 12, the three plant remedies showed 100% efficacy while albendazole had an efficacy of 63%. The extract of Eugenia uniflora, Acacia atraxacantha, Terminalia ivorensis, T. superba and Alchornea cordifolia showed trypanicidal activity (Adewunmi, et al., 2001). The whole plant of Artrmisia brevifolia was administered as crude powder (CP) crude aqueous extract (CAE) and crude methanolic extract (CME) at graded doses (1, 2 and 3g/kg body weight) to sheep naturally infected with mixed species of gastrointestinal nematodes. Maximum reduction (67.2%) in egg per gram (EPG) of faeces was recorded on day 14 post treatment in sheep treated with Artemisia brevifolia CAE at 3g/kg. While, levamisole produced 99.2% reduction EPG. However, increase in EPG reduction was noted with an increase in the dose of Artemisia brevifolia administered as CP, CAE and CME (Igbal et al., 2004).
Dawo and Tibbo (2005) evaluated the efficacy of *Halothamus somalensis* in goats naturally infected with mixed species of gastrointestinal nematodes. The goats were drenched with crude preparation of the plant at doses (0.5 and 2g/kg body weight). The dose of 2g/kg reduced faecal egg count by 50%. The anthelmintic activity of *Calotropis procera* flowers in sheep naturally infected with mixed species of gastrointestinal nematodes was tested by Igbal *et al.*, (2005). They revealed reduction in egg count percent of 88.4 and 77.8% in sheep treated with CAE and CP at 3g/Bwt on day 7 and 10 post treatment respectively.

Igbal, *et al.*, (2006a) evaluated efficacy of ginger in sheep naturally infected with mixed species of gastrointestinal nematodes. CP and CAE of dried ginger (1-3g/kg) both exhibited a dose and a time dependent anthelmintic effect with respective maximum reduction of 25.6% and 66.6% in EPG of faeces on day 10 post treatment. The whole plant powder of *Fumaria praviflora* (at dose 2gkg), its water extract, ethanol extract and Morantel tartrate (at dose 0.01g/kg) were compared for their efficacy against *Trichostrongylus*, *Haemonchus* and *Trichuris* nematodes in sheep. The respective reduction in EPG was 99.6, 99.8 and 99.8% (Aktar and Javed, 1985). *Saussurea lappa* roots powder (at dose 2k/kg), its equivalent water extract, methanolic extract and morantel tartrate (at dose 0.01g/kg) decreased EPG by 99±21, 48±32, 100±21 and 100±36 % in sheep infected with mixed species of nematodes (Aktar and Hassan, 1985). Glycosides (300mg/kg) extracted from root of *S. lappa* and morantel tartrate (0.01g/kg) resulted in reduction of EPG by 93±11 and 92±8 % in sheep and 93±4 and 97±8% in buffalo calves infected with mixed species of nematodes respectively (Aktar and Makhoom, 1988).

Aktar and Riffat (1984) evaluated the efficacy of *Melia azedarach* against gastrointestinal nematodes of goats. They have reported 99.4 and 90.2% reduction in EPG using *M. azedarach* fruit powder (30mg/kg) and Morantel tartrate (0.01g/kg) treated animals respectively. In another study *M. azedarach* fruit powder
(20mg/kg), its equivalent water extract, methanol extract, ethanol extract and piperazine (200mg/kg) were found to reduce EPG count of Acaridae galli infected chickens by 57.8, 15.7, 18.5, 67.8 and 75%, respectively (Aktar and Riffat, 1985).

Study in buffalo calves infected with Neoascaris vitulorum, on day 15 post treated with powered C. crista seeds (4g/kg) or its equivalent methanol extract, water extract and morantel tartrate (0.01g/kg) resulted in 100, 100, 81, 100% reduction in EPG count respectively (Akhtar, et al., 1985). In another study, glycosides (200mg/kg) extracted from C. crista seeds and moranted tartrate (10mg/kg) caused 94 and 100% reduction in EPG on day 15 post treatment in sheep having mixed nematode infection (Aktar and Aslam 1989). The anthelmintic activity of powdered C.crista seeds and its water and methanol extract was also reported in chicken infected with Ascaridia galli by Javed et al., (1994). That study reported 94±3, 98±1 and 100±0 % reduction in EPG by day 15 post treatment treated with powderd C.crista seeds (50mg/kg), its equivalent methanol extracts and piperazine adipate (200mg/kg) respectively. The Psoralea coylifolia seed powder (2g/kg), its equivalent water extract, methanol extract, and morantel tratrare (0.01g/k) caused reduction in EPG of mixed gastrointestinal nematodes in sheep in day 15 post treatment by 98±0.1, 99±0.09, 181±2 and 99±0.6% respectively( Javed and Aktar, 1986). Akhtar and Riffat (1986) reported anthelmintic efficacy of Peganum harmala against gastrointestinal cestodes of goats. The treatment; P.harmala seed powder 3g/kg, its equivalent water and methanol extract and Nilzan (levamisole hydrochloride + oxyclozanide) (5ml/15kg) resulted in 100±0, 89±3.2, 92±4.1 and 98±6.2 % reduction in EPG, respectively. Morus albo_stem bark powder (3g/kg), its equivalent water extract, methanol extract, and morantel tatrare (0.01g/k) were found to reduce EPG by 82±4.7, 79±6.9, 81±6.7 and 98±3.2% respectively in sheep infected with mixed species of nematodes. Similar treatments except with morantel tartrate replaced by Nilzan (5ml/15kg) were used to treat cestode
infection in sheep. This resulted in reduction in EPG by 85±6.6, 70±3.3, 79±4.2 and 99±2.9% in respective treatment groups (Riffat et al., 1986) Kailami, et al., (1995) evaluated antifasciolic efficacy of powdered *Nigella sativa* seeds, *Fumaria praviffora* aerial parts and *caesalpinia crista* seeds in buffaloes. Maximum antifasciolic efficacy, judged on the basis of percent reduction in EPG was shown by *F.paraviflora* (60mg/kg) 93.2±0.5% followed by *C.crista* (40mg/kg) 89.7±1.7% and N. sativa (25mg/kg) 88.2±0.4% at day 15 post-treatment.
1-4: **Haemonchosis:**
Parasitic infections are important economic problem of livestock. They reduce productivity and cause sterility, abortion and death of producing animals. Infection by gastrointestinal (GI) helminth parasites of livestock are among the most common and economically important diseases of grazing livestock (Perry, et al., 2002). They are characterized by lower out puts of animal products (Meat, milk, hides and skins), which all impact on the livelihood of smallholder farmers (Perry & Randolph, 1999). The greater losses associated with nematode parasite infections are sub-clinical, and economic assessments show that financial cost of internal parasitism are enormous (Preston & Allonby, 1979, Mcleod, 1995). One exception to this is the highly pathogenic nematode parasite of small ruminant, *Haemonchus contortus*, which is also capable of causing acute disease and high mortality in all classes of stock (Allonby & Urquhart, 1975). Haemonchosis has been identified as one of the top ten constraints to sheep and goat rearing in East Africa (Perry, *et al.*, 2002).

1.4.1: **Haemonchus contortus**

1.4.1.1: **Classification:**

- **Class:** Secernentea
- **Subclass:** Rhabditia
- **Order:** Strongylida
- **Family:** Trichostrongylidae
- **Scientific name** – *Haemonchus contortus*
- **Common name** – Barber pole worm

**Hosts:** Sheep, Goats, cattle, camels and Wild ruminants.
1.4.1.2: characteristics:-
The males are 10 to 20mm and females 18 to 30mm long. The white uteri and ovaries winding around the red blood-filled intestine give a twisted or barber pole appearance. The small buccal capsule bears a curved dorsal tooth. There are two prominent lateral spike-like cervical papillae near the junction of the first and second quarters of the oesophagus. The male bursa has long lateral lobes and slender rays with a flap-like dorsal lope located a symmetrically near the base of the left lateral lobe. The spicules are 450 to 500um long, each with a terminal barb. Usually, the vulva is covered by an anterior thumb-like flap, which may be reduced to a mere knob in some individuals. The oval eggs are 70 to 85 um long by 41 to 44 um wide and in early stages of cleavage when laid. They are somewhat yellowish (Soulsby, 1982).

1.4.1.3: Life cycle:-
No intermediate host is required. Adult male and female worms live in the abomasum of ruminant animals. The female deposits 5,000 to 10,000 eggs per day, which pass out of the host with feaces. First stage juveniles hatch from the eggs. First and second stage juveniles feed on bacteria. Third stage juveniles retain the second stage cuticle as a sheath. Third stage juveniles do not feed and are infective for the vertebrate host. The ruminant becomes infected while grazing by eating the third stage juveniles. Exsheathment occurs in the rumen, anterior to the abomasum, and the young worms pass in to the abomasum where they burrow into the mucosa. Here they undergo another molt, and the fourth-stage juveniles come back into the para mucosa lumen of the abomasum, and begin to feed and under go another molt before reaching adulthood. Mating of adult occurs and egg production commences. The egg hatch in soil or water and develop directly to infective third-stage juveniles. Enormous numbers of juveniles may accumulate on heavily grazed pasture (Bowman and Lynn, 1995)
1.4.2: Clinical signs and pathogenicity:-
Anemia, emaciation, edema and intestinal disturbances resulting principally from the loss of blood and injection hemolytic proteins in the host's system. Heavy infections may be fatal (Georgi and Georgi 1990).

1.4.3: Epidemiology:-
The epidemiology of the *H. contortus* is best considered separately depending on whether it occurs in tropical and subtropical or in temperate area.

1.4.3.1: Tropical and subtropical areas:-
Because larval development of *H. contorts* occurs optimally at relatively high temperature, haemonchosis is primarily a disease of sheep in warm climate however, since high humidity, at least in the microclimate of the faeces and the herbage, is also essential for larval development and survival. The frequency and severity of out breaks of disease is largely dependent on the rainfall in any particular area.

Given these climatic conditions, the sudden occurrence of acute clinical haemonchosis appears to depend on two further factors. First, the high faecal worm eggs output of between 2,000 and 20,000.egg. Even in moderate infections, means that massive pasture population of L₃ may appear very quickly. Second, in contrast to many other helminth infections, there is little evidence that sheep in endemic area develop an effective acquired immunity to *Haemonchus*, so that there is continuous contamination of pasture.

In certain area of the tropics and subtropics such as Australia, Barazil, the Middle East and Nigeria, the survival of the parasite is also associated with ability of *H. controtus* larvae to under go hypobiosis. Although, the trigger for this phenomenon is unknown, hybobiosis occurs at the start of a prolonged dry season and permit the parasite to survive in the host as arrested l₄ instead of maturing and producing
eggs, which would inevitably fail to develop on the arid pasture. Resumption of development occurs just before the onset of seasonal rains. In other tropical area such as East Africa, no significant degree of hypobiosis has been observed and this may be due to more frequent rainfall in this area making such an evolutionary development unnecessary (Besier and Dunsmore, 1933).

The survival of *H. contortus* infection on tropical pastures is variable depending on the climate and degree of resistant to desiccation and some may survive for 1-3 month on pasture or in faeces.

1.4.3.2: Temperate areas:-

In the British Isles, the Netherlands and presumably in other parts of northern Europe and in Canada, which are among the least favorable for the survival of *H. contortus*, the epidemiology is different from that of tropical zones. From the information available, infections seem to develop in two ways. Perhaps most common is the single annual cycle. Infective larvae which have developed from eggs deposited by ewes in the spring are ingested by ewes and lambs in early summer. The majority of these become arrested in the abomasum as L4 and does not complete development until the following spring. During the period of maturation of these hypobiotic larvae, clinical signs of acute haemonchosis may occur in the ewes, this often coincides with lambing (Eckert and Hertzberg, 1994).

1.4.4: Treatment:-

When an acute outbreak occurs, the animals should be treated with one of the anthelmintics, Benzimidazoles, levamisole or ivermectin and immediately moved to pasture not recently grazed (Bowman and Lynn, 1995).
1.4.5: Control:-
The main methods for control of gastrointestinal nematode parasites are prophylactic treatment with synthetic anthelmintics in combination with grazing management.

1.4.5.1: Anthelmintics:-
The broad-spectrum anthelmintics, which remove parasites in different stages of development within the host species, are the cornerstone of parasite control in G1 nematode infections. The major classes of synthetic anthelmintics used for control of G1 nematode parasites of ruminant livestock are:-

- The benzimidazole / probenzimidazoles group. The mode of action of BZs is by interference with polymerization of microtubules (Harder, 2002). These drugs bind to the protein tubulin of the parasite, therefore causing death by starvation (Roos, 1997).
- The tetrahydropyrimidines / imidazothiazoles group (levamisole / pyrantel, morantel) these drugs affect acetylcholine neuro-transmission by interfering with nicotinic acetylcholine receptors (Roos, 1997, harder, 2002).
- The macrocyclic lactones (MLs) or avermectins / milbemycins group. The MLs are thought to interact with chloride channels on helminth gamma-aminobutyric acid (GABA) receptor complexes, and also inhibit pharyngeal pumping, fecundity and motility in susceptible nematodes resulting in paralysis and ultimately elimination from the host (Harder, 2002; Yates et al., 2003).
- There are other anthelmintics referred to as narrow spectrum compounds, which have activity against fewer species of parasites and/ or lack high levels of efficiency against all stages of the parasites (Bowman et al., 2003). Examples of these anthelmintics include salicylanilrdes substituted phenols (closantel, oxyclozanide and nitroxnil) and triclabendazole.
1.4.5.2: Grazing management:-
Pasture management is designed to prevent infection of ruminants with internal parasites, and requires long-term planning. The main methods of parasite control through exploiting pasture management have been defined as preventive, evasive and diluting (Michel, 1985). It is by vary such factors as the density and age groups of animals and the time and intensity of grazing that serious infections can be avoided.

1.4.5.3: Nematocide plants:-
Several plants have anthelminthic properties and were in fact a part of the traditional husbandry before synthetic dewormers were commonly adopted. Veterinary research zeroed in deworming plants, also called anthelmintic plants, particularly before the Second World War in western countries then, subsequently, mainly in Eastern countries and India, there is reliable data available on the effects of several plants or plant extract on certain parasites. More potent botanical dewormers include wormwoods, snakeroot, cucurbits, umbelliferae and tansy. Goose food, fern lupine and tobacco have serious side effects that discourage their use (Duval, 1994) Garlic is a common plant dewormer readily available in many parts of the world. Anonymous, (1953) reported that garlic could be effective against Ascaris, Enterobius and, of particular interest for ruminants, against lungworm in general. Garlic does not prevent the production of eggs but prevent the eggs of certain parasites from developing into larvae (Bastidas, 1969).
1.5: Anthelmintic plants:-

1.5.1: *Balanites aegyptiaca*:-

1.5.1.1: Classification:-

Kingdom: *Plantae*
Subkingdom: *Tracheobionta*
Super division: *Spermatophyta*
Division: *Magnoliophyta*
Subclass: *Rosidae*
Family: *Zygophyllaceae*
Genus: *Balanites Delile*
Species: *Balanites aegyptiaca* (L.) Delile

*Balanites aegyptiaca* (L.) Delile is the only species of the genus *Balanites* present in Sudan (Andrews 1952).

Common name: Arabic: Heglig (tree), Laloub (fruits) English: Thorn tree, Desert date, Egyptian balsam, Indian *Balanites*

*Balanites aegyptiaca*  
*Balanites aegyptiaca* kernel
1.5.1.2: Distribution:–

*Balanites aegyptiaca* is a large savanna tree widely distributed throughout Africa, along the tropical belt from Tanzania in the East to Ivory coast in the west. It is also found in the relatively drier regions of northern Africa from Mauritania to Nigeria and Ghana, to Egypt, across Palestine, Saudi Arabia and India. The drier regions of Kenya, Uganda and Zaire, carry scattered open forests of *B.aegyptiaca* (Suliman & Jackson 1959). In the Sudan, the tree is wide spread throughout the Northern and Central provinces (Wickens, 1976)

1.5.1.3: Botanical description

The tree is common in the low rain full savanna and semi-desert vegetation of types (Harrison & Jackson 1952). It grows in various soil types such as clay, dark cracking clay, sand, hard-surfaced sandy clay, etc. It flourishes in habitats of clay soils receiving 500-1000 mm of annual rain full as well as on sandy clay soil where rain full exceeds 250 mm annually. However, the most luxurious *Balanites* forests are typically found on slightly elevated dark cracking clay under a rainfall of 500 mm and upwards annually.

1.5.1.4: Active constituents:–

**Fruit:** - The fruits of *B.aegyptiaca* were found to contain isorhamnetin 3-rutinoside and 3-rhamnogalactoside (Maksoud and AL-Hadidi, 1988). The alcoholic extract of the pulp and kernel contained sterols, terpenes and saponins as predominant compounds where as tannins, alkaloids and resins were found in slightly small amounts (AbdelRahim, *et al.*, 1986). Five saponins were isolated from the pulp and named as balanitisisins A, B, C, D and E. (Varshney *et al.*, 1977, Varshney and Jain, 1979). Two other saponins named, as Balanitisins F and G were isolated from the kernel (Varshney and Vyas, 1982). The total saponin content was found to be 7.2% in the mesocarp and 6.7 in the kernel (Watt and Breyer-Brandwijk, 1962). The oil
extracted from the kernel constituted 44-51% w/w and is composed mainly of triglycerides and with small quantities of diglycerides, phytosterols, sterolesters and tocopherhols. The oil contains palmitic acid 10-12%, stearic acid 9-10%, oleic acid 30-40% and linoleic acid 40-48% w/w (Abu-Al-Futuh, 1983).

Leaf: - Six flavonoid glycosides identified as quercetin 3-glucoside, quercetin 3-rutinoside, 3, 7, digluscoside and 3-rhamnogalactoside, isorhamnetin, were isolated from the leaves and branches of the plant (Maksoud and El-Hadidi, 1988).

Stem bark: - three saponins kown as Balanitisins l, 2 and 3 were isolated from the East African specimen of *B.aegyptiaca* (Liu and Nakanishi, 1982). In addition to the above saponins two other saponins were isolated and identified as the previously known saponins deltonin and protodeltonin. A Furancoumarin, bergapten and adihydrofuranocoumarin, marmesin were isolated from the chloroform extract of the bark (Ahmed, et al., 1981). Balanitol was isolated from the bark of the Indian specimen (Cordano, *et al.*, 1978).

Stem wood: - Balanitisin 1 was isolated from the stem wood of the Indian specimen (Varshney and Vyas, 1982).

Root: - Balanitisin 1, 2 and 3 were isolated from the East Africa specimen (Liu and Nakanishi, 1982). Alkaloids were reported in the root bark (El-khier, 1987).

Root wood: - Balanitisin H was isolated from the root wood of the plant (Varshney and Vyas, 1982).
1.5.1.5: Medicinal Folk-uses

The tree has many folk uses in various African countries. The fruit is used as fumigatory in liver disease in Chad (Croach, 1962, Watt and Breyers Brand Wijk, 1962) and as a purgative and sucked by schools children as a confectionery in Sudan (Abu-El-Futuh, 1983). In Tanzania the bark used in treatment of syphilis, round worm infections and a fish poison, (Bailey, 1962).

1.5.1.6: Pharmacological studies:

The root, bark, seed kernel, Fruit and branch of *Balanites aegyptiaca* were lethal to snails, miracidia and cercariae of schistosomes (Archibald, 1933). Also the aqueous extract of Fruit pulp, seed, kernel, root, bark and leaves of *Balanites aegyptiaca* had larvacidal effect against larvae of culex (Chapain & Wiesman, 2005).

The fruits are used as an oral hypoglycemic (Kamel, 1998) and an antidiabetic (Saker, *et al.*, 2000). Additionally, extracts of the plant display abortive and antiseptic properties (Speroni, *et al.*, 2005). Koko *et al* (2005) showed that *Balanites aegyptiaca* fruit mesocarp is highly effective as antischistosomal remedy. The aqueous extract of the bark is widely used as anti-jaundice (Saker, *et al.*, 2000) and antibacterial (Bashir, *et al.*, 1984).

The mixture of steroid saponin balatin6 and balatin7 isolated from *Balanites aegyptiaca* kernel has anticancer effects in human cancer cell in vitro (Gnoula, 2008). The aqueous extract and saponins isolated from kernel cakes of *Balanites aegyptiaca* have a potent mosquito larvicidal activity (Zarroug, *et al* 1988).

The ethanolic extract of the leaves, showed that it has a considerable anti-inflammatory and moderate antipyretic activities. It also inhibited the growth of *Bacillus subtilis*. However, no analgesic or diuretic activity could be demonstrated (Tanira *et al*, 1988).
The anthelmintic activity of Balanites aegyptiaca was evaluated in vitro using Caenorhabditis elegans as biological model by Gnoula, et al (2007) and Ibrahim (1992) they reported that the plant had nematocidal activity. In vivo Koko et al (2000) showed that Balanites aegyptiaca was lethal to Fasciola gigantica in goats.

1.5.2: Peganum harmala

1.5.2.1 Classification:

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Magnoliopsida  
Order: Sapindales  
Family: Nitrariaceae  
Genus: Peganum  
Species: Peganum harmala  

Common name: - Surian rue, African rue, Wild rue, Harmal, Harmala

1.5.2.2: Distribution:-

The plant is widely distributed in the central Asia, North Africa and Middle East and has been introduced in America and Australia.
1.5.2.3: **Botanical Description**:-

*Peganum harmala* L. (Zygophyllaceae) is a perennial herbaceous, glabrous plant, which may grow to 30-100cm. Its normal habitat is semi-arid rangeland, steppe areas and sandy soils. It has alternately spaced thorny-like leaves, which has a strong deterrent odor when rumpled. Opposite to the leaves are solitary white flowers with green veins. The fruits are globose capsules with 3 chambers containing many angular blackish seeds (Rechinger, 1982). The plant is not usually grazed. Its bitter taste repels animals. However, when pasture is parsing, donkeys and other domestic animal may be attracted to and graze intermittent on this plant. All species are susceptible to poisoning from this plant, but dromedaries (camels) are the most often affected (El Bahri and Chemli, 1991).

1.5.2.4: **Active constituents**:-

The plant is rich in alkaloid and contains up to 4% total alkaloids (Abdel-Fattah *et al.*, 1997; Chopra *et al.*, 1995). The principle alkaloid present are harmine, harmaline (identical with harmidine), harmalol and harman and quinazoline derivatives: vasicine and vasicinone. The alkaloidal content of unripe seeds is less than the ripe ones (Kamel, *et al.*, 1970)

**Harmaline (harmadine):** first isolated by Göbel (1841) from the seeds and roots of *P.harmala*, this is the major alkaloid of this plant. This compound is slightly soluble in water, alcohol and ether, quite soluble in hot alcohol and dilute acids. Its hydrochloride dehydrate, which crystallizes as yellow needles, is moderately soluble in water and alcohol. Harmaline is almost twice as toxic as harmine and in moderate doses causes tremors and colonic convulsions but with no increase in spinal reflex excitability (Budavari & O Neil, 1996). Lethal doses bring about convulsion, which are soon followed by motor paralysis due to the marked depressive action upon the central nervous system. Respiration is paralyzed and a decrease in body temperature occurs. The perfused heart arrested in diastolic
phase and the contraction of smooth muscle is diminished with exception of the uterus, which may be made to contract more powerfully. Over a wide range of doses, there is a reduction in blood pressure due to a pronounced weakening of the heart muscle.

**Harmine:** - The alkaloid is optically inactive and form colorless rhombic prism from methanol. It is slightly soluble in water, alcohol or ether. Solution of its salt shows a deep blue fluorescence. Pharmacologically, harmine resemble harmaline in its action but is less toxic. The hydrochloride has been found to be highly active against Mycobacterium tuberculosis (Glasby, 1978).

**Harmalol:** - It is freely soluble in hot water; acetone or chloroform but only sparingly soluble in benzene. The alkaloid is unstable when exposed to air. Its methyl ether is harmaline (Glasby, 1978).

**Harman:** - The alkaloid is crystallized from several organic solvents as colorless prism. It is readily soluble in methanol, alcohol, acetone, chloroform, or ether but only moderately so in hot water. It dissolves in mineral acids and exhibits a blue-violet fluorescence. (Glasby, 1978).

**Vasicine (peganine):** - This quinazoline alkaloid was first isolated from the leaves of *Adhatoda vasica Nees* and subsequently discovered in *P.harmala* under the name of Peganine. The crude drug from *A.vasica* is used in India as a remedy for asthma and the pure alkaloid act as bronchodilator (Glasby, 1978).

**Vasicinone:** - A further alkaloid present in *Adhatoda vasica Nees* and *P.hamala*. The alkaloid yield crystalline salts with mineral acid. It are an active bronchodilator (Glasby, 1978).
1.5.2.5: Pharmacological uses:

There are several reports, which indicate the great variety of pharmacological and biological activities of *Peganum harmala* such as antibacterial, antifungal and MAO inhibition (Abdel-Fattah *et al.*, 1997), to be effective in the treatment of dermatitis (Saad and Rifai, 1980), hypothermic (Abdel-Fattah *et al.*, 1995) and cancer (Adams, 1983). The aqueous extract of *Peganum harmala* possesses anti-conceptive, analgesic and anti-inflammatory properties (Monsef *et al.*, 2004).

Akhtar and Riffat (1986) reported anthelmintic efficacy of *peganum_harmala* against gastrointestinal cestodes of goats.

The seed has been used as an anthelmintic in order to rid body of tapeworms (Chopra, *et al.*, 1986). The root has been used as a parasiticide in order to kill body lice (Chopra, *et al.*, 1986). It is also used internally in the treatment of rheumatism and nervous condition (Chevallier, 1996).

The fruit and seed are digestive, diuretic, hallucinogenic, narcotic and uterine stimulant (Emboden 1979, Bowman, 1995). They are taken internally in the treatment of stomach complaints, urinary and sexual disorders, epilepsy, menstrual problems, mental and nervous illnesses (Bowman, 1995). This remedy should be used with caution and preferably under the guidance of a qualified practitioner since excessive doses cause vomiting and hallucinations (Bowman, 1995). The seeds contain the substance harmine which is being used in research in to mental disease, encephalitis and inflammation of the brain. Small quantities stimulate the brain and said to be therapeutic, but in excess harmine depress the central nervous system. A crude preparation of the seed is more effective than an extract because of the presence of related in doles (Emboden, 1979). The seed is used externally in the treatment of hemorrhoids and baldness (Bowman, 1995).
The whole plant is said to be abortifacient, aphrodisiac, emmenagogue and galactogogue. The leaves are used in the treatment of rheumatism (Chopra, et al., 1986).

1.5.2.6: Animal toxicity:-

All parts of plant are thought to be toxic. Intravenous injection of harmine and harmaline (9mg/kg) into cattle has shown toxic effects such as accelerated breathing and pulse and colonic muscular spasm (Puzii, et al., 1980). The toxic doses of various alkaloids in different species of animals (harmaline in rats LD50-SC 120mg/kg, harman in rabbits LD50-SC 200mg/kg, harmine in mice LD50-IV 38mg/kg, harmine LD50-SC in rats 200mg/kg) (Budavari, et al., 1996). All domesticated animals are susceptible to poisoning from *P. harmala*, camels especially young animals are the most affected in dry seasons (El-Bahri & Chemli, 1991), followed by donkeys (Bailey & Damn, 1981), sheep and horses (Bailey 1986). Digestive and nervous syndromes have been reported in animals that consume a sub-lethal amount of the plant (Billil, 1983). The animal initially becomes prostrate and then anorexia, hyper-salivation, vomiting and diarrhea occur. The first signs are excitability followed by muscular trembling, accelerated breathing, standing is impossible, and the animal goes into recumbency. The animal appears in a narcotic state interrupted by occasional short periods of excitement. After a few hours, dyspnea and mydriasis are noted. Frequent urination and subnormal temperature has also been reported in cattle (Bailey, 1979). Abortion frequently occurs. The course of the nervous syndrome is usually short and death follows within 30-36 hours after the onset of signs of CNS intoxication. Anorexia, restlessness, and weakness of the hind limbs and knocking of the fetlock joint characterize the chronic intoxication of cattle. In postmortem examination of animal, no distinctive lesion is observed. Rapid rigor mortis has been noted. The
heart, pulmonary, renal and gastrointestinal systems are reported to be congested and sub-capsular hemorrhage in the liver has been observed (Bailey, 1979)

1.5.3:  **Zizyphus spina-christi:**

1.5.3.1: **Classification:**

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Rosales
- **Family:** Rhamnaceae
- **Genus:** Ziziphus
- **Species:** Zizyphus spina-christi

The genus *ziziphus* belongs to buckthorn family (Rhamnaceae). It is genus of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world.

Latin names:  
- *zizyphus* spina-Christi (syn.z.splnosa), *Rhamnus* spina-Christi,  
- *Paliurus* spina-Christi, *paliuris virgatus, paliuris australis, paliuris aculeatus*. The literature is quite confused on the other possible synonyms *Rhamnus paliurus, zizyphus Spinoza.*
Common names:-The Arabs call it Nubka. Sudan: Nabag, Nabak, Cidir. Nigeria: Kurna. In English it is known as Dom, Jerusalem-thorn or Christ thorn. In French it is called paliure, epine du Christ, porte-chapeau, capelets, argolou and arnaves.

1.5.3.2: Distruptions:

*Zizyphus spina-christi* grows wild in tropical Africa and Asia (Adzu and Haruna, 2007). In Sudan distributed in northern Kordofan and throughout northern and central Sudan (El-Ghazali *et al*, 1997).

1.5.3.3: Botanical description:-

It is a deciduous shrub growing to 5m by 5m at a medium rate. It is hardly. It is in flower from July to August, and the seeds ripen from October to December. The scented flowers are hermaphrodite. The plant has small white woolly flowers that are highly scented and honey-like (Levy, 1991).

1.5.3.4: Chemical composition:-

The plant has been extensively studied (Ikram and Tomlinson, 1976; Aynchchi and Mahoodian, 1973), and its chemical composition is well known (Younes, *et al.*, 1996, Mahran, *et al.*, 1996). The main constituents of the essential oil were Alpha-terpineol (16.4%) and linalool (11.5%). The main neutral hydrocarbons were n-pentacosane forms (81%). Methyl esters isolated from leaves included methyl palmitate, methyl stearate, methyl myristate, Beta-sitosterol, oleanolic acid and maslinic acid were the main aglycones of the glycosides present in leaves. The plant also contains four saponin glycosides (Mahran, *et al* 1996). The highest flavonoid content was found in the leaves (0.66%). Quercetin 3-O-rhamnogluconside, 7-O-rhamnose and rutin are main flavonoid compounds present in all plant parts investigated (Branter, *et al.*, 1999). Alkaloids of *zizyphus* are, zizphine-F, jubanine-A, and amphine-H, a new peptide alkaloid spinanine-A has been isolated from the stem bark of *zizyphus spina-christi*. Spinanine-A is a 14-

1.5.3.5: Folkloric uses:-
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 Sudan, the maceration of the fruit is used against constipation and as antidiabetic (El-Ghazali, et al 1997). In Saudi Arabia, folk medicine, the leaves are used to heal wounds, treat some skin diseases, some inflammatory conditions, sores, against ringworm, fever and ulcer (Blatter, 1978; Shahat et al., 2001). Plant leaves are also used in Iranian folk medicine as an antiseptic, antifungal and anti-inflammatory agent, and for healing skin diseases such as topic dermatitis (Amin, 1991, Nafisy, 1989).

1.5.3.6: Pharmacological studies:-
The leaves are applied locally to sores (Dalziel, 1937) as wound powder and antiseptic (Fleurentin and Plet, 1982) and was reported to exhibit hypoglycemic activity against streptozotocin diabetic rats (Glommbitza et al., 1994; Abdel-Zaher et al., 2005) and antibacterial efficacy against Gram-positive strains (Ali et al., 2001; Suksamrarn et al., 2006). It has been described as anticathartic, astringent, diuretic and tonic (Duke and Ayensu, 1985). Moreover, its root bark is used in folk medicine as remedy against pain (Adzu et al., 2002; Adzu et al., 2003).

Alsudani, (2007) found that Aqueaus leaf extract of Zizyphus spina-christi (1mg/kg) kill all protoscolices (larval stage of Echinococcus granulosus) in 72 hrs. Aqueous leaf extract of Zizyphus spina-christi possess anti-ceptive properties in the rat and have a calaming effect on the central nervous system (Effraim, et al., 1998)

Adzu et al , (2002), studied the effect of Zizyphus spina-christi aqueous extract (100, 200 mg/kg, i.p) on the central nervous system in mice. They observed that aqueous extract of Zizyphus spina-christi root bark may have sedative activity
That was evident from the market inhibition of the exploratory behavior spontaneous motor activity and prolonged sleeping time in mice. Those results suggest that the extract contained some constituents that depress the central nervous system. Such finding correlates with observation of Morishita et al, (1987), on the aqueous extract of *Zizyphus* seed. In addition, Waggas (2007), observed that *Zizyphus spina-christi* leaves extract (100 mg/kg, i.p) caused increased in epinephrine, norepinephrine, dopamine, serotonin, and 5-hydroxyindoleacetic and decrease in gamma-aminobuteric acid content in different brain area of rats.
CHAPTER TWO
MATERIALS AND METHODS

2.1: Plants:
The experimental plants used in the present study were mainly the kernel of *Balanites aegyptiaca*, the seeds of *Peganum harmala* and the leaves of *Zizyphus spina-christi*. The three plants were obtained from Omdurman local market.

2.2: Plant extracts:
Each of the plants was studied in terms of two extracts (aqueous and methanolic).

- Methanolic extracts of the plants were performed at the Medicinal and Aromatic Plants Research Institute (MAPRI) – National Centre for Researches (Khartoum) according to the method of Harborne (1984).

- Aqueous extracts of the plants were performed at the research laboratory – College of Veterinary science–University of Bahar El Ghazal, according to the method of Fenado *et al* (1989).

2.3: Animals:
Animals used in the present study were:

- A total of 93 male Nubian goats (6-8 month old) weighing 10-12kg were purchased from Bahry (Khartoum North) livestock market. They were housed in pens in the premises of the College of Veterinary Science, University of Bahr El Ghazal. The goats were healthy and were given ivermectin (ivomec1% ) at dose of 1ml/50kg to remove subclinical internal worm infections. The animals were given a three weeks preliminary period during which they were fed on Lucerne and sorghum grains *ad libitum* and had free access to drinking water. After ascertaining that the goats were not
shedding nematode eggs, they were inoculated with 4000 *H. contortus* infective larvae.

- 120 different sexes’ Wister rats, weighing 100-200 grams were used for toxicological study. All rats obtained from the Faculty of Pharmacy; University of Khartoum.
- 12 healthy rabbits (local breed), weighing 1-3 kg were obtained from Bahry market. Rabbits were used for pharmacological studies.

2.4: Parasites

Adult parasites of *H. contortus* were collected from abomasa of infected sheeps obtained from El Kadro abattoir.

2.5: Samples collection:

2.5.1: Blood samples:

Blood samples were taken in EDTA coated vacutainer tubes, from the jugular vein of the goats at day zero pretreatment and at days 7, 14, 21 post treatment for hematological examination and plasma separated for biochemical analysis.

2.5.2: Faecal samples:

Faecal samples were collected from the goats directly from the rectum into a clean Petri dish. The samples from each animal were collected in the morning starting from day zero pretreatment and at day 7, 14, 21 post treatment to determine the count of the parasite eggs.

2.6: Preparation of the aqueous extract of the plants:

The aqueous extracts of the three plants (*Balanites aegyptiaca*, *Peganum harmala* and *Zizyphus spina-christi*) were prepared according to the standard methods of Fenado *et al* (1989) as follows:

One hundred grams of the powdered plant material were mixed with 500ml of distilled water in a 1 L flask and boiled for 1.5 hrs, allowed to cool to 40 °C, and then filtered using Whatman paper No.1. The filtrates were then concentrated in
rotary evaporator and the extract stored at 4°C until required for use.

2.7: Preparation of methanolic extract of the plants:
Sixty grams of each plant were weighed and packed in Soxhlet apparatus. 500ml of petroleum ether was used, as a solvent for each to separate lipids and terpenoids. The samples were repacked in Soxhlet and chloroform was used as a solvent for the same purpose (to separate lipids and terpenoids). The samples were again unpacked, dried and repacked, this time with methanol as a solvent to get the polar constituents of the plants. The extracts were evaporated till dryness using a rotatory evaporator (Harborne, 1984).

2.8: In vitro anthelmintic Activity:
Four in vitro experiments were conducted to determine anthelmintic effects of methanolic and aqueous extracts of the three plants (Balanites aegyptiaca, Peganum harmala, Zizyphus spina-christi) and Ivomec on adult H. contortus. Adult H. contortus were collected from the abomasum of infected goats. Immediately after slaughtering, the abomasum were collected from slaughter house and transported to the laboratory. The parasites were then collected after opening the abomasum, washed and kept in phosphate buffer saline (PBS). Ten actively moving worms were placed in Petri dishes containing 25mg/ml of the aqueous and alcoholic extracts for each of the 3 plants extracts in PBS and PBS alone for the control group in total volume of 4 ml. Ivermectin diluted in PBS at the concentration of 1% was used as control. Three replications per each treatment were employed. The inhibition of motility of the worms was used as criterion for anthelmintic activity. The motility was observed after 0, 1, 2, 3, 6, 12 and 24h intervals. After 24 hrs the plant extracts and ivermectin were washed away and the parasites were suspended in PBS for 30 minutes for possible recovery of the parasite motility. The number of motile (alive) and non motile (dead) worms were counted under dissecting microscope, and
recorded for each treatment. Mortality index was calculated as a number of dead worms divided by the total number of worms per Petri dish (Sharma et al., 1971).

2.9: In vivo anthelmintic activity:-

Four in vivo experiments were conducted to determine anthelmintic effects of methanolic and aqueous extracts of the three plants (Balanites aegyptiaca, Peganum harmala, Zizyphus spina-christi) and Ivomec on goats infected with Haemoncus contortus.

2.9.1: Infective material-Culture of eggs:

Adult parasites of H. contortus were collected from abomasum of infected sheep obtained from El Kadro abattoir. The worms were washed and crushed to liberate eggs. The eggs were then cultured in a glass jar filled with autoclaved goat faeces for eight days at room temperature. At the end of the 8th day, infective larvae (L3) were harvested by using Baermann apparatus.

2.9.2: Experimental animals:-

A total of 93 (6-8 month old) healthy male Nubian goats weighing 10-12kg were used in this study.

2.9.3: Experimental design

Four experiments were conducted in this study. Goats infected with a single dose of 4000 L3 Haemoncus contortus.

Experiment 1: test of anthelmintic effect of Balanites aegyptiaca

31 goats were used in this experiment divided into 4 groups.

Group 1: three non-infected goats served as negative control.

Group 2: three infected goats with Haemoncus contortus received no treatment served as positive control.

Group 3: 10 goats infected with Haemoncus contortus divided into 2 sub groups each of five animals treated orally with two doses of aqueous extract of
**Balanites aegyptiaca** (100&400mg /kg)

Group 4: 15 goats infected with *Haemoncus contortus* divided into 3 subgroups each of five animals treated orally with three doses of methanolic extract of *Balanites aegyptiaca* (100&400&800mg /kg).

**Experiment 2:** test of anthelmintic effect of *Peganum harmala*

26 goats were used in this experiment divided into 4 groups.

Group 1: three non-infected goats served as negative control.

Group 2: three infected goats with *Haemoncus contortus* received no treatment served as positive control.

Group 3: 10 goats infected with *Haemoncus contortus* divided into 2 subgroups each of five animals treated orally with two doses of aqueous extract of *Peganum harmala* (100&400mg /kg)

Group 4: 10 goats infected with *Haemoncus contortus* divided into 2 subgroups each of five animals treated orally with two doses of methanolic extract of *Peganum harmala* (100&400mg /kg).

**Experiment 3:** test of anthelmintic effect of *Zizyphus spina-christi*

31 goats were used in this experiment divided into 4 groups.

Group 1: three non-infected goats served as negative control.

Group 2: three infected goats with *Haemoncus contortus* received no treatment served as positive control.

Group 3: 10 goats infected with *Haemoncus contortus* divided into 2 subgroups each of five animals treated orally with two doses of aqueous extract of *Zizyphus spina-christi* (100&400mg /kg)

Group 4: 15 goats infected with *Haemoncus contortus* divided into 3 subgroups each of five animals treated orally with three doses of methanolic extract of *Zizyphus spina-christi* (100&400&800mg /kg).
**Experiment 4: Ivermectin**

Five goats infected with *Haemoncus contortus* injected with ivermectin 10 mg/kg were used in this experiment.

**2-9-4: Faecal samples:**

Animals were observed for clinical signs. Faecal samples from each animal were collected in the morning starting from day zero pretreatment and at day 7, 14, 21 post treatment and examined for the presence of worm eggs by flotation technique (MAFF, 1979). The eggs count was performed by Mc Master Method (Soulsby, 1982). The percent reduction in egg count was calculated using the following formula (Pankavich *et al.*, 1973):

\[
\text{ECR\%} = \frac{\text{Pre-treated EC/g} - \text{post treated EC/g}}{\text{Pretreatment EC/g}} \times 100
\]

**ECR\% =** percent reduction in egg count per gram

**2-9-5: Egg count procedure:**

Fresh fecal samples were collected into a clean Petri dish. The EPG counting was determined using a Mc Master technique (Soulsby, 1982) and expressed as faces with lower limit of determination of 100 parasites of eggs. Three grams of faecal sample were grounded and mixed with 87ml of flotation fluid (a saturated salt solution in water). After filtering through a tea strainer, a sub-sample was transferred to both compartments of McMaster counter chamber and allowed to stand for 5 minutes. All helminth eggs were counted under a microscope at 10X magnification. Since 3 g of faeces yielded 90ml of suspension (1 g per 30 ml suspension) and the volume of suspension examined was 0.3ml (0.15ml under each square of the counting chamber) the number of egg per gram of faeces is obtained by multiplying the total number of eggs in the two squares by 100.
2-9-6: Mature worms count procedure:
The animals were humanely slaughtered and the abdomen was ligated at the junction of the abomasum to omasum and abomasum to the small intestine, the abomasum was removed, and opened up with a blunt tipped pair of scissors and the contents were emptied into a bucket. The abomasal mucosa was washed gently with running tap water and the parasites washed off into the bucket. Then the numbers of adult *H. contortus* in the aliquots were counted.

2-9-7: Blood samples:
Blood samples were taken in EDTA coated vacutainer tubes, from the jugular vein of the goats from day zero pretreatment and at days 7, 14, 21 post treatment for hematological examination and plasma separated for biochemical analysis.

Preparation of the plasma:
Samples were collected in EDTA coated vacutainer tubes and after mild shaking were centrifuged at 3000 revolutions/minute (rpm) for 15 minutes. The fluid part (plasma) was separated from the cellular part using a dropper and the plasma was placed in a new plane sample container labeled according to the study group, goat number, time and date of collection and stored at -20C waiting analyses.

2.10: Blood and biochemical analysis:

2.10.1: Haemoglobin concentration (Hb)
The concentration of haemoglobin was measured by the cyanomethaemoglobin technique (Kelly, 1984) the method is based on the conversion of haemoglobin by Drabkin's solution to cyanomethaemoglobin.

Method:
20 µl of blood was added to 5ml of a modified Drabkin reagent (potassium cyanide 0.2g, potassium ferrocyanide 0.2g, sodium bicarbonate 1.08g dissolved in distilled water to 1 L). After 10 minutes, the solution cyanomethaemoglobin is compared against a standard in a colorimeter (wavelength 540 nm).
Calculation:

Concentration of Hb = OD Test / OD standard

OD = Optical density

2-10-2: **Packed cell volume (PCV%)**

PCV was measured by using haematocrit centrifugation method. The tubes which are sealed at one end with special clay (cristaseal), were centrifuged in a microhaematocrit centrifuge for 5 minutes and PCV was measured from the scale on the microhaematocrit reader (Scham, 1975).

2-10-3: **Glutamate oxalate Transaminase (GOT)**

The activity of GOT was determined by the colorimetric method as described by Karmen, 1955 and Braunstein, 1973 using a kit (Linear chemical .S.I).

**Test Principle:**

The enzyme catalyzes the intermolecular transfer of an amino group from aspartate to α-oxaloglutarate according to the following reaction

α–oxalglutarate + L-aspartate → L-glutarate + oxaloacetate

The oxaloacetate formed reacts with 2, 4 dinitrophenylhydrazine to form oxaloacetate hydrazone which subsequently reacts with sodium hydroxide to form a coloured solution. The intensity of the colour is proportional to GOT concentration.

Absorbance of sample and standard were read against a reagent blank at wave length 500nm. The value of enzyme activity was obtained from the table provided with the kit.
2-10-4: Glutamate Pyruvate transaminase (GPT):

The enzyme activity of GPT was determined by colorimetric method described by Braunstein (1973) using a kit (Linear chemical .S.I).

**Test principle:**

It is the same as that of GOT in which an amino group is transferred from an amino acid alanine to a \(\alpha\)-oxoglutaric acid according to the following reaction:

\[
\alpha\text{-oxoglutarate + L –amino acid } \rightarrow \ L \text{ glutamate + pyruvate}
\]

The oxaloacetate formed reacts with 2, 4 dinitrophenylhydrazine to form pyruvate hydrazone which subsequently reacts with sodium hydroxide to form a coloured solution. The intensity of this colour is proportional to GPT concentration. Absorbance of sample and standard were read against a reagent blank at wave length 500nm. The value of enzyme activity was obtained from the table provided with the kit.

2-10-5: Urea Determination:-

Enzymatic colorimetric method was used

**Principle:**

Urea is hydrolyzed by urease into ammonia and carbon dioxide. Ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield blue cromophore the intensity of the color formed is proportional to the concentration of urea in the sample.

Reagent composition:-

R1: enzyme reagent urease 500mg/ml stablizer.

R2: bufferd chrpmogen. Phosphate buffer 20mmol/l .PH 6.9, EDTA 2mmol/l sodium, salycilate60mmol/l, sodium nitropusside 3.4mmol/l

R3:Alkalinehypochlorite:sodium hypochlorite 10mmol/l.NaOH150mmol/l

Urea standard: urea standard 50mg/dl (8.3mmol/l)
Procedure:-
Into three test tubes containing 1.0ml from R1 (tube 1 for blank, tube 2 for sample and tube 3 for standard) for sample tube 10µl from plasma sample was added. For tube 3 10µl from standard was added. The tube were mixed well and incubated at 37°C for 10 minutes. Then for each tube 1.0 ml were added from R1. The tubes were mixed well and incubated at 37°C for 5 minutes. The absorbance (A) of the samples and the standard were read at 600nm in colorimeter against the reagent blank.

Calculation:-
\[
\frac{A_{\text{sample}} \times C_{\text{standard}}}{A_{\text{standard}}} = \text{g/dl albumin}
\]

2-10-6: Sodium Determination
Sodium was determined by flame emission spectrophotometer at 589nm.

2-10-7: Potassium Determination
Potassium was determined by flame emission spectrophotometer at 768 nm.

2-10-8: total protein:-
Colorimetric method was used.

Principle:-
In the biuret reaction, a chelate is formed between cupper ions and the peptide of the protein in alkaline solution to form a violet colored complex whose absorbance is measured photometrically. The intensity of the color produced is proportional to the concentration of protein of the sample (Gomall et al., 1949 & Falkner and Meit., 1982).

Reagent composition:-
1-Biuret reagent:-cupric sulphate 6 m mol/L, potassium tartrate 21 m mol /L, potassium iodide 6m mol /L, sodium hydroxide 0.75mol/l.
2-Protein standard; Bovine serum albumin 7g/dl.
Procedure:-
Into three test tubes containing 1.0ml from biuret( tube1 for blank, tube2 for sample, tube3 for standard) for sample tube 20ml from plasma sample was added and for tube 3, 20ml from standard was added. The tubes were mixed well and incubated and incubate for 10 minutes. The absorbance of the samples and the standard were read at 540nm against the reagent blank.

Calculation: \[
\frac{\text{Absorbance sample} \times C_{\text{standard}}}{\text{Absorbance standard}}
\]

2-10-9: Albumin determination: -

Colorimetric method was used

Principle:-
the method is based on the specific binding of bromocresol green (BCG); an ionic dye, and the protein at acid pH with resulting shift in the absorption on wavelength of the complex. The intensity of color formed is proportional to the concentration of albumin in sample (Doumas et al, 1971)

Reagent composition:-

**R1:** Bromocresol reagent: succinate buffer75mmol/l BCG 0.12mmol/l tensionactive 2g/l (w/v)

**R2:** albumin Standard: Bovine serum albumin 5g/dl

Procedure:-
Into three test tubes containing 2.0ml from R1 (tube1 for blank, tube2 for sample tube3 for standard) for sample tube 10ml was added from plasma sample and for tube3 10ml from standard was added. The tubes were mixed and incubated at 37C for 10 minutes. The absorbance (A) of the samples and the standard were read at 630nm against the reagent blank.
Calculation:-
\[
\frac{A \text{ sample} \times C \text{ standard}}{A \text{ standard}} = \text{g/dl albumin}
\]

2-11: Pharmacological experiments:

2-11-1: The effect of the plants extracts on the intestinal motility:

For investigation of the effects of different concentration of the *Balanites aegyptiaca*, *Peganum harmala*, and *Zizyphus spina-christi* on the intestinal motility, isolated rabbit jejunum was used.

Apparatus:
The glass jar bath apparatus connected to an ink-writing Harvard universal oscillograph was used.

Procedure:
A rabbit was slauter and then the neck was dislocating, exsanguinated, abdomen opened and jejunal part of the intestine was located (this is the proximal part where the mesenteric supply is perfuse). Jejunum was removed and cut into segments (4cm in length), a section of intestine was transferred to Petri dish containing Tyrode solution. The mesentery and fat surrounding the muscle was trimmed away and a thread was passed through one wall of the jejunum at both top and bottom. The bottom thread was attached to the tissue holder then the mounted tissue was transferred to the organ bath and attached to isotonic transducer. The tissue was let for 15 minutes to adapt to the new environment and washed several times by outflow (Ian Kitchin,1984). Then the extract of the plant was added to determine the effects.

Depending on the response showed to extracts, different known antagonists were used to determine the mechanism of response. Contraction response blocked by atropine in a dose of 400mg/ml. Relaxation response blocked by propanalol and tolazoline in a dose of 500µg/ml.
2-11-2: Determination of mean lethal dose (LD50) of plants extract:

The mean lethal dose (LD50) of the methanolic extract of *Balanites aegyptiaca*, *Peganum harmala*, and *Zizyphus spina-christi* was monitored in groups of Wister rats of different sexes, equally distributed in each treatment group, with 4 rats assigned to each treatment. The rats were observed for 24 hours after dosing with appropriate plant extracts. The signs of toxicity and number of dead animals in each group were recorded. The finding was tabulated as follow:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/rats)</th>
<th>No. of rats in group</th>
<th>No. of dead animals</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4000</td>
<td>4</td>
<td>-</td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>6000</td>
<td>4</td>
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<td>3</td>
<td>8000</td>
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<td>200</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>12000</td>
<td>4</td>
<td>-</td>
<td>200</td>
<td>-</td>
</tr>
</tbody>
</table>

The LD50 was calculated from the table as described by Stewart and Stolman (1961) in the following way:

$$LD50 = \text{the largest dose} - \frac{\sum a \cdot b}{N}$$

- $a$ = the constant factor between two consecutive doses
- $b$ = the mean of the dead animals in two successive groups
- $n$ = number of animals in the group
- $\sum$ = the sum of $a$ and $b$
2.12: **Statistical Analysis:**

The mean of pre and post-treated egg counts per gram and the number of adult worms counted at necropsy were analyzed with the independent t-test by the help of SPSS (version 11.5) computer program.

Results were expressed as mean ±s.d. \( P < 0.05 \) was considered as significant.

The percent reduction in egg count per gram of faeces was calculated using the following formula:

\[
\text{ECR\%} = \frac{\text{Pre-treated EC/g} - \text{post treated EC/g}}{\text{Pretreatment EC/g}} \times 100
\]

EC/g = egg count per gram
3.1: *Balanites aegyptiaca*

Anthelmintic effects of aqueous extracts (AE) and methanolic extracts (ME) of *Balanites aegyptiaca* were tested *in vitro* and *in vivo*.

3.1.1: *In vitro* anthelmintic activity:

The *in vitro* trials demonstrated anthelmintic activity of crude aqueous and methanolic extracts of *Balanites aegyptiaca* against adult *H. contortus* as evident from mortality rate of the worms.

The AE of *Balanites aegyptiaca* produced mortality rate of the worms of 20%, 40%, 40%, 90% at 1, 3, 6, 12 hours post treatment respectively, while at 24h all worms exposed to extract were found dead. The ME produced week mortality rate (1%) at the first six hours, but mortality rate increased to 25% and 90% at 12, 24 hours post treatment respectively; whereas, 10%&30% of the worms were found dead at 12 & 24 hrs respectively in phosphate buffer saline (PBS) (Fig.1).

The AE of *Balanites aegyptiaca* produced mortality rate of the worms significantly greater (p ≤ 0.05) than ME of *Balanites aegyptiaca* at the first 12 hours post exposure to extracts.

3.1.2: *In vivo* anthelmintic activity:

*In vivo* experiments were conducted to determine anthelmintic effects of methanolic and aqueous extracts of *Balanites aegyptiaca* on goats infected with *Haemoncuncus contortus*.

All Haemoncunc infected goats manifested inappetence and dullness, but this was less marked in the animals treated with the plant extracts. This observation was supported by reduction in the number of *Haemoncunc* eggs in faeces and adult worms in the abomasa at necropsy.
3.2.2.1. Aqueous extracts of *Balanites aegyptiaca* anthelmintic effects:
The anthelmintic activity of two doses (100, 400mg/kg) of aqueous extract of *Balanites aegyptiaca* in goats infected with *H.contortus* shown in table (3-1).
The dose of 100mg/kg showed no significant reduction (p≤0.05) in EPG, while, the dose of 400mg/kg revealed a time dependent anthelmintic effect and significant reduction (p≤0.05) in the EPG, which were 42.8%, 55.1%, and 61.2% at days 7, 14 and 21 post treatments respectively.
The control group and animals treated with 400mg/kg slaughtered at day 21 post treatments. The numbers of adult *H.contortus* worms found in abomasa of the animals counted. The result revealed that, worms were significant reduced (p ≤ 0.05) in treated animals compared to controls (Table 3-1).

3.1.2.2. Methanolic extracts of *Balanites aegyptiaca* anthelmintic effects:
The anthelmintic activity of three doses (100, 400,800mg/kg) of ME of *B. aegyptiaca* in goats infected with *H.contortus* shown in Table (3-2).
The three doses of ME of *B. aegyptiaca* exhibited different anthelmintic effects. The dose of 100mg/kg showed a maximum reduction of 20% in EPG on day 21. While, the dose of 400mg/kg revealed a time dependent anthelmintic effect and significant reduction (p≤0.05) in EPG of 33.3%, 48.2% and 74.7% at days 7, 14 and 21 post treatment respectively. In addition, the dose of 800mg/kg showed a time dependent effect and significant reduction (p≤0.05) in EPG of 30.4%, 60%, and 79.2% at days 7, 14, and 21 post treatments respectively.
Fig 1: Comparison of mean percentage of survival adult *H. contortus* for 24 hours exposure to methanolic and aqueous extracts of *Balanites aegyptiaca* with control (phosphate buffer saline; PBS)

MB : methanolic extract of *Balanites aegyptiaca*

AB : aqueous extracts of *Balanites aegyptiaca*

PBS: phosphate buffer saline
Table 3-1: Effect of different doses of aqueous extract of *Balanites aegyptiaca* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemoncus contortus*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Dose 100mg/kg</th>
<th>FECR%</th>
<th>Dose 400mg/kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>6600 ±636</td>
<td>4300±561</td>
<td>-</td>
<td>4900±172</td>
<td>-</td>
</tr>
<tr>
<td>Day 7</td>
<td>6600 ±495</td>
<td>3800±521</td>
<td>11.6%</td>
<td>2800±294</td>
<td>42.8%</td>
</tr>
<tr>
<td>Day 14</td>
<td>6900 ±353</td>
<td>4000±850</td>
<td>6.9%</td>
<td>2200±258</td>
<td>55.1%</td>
</tr>
<tr>
<td>Day 21</td>
<td>6100±141</td>
<td>3900±54</td>
<td>9.3%</td>
<td>1900±148</td>
<td>61.2%</td>
</tr>
<tr>
<td>TWC</td>
<td>409±58.0</td>
<td>-</td>
<td>137±6.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FECR%: Faecal egg count percent reduction
TWC: Total worm count
Values in table are means ± s.d
Significantly different from day zero values (p ≤ 0.05)
Significantly different from control values (p ≤ 0.05)
Table 3-2: Effect of different doses of methanolic extract of *Balanites aegyptiaca* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemonchus contortus*.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>Dose 100mg/Kg</th>
<th>FECR%</th>
<th>Dose 400mg/Kg</th>
<th>FECR%</th>
<th>Dose 800mg/kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 0</td>
<td>8100±1484</td>
<td>10500±1202</td>
<td>-</td>
<td>8700±1475</td>
<td>-</td>
<td>8200±988</td>
<td>-</td>
</tr>
<tr>
<td>day 7</td>
<td>8500±424</td>
<td>9200±1154</td>
<td>12.3%</td>
<td>5800±1301</td>
<td>33.3%</td>
<td>5700±637</td>
<td>30.4%</td>
</tr>
<tr>
<td>day 14</td>
<td>8500±636</td>
<td>8500±968</td>
<td>19.1%</td>
<td>4500±955</td>
<td>48.2%</td>
<td>2200±952</td>
<td>60%</td>
</tr>
<tr>
<td>day 21</td>
<td>8600±212</td>
<td>8400±680</td>
<td>20%</td>
<td>2200±1583</td>
<td>74.7%</td>
<td>1700±204</td>
<td>79.2%</td>
</tr>
<tr>
<td>TWC</td>
<td>430±40.9</td>
<td>-</td>
<td>102±13.7</td>
<td>33±13.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FECR%: Fecal egg count percent reduction
TWC: Total worm count
Values in table are means ± s.d
   Significantly different from day zero values ( p≤0.05)
   Significantly different from control values ( p≤0.05)
There was a significant difference ($p \leq 0.05$) in EPG reduction percentage at day 14 between two doses (400 & 800 mg/kg) of the ME of *B. aegyptiaca* (48.2% for 400 mg/kg and 60% for 800 mg/kg).

The control group and animals which treated with 400 & 800 mg/kg were slaughtered at day 21 post treatments. Then the numbers of adult *H. contortus* worms found in abomasum of the animals were counted. The result revealed that, the worms were significant reduced ($p \leq 0.05$) in treated animals compared to the control animals (Table 3-2).

The ME of *Balanites aegyptiaca* revealed significant greater anthelmintic effects than AE of *Balanites aegyptiaca*. Maximum activity exhibited by ME at the dose (400 mg/kg) was 74.7% reduction in EPG on day 21 post treatment, while CAE using the same dose, showed maximum inhibitory effect in EPG 61.4%.

### 3.1.3: Haematological findings:

The blood samples were taken at day zero pretreatment and at days 7, 14, 21 post treatment with methanolic and aqueous extracts of *B. aegyptiaca* for haematological examinations. Packed cell volume (PCV) (Fig.2) and hemoglobin concentration (Hb) (Fig.3) were indicative of the degree of anaemia in control animals and infected treated animals, they significantly unchanged during post treatments period of the experiments.

### 3.1.4: Biochemical finding:

The result of biochemical assays revealed that, there were no significant differences in plasma Na$^+$, K$^+$, GPT, GOT, Urea, total protein, and albumin in control infected animals during the period of the experiment (Table 3-3). The animals treated with CAE of *Balanites aegyptiaca* at the dose (400 mg/kg) showed no significant difference in plasma N+, K+, GPT, GOT, urea, total protein, and albumin during the post treatments period of the experiment (Table 3-4). While those treated with the CME of *Balanites aegyptiaca* at the dose (400 mg/kg) and the
dose of (800mg/kg) showed no significant difference in plasma Na⁺, K⁺, GPT, GOT, urea, and albumin (Tables 3-5&3-6), but they showed significant increase in total protein at days 14&21 during the post treatments period of the experiment (Fig.4).
Cp: control positive
Ab: aqueous Balanites extract (400mg/kg)
Mb1: methanolic Balanites extract (400mg/kg)
Mb2: methanolic Balanites extract (80mg/kg)

Fig. 2: Effect of AE & ME of *Balanites aegyptiaca* on PCV% in goats infected with *H.contortus*
Fig. 3: Effect of treatment with AE&ME of *Balanites aegyptiaca* on Hb concentration in goats infected with *H.contortus*
Table 3-3: Plasma constituents of goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>Total p g/l</th>
<th>Albumin g/l</th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>49.8±5.15</td>
<td>58.0 ±2.4</td>
<td>25.0± 0.5</td>
<td>164± 0.0</td>
<td>5.1±0.10</td>
<td>8.7 ±0.8</td>
<td>8.2±2.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>50.5± 9.25</td>
<td>52.0±0.2</td>
<td>24.0±0.2</td>
<td>158±4.5</td>
<td>5.2±0.16</td>
<td>7.5±0.5</td>
<td>6.0±0.8</td>
</tr>
<tr>
<td>Day 14</td>
<td>53.5± 6.60</td>
<td>54.2±0.4</td>
<td>24.3±0.3</td>
<td>164±0.0</td>
<td>5.1±0.00</td>
<td>7.7±0.5</td>
<td>7.5±1.2</td>
</tr>
<tr>
<td>Day 21</td>
<td>48.7± 5.18</td>
<td>48.7±2.0</td>
<td>23.5±0.1</td>
<td>162±5.9</td>
<td>4.8±0.10</td>
<td>8.5±1.0</td>
<td>8.5±1.0</td>
</tr>
</tbody>
</table>

Values are mean ± s.d

Table 3-4: Effect of treatment with 400 mg/kg of aqueous extract of *Balanites aegyptiaca* on plasma constituents of infected goats by *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>T-Protein g/l</th>
<th>Albumin g/l</th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4.4±0.6</td>
<td>65±3.1</td>
<td>25.6±1.5</td>
<td>172±18.2</td>
<td>5.1±0.7</td>
<td>11.3±1.5</td>
<td>7.3±0.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>4.2±0.8</td>
<td>65±7.5</td>
<td>26.3±1.5</td>
<td>177±8.0</td>
<td>5.2±0.3</td>
<td>8.6±0.5</td>
<td>6.6±0.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.6±0.2</td>
<td>68±6.3</td>
<td>30.3±3.7</td>
<td>165±2.0</td>
<td>4.9±0.5</td>
<td>8.8±0.5</td>
<td>7.3±0.5</td>
</tr>
<tr>
<td>Day 21</td>
<td>4.2±0.4</td>
<td>70±9.1</td>
<td>30.3±1.5</td>
<td>164±2.4</td>
<td>5.2±0.6</td>
<td>8.6±1.0</td>
<td>8.0±1.0</td>
</tr>
</tbody>
</table>

Values are mean ± s.d
### Table 3-5: Effect of treatment with 400 mg/kg of methanolic extract of *Balanites aegyptiaca* on plasma constituents of infected goats with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>T. Protein g/l</th>
<th>Albumin g/l</th>
<th>+ Na mmol/l</th>
<th>K mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.0±0.3</td>
<td>61±1.3</td>
<td>24.6±4.5</td>
<td>163±3.4</td>
<td>5.6±0.8</td>
<td>9.5±1.2</td>
<td>7.7±0.9</td>
</tr>
<tr>
<td>Day 7</td>
<td>4.6±0.6</td>
<td>64±4.9</td>
<td>25.3±1.5</td>
<td>163±4.5</td>
<td>4.9±0.7</td>
<td>8.7±1.2</td>
<td>7.7±0.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.8±0.4</td>
<td>69±2.6</td>
<td>26.4±3.7</td>
<td>163±4.6</td>
<td>4.5±0.5</td>
<td>10.5±1.9</td>
<td>9.7±1.7</td>
</tr>
<tr>
<td>Day 21</td>
<td>5.7±1.0</td>
<td>72±4.4</td>
<td>28.3±1.5</td>
<td>172±7.3</td>
<td>4.9±0.3</td>
<td>8.5±0.5</td>
<td>9.0±1.8</td>
</tr>
</tbody>
</table>

Values in table are means ± s.d
Significantly different from day zero values (p<0.05)

### Table 3-6: Effect of treatment with 800 mg/kg of methanolic extract of *Balanites aegyptiaca* on plasma constituents of infected goats with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>T. Protein g/l</th>
<th>Albumin g/l</th>
<th>Na+ mmol/l</th>
<th>K+ mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.2±2.3</td>
<td>60±1.3</td>
<td>25.5±3.3</td>
<td>163±5.4</td>
<td>5.2±1.4</td>
<td>12±2.0</td>
<td>7.0±2.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.2±2.4</td>
<td>66±3.7</td>
<td>25.8±2.3</td>
<td>168±5.5</td>
<td>4.9±2.3</td>
<td>8.0±2.2</td>
<td>6.0±3.1</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.0±8.1</td>
<td>69±2.2</td>
<td>27.0±1.5</td>
<td>161±3.2</td>
<td>5.2±2.2</td>
<td>7.0±3.5</td>
<td>8.0±1.0</td>
</tr>
<tr>
<td>Day 21</td>
<td>4.8±5.7</td>
<td>73±3.3</td>
<td>29.1±4.0</td>
<td>164±2.0</td>
<td>5.1±3.1</td>
<td>9.0±1.5</td>
<td>9.0±2.0</td>
</tr>
</tbody>
</table>

Values in table are means ± s.d
Significantly different from day zero values (p<0.05)
Fig. 4: Effect of different doses of methanolic extract of *Balanites aegyptiaca* on plasma total protein concentration in goats infected with *H. contortus*.
3.1.5: Pharmacological studies:

3.1.5.1: Effect of *Balanites aegyptiaca* extracts on isolated rabbit jejunum:
The effects of methanolic and aqueous extracts of *Balanites aegyptiaca* were tested using rabbit jejunum tissue. The methanolic and aqueous extracts produced a dose dependant contraction on isolated rabbit jejunum at different doses (2.5, 5, 7.5mg/ml) (Fig.5&6). Atropine as antagonist was used to determine the mechanism of this contraction response. The result revealed that, the contraction activity was blocked by atropine (5µg/ml) (Fig. 7&8).

3.1.5.2: LD50 of *Balanites aegyptiaca* kernel methanloic extract:
The mean lethal dose of the methanolic extract of *Balanites aegyptiaca* was tested in groups of Wister rats of different sexes.
Oral administration of *Balanites aegyptiaca* kernel methanolic extract to rats in single different doses started from dose of 400mg up to 25g/kg did not induce any observable toxicity, so the oral LD50 of *Balanites aegyptiaca* kernel methanolic extract in rats was suggested to be more than 25g/kg.
Fig. 5: Contraction effect of methanolic extract of *Balanites aegyptiaca* (B) at different doses of (2.5, 5, 7.5 mg/ml) on isolated rabbit jejunum (N=normal)

Trace Keys:
- Chart speed = 0.25 mm/sec (6 square=2 min)
- Gain sensitivity = 8
- Polarity = (+)
- Transducer (Isometric)
Fig. 6: Contraction effect of aqueous extract of *Balanites aegyptiaca* (B) at different doses (1.2, 2.5, 5, 7.5 mg/ml) on isolated rabbit jejunum. (N=normal).
**Fig. 7:** Contraction effect of methanolic extract of *Balanites aegyptiaca* (B) at different doses (2.5, 5 mg/ml) on isolated rabbit jejunum; that blocked by atropine

(N=normal, A= atropine)
**Fig. 8:** Contraction effect of aqueous extract of *Balanites aegyptiaca* (B) at a dose of 5 mg/ml on isolated rabbit jejunum, the contraction blocked by atropine (5µg/ml)
(N=normal, A=atropine)
3. 2: Peganum harmala:
The anthelmintic effects of aqueous (AE) and methanolic extracts (ME) of Peganum harmala seed were tested in vitro and in vivo.

3.2.1: In vitro anthelmintic activity:
In vitro experiments were conducted to determine anthelmintic effects of aqueous (AE) and methanolic extracts (ME) of Peganum harmala seed on adult H. contortus. The in vitro trials demonstrated time dependent anthelmintic activity of crude aqueous and methanolic extracts of Peganum harmala against adult H. contortus as evident from mortality rate of the worms. Methanolic extract of Peganum harmala (25mg/ml) produced mortality rate of 14%, 50%, 75%, 75% and 100% at hour 1, 3, 6, 12, 24 post treatment respectively. While aqueous extract (25mg/ml) produced mortality rate of 20%, 55%, 70%, 95% and 100% at 1, 3, 6, 12, 24 hours post treatment respectively (Fig.9). The ME and AE produce mortality rate of 70% & 95% at 12h respectively. While at 24h all the worms were found dead. Whereas 10% & 30% of the worms were found dead at 12h & 24h respectively in PBS.

3.2.2: In vivo anthelmintic activity:
In vivo experiments were conducted to determine anthelmintic effects of methanolic and aqueous extracts of Peganum harmala on goats infected with Haemoncus contortus. The infected goats in different groups had reduced appetite, passed soft faeces, and appeared depressed from day 21 post infection. Treatments by single doses of different extracts revealed that, at day 7 post treatments the general condition of goats started to improve as indicated by return of normal appetite and normal defication.
3.2.2.1 Aqueous extracts of *Peganum harmala* anthelmintic effects:
The anthelmintic activity of two doses (100 & 200mg/kg) of aqueous extract of *P. harmala* in goats infected with *H. contortus* were shown in Table (3-7). The dose of 100mg/kg showed a significant reduction (P≤0.05) in EPG of 75.5% on day 7 and 14 days post treatment and 85.4% on day 21 post treatment. The dose of 200mg/kg revealed a time dependent anthelmintic effect and showed a significant reduction (P≤0.05) in EPG of 67.9%, 75.5% & 86.6% at day 7, 14 & 21 post treatments respectively.
The two doses of AE gave almost similar reduction in percentage of EPG on different days of experiments. The two doses of extract were reduced the egg production but were not completely suppressed it.
The control group and animals treated with 200 mg/kg of AE *P. harmala* were slaughtered at day 21 post treatments. Then the numbers of adult *H. contortus* worms found in abomasa of the animals were counted. The result revealed that, the worms were significant reduced (p≤0.05) in treated animals compared to the control group (Table 3-7).

3.2.2.2 Methanolic extracts of *Peganum harmala* anthelmintic effects:
The anthelmintic activity of two doses (100 & 200mg/kg) of ME of *P. harmala* in goats infected with *H. contortus* shown in Table (3-8). The dose of 100mg/kg revealed a maximum significant reduction (P≤0.05) in EPG of 23.9% on day 21 post treatment, while the dose of 200mg/kg showed a time dependent anthelmintic effect and reduced the EPG to 56.2%, 64.1% & 70.3% at 7, 14 & 21 days post treatment respectively.
The control group and animals treated with 200 mg/kg ME of *P. harmala* were slaughtered at day 21 post treatments. Then the numbers of adult *H. contortus* worms found in abomasa of the animals were counted. The result revealed that,
the worms were significantly reduced (p≤0.05) in treated animals compared to the control group (Table 3-8).
AE and ME revealed anthelmintic effect. Maximum activity exhibited by AE of *P.harmala* (200mg/kg) was 67.9% reduction in EPG on day 7 post treatment. While ME at the same dose showed inhibitory effect of 56.2% reduction in EPG on day 7 post treatment. Also after three week of treatment AE (200mg/kg) revealed significantly greater reduction in EPG (86.6%) compared with ME at the same dose (70.3%).
Fig. 9: Comparison means percentage of survival adult *H. contortus* for 24 hours exposure to methanolic and aqueous extracts of *Peganum harmala* comparison with control (*phosphate buffer saline*).
**Table 3-7:** Effect of different doses of aqueous extract of *Peganum harmala* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Dose 100mg/kg</th>
<th>FECR%</th>
<th>Dose 200mg/kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>7500±778</td>
<td>4100±650</td>
<td>-</td>
<td>5300±817</td>
<td>-</td>
</tr>
<tr>
<td>Day 7</td>
<td>6100 ±1202</td>
<td>1000±151</td>
<td>75.5%</td>
<td>1700±212</td>
<td>67.9%</td>
</tr>
<tr>
<td>Day 14</td>
<td>9200 ±1909</td>
<td>1000±153</td>
<td>75.5%</td>
<td>1300±14</td>
<td>75.5%</td>
</tr>
<tr>
<td>Day 21</td>
<td>8500±1202</td>
<td>600±258</td>
<td>85.4%</td>
<td>700±158</td>
<td>86.6%</td>
</tr>
<tr>
<td>TWC</td>
<td>490±87</td>
<td>-</td>
<td></td>
<td>60±22.5</td>
<td></td>
</tr>
</tbody>
</table>

FECR%: Faecal egg count percent reduction  
TWC: Total worm counts  
Values in table are means ± s.d  
Significantly different from day zero values (p≤0.05)  
Significantly different from control values (p≤0.05)
Table 3-8: Effect of different doses of methanolic extract of *Peganum harmala* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dose100mg/K</th>
<th>FECR%</th>
<th>Dose200mg/K</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td>4700±848</td>
<td>4600±941</td>
<td>-</td>
<td>6400±2281</td>
<td>-</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td>5100±141</td>
<td>4100±1128</td>
<td>10.3%</td>
<td>2800±1184</td>
<td>56.2%</td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td>5100±777</td>
<td>3900±730</td>
<td>15.2%</td>
<td>2300±1192</td>
<td>64.1%</td>
</tr>
<tr>
<td><strong>Day 21</strong></td>
<td>4300±1060</td>
<td>3500±756</td>
<td>23.9%</td>
<td>1900±777</td>
<td>% 70.3</td>
</tr>
<tr>
<td><strong>TWC</strong></td>
<td>312±50.3</td>
<td>-</td>
<td></td>
<td>119±21</td>
<td></td>
</tr>
</tbody>
</table>

FECR%: Faecal egg count percent reduction  
TWC: Total worm count  
Values in table are means ± s.d  
Significantly different from day zero values (p<0.05)  
Significantly different from control values (p<0.05)
3.2.3: Haematological findings:

The blood samples were taken at day zero pretreatment and at days 7, 14, 21 post treatment with methanolic and aqueous extracts of *Peganum harmala* for hematological examinations. Packed cell volume (PCV) and hemoglobin concentration (Hb) were indicative of the degree of anemia in infected control animals and infected treated animals. They did not obtain values statistically different from the controls during the post treatment period of experiment (Figs. 10&11).

3.2.4: Biochemical findings:

The result of biochemical assays showed that, there were no significant differences in plasma Na, K, GPT, GOT, urea, total protein, and albumin in control-infected animals during the period of the experiment table (3-9). The animals treated with AE of *Peganum harmala* at the dose (200mg/kg) showed significant reduction in concentration of urea at day 14 post treatment. While the result of Na, K, GPT, GOT, and total protein concentration revealed no significant difference post treatment (Table 3-10).

ME of *Peganum harmala* at dose 200mg/kg revealed significant decrease in concentration of urea at day 14 post treatment. While the result of Na, K, GPT, GOT, albumin and total protein concentration revealed no significant difference post treatment (Table 3-11).

The aqueous extract and methanolic extract of *Peganum harmala* revealed significant decrease in concentration of urea at day 14-post treatment Fig. (12).
Fig. 10: Effect of treatment with AE & ME of *Peganum harmala* on PCV % in goats

Cp = control positive
Ap = aqueous extract of *Peganum harmala*
Mp = methanolic extract of *Peganum harmala*
Cp = control positive  
Ap = aqueous extract of *Peganum harmala*  
Mp = methanolic extract of *Peganum harmala*

**Fig. 11:** Effect of treatment with AE & ME of *Peganum harmala* on Hb concentration of *H. contortus* infected goats
Table 3-9: Plasma constituents in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>Total p g/l</th>
<th>Albumin g/l</th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>6.0 ± 1.5</td>
<td>61 ± 3.8</td>
<td>25.5 ± 3.5</td>
<td>184 ± 9.1</td>
<td>5.6 ± 2.0</td>
<td>11 ± 0.5</td>
<td>7.6 ± 2.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.1 ± 0.2</td>
<td>61 ± 2.3</td>
<td>23.5 ± 1.9</td>
<td>161 ± 14.2</td>
<td>5.0 ± 0.5</td>
<td>11 ± 2.3</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>5.0 ± 0.8</td>
<td>58 ± 4.6</td>
<td>23.3 ± 1.5</td>
<td>175 ± 1.4</td>
<td>4.9 ± 0.7</td>
<td>12 ± 2.5</td>
<td>8.5 ± 0.7</td>
</tr>
<tr>
<td>Day 21</td>
<td>6.4 ± 1.4</td>
<td>51 ± 5.6</td>
<td>22.5 ± 1.7</td>
<td>169 ± 9.8</td>
<td>5.2 ± 0.5</td>
<td>10 ± 1.7</td>
<td>7.5 ± 0.7</td>
</tr>
</tbody>
</table>

Values are mean ± s.d
Table 3-10: Effect of treatment with 200mg/kg aqueous extract of *Peganum harmala* on plasma constituents in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>T.Protein g/l</th>
<th>Albumin g/l</th>
<th>Na+ mmol/l</th>
<th>K+ mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.2±0.9</td>
<td>61±9.8</td>
<td>25.3±4.0</td>
<td>170±10.5</td>
<td>5.3±0.3</td>
<td>9.0±2.4</td>
<td>7.6±0.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>4.1±0.4</td>
<td>61±5.6</td>
<td>25.5±2.1</td>
<td>157±5.6</td>
<td>5.1±0.1</td>
<td>9.0±2.3</td>
<td>7.6±1.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>2.0±1.0</td>
<td>68±2.8</td>
<td>26.3±1.5</td>
<td>159±4.9</td>
<td>5.0±0.2</td>
<td>10.3±1.0</td>
<td>9.6±0.5</td>
</tr>
<tr>
<td>Day 21</td>
<td>3.4±1.4</td>
<td>68±7.0</td>
<td>26.5±3.5</td>
<td>151±1.5</td>
<td>5.1±0.1</td>
<td>9.5±2.7</td>
<td>7.3±2.0</td>
</tr>
</tbody>
</table>

Values in table are means ± s.d

Significantly different from day zero values (p≤0.05)

Table 3-11: Effect of treatment with 200mg/kg methanolic extract of *Peganum harmala* on plasma constituents in goats infected with *Haemoncus contortus*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>T.Protein g/l</th>
<th>Albumin g/l</th>
<th>Na+ mmol/l</th>
<th>K+ mmol/l</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.1±0.2</td>
<td>47.6±7.0</td>
<td>26.0±3.5</td>
<td>162±9.5</td>
<td>5.1±0.7</td>
<td>7.3</td>
<td>6.6</td>
</tr>
<tr>
<td>day 7</td>
<td>5.6±0.4</td>
<td>47.0±7.0</td>
<td>27.6±3.5</td>
<td>168±19.0</td>
<td>5.2±0.5</td>
<td>6.6</td>
<td>7.6</td>
</tr>
<tr>
<td>day 14</td>
<td>1.7±0.4</td>
<td>49.0±1.7</td>
<td>28.0±1.0</td>
<td>170±17.7</td>
<td>5.4±0.4</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>day 21</td>
<td>2.4±0.6</td>
<td>52.0±3.6</td>
<td>29.6±2.5</td>
<td>168±19.2</td>
<td>5.1±0.5</td>
<td>7.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Values in table are means ± s.d
Significantly different from day zero values (p ≤ 0.05)

Concentration of urea (mmol/l)

Days post treatments

Cp = control positive
Ap = aqueous extract of *Peganum harmala*
Mp = methanolic extract of *Peganum harmala*

**Fig. 12:** Effect of aqueous and methanolic extracts of *Peganum harmala* on plasma urea concentration in goats infected with *H. contortus*
3.2.5: Pharmacological studies:
3.2.5.1: Effect of extracts on isolated rabbit jejunum:
The effects of methanolic and aqueous extracts of *Peganum harmala* were tested using rabbit jejunum tissue. The methanolic and aqueous extracts produced a dose dependent relaxation on isolated rabbit jejunum at different doses (2.5, 5, 7.5mg/ml) (Fig. 13&14). Two antagonists were used to determine the mechanism of this relaxation response (propranolol and tolazoline). The result revealed that, the relaxation activity was not blocked by different doses (5mg/ml & 1mg/ml) of propranolol (known non-selective β blocker) (Fig. 15), but blocked by tolazoline (known non-selective α blocker) (Fig. 16).

3.2.5.2: LD50 of methanolic extract of *Peganum harmala*:
The mean lethal dose of the methanolic extract of *Peganum harmala* was tested in groups of Wister rats of different sexes.
Tremor appeared in most of rats treated by different doses. Convulsions in some treated rats specially those die after treatment.
The following table showed the mortality rate induced by different doses of the methanolic extract in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Dose(mg/rat)</th>
<th>No. of dead animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4000</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6000</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>8000</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>10000</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>12000</td>
<td>4</td>
</tr>
</tbody>
</table>
The LD50 was calculated from the table as described by Stewart and Stolman (1961) in the following way:

\[ \text{LD50} = \text{the largest dose} - \frac{\sum a \cdot b}{N} \]

- \( a \) = the constant factor between two consecutive doses
- \( b \) = the mean of the dead animals in two successive groups
- \( n \) = number of animals in the group
- \( \sum \) = the sum of \( a \) and \( b \)

From the table, the oral LD50 of *Peganum harmala* was calculated to be 7g/kg.
Fig. 13: Relaxation effect of methanolic extract of *Peganum harmala* seed (H) at different doses (2.5, 5, 7.5 mg/ml) on isolated rabbit jejunum.

(N=normal, W=wash)
Fig. 14: Relaxation effect of aqueous extract of *Peganum harmala* seed at different doses (2.5, 5, 7.5 mg/ml) on isolated rabbit jejunum (N=normal).
**Fig. 15**: Relaxation effect of methanolic extract of *Peganum harmala* seed (H) at different doses (5, 7.5 mg/ml) on isolated rabbit jejunum; the relaxation was not blocked by a dose of 0.5mg/ml of propranolol (N = normal, P= propranolol)
Fig. 16: Relaxation effect of methanolic extract of *Peganum harmala* seed (H) at different doses (5, 7.5 mg/ml) on isolated rabbit jejunum, that blocked by doses of 0.5, 1 mg/ml of tolazoline.

(N = normal, T = tolazoline)
3.3: *Zizyphus spina-christi*

The anthelmintic effects of aqueous extracts (AE) and methanolic extracts (ME) of *Zizyphus spina-chisti* leaf were tested, *in vitro* and *in vivo*.

3.3.1 *In vitro* anthelmintic activity:

The *in vitro* trial for anthelmintic activity of the aqueous extract (AE) and the methanolic extract (ME) of the leaf of *Zizyphus spina-christi* were conducted on mature live *Haemoncous contortus*.

The AE and ME exhibited significant activities (p≤0.05) against *H. contortus* as evident from the mortality rate of worms. After 24 hours exposure of adult worms to aqueous and methanolic extracts significant time dependent reduction in motility/mortality was observed. Aqueous extract of *Zizyphus spina-christi* (25mg/ml) produced mortality rate of 10%, 14%, 14%, 20% and 74% at 1, 3, 6, 12, 24 hours post treatment respectively. While methanolic extract (25mg/ml) produced mortality rate of 10%, 54%, 84%, 100% and 100% at hour 1, 3, 6, 12, 24 hours post treatment. Whereas, 10% & 30% of the worms were found dead at 12 & 24 respectively in PBS (Fig. 17).

3.3.2 *In vivo* anthelmintic activity:

*In vivo* experiments were conducted to determine anthelmintic effects of methanolic and aqueous extracts of *Zizyphus spina-chisti* on goats infected with *Haemoncous contortus*.

3.3.2.1 Aqueous extracts of *Zizyphus spina-christi* anthelmintic effects:

The anthelmintic activity of two doses (100&400mg/kg) of aqueous extract of *Zizyphus spina-chisti* in goats infected with *H. contortus* was shown in Table (3-12).

The dose of 100mg/kg showed a time dependent anthelmintic effect with significant reduction (p≤0.05) in EPG to 39.2%, 50.3% & 61.5% at 7, 14 and 21 days post treatment respectively. Likewise, the dose of 400mg/kg revealed a time
dependent anthelmintic effect with significant reduction (p≤0.05) in EPG to 46, 2%, 71.2%, and 78.7% at 7, 14 and 21 days post treatment respectively. The control group and animals treated with 400mg/kg were slaughtered at day 21 post treatments. Then the numbers of adult *H. contortus* worms found in abomasa of the animals were counted. The result revealed that, the worms were significantly reduced (p≤0.05) in treated animals compared to the control group (Table 3-12).

### 3.3.2.2 Methanolic extracts of *Zizyphus spina-christi* anthelmintic effects:

The anthelmintic activity of three doses (100, 400 & 800mg/kg) of ME of *Zizyphus spina-christi* in goats infected with *H. contortus* shown in Table (3-13).

The dose of 100mg/kg showed a maximum reduction of 24.4% in EPG on day 21. While, the dose of 400mg/kg revealed a time dependent anthelmintic effect and were significantly reduced (p≤0.05) the EPG to 48.7%, 63.4% and 73.1% at 7, 14 and 21 days post treatment respectively. Also the dose of 800mg/kg showed a time dependent effect and significantly reduced (p≤0.05) in the EPG 37%, 62.9%, and 85% for 7, 14, and 21 day post treatment respectively.

The two different doses (400 & 800 mg/kg) of CME gave almost same reduction percentage (63.4% & 62.9%) on day 14, but there was significant difference (p≤0.05) in EPG reduction percentage on day 21 (73.1% for 400mg/kg and 85.1% for 800mg/kg).

The control group and animals treated with 400&800mg/kg were slaughtered at day 21 post treatments. Then the numbers of adult *H. contortus* worms found in abomasa of the animals were counted. The result revealed that, the worms were significantly reduced (p≤0.05) in treated animals compared to the control group (Table 3-13).
The CAE and the CME revealed anthelmintic effect and the maximum activity exhibited by CAE (400mg/kg) was 78.7% reduction in EPG on day 21 post treatment, however, CME (400mg/kg) showed a maximum inhibitory effect of 73.1 % reduction in EPG on day 21 post treatment.
Fig 17: Comparison of mean percentage of survival adult *H. contortus* for 24 hours exposure to methanolic and aqueous extracts of *Zizyphus spina-christi* with control (phosphate buffer saline)

MZ: Methanolic extract of *Zizyphus spina-christi*
AZ: Aqueous extract of *Zizyphus spina-christi*
PBS: phosphate buffer saline
Table 3-12: Effect of different doses of aqueous extract of *Zizyphus spina-christi* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Dose 100mg/kg</th>
<th>FECR%</th>
<th>Dose 400mg/kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>12550±3464</td>
<td>13500±1060</td>
<td>-</td>
<td>13220±1136</td>
<td>-</td>
</tr>
<tr>
<td>Day 7</td>
<td>13250±2474</td>
<td>8200±710</td>
<td>39.2%</td>
<td>7100±620</td>
<td>46.2%</td>
</tr>
<tr>
<td>Day 14</td>
<td>11200±2687</td>
<td>6720±363</td>
<td>50.3%</td>
<td>3800±940</td>
<td>71.2%</td>
</tr>
<tr>
<td>Day 21</td>
<td>10250±1767</td>
<td>5240±680</td>
<td>61.5%</td>
<td>2880±657</td>
<td>78.7%</td>
</tr>
<tr>
<td>TWC</td>
<td>356±49.2</td>
<td>-</td>
<td></td>
<td>142±18.3</td>
<td></td>
</tr>
</tbody>
</table>

FECR%: Faecal egg count percent reduction  
TWC: Total worm count  
Values in table are means ± s.d  
Significantly different from day zero values (p≤0.05)  
Significantly different from control values (p≤0.05)
Table 3-13: Effect of different doses of methanolic extract of *Zizyphus spina-christi* on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemoncus contortus*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Dose100 mg/Kg</th>
<th>FECR%</th>
<th>Dose400 mg/Kg</th>
<th>FECR%</th>
<th>Dose800 mg/kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± S.D</td>
<td>Mean± S.D</td>
<td>FECR%</td>
<td>Mean± S.D</td>
<td>FECR%</td>
<td>Mean± S.D</td>
<td>FECR%</td>
</tr>
<tr>
<td>Day 0</td>
<td>2900±565</td>
<td>4520±443</td>
<td>-</td>
<td>3800±986</td>
<td>-</td>
<td>2700±364</td>
<td>-</td>
</tr>
<tr>
<td>Day 7</td>
<td>3100±707</td>
<td>4140±304</td>
<td>8.8%</td>
<td>1500±258</td>
<td>48.7%</td>
<td>1700±461</td>
<td>37%</td>
</tr>
<tr>
<td>Day 14</td>
<td>3000±636</td>
<td>3940±808</td>
<td>13.3%</td>
<td>1500±554</td>
<td>63.4%</td>
<td>1000±258</td>
<td>62.9%</td>
</tr>
<tr>
<td>Day 21</td>
<td>3200±282</td>
<td>3440±638</td>
<td>24.4%</td>
<td>1100±383</td>
<td>73.1%</td>
<td>400±151</td>
<td>85.1%</td>
</tr>
<tr>
<td>TWC</td>
<td>182±9.2</td>
<td>73±21</td>
<td>33±13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FECR%: Faecal egg count percent reduction  
TWC: Total worm count  
Values in table are means ± s.d  
Significantly different from day zero values (p≤0.05)  
Significantly different from control values (p≤0.05)
3.3.3: Heamatological findings:
The blood samples were taken at day zero pretreatment and at days 7, 14, 21 post treatment with methanolic and aqueous extracts of *Peganum harmala* for hematological examinations. Packed cell volume (PCV) and haemoglobin concentration (Hb), which were indicative of the degree of anaemia in infected control groups, showed no significant difference during the post treatment period of the experiment. However, animals treated with AE&ME of *Zizyphus spin-a-christi* (400mg/kg) revealed significant increase in PCV& Hb concentration at the day 21 post treatment (Figs.18&19).

3.3.4: Biochemical findings:
The results of biochemical assays revealed no significant difference in plasma Na+, K+, GPT, GOT, Urea, total protein, and albumin in control infected animals during the period of the experiment (Table3-14).
The animals treated with AE of *Zizyphus spina-christi* showed significant increased in sodium concentration at day 21 post treatment. While the result of, K, GPT, GOT, urea, total protein, and albumin concentration revealed no significant difference post treatment (Table 3-15).
The ME of *Zizyphus spina-christi* also showed significant increase in sodium concentration at days 7, 14, 21 post treatments. The result of, K, GPT, GOT, urea, total protein, and albumin concentration revealed no significant difference post treatment (Table 3-16 &3-17).
The aqueous extract and methanolic extract of *Zizyphus spina-christi* revealed significant increase in sodium concentration (Fig 20 ).
Cp  = control positive
Az   = aqueous extract of *Zizyphus spina-christi* (400mg/kg)
Mz1  = methanolic extract of *Zizyphus spina-christi* (400mg/kg)
Mz2  = methanolic extract of *Zizyphus spina-christi* (800mg/kg)

**Fig. 18:** Effect of AE&ME of *Zizyphus spina-christi* on PCV% in goats infected with *H. contortus*
Fig 19: Effect of AE&ME of *Zizyphus spina-christi* on Hb concentration in goats infected with *H. contortus*
### Table 3-14: Plasma constituents in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>Total p g/l</th>
<th>Albumin g/l</th>
<th>Na+ mmol/l</th>
<th>K+ mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4.6± 1.5</td>
<td>63 ± 1.6</td>
<td>25.0 ± 3.5</td>
<td>164 ±5.1</td>
<td>5.1±  0.2</td>
<td>9 ±0.5</td>
<td>8.6±2.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.1± 0.4</td>
<td>62± 2.6</td>
<td>25.1 ± 0.9</td>
<td>156±6.2</td>
<td>4.7± 0.5</td>
<td>9± 2.3</td>
<td>8.5±0.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.3 ±0.9</td>
<td>61 ± 3.3</td>
<td>23.3 ± 1.5</td>
<td>162±3.4</td>
<td>5.4±0.5</td>
<td>12± 2.5</td>
<td>7.5±0.7</td>
</tr>
<tr>
<td>Day 21</td>
<td>5.2 ±0.8</td>
<td>60± 3.8</td>
<td>22.5 ±1.7</td>
<td>169±2.8</td>
<td>5.1 ± 0.1</td>
<td>9 ± 2.7</td>
<td>7.9±0.7</td>
</tr>
</tbody>
</table>

Values are mean ± s.d

### Table 3-15: Effect of treatment with 400 mg/kg of aqueous extract of *Zizyphus spina-christi* on plasma constituents in goats infected with *Haemoncus contortus.*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>T. Protein g/l</th>
<th>Albumin g/l</th>
<th>Na+ mmol/l</th>
<th>K+ mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4.4±0.2</td>
<td>59.0±6.0</td>
<td>24.3±2.3</td>
<td>158±5.7</td>
<td>4.9±0.2</td>
<td>10±1.5</td>
<td>10±2.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.2±0.3</td>
<td>55.6±8.0</td>
<td>24.6±8.1</td>
<td>158±1.5</td>
<td>4.8±0.1</td>
<td>9±0.0</td>
<td>6±1.0</td>
</tr>
<tr>
<td>Day 14</td>
<td>3.6±0.9</td>
<td>63.0±12.4</td>
<td>25.0±1.7</td>
<td>169±0.5</td>
<td>4.6±0.4</td>
<td>12±1.0</td>
<td>12±0.5</td>
</tr>
<tr>
<td>Day 21</td>
<td>3.8±0.7</td>
<td>64.6±5.5</td>
<td>28.3±4.5</td>
<td>179±1.1</td>
<td>4.8±0.2</td>
<td>10±1.0</td>
<td>83±1.1</td>
</tr>
</tbody>
</table>

Values in table are means ± s.d

Significantly different from day zero values (p≤0.05)
Table 3-16: Effect of treatment with 400 mg/kg of methanolic extract of *Zizyphus spina-christi* on plasma constituents in goats infected with *Haemoncus contortus*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>Total p g/l</th>
<th>Albumin g/l</th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.0 ± 0.4</td>
<td>51.0 ± 4.7</td>
<td>25.0 ± 2.3</td>
<td>160 ± 4.1</td>
<td>5.2 ± 0.2</td>
<td>9.5 ± 1.7</td>
<td>7.7 ± 0.9</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.2 ± 0.3</td>
<td>51.7 ± 5.7</td>
<td>26.5 ± 1.1</td>
<td>170 ± 5.4</td>
<td>5.1 ± 0.5</td>
<td>8.0 ± 0.8</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.6 ± 0.5</td>
<td>59.5 ± 5.5</td>
<td>27.7 ± 1.8</td>
<td>168 ± 4.5</td>
<td>5.1 ± 0.3</td>
<td>8.5 ± 1.2</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td>Day21</td>
<td>4.6 ± 0.3</td>
<td>66.2 ± 3.4</td>
<td>32.7 ± 2.9</td>
<td>169 ± 1.9</td>
<td>5.2 ± 0.2</td>
<td>7.7 ± 0.9</td>
<td>9.5 ± 2.3</td>
</tr>
</tbody>
</table>

Values in table are means ± s.d
Significantly different from day zero values (p ≤ 0.05)

Table 3-17: Effect of treatment with 800 mg/kg of methanolic extract of *Zizyphus spina-christi* on plasma constituents in goats infected with *Haemoncus contortus*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea mmol/l</th>
<th>Total p g/l</th>
<th>Albumin g/l</th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4.6 ± 2.4</td>
<td>65.0 ± 4.0</td>
<td>25.0 ± 2.1</td>
<td>160 ± 5.6</td>
<td>5.1 ± 3.1</td>
<td>9.0 ± 1.1</td>
<td>8.0 ± 3.1</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.0 ± 4.4</td>
<td>65.7 ± 5.0</td>
<td>26.5 ± 3.0</td>
<td>177 ± 2.1</td>
<td>4.7 ± 2.4</td>
<td>9.0 ± 2.2</td>
<td>8.5 ± 2.2</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.8 ± 3.5</td>
<td>67.5 ± 2.8</td>
<td>26.7 ± 5.1</td>
<td>168 ± 3.1</td>
<td>5.4 ± 4.3</td>
<td>10.0 ± 1.2</td>
<td>10.5 ± 3.4</td>
</tr>
</tbody>
</table>
Day21 | 5.2 ±5.3 | 67.2±3.1 | 28.7±3.3 | 169±1.2 | 5.1 ±1.1 | 9.0 ±2.4 | 9.0 ±2.3

Values in table are means ± s.d

Significantly different from day zero values (p≤0.05)

**Fig 20**: Effect of aqueous and methanolic extracts of *Zizyphus spina-christi* on plasma sodium concentration in goats infected with *H.contortus*
3.3.5: Pharmacological studies:

3.3.5.1: Effect of *Zizyphus spina-christi* extracts on isolated rabbit jejunum:
The effects of methanolic and aqueous extracts of *Zizyphus spina-christi* were
determined using rabbit jejunum tissue. The methanolic and aqueous extracts of
*Zizyphus spina-christi* exhibited a dose dependent relaxation on isolated rabbit
jejunum at different doses (2.5, 5, 7.5mg/ml) (Fig. 21&22). This relaxation activity
was not blocked by a dose (5mg/ml &1mg/ml) of propanolol (known non-selective
β block) (Fig. 23), and not blocked by tolazoline (known non selective α
blocker) (Fig. 24).
The contraction caused by acetylcholine (0.5mg/ml) on the tissue was reversibly
inhibited by 5mg/ml of the methanolic extract of *Zizyphus spina-christi* (Fig.
25).

3.3.5.2: LD50 of methanolic extract of *Zizyphus spina-christi*:
The mean lethal dose of the methanolic extract of *Zizyphus spina-christi* was tested
in groups of Wister rats of different sexes.
Oral administration of *Zizyphus spina-christi* kernel methanolic extract to rats in
single different doses started from dose of 400mg up to 8g/kg did not induce any
observable toxicity, so the oral LD50 of *Zizyphus spina-christi* kernel methanolic
extract in rats was suggested to be more than 8g/kg.
Fig. 21: Relaxation effect of methanolic extract of *Zizyphus spina-christi* leaf (Z) at different doses (1.2, 2.5, 5, 7.5 mg/ml) on isolated rabbit jejunum. 
(N = normal)
Fig. 22: Relaxation effect of aqueous extract of *Zizyphus spina-christi* leaf at different doses (1.5, 2.5, 5, 7.5 mg/ml) on isolated rabbit jejunum. (N=normal)
Trace keys:
Chart speed = 0.25 mm/sec (6 square = 2 min)
Gain sensitivity = 8
Polarity = (+)
Transducer (Isometric)

N * 5 mg/ml * N * 7.5 mg/ml * N * 0, 5 mg/ml of p * 5 mg/ml
**Fig. 23**: Relaxation effect of methanolic extract of *Zizyphus spina-christi* leaf (Z) at different doses (5, 7.5 mg/ml) on isolated rabbit jejunum; that not blocked by a dose of 0.5 mg/ml of propranolol. (N = normal, P = propranolol)

**Fig. 24**: Relaxation Effect of methanolic extract of *Zizyphus spina-christi* leaf (Z) in different doses (1.2, 2.5, 5 mg/ml) on isolated rabbit jejunum, that not blocked by a dose of 0.5 mg/ml of tolazoline. (N = normal, T = tolazoline)

Trace keys: Chart speed = 0.25 mm/sec   (6 square = min) 
Gain sensitivity = 8   Polarity = (+) 
Transducer (Isometric)
Trace keys:
Chart speed = 0.25 mm/sec (6 square = 2 min)
Gain sensitivity = 8
Polarity = (+)
Transducer (Isometric)
Fig. 25: Effect of methan (5 mg/ml) on isolated rabbit jejunum; the extract diminished the effect of acolic extract of Zizyphus spina-christi leaf (Z) at dose etylcholine (5 µg/ml)
(N= normal, Ac= acetylcholine)

3.4: Ivermectin (ivomec 1%)
The anthelmintic effect of ivermectin was tested in vitro and in vivo to compare with investigated plants (Balanites aegyptiaca, Peganum harmala and Zizyphus spina-Christi).

3.4.1: In vitro anthelmintic activity:
In vitro experiment was conducted to determine anthelmintic effects of ivomec on adult H.contortus.
Ivermectin (25mg/ml) produced mortality rate of 100% at the first hour post treatment. While crude aqueous extract of the three plants produced mortality rate of 40% (Balanites aegyptiaca) 75% (Peganum harmala) and 84% (Zizyphus spina-Christi) at six hour post treatment. Whereas, the crude methanolic extract produced mortality rate of 1% (Balanites aegyptiaca) 70% (Peganum harmala) and 14% (Zizyphus spina-Christi) at six hour post treatment (Table 3-18).
Maximum mortality rate at six hour post treatments with ivermectin and the three plants was exhibited by ivermectin (100%) followed by methanolic extract of Zizyphus spina-Christi (84%), aqueous extract of Peganum harmala(75%)and aqueous extract of Balanites aegyptiaca(40%).
The aqueous and methanolic extracts of Peganum harmala gave almost same effect 75%and 70% mortality rate at six hours post treatment.
Table 3-18: *In vitro* effect of aqueous and methanolic extracts of *Balanites aegyptiaca*, *Peganum harmala* and *Zizyphus spina-christi* on *Haemoncus contortus* in comparison with ivermectin.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0h</th>
<th>1h</th>
<th>3h</th>
<th>6h</th>
<th>12h</th>
<th>24h</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivomic</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PBS</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9.0</td>
<td>9.0</td>
<td>7.0</td>
<td>7</td>
</tr>
<tr>
<td>AE of <em>Balanites</em></td>
<td>10</td>
<td>8</td>
<td>6.0</td>
<td>6.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>ME of <em>Balanites</em></td>
<td>10</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>7.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>AE of <em>Peganum</em></td>
<td>10</td>
<td>8.0</td>
<td>4.5</td>
<td>2.5</td>
<td>0.5</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>ME of <em>Peganum</em></td>
<td>10</td>
<td>9.6</td>
<td>5.0</td>
<td>3.0</td>
<td>2.5</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>AE of <em>Zizyphus</em></td>
<td>10</td>
<td>10</td>
<td>8.6</td>
<td>8.6</td>
<td>6.0</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>ME of <em>Zizyphus</em></td>
<td>10</td>
<td>9.0</td>
<td>4.6</td>
<td>1.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

AE: aqueous extract  
ME: methanolic extract  
PBS: phosphate buffer saline
3.4.2: *In vivo* anthelmintic activity:

*In vivo* experiments were conducted to determine anthelmintic effects of ivermectin on goats infected with *Haemoncus contortus*. The infected goats had reduced appetite, passed soft faeces, and appeared depressed from day 21 post infection. After treatments by single dose of ivermectin, the general condition of goats started to improve as indicated by return of normal appetite and normal defication.

The single dose of ivermectin (1ml/50kg) showed a significant reduction ($P \leq 0.05$) in EPG of 63%, 98.7%, and 98.7% at 7 and 14, 21 days post treatment respectively (Table 3-19).

The control group and treated animals were slaughtered at day 21 post treatments. Then the numbers of adult *H. contortus* worms found in abomasum of the animals were counted. The result revealed that, the worms were significantly reduced ($p \leq 0.05$) in treated animals compared to the control group (Table 3-19).
Table 3-19: Effect of ivermectin (1ml/50kg) on faecal egg counts and total worms recovered at necropsy in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>Dose 1ml/50kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td>7500±778</td>
<td>7800±1216</td>
<td>-</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td>6100±1202</td>
<td>300±63</td>
<td>96.1%</td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td>9200±1909</td>
<td>100±40</td>
<td>98.7%</td>
</tr>
<tr>
<td><strong>Day 21</strong></td>
<td>8500±1202</td>
<td>100±23</td>
<td>98.7%</td>
</tr>
<tr>
<td><strong>TWC</strong></td>
<td>490±87</td>
<td>26±2</td>
<td></td>
</tr>
</tbody>
</table>

FECR%: faecal egg count percent reduction  
TWC: Total worm counts  
Values in table are means ± s.d  
Significantly different from day zero values (p≤0.05)  
Significantly different from control values (p≤0.05)
3.4.3: Haematological findings:
The blood samples were taken at day zero pretreatment and at days 7, 14, 21 post treatment with ivermectin for hematological examinations. Packed cell volume (PCV) and hemoglobin concentration (Hb) were indicative of the degree of anemia in infected control animals and infected treated animals. They did not obtain values statistically different from the controls during the post treatments period of experiment (Fig.26&27).

3.4.4: Biochemical findings:
There were no significant differences in plasma Na, K, GPT, GOT, urea, total protein, and albumin in control infected animals and animals treated with ivermectin during the period of the experiment (Table3-20).
Fig. 26: Effect of ivermectin on PCV percentage in goats infected with
Fig. 27: Effect of ivermectin on Hb concentration in goats infected with *H. contortus*
Table 3-20: Effect of ivermectin on plasma constituents in goats infected with *Haemoncus contortus*

<table>
<thead>
<tr>
<th>Time</th>
<th>Urea Mmol/l</th>
<th>Total p g/l</th>
<th>Albumin g/l</th>
<th>Na+ Mmol/l</th>
<th>K+ Mmol/l</th>
<th>GOT IU</th>
<th>GPT IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4.3±1</td>
<td>57.3±3.0</td>
<td>30.6±7.6</td>
<td>166.0±8.8</td>
<td>4.8±4.5</td>
<td>7.6±1.5</td>
<td>7.6±0.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>3.9±1.1</td>
<td>56.0±0.5</td>
<td>31.6±3.0</td>
<td>172±7.5</td>
<td>5.1±4.0</td>
<td>9±1.0</td>
<td>9±1</td>
</tr>
<tr>
<td>Day 14</td>
<td>5.2±7.0</td>
<td>61.0±0.0</td>
<td>33.3±4.0</td>
<td>152±6.4</td>
<td>4.0±4.0</td>
<td>11±1.0</td>
<td>9±1.0</td>
</tr>
<tr>
<td>Day 21</td>
<td>5.0 ±4.7</td>
<td>62.6±5.5</td>
<td>33.6±2.0</td>
<td>158±3.7</td>
<td>4.2±3.2</td>
<td>9.3±2.0</td>
<td>9±1.4</td>
</tr>
</tbody>
</table>

Values are mean ± s.d
CHAPTER FOUR

Discussion

The most promising plants should be scientifically validated through systematic experiments, using indicator helminths in laboratory animal and toxicity studies and, if there are positive, screening in domestic animals (Hammond et al, 1997). In this study, aqueous and methanolic extracts of Balanites aegyptiaca kernel, Peganum harmala seeds and Zizyphus spina-christi leaves were screened for their anthelmintic activity against Haemoncus contortus in vitro and in vivo.

The present research elaborated on the anthelmintic efficacy of aqueous and methanolic extract of Balanites aegyptiaca kernel in vitro and in vivo trial. In vitro study using a dose of 25mg/ml of aqueous and methanolic extracts of Balanites aegyptiaca kernel extracts was cause death of adult worms of H. contortus. The AE and ME produced mortality rate of 90% & 25% at 12h respectively. At 24 hours, all worms exposed to aqueous extract were found dead, while 90% of the worms exposed to methanolic extract were found dead.

Study in Sudan showed that the AE of Balanites aegyptiaca seed revealed in vitro anthelmintic activity against Caenorhabitis elegans that a dose of 25mg/ml resulted in 100% death of C. elegan adult worms after 6 hours (Ibrahim, 1992). Also Gnoula et al (2006) revealed that aqueous extract of Balanites aegyptiaca at a dose of 1mg/ml killed 50 and 75 % of C.elegans worms in 5 and 7 days, respectively. Another study using aqueous extract of root bark of Balanites aegyptiaca showed that significant anthelmintic activity against adult earthworms Phertima prosthuma and the aqueous extract revealed marked and potent anthelmintic activity than the standard drug albendazol (Dwivedi et al, 2009).
Chapagain and Wiesman (2005) demonstrated the potency of *Balanites aegyptiaca* aqueous extract in control of *Culex pipens* (mosquito larvae). Various part of the plant (root, bark, fruit pulp, seed kernel, and leaves) showed larvicidal properties. Seed kernel revealed larvicidal activity of 95% at 3rd day post treatment. The finding of Ibrahim (1992), Gonoula *et al* (2006), and Dwrivedi *et al* (2009) agreed with our finding in that *Balanites aegyptiaca* has vermicidal activity, which is dose dependent.

*In vivo* the anthelmintic activity of *Balanites aegyptiaca* kernel was also confirmed by graded dose response in goats treated with CAE and CME. The CAE of *B. aegyptiaca* at a dose of 100mg/kg showed no significant reduction in EPG, while, a dose of 400mg/kg revealed a time dependent anthelmintic effect and significant reduction in EPG to 42.8%, 55.1%, and 61.2% at 7, 14 and 21 days post treatment respectively. Treatment with ME of *B. aegyptiaca*, the three doses (100, 400 and 800mg/kg) exhibited different anthelmintic effect. The dose 100mg/kg showed a maximum reduction of 26.3% in EPG on day 21, while, the dose of 400mg/kg revealed a time dependent anthelmintic effect with significant reduction in the EPG to 33.3%, 48.2% and 74.7% at 7, 14 and 21 days post treatment respectively. Also the dose of 800mg/kg showed a time dependent effect with also significant reduction of the EPG to 30.4%, 60%, and 79.2% at 7, 14, and 21 day post treatment respectively. In this study, the reduction in EPG increased with time after treatment, suggesting that the chemical responsible for the effect on the parasite might be released slowly or has delayed effect. This increase in the activity of medicinal plants over time was reported previously (Hammond *et al* 1997, Usharani Dive *et al* 2000, and Dawo *et al* 2001).
In literature, there are only a few solitary reports available for anthelmintic activity of *Balanites aegyptiaca* in vivo. Koko *et al* (2000) studied the effect of water extract of *Balanites aegyptiaca* against *Fasciola gigantica* in goats. The result showed that a dose of 9g/k of *Balanites aegyptiaca* revealed efficacy of 93.2% reduction of the EPG of faeces. Other study by Oliver (1960) revealed that the kernel oil of *Balanites aegyptiaca* proved highly effective against ecto-parasite infestation of camels. The current study result was considered as first report for the anthelmintic property by both aqueous and methanolic extract of *Balanites aegyptiaca* against *H. contortus* in goats based on reduction of the number of eggs per gram faeces after treatment and the number of adult worms recovered at necropsy. It can be concluded that the extracts of *Balanites aegyptiaca* posses appreciable anthelmintic activity.

Photochemical screening of the medicinal plants showed good anthelmintic activity as it may contain secondary metabolites like alkaloids and flavonoids. These classes of plant, which possess secondary metabolites, are considered the sources of chemicals, which are responsible for wide therapeutic activities of several plants (Debella, 2002).

Phytochemical investigation on *B. aegyptiaca* yielded several classes of secondary metabolites such as coumarins, flavonoids and steroidal saponins, many of which express biological activities (Saker *et al*, 2000). The alcoholic extract of kernel of *B.aegyptiaca* contains sterol, terpens, and saponin as predominant compounds whereas tannins, alkaloid, and resin are in slightly small amounts (Abdel Rahi *et al*, 1986). The presence of tannin in *B.aegyptiaca* might be responsible for the observed anthelmintic activity. Previous studies revealed that plants with higher content of condense tannin have shown anthelmintic effect on different gastrointestinal nematode parasites of sheep. (Athanasiadou *et al*, 2001,
Molan et al, 2000). *Balanites aegyptiaca* known for a wide range of biological activities, which were mainly, attributed to its saponin constituents (Spragi et al, 2004). The total saponin content was found to be 6.7% in kernel (Watt and Breyer-Brand wijk, 1962). Gonoula et al, (2006) isolate blanitin-7 a steroidal saponin with high potential of anthelmintic activity. Blanitin-7 at concentration of 52µg/ml it killed about 75% of larval L3 stage of *C. elegan* in 7 days. While at 8.3µg/ml, it killed about 100% of adult stage worms in 7 hrs. The activity then strongly stage dependent (Gonoula et al 2007). From all the mentioned studies, it is clear that the anthelmintic effect of *B. aegyptiaca* is attributed to the presence of saponin and tannin.

The result of biochemical assays revealed no significant differences in Na+, K+, GPT, GOT, urea, total protein, and albumin concentration in control infected animals during the period of the experiment. The animals which were treated with CAE of *Balanites aegyptiaca* at dose (400mg/kg) showed no significant difference in plasma Na+, K+, GPT, GOT, urea, total protein, and albumin concentration during the period of the experiment. While those treated with CME of *Balanites aegyptiaca* at doses (400&800 mg/kg) gave the same biochemical parameters except for total protein which showed increased in concentration(from 60 to73g/l) and this increase was in normal range of total protein concentration in goats( 66-75g/l).

Study conducted by Mohamed et al (1999) in rat treated with *Balanites aegyptiaca*, the hematological parameters( Hb and PCV) and the plasma level of total protein, albumin, urea, GPT, and GOT gave similar result to control group. From these studies we can concluded that, the biochemical assays of treated animals with
Balanites aegyptiaca revealed no alteration in the biochemical parameters which may be attributed to the safety of Balanites aegyptiaca.

Regarding the pharmacological screening of Balanites aegyptiaca kernel, both methanolic and aqueous extracts showed contraction when tested on isolated rabbit jejunum in different doses (2.5, 5, 7.5mg/ml). This contraction activity was blocked by atropine (1µg/ml). This result was in line with the study of Iskender (1982) which revealed that the alcoholic and watery extracts of Balanites aegyptiaca Kernel resulted in stimulation of intestinal motility in rabbit.

In present study, experiment conducted to determine the oral LD50 of methanloic extract Balanites aegyptiaca kernel in mice and it was found to be more than 25g/kg. This result goes in parallel with the study of Iskender (1982) who found that the oral LD50 of Balanites aegyptiaca kernel in mice was 27.7g/k. Both studies proved that Balanites aegyptiaca is highly safe. There are several reports, which indicated the great variety of pharmacological and biological activities of Peganum harmala such as antibacterial, antifungal and MAO inhibitor (Abdel-Fattah et al, 1997). It has also been used as narcotic analgesic, antispasmodic and against tapeworm infection in man and animals, (Chopra et al, 1956, Said, 1969). The seed has been used internally in the treatment of intestinal parasites and the root has been used externally as a parasiticide in order to kill body lice (Chopra, et al., 1986).

The present research elaborated on the anthelmintic efficacy of aqueous and methanolic extract of Peganum harmala seed in vitro and in vivo trial.
In *vitro* a dose of 25mg/ml of *Peganum harmala* seed extracts was reported to cause the death of adult worms of *H. contortus*. The AE and ME produced mortality rate of 95% & 75% at 12h respectively. While at 24h all worms exposed to extracts were found dead.

In *vivo* the anthelmintic activity of *Peganum harmala* seed was also confirmed by graded dose response in goats treated with AE and ME. The AE of *Peganum harmala* at dose of 100mg/kg showed 75.5% reduction in EPG of faeces on day 7&14, and 85.4% on day 21 post treatment. While, the dose of 200mg/kg revealed reduction in the EPG of faeces of 67.9%, 75.5%, and 86.6% on day 7, 14 and 21 day post treatment respectively. The ME of *Peganum harmala*, the two doses (100, 200 /kg) exhibited anthelmintic effect. The dose of 100mg/kg showed a maximum reduction of 23.9% in EPG on day 21. While, dose 200mg/kg revealed a time dependent anthelmintic effect and reduced the EPG to 56.2%, 64.1% and 70.3% for 7, 14 and 21 day post treatment respectively.

In contrast the AE of *Peganum harmala* revealed maximum reduction in EPG of 85.4 and 86.6 at doses 100&200mg/kg respectively. While The ME of *Peganum harmala* showed maximum reduction in EPG of 23.9% and 70.3% at the doses of 100&200mg/kg respectively, so the aqueous extract of *Peganum harmala* have shown better activity in reduction of EPG of faeces than methanolic extract. The variation in activities of extract types of the plant might be due to difference in the proportion of the active component responsible for the tested anthelmintic activity resulting from the difference in the solubility either in water or in methanol. In our study, the active ingredients may be more soluble in water than in alcoholic so giving better anthelmintic activity. Eloff (1998) stated that the activity of botanical compound found in plant material depends on the type of extract and the method of extraction supported this.
The findings from the current study revealed that, the AE and the ME of *Peganum harmala* had anthelmintic effect. This result was supported by that of Akhtar and Rifat (1986) who reported anthelmintic efficacy of *Peganum harmala* against gastrointestinal cestodes of goats. In addition, another study by Chopra *et al* (1986) revealed that the seed of *Peganum harmala* has been used as anthelmintic in order to get rid of the body tapeworms. Another Study on cattle naturally infected with *Theileria annulata* treated with the extract of *Peganum harmala*, revealed a recovery of 78% (Marzaei, 2007). The finding of Akhtar and Rifat (1986), Chopra *et al* (1986) and Marzaei (2007) agreed with our finding that the *Peganum harmala* has parasiticidal effects.

The pharmacologically active compounds of *Peganum harmala* are several alkaloids, which are found specially in the seed and the root. These include, harmine, harmalol, harmin, and quinazoline derivatives, vasicine and vasicinone (Kamel *et al.*, 1960). The active principles that induced the observed anthelmintic activity might be found in these classes of chemical. This was supported by Debella (2002) who stated that, medicinal plants showed good anthelmintic activity have secondary metabolites like alkaloids and flavonoids and these classes of plant secondary metabolites are consider the sources of chemicals responsible for wide therapeutic activities of several plants.

Regarding the result of biochemical assays, there were no significant difference in plasma Na+, K+, GPT, GOT, Urea, total protein, and albumin in control infected animals during the period of the experiment. The animals that were treated with CAE and CME of *Peganum harmala* at the dose of (200mg/kg) revealed significant decrease in concentration of urea at day 14&21 post treatment. While the result of Na+, K+, GPT, GOT, and total protein concentration revealed no
significant difference post treatment. Another study by Muhi-eldin et al (2008) showed no changes in these values in treated rats with aqueous extract of *Peganum harmala* compare to the control group.

In pharmacological screening, the methanolic and aqueous extracts of *Peganum harmala* produced a dose dependant relaxation on isolated rabbit jejunum at different doses (2.5, 5, 7.5mg/ml). This result was same with these obtained by Aqel and Hadidi (1991) using aqueous extract of *Peganum harmala* seed on the smooth muscles of rabbit and guinea pig. The extract inhibited the spontaneous movement of the rabbit jejunum and guinea pig ileum. In addition, harmine and other harmala alkaloids are known to inhibit the contractile responses induced by ouabain and acetylcholine in guinea pig ilea smooth muscle (Hider *et al*., 1981). Relaxation activity was not blocked by different doses (0.5mg/ml) of propanolol (known non-selective β blocker) but blocked by tolazoline (known non-selective α blocker).

The oral LD50 of *Peganum harmala* methanolic extract in this study was calculated to be 7g/kg. Another study by Lamchouri *et al* (2002) revealed that, the oral LD50 of *Peganum harmala* aqueous extract in rats was 2.7g/kg. The LD50 value of aqueous extract of *Peganum harmala* given IM to rat was found to be 420mg/kg (Barnes and Eltherington, 1973, Muuhi-eldeen, 2008). From all mentioned studies, it is clear that the *Peganum harmala* seems to be safer when administered orally and as methanolic extract.
The present research elaborated on the anthelmintic efficacy of aqueous and methanolic extracts of *Zizyphus spina-christi* in vitro and in vivo trial.

In vitro, the mortality percentage effect of *Z.spina-christi* on mature *H.contortus* was tested. The result showed that, the aqueous extract of *Z.spina-christi* produced mortality of 40% at 12h and 74% at 24h, while methanolic extract produced mortality of 100% at 12h. However the crude methanolic extract of *Z.spina-christi* revealed anthelmintic activity better than aqueous extract.

The in vivo test screened the anthelmintic activity of aqueous and methanolic extract of *Z.spina-christi* on goats infected with *H. contortus*. All *Haemoncus* infected goats manifested in appetite and dullness but these were less marked in the animal treated with *Z.spins-christi* at 100, 400, or 800 mg/kg. This observation was supported by reduction in the numbers of heamoncus faecal eggs count and of adult worms recovered from abomasa at necropsy.

Maximum activity exhibited by AE of *Z.spina-christi* at the doses of (100mg/kg) and (400mg/kg) was 61.5% and 78.7% reduction in EPG of faeces on day 21 post treatment respectively. While ME at the doses of (100mg/kg, (400mg/kg) and (800mg/kg) showed maximum of 24.4%, 73.1% and 85.1% reduction in EPG of faeces at day 21 post treatment. The aqueous of *Z.spina-christi* at the dose of 400mg/kg exhibited better reduction in EPG of faeces than methanolic extract (78.7% &73.1%). These results revealed that active ingredients in *Z.spina-christi* that have anthelmintic activity might be more soluble in water than alcoholic extracts.

The two different doses (400 &800 mg/kg) of ME of *Z.spina-christi* give almost same reduction percentage (63.4%, 62%) on day14, but there was significant (p≤0.05) difference in EPG reduction percentage on day 21 post treatment (78% for 400mg/kg and 85.2% for 800mg/kg). These results revealed that, the ME of *Z.spina-christi* had dose and time dependents effect. Previous result by Alsudani
(2007) showed that the leaves of *Z.spina-christi* kill protoscolices (larval stage of Echinococcus). Another study by Ali and Hamid (2006) showed that ethanolic extract of the *Z.spina-christi* roots against bilharzias infestation reduced the number of worm burdens, ova count, granuloma size and count. The finding of Alsudani (2007), Ali, and Hamid (2006) agreed with our finding that *Z.spina-christi* has anthelmintic effects.

*Zizyphus spina-christi* was shown to contain cyclopeptide alkaloid as well as four saponin glycoside (Mahran *et al*, 1996) and several flavonoids which have been isolated from the leaves *Zizyphus* (Amos *et al*, 2001). The active principles that induced the observed anthelmintic activity might be found in these classes of chemical. Photochemical constituents such as tannins, flavonoid, alkaloids, and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanism against predation by many micro-organisms, insects and herbivores.

Regarding the result of biochemical assays, there were no significant differences in plasma Na, K, GPT, GOT, Urea, total protein, and albumin in control-infected animals during the period of the experiment. The animals that were treated with CAE&CME of *Zizyphus spina-christi* showed no significant change in concentration of K, GPT, GOT, urea, total protein, and albumin, but there was increase in sodium concentration during the period of the experiment.

Study by Islam *et al* (2001) showed that there were no significant change in biochemical parameters studied (GPT, GOT, urea, total protein, and albumin, creatinine, iron, chloride calcium, magnesium, sodium, potassium in mice treated with *Zizyphus spina-christi*.)
In pharmacological screening, the methanolic and aqueous extracts of *Zizyphus spina-christi* exhibited a dose dependent relaxation on isolated rabbit jejunum at different doses (2.5, 5, 7.5mg/ml). The contraction caused by acetylcholine (0.5mg/ml) on the tissue was reversibly inhibited by 5mg/ml of the methanolic and aqueous extracts of *Zizyphus spina-christi*. These results supported by Gharib *et al* (2003) who found that, *Zizyphus spina-christi* leaf extract induced relaxation in rat ileum. Another study on the methanolic extract of *zizyphus* root showed inhibition of spontaneous movement of isolated rabbit jejunum and inhibited acetylcholine induced contraction of rat ileum (Dahiru *et al* 2006).

The oral LD50 of *Zizyphus spina-christi* in rats was found to be more than 8g/kg. This result supported by that of Islam *et al* (2001) who showed that, *Zizyphus spina-christi* leaf extract oral LD50 in mice was more than 6.4 g/kg. While the oral LD50 of butanol extract of *Zizyphus spina-christi* leaf in mice was 3.82g/kg (Abdel-zaher *et al.*, 2005). The difference in oral LD50 seems to be due to the difference in the activity of botanical compound extracted from plant material depend on the type of extractant and the method of extraction (Eloff, 1998). Study by Adzu and Haruna (2007) revealed that, i.p LD50 of *Zizyphus spina-christi* methanolic extract in mice was established to be 783.3 mg/kg. From all mentioned studies, it is clear that the *Zizyphus spina-christi* seems to be safer when administered orally.

In general, *Balanites aegyptiaca, Peganum harmala* and *Zizyphus spina-christi* revealed *in vitro* anthelmintic activity against adult *H. contortus*, these results are in line with previous studies of different anthelmintic plants against adult *H. contortus*. Anthelmintic effect of aqueous and hydro alcoholic extract of the seeds of *Croton macrostachyus* and *Ekebergia capensis*, on adult *H.contortus* was investigated. Hydroalcoholic extracts of *C.macrostachus* and *E.capensis* produced
significantly mortality of 90 and 60% of adult *H. contortus* at concentration of 8mg/ml while aqueous extract of these plants produced only 36.67 and 43.33% mortality respectively at the same concentration (Equale, *et al.*, 2006).

Sharma, *et al.*, (1971) have reported significant *in vitro* effect of extract of *Cucurbita pepo, Calotropis gigantea, Juglans regia, Momordica charantia, Musu paradisaca* and *Scindapsus officinalis* on the motility of mature *H. contortus* of goat origin. Crude aqueous and hydro-alcoholic extracts of the seeds of *Croton macrostchyus, Ekebergia capensis* induced significant dose dependent mortality of adult *H. contortus* (Equale, *et al.*, 2006).

*In vitro* anthelmintic of crude aqueous and hydro-alcoholic extract of the seeds of *Coriandrum Staivum* were investigated on adult nematode parasite *H. contortus*. The hydro-alcoholic extract showed better in vitro activity against adult parasites than the aqueous one (Equale *et al.*, 2007). Crude extract of the ripe and fruits of *Hedera helix* investigated against adult *H. contortus*. Hydro-alcoholic extract showed better *in vitro* activity against adult parasites compared to the aqueous extract.( Equale *et al.*, 2007).

In the current study, the results obtained by *in vitro* study ascertained by *in vivo* study of *Balanites aegyptiaca, Peganum harmala* and *Zizyphus spina- Christi* in goats infected with *H. contortus*. The results also revealed anthelmintic activity of the three plants, these results are in line with several investigations that assessed *in vivo* plants efficacy against *H. contortus* in sheep and goats. Frenandez, (1999) studied the potential of *Tinspora rumphic* as anthelmintic against *H. contortus* in goats. The study showed the crude extract of *T. rumphic* given at a dose of 40mg/kg body weight significantly reduced the number of worm eggs in faces of naturally infected goats. The anthelmintic activity of *Calotropics procera* latex was investigated in sheep infected with *H. contortus* by Al-Qurawi, *et al.*, (2001). The
egg production was significantly reduced, but not completely suppressed and fewer adult *Haemonchus* worms were found in the abomasum. Aqueous extract of *Hedera helix* was evaluated for *in vivo* anthelmintic activity at dose of 1.13 and 2.25g/kg in sheep artificially infected with *H. contortus*. Significant faecal egg counts reduction was detected in the groups treated with both doses of *H. helix* and significant, dose dependent reduction in total worm count was observed (Equale, *et al*., 2007). The anthelmintic efficacy of *Artemisia herba-alba* was investigated in experimental haemonchosis in Nubian goats. The results revealed that *aherba-alba* had overall efficacy of 66.6% (Idris, *et al*., 1982). Githiori, *et al*., (2003) investigated the anthelmintic efficacy of *Albizia anthelmintica* against *Haemonchus contortus* of sheep. The study revealed significant reductions in faecal egg counts, but the efficacy levels achieved were well below the 70% reduction required.

**General overview:**

The use of medicinal plants for the prevention and treatment of gastro-intestinal parasitism has its origin in ethnoveterinary medicine. Although until recently the majority of the evidence on the antiparasitic activity of medicinal plants was anecdotal and lack scientific validity. However, there are currently an increasing number of controlled experimental studies, which aim to verify and quantify such plants activity. There are indeed a large numbers of plants whose anthelmintic activity has been demonstrated under controlled experimentation, either through feeding the whole plants or administering plants extracts to parasitised hosts. There is a concurrent trend towards using plant extracts to asses the anthelmintic activity of medicinal plants. Extraction procedure use for this purpose vary from simple water extractions to very complicated ones, where a series of organic solvents is used. The aim is to extract the active compounds from the medicinal plants, and then test their anthelmintic activity through *in vitro* and *in vivo*. 
In the present study aqueous and methanolic extracts of *Balanites aegyptiaca*, *Peganum harmala* and *Zizyphus spina-Christi* has been demonstrated *in vitro* and *in vivo* to assess the anthelmintic activity of them.

*In vitro* study revealed that the mortality rate of *H.contortus* worms at 12 hours post treatments with aqueous and methanolic extracts of *Balanites aegyptiaca* was 90% and 25% respectively so aqueous extract of *Balanites aegyptiaca* gave better anthelmintic effect than methanolic extract. While crude aqueous and methanolic extracts of *Zizyphus spina-Christi* revealed mortality rate of 84%, 100% at 12 hours post treatments respectively, although methanolic extract of *Zizyphus spina-Christi* gave more anthelmintic effect than aqueous extract. The aqueous and methanolic extracts of *Peganum harmala* revealed mortality rate of 95%, 75% at 12 hours post treatments respectively, so aqueous extract of *Peganum harmala* gave the most efficient anthelmintic effect than methanolic extract.

*In vivo*, the ME of *Balanites aegyptiaca* revealed significantly greater anthelmintic effects than AE of *Balanites aegyptiaca*. Maximum activity exhibited by ME of *Balanites aegyptiaca* at the dose (400mg/kg) was 74.7% reduction in EPG of faeces on day 21 post treatment, while CAE using the same dose, showed maximum reduction in EPG was 61.2%. The AE of *Peganum harmala* (200mg/kg) revealed significantly greater reduction in EPG of faeces (86.6%) compared to CME extract (70.3%) at the same dose. The AE of *Zizyphus spina-Christi* revealed better anthelmintic effect than the ME of *Zizyphus spina-Christi* at the same dose (400mg/k), maximum activity exhibited by AE of *Zizyphus spina-Christi* was 78.7% reduction in EPG and ME of *Zizyphus spina-Christi* was 73.1 % reduction in EPG on day 21 post treatment.
In vitro and in vivo studies revealed that aqueous extracts of *Peganum harmala* showed significant anthelmintic effect than methanolic extracts. However, aqueous and methanolic extracts of *Zizyphus spina-Christi* gave different results at in vivo and in vitro tests. In vitro, methanolic extract of *Zizyphus spina-Christi* showed significant anthelmintic effect than aqueous extract, while in vivo the aqueous extract of *Zizyphus spina-Christi* revealed significant greater anthelmintic effects than methanolic extract. The variation in activity of extract type of the plant might be due to difference in the proportion of the active component responsible for the tested anthelmintic activity resulting from the difference in the solubility either in water or in methanol. Elof (1998) stated that the activity of botanical compound found in plant material depends on the type of extract and the method of extraction supported this. Also in vitro results are not always verified by in vivo experimentation (Athanasiadou et al., 2007) that might be related to bioavailability of the active compounds at different parts of the gastro-intestinal tract, the host-plant interaction, metabolic biotransformation and the parasite specificity also increase the degree of variability observed in this study.

*Balanites aegyptiaca, Peganum harmala* and *Zizyphus spina-Christi* showed anthelmintic activity, these plants have secondary metabolites like alkaloids, terpenes, saponins and lactones. These classes of plant secondary metabolites are consider the sources of chemicals responsible for wide therapeutic activities of several plants (Debella, 2002). Although the majority of compounds with anthelmintic properties have yet to be isolated from plants, in most cases such compounds have been identified, they are plants secondary metabolites. For example, lactone such as santonin isolated from *A. maritima*, have shown a strong anthelmintic activity towards *Ascaris spp.*, nematodes species of livestock and humans (Waller et al., 2001). Alkaloids have also demonstrated strong nematocidal activity towards *Strongoliodes ratti* (Satou et al., 2002). Alkaloids hydrochlorides
extracted from seed of *Butea frondosa* at 20mg /ml proved 100% lethal to earthworms within 24 hours (Kalesaraj and Kurup 1962).

The nematocidal activity of tannin extracts has been reported as early as the 1960s (Taylor and Murant, 1966). More recently, evidence on the anthelmintic properties of condensed tannins has been supported by a series of *in vitro* (Dawson *et al*., 1999; Athanasiadou *et al*., 2001; Molan *et al*., 2003; Ademla and Idowu, 2006) and *in vivo* studies (Athanasiadou *et al*., 2000; Butter *et al*., 2001; Paolini *et al*., 2003a and 2003b). The tannins contained in plants have been reported to posses antiviral (Cheng *et al*., 2002), antibacterial (Perum *et al*., 1998) and anthelmintic (Paolin *et al*., 2003, 2005, Ademola *et al*., 2004, 2005) activities.

Regarding the result of biochemical assays, the animals which were treated with AE of *Balanites aegyptiaca* at dose (400mg/kg) showed no significant difference in plasma Na+, K+, GPT, GOT, urea, total protein, and albumin concentration during the period of the experiment. While those treated with ME of *Balanites aegyptiaca* at doses (400&800 mg/kg) gave the same biochemical parameters except for total protein which showed increased in concentration(from 60 to 73g/l) and this increase was in normal range of total protein concentration in goats (66-75g/l). The animals which were treated with AE and ME of *Peganum harmala* at the dose of (200mg/kg) revealed significant decrease (from 5.1 to 1.4 mmol/l) in concentration of urea at days 14&21 post treatment and this decrease is more or less comparable to the normal concentration of urea in goats (2.6-6.6mmol/l). While the result of Na+, K+, GPT, GOT, and total protein and albumin concentration revealed no significant difference post treatment. The animals treated with AE&ME of *Zizyphus spina-chisti* showed no significant change in concentration of K, GPT, GOT, urea, total protein, and albumin, but there was an increase in sodium concentration(from 160 to 169mmol/l) during the period of experiment.
In our study, the oral LD50 of methanloic extract of *Balanites aegyptiaca* kernel in rats was found to be more than 25g/kg. This result agreed with the study of Iskender (1982) who found that the oral LD50 of *Balanites aegyptiaca* kernel in mice was 27.7g/k .While the oral LD50 of methanloic extract of *Zizyphus spinachristi* in rats was found to be more than 8g/kg. This result in accord with Islam et al (2001) who showed that, oral LD50 of *Zizyphus spina-christi* leaf extract in mice was more than 6.4 g/kg.

The oral LD50 of methanolic extract of *Peganum harmala* in our study was calculated to be 7g/kg. Another study by Lamchouri et al (2002) revealed that, the oral LD50 of aqueous extract of *Peganum harmala* in rats was 2.7g/kg. the variation in result may be related to the type of the extracts. Thus, it appeared that methanolic extract of *Peganum harmala* was safer than the aqueous extract.
CONCLUSION

In the current study, *in vitro* and *in vivo* studies revealed that, aqueous and methanolic extracts of *Balanites aegyptiaca*, *Peganum harmala* and *Zizyphus spina-christi* possess appreciable anthelmintic effects against *Haemoncus contortus* depending on the dose size and time after dosing. The extracts of the three plants showed significant reduction in egg count per gram of faeces and the number of adult *Haemoncus contortus* found in abomasa of goats. The aqueous extracts of *Peganum harmala* showed significant anthelmintic effect than methanolic extract *in vitro* and *in vivo*. Aqueous and methanolic extracts of *Balanites agyptiaca* and *Zizyphus spina-christi* gave different results. *In vitro*, aqueous extract of *Balanites agyptiaca* showed significant anthelmintic effect greater than methanolic extract, while *in vivo*, the methanolic extract of *Balanites aegyptiaca* revealed significantly greater anthelmintic effects than aqueous extract. *In vitro*, methanolic extract of *Zizyphus spina-Christi* showed significant anthelmintic effect than aqueous extract, while *in vivo* the aqueous extract of *Zizyphus spina-Christi* revealed significant greater anthelmintic effects than methanolic extract.

Biochemical assays of the three plants pertaining to liver function tests none of the aqueous or methanolic extracts showed change. However, kidney function tests showed slight decrease in urea concentration with respect to *Peganum harmala*.

In mice, the oral LD50 of methanloic extracts for *Balanites aegyptiaca* kernel was found to be more than 25g/kg, for *Peganum harmala* seed was 7g/kg and for *zizyphus spina-christi* more than 8g/kg.

The AE and ME of *Balanites aegyptiaca* showed contraction of the smooth muscle of rabbit jejunum. The contraction was blocked by atropine. The AE and ME of
*Peganum harmala* inhibited the spontaneous movement of the rabbit jejunum, these inhibitions were non-responsive to the adrenergic blockers (propranolol and tolazoline). The AE and ME of *Zizyphus spina-christi* showed relaxation of the rabbit jejunum. These relaxations were blocked by the adrenergic blocker tolazoline.
Recom**mendation:-**

Further research on a large scale should be carried out on larger numbers of animals and on higher doses than those used in the current study with a view to determining optimal levels of the three plants, and to identify and isolate the effective anthelmintic ingredients of the plants.
REFERENCES


