Anthelmintic Activity of Zizyphus Spina-Christi Leaves

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Abstract—Helminthosisis one of the major problems of livestock production throughout the world, particularly in tropical and subtropical areas. The present work is an attempt to carry out evaluation of anthelmintic efficacy of aqueous and methanolic extracts of Zizyphus spina-christi leaves, in vitro using live Haemoncus contortus and in vivo using experimentally induced Haemoncus contortus infection in Nubian goats. The in vitro study revealed significant anthelmintic effects (p≤0.05) for both crude aqueous extract (CAE) and crude methanolic extract (CME) of Zizyphus spina-christi leaves on live Haemoncus contortus as evident from their mortality or temporary paralysis. CAE of Zizyphus spina-christi (25mg/ml) produced mortality rate of 14%, 20% and 74% at 6, 12, 24 hours post treatment respectively. While, CME (25mg/ml) produced mortality rate of 84%, 100% and 100% at 6, 12, 24 hours post treatment respectively. For in vivo studies, the CAE of Zizyphus spina-Christi, the doses of 100mg/kg and 400mg/kg showed 61.5% and 78.7% reduction in egg count percent (EPG) of faeces on day 21 post treatments respectively. While CME 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 treatments, respectively. This study shows that Zizyphus spina-christi leaves possess in vitro and in vivo anthelmintic activity.

Index terms: Anthelmintic, Haemoncus contortus, Goats, In vitro, In vivo Zizyphus spina-christi, Sudan.

I. INTRODUCTION
Infections by gastrointestinal helminth parasites of livestock are among the common and economically important diseases of grazing livestock worldwide [1]. Gastrointestinal parasites are world wide problem, manifested by reduced weight, lowered meat and milk production worldwide [2]. In small ruminants Haemoncus contortus is one of most pathogenic roundworms. Development of resistance to most of the commercially available anthelmintic became a severe problem worldwide [3]. Moreover, these drugs are unavailable especially in rural areas and to the poor farmers of the developing countries [4]. These factors paved the way for herbal remedies as alternative anthelmintics [5]. Herbal medicine have gained much importance in recent years due to the good efficacy and cost effectiveness worldwide [6]. Zizyphus spina-christi belongs to family (Rhamnaceae). It is a shrub, sometimes a tall tree, reaching a height of 20 m and a diameter of 60 cm, grows wild in tropical Africa and Asia worldwide [7]. In Sudan distributed in northern Kordofan and throughout northern and central Sudan worldwide [8]. For a long time, Z. spina-christi has been used in alternative medicine for the treatment of fever, pain, dandruff, wounds and ulcers, inflammatory conditions, asthma and to cure eye diseases. Z. spina-christi has recently been shown to have antibacterial, antifungal, antioxidant, anti-hyperglycemic, and antinoceptive activities worldwide [9], also used as anthelmintic in ethno veterinary medicinal system in Pakistan worldwide [10]. Flavonoids, alkaloids and saponins are the main phytochemicals that are reported from this plant. Geranyl acetone, methyl hexadecanoate, methyl octadecanoate, farnesyl acetone C, hexadecanol and ethyl octadecanoate are characterized as the major components of the leaves worldwide [11]. This study was carried to investigate the anthelmintic activity of Zizyphus spina-christi leaves.
II. MATERIALS AND METHODS

Plant material
The leaves of Ziziphus spina-christi were obtained from Omdurman local market and identified by a botanist, Department of Botany, Medicinal and Aromatic Plant Research Institute. The leaves were ground and kept until processed.

Plant extracts
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 [12]. 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. [13].

In vitro anthelminthic Activity
The in vitro trials for anthelminthic activity of methanolic and aqueous extracts of Ziziphus spina-christi were conducted on mature live H. contortus of sheep as described by Lal et al [14]. The adult H. contortus were collected from the abomasum of infected sheep. Immediately after slaughtering, the aboma were collected from El Kadro 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 methanolic extracts of the plant extracts in PBS and PBS alone for the control group in total volume of 4 ml. Three replications per each treatment were employed. The inhibition of motility of the worms was used as a criterion for anthelminthic activity. The motility was observed after 0,1,2,3,6,12 and 24h h intervals. The numbers 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 [15].

In vivo anthelminthic activity
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. Twenty six (6-8 month old) healthy male Nubian goats weighing 10-12kg 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 Ziziphus spina-christi (1000mg /kg)
- Group 4: 10 goats infected with Haemoncus contortus divided into 2 sub groups each of five animals treated orally with three doses of methanolic extract of Ziziphus spina-christi (100,400,800mg /kg).

Faecal samples
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 [15].

Egg count procedure
Fresh fecal samples were collected into a clean Petri dish. The eggs count was determined using a Mc Master technique [16] and expressed as faces with lower limit of determination of 100 parasites of eggs.
Three grams of faeces 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 eggs per gram of faeces is obtained by multiplying the total number of eggs in the two squares by 100.
The percent reduction in egg count per gram of faeces was calculated using the following formula:

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

**Mature worms count procedure**
The animals were slaughtered and the abdomen was ligated at the junction of the abomasum to omasum and abomasum to the duodenum. 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.

**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 using Statistical Packaging for Social Sciences (SPSS version 11.5 for windows).

### III. RESULTS AND DISCUSSION

#### In vitro anthelmintic activity

The *in vitro* trial for anthelmintic activity of the crude aqueous extract (CAE) and the crude methanolic extract (CME) of the leaf of *Ziziphus spina-christi* were conducted on mature live *Haemoncus contortus*. The CAE and CME 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 *Ziziphus 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. 1).

Many researchers studied anthelmintic activity of genus *Ziziphus in vitro*. Crude methanolic extract of bark for *Ziziphus nummularia* (Rhamnaceae) was studied adjacent to trichostrongylid nematodes of sheep by conducting adult motility assay, egg hatch test and the larval development assay. These revealed the dose and time-dependent anthelmintic effects [17]. Veeresh and Kambhoja [18] screened anthelmintic activity of *Ziziphus jujuba* (Rhamnaceae) on adult earthworms *Pheritima posthuma*, using piperazine citrate as standard drug. Both methanol and aqueous extract showed significant anthelmintic activity compared to standard drug Piperazine citrate. Aqueous extract as well as methanolic extracts of *Ziziphus mauritiana*(Rhamnaceae) exhibited anthelmintic activity by inhibiting hatching of nematode eggs [19]. Ethanolic extract of *Ziziphus oenoplia* (Rhamnaceae) roots showed good anthelmintic activity against *Pheritima posthuma* [20]. *Ziziphus. nummularia* (Rhamnaceae) leaves were evaluated for the control of snails which exhibit that both ethanolic and n-hexane extracts were highly molluscicidal effect[21]. Alsudani [22] showed that leaves of *Z. spina-christi* kill protozoalces (larval stage of Echinococcus). Another study by Ali et al [23] 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.

#### In vivo anthelmintic activity

**Aqueous extracts of *Ziziphus spina-christi* anthelmintic effects**
The anthelmintic activity of two doses (100&400mg/kg) of aqueous extract of *Ziziphus spina-christi* in goats infected with *H. contortus* was shown in Table (1). 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 abomas 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 1).
Methanolic extracts of *Ziziphus spina-christi* anthelmintic effects

The anthelmintic activity of three doses (100,400& 800mg/kg) of CME of *Ziziphus spina-christi* in goats infected with *H.contortus* shown in Table (2).

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 21days 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 21day 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 2).

*In vivo* there is one report available on an anthelmintic activity of genus *Ziziphus*. Bachaya *et al* [17] evaluated anthelmintic activity of *Ziziphus nummularia* in sheep naturally infected with gastrointestinal nematodes. The plant exhibited maximum fecal egg count reduction (84.7%) on day 13 post-treatment in sheep.

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**Fig 1.** Comparison of mean percentage of survival adult *H. contortus* for 24 hours exposure to methanolic and aqueous extracts of *Ziziphus spina-christi* with control (phosphate buffer saline)

<table>
<thead>
<tr>
<th>PBS</th>
<th>AZ</th>
<th>MZ</th>
</tr>
</thead>
</table>

PBS: phosphate buffer saline
AZ: Aqueous extract of *Ziziphus spina-christi*
MZ: Methanolic extract of *Ziziphus spina-christi*
Table 1: Effect of different doses of aqueous extract of *Ziziphus 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>
</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 2. Effect of different doses of methanolic extract of *Ziziphus 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>Dose100mg/Kg</th>
<th>FECR%</th>
<th>Dose400mg/Kg</th>
<th>FECR%</th>
<th>Dose800mg/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)

IV. CONCLUSION

The current study, revealed that, aqueous and methanolic extracts of *Ziziphus spina-christi* had appreciable anthelmintic effects against *Haemoncus contortus* depending on the dose size and time after dosing. More investigation about the active components of the plants well helps to determine the mode of action of this plant.

REFERENCES


