In Vitro and In Vivo Anthelmintic Activity of Peganum harmala Seeds against Haemoncus contortus in Goats

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Abstract—There is an increased awareness among medical and scientific communities that the importance of medicinal plant studies have gone beyond anthropological curiosity. Plant anthelmintics have been in the forefront of this growing awareness. The present work is an attempt to carry out evaluation of anthelmintic efficacy of aqueous and methanolic extracts of Peganum harmala seeds, 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 Peganum harmala seeds on live Haemoncus contortus as evident from their mortality or temporary paralysis. For in vivo studies, the CME and CAE of Peganum harmala revealed dose dependent anthelmintic effects. The CAE at dose of 100mg/kg and 200mg/kg showed 85.4% and 86.6% reduction in egg count per gram (EPG) of faeces at day 21 post treatments, while the CME 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.

Index Terms — Anthelmintic, Haemoncus contortus, In vivo, in vitro, Goats, Peganum harmala, Sudan.

I. INTRODUCTION

Helminthiasis is one of the most important animal health problems, which inflict heavy production losses in grazing animals. The disease is highly prevalent particularly in developing countries[1]. Infections by gastrointestinal (GI) helminth parasites of livestock are among the most common and economically important diseases of grazing livestock [2]. They are characterized by lower outputs of animal products (Meat, milk, hides and skins), which all impact on the livelihood of smallholder farmers [3]. Haemonchosis has been identified as one of the top ten constraints to sheep and goat rearing in East Africa [2]. Development of resistance to most of the commercially available anthelmintic became to a severe problem worldwide [4]. Moreover, these drugs are unavailable especially in rural areas and to the poor farmers of the developing countries [5]. These factors paved the way for herbal remedies as alternative anthelmintics [6].

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 is widely distributed in the central Asia, North Africa and Middle East and has been introduced in America and Australia. 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 [7]. Many pharmacological surveys have shown different effects of Peganum harmala and/or its active alkaloids (particularly harmaline) [8].

Various studies have shown different antiparasidal [9-11], antifungal [12,13], antibacterial[12,14] and insecticidal[15,16] effects of the alkaloids derived from P. harmala seeds. Moreover, its powdered seeds and various extracts have been used as a remedy against tapeworm infections in men and animals.[17] Akhtar and Riffat [18] reported anthelmintic efficacy of Peganum harmala against gastrointestinal cestodes of goats. Seeds of Peganum harmala contain an active constituent-Tetra-hydro-harmine that has been claimed to be active against mixed gastrointestinal nematode infection in goats [19] also the plant has shown a potent anthelmintic activity against Fasciola gigantica [20]. However, there are only a few solitary reports available for anthelmintic activity of Peganum harmala. Therefore, the aim of the present study was to determine the anthelmintic activity of aqueous and methanolic extracts of Peganum harmala in vitro using live Haemoncus contortus and in vivo using experimentally induced haemoncus contortus infection in Nubian goats.

II. MATERIALS AND METHODS

Plant material

The seed of Peganum harmala were obtained from Omdurman local market and identified by a botanist, Department of Botany, Medicinal and Aromatic Plant Research Institute. The kernel was grounded 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 [21].
Aqueous extracts of the plants were performed at the research laboratory –College of Veterinary science–University of Bahr El Ghazal, according to the method of Fenado et al. [22].

In vitro anthelmintic Activity
The in vitro trials for anthelmintic activity of methanolic and aqueous extracts of Peganum harmala were conducted on mature live H. contortus of sheep as described by Lal et al.[23]. The adult H. contortus were collected from the abomasum of infected sheep. Immediately after slaughtering, the abomasum 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 anthelmintic activity. The motility was observed after 0,1,2,3,6,12 and 24h 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 [24].

In vivo anthelmintic 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 weighting 10-12kg were used in this experiment divided into 4 groups:
Group I: 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&200mg /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&200mg /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 [25].

Egg count procedure
Fresh fecal samples were collected into a clean Petri dish. The eggs count was determined using a McMaster technique [26] 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:

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

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

III. RESULTS AND DISCUSSION
The anthelmintic effects of aqueous (AE) and methanolic extracts (ME) of Peganum harmala seeds were tested in vitro and in vivo.

In vitro anthelmintic activity
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.1)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.

In vivo anthelmintic activity
1-Aqueous extracts of Peganum harmala anthelmintic effects
The anthelmintic activity of two doses of aqueous extract of Peganum harmala in goats infected with H. contortus were shown in Table (1). 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 200 mg/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 abomasum 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 1).

2- Methanolic extracts of Peganum harmala anthelmintic effects
The anthelmintic activity of two doses of ME of Peganum harmala in goats infected with H. contortus shown in Table (2).

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 Peganum harmala 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≤0.05) in treated animals compared to the control group (Table 2).

Maximum activity exhibited by AE of Peganum harmala (200mg/kg) resulted in 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 treatments. Also after three week of treatment AE (200mg/kg) revealed a significantly greater reduction in EPG (86.6%) compared to ME at the same dose (70.3%).

PBS: Phosphate buffer saline
MP: Methanolic extract of Peganum harmala
Ap: Aqueous extract of Peganum harmala

Figure 1. Comparison of means percentage of survival adult H. contortus for 24 hours exposure to methanolic and aqueous extracts of Peganum harmala in comparison with control (phosphate buffer saline)

Table 1. 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>60±22.5**</td>
<td></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 2. 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>Dose 100mg/kg</th>
<th>FECR%</th>
<th>Dose 200mg/kg</th>
<th>FECR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4700±848</td>
<td>4600±941</td>
<td>-</td>
<td>6400±2281</td>
<td>-</td>
</tr>
<tr>
<td>Day 7</td>
<td>5100±141</td>
<td>4100±1128</td>
<td>10.3%</td>
<td>2800±1184*</td>
<td>56.2%</td>
</tr>
<tr>
<td>Day 14</td>
<td>5100±777</td>
<td>3900±730*</td>
<td>15.2%</td>
<td>2300±1192*</td>
<td>64.1%</td>
</tr>
<tr>
<td>Day 21</td>
<td>4300±1060</td>
<td>3500±756*</td>
<td>23.9%</td>
<td>1900±777*</td>
<td>% 70.3</td>
</tr>
<tr>
<td>TWC</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)

The present research elaborated on the anthelmintic efficacy of aqueous and methanolic extracts of *Peganum harmala* seed in vitro and in vivo trials in goats. In vitro, the AE and ME produced mortality rate of 95% &75% respectively. In vivo the anthelmintic activity of *Peganum harmala* seeds was also confirmed by graded dose response in goats treated with AE and ME. 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. The aqueous extract of *Peganum harmala* showed better activity in reduction of EPG of faeces than methanolic extract in vitro and in vivo. This 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. This is in line with Eloff [27] who stated that the activity of botanical compound found in plant material depends on the type of extract and the method of extraction. The aqueous and methanolic extracts of *Peganum harmala* seed showed significant reduction in egg count per gram of faeces and the number of adult *Haemoncus contortus* found in abomasum of goats.. This result was in accordance with Akhtar and Rifat [18] who reported that *Peganum harmala* had anthelmintic effect against gastrointestinal cestodes of goats. Moreover, Koko *et al* [20] reported that *Peganum harmala* plant has a potent anthelmintic activity against *Fasciola gigantica*.

Chopra *et al* [28] revealed that the seed of *Peganum harmala* has been used as anthelmintic in order to get rid of the body tapeworms. There have been several studies indicating effectiveness of *P. harmala* extract against theileriosis.[29,30] Two studies were conducted in Iran on the effect of *P. harmala* extract with a dose of 5mg/kg body weight once daily for 5 days on cattle[30] and sheep[29] theileriosis that showed a significant recovery rate of respectively 78% and 65%. Beta-carbolines from the seeds of *P. harmala* showed strong trypanosomicidal activity [31].

IV. CONCLUSION

The current study, revealed that, aqueous and methanolic extracts of *Peganum harmala* 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 help to determine the mode of action of this plant.

V. REFERENCES


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