ABSTRACT

Introduction: The perennial herb Phyllanthus niruri, belonging to the family Euphorbiaceae is commonly known as Bhuiavala in Marathi and possess antibacterial, analgesic, antipyretic, anti-hepatotoxic, antifungal and anthelmintic activity.

Objective: The present study was carried out to validate the in vitro anthelmintic activity of hot aqueous whole plant extract of Phyllanthus niruri against Paramphistomes by petri-dish method.

Materials & method: The in vitro trials for anthelmintic activity of aqueous whole plant extract of Phyllanthus niruri was conducted on mature live Paramphistomes by petri-dish method. The extracts were used at the concentration of 5 and 10 mg/ml and observation were made on their viability at room temperature.

Result: The hot aqueous whole plant extract of Phyllanthus niruri was found to cause cessation of motility (paralysis) of Paramphistomes after 2:15 and 1:45 hours of exposure while the complete cessation of motility (death) was observed after 4:35 and 4:15 hours of exposure in the concentration of 5 and 10 mg/ml respectively.

Conclusion: The hot aqueous whole plant extract of Phyllanthus niruri exhibited significant in vitro anthelmintic activity in a dose dependant manner.

Keywords: Phyllanthus niruri, Anthelmintic, Paramphistomes, Aqueous extract.

INTRODUCTION

Parasitic diseases are distributed worldwide and are a major constraint for profitable livestock industry. It causes approximately US $ 300–400 millions/yr in USA and in India, losses due to parasitic diseases have not been estimated and cause a considerable loss to the developing world[1].

Among these parasitic diseases gastrointestinal helminthic infection in livestock is common and the economic losses due to it are unaccountable, mainly due to morbidity and mortality. Moreover, the geographical and agro-ecological conditions of India are endowed with hot and humid climate, multiple riverbanks, and floods during monsoon, less urbanization and large agricultural lands. These conditions are very congenial for the propagation of parasites particularly helminthes in livestock.

Among various methods of controlling helminthiasis, anthelmintics are used since long to combat the problem of gastrointestinal parasitism. Anthelmintics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. Anthelmintics act either locally or systemically. They expel worms directly from the Gastro intestinal tract (GIT) or destroy helminths that invade organs and tissues. To be an effective anthelmintic, a drug must be able to penetrate the cuticle of the worm or enter its alimentary tract[2]. But the extensive and sole reliance on anthelmintics with their indiscriminate use is under serious threat due to rapid and widespread emergence of anthelmintic resistant strains of parasites throughout world including India[3]. Moreover, these drugs are unaffordable, inaccessible or inadequately available to the resource- poor farmers of the developing countries. Apart from this, the high cost of conventional anthelmintic drugs paved the way for herbal remedies as an alternative source of anthelmintics.

Herbal drugs with different modes of action could be of clinical significance being eco-friendly and biodegradable and have been in use since ancient times for the treatment of various acute and chronic ailments including parasitic diseases both in human and animals.

Many unknown and lesser known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not much known to the scientific world[4].

It has been well evidenced that the traditional medicines including plants and plant-derived preparations hold a great promise as source of easily available effective anthelmintic agents to the people[5,6,7][8].

The plant Phyllanthus niruri, commonly known as Bhuiavala in Marathi is a perennial herb and belongs to the family Euphorbiaceae. It is reported to possess antibacterial, analgesic, antipyretic, anti-hepatotoxic[9], antifungal and anthelmintic activity[10]. The present study was carried out to validate the in vitro anthelmintic activity of hot aqueous whole plant extract of Phyllanthus niruri against Paramphistomes by petri-dish method.

MATERIALS AND METHOD

Herbal Medicine: Hot aqueous whole plant extract was prepared and was used as herbal medicine.

Method

The in vitro trials for anthelmintic activity of aqueous whole plant extract of Phyllanthus niruri was conducted on mature live Paramphistomes as described briefly[11]. The mature worms were collected from the stomach of freshly slaughtered goat from local abattoir, governed by Nagpur Municipal Corporation. The worms were collected by blunt forceps then washed and finally suspended in tyrode solution. The worms were identified from department of Parasitology, Nagpur Veterinary College, Nagpur. These worms were used for screening the anthelmintic activity of extract by petri-dish method.

The activity of extracts was tested at three different dilutions. The extract was diluted to 20 ml of tyrode solution alone and fenbendazole dissolved in tyrode solution was used as negative control respectively. The fenbendazole was used in concentration of 5 mg/ml of tyrode solution. The extract of Phyllanthus niruri was used in the concentration of 5 and 10 mg/ml respectively. Ten
worms were exposed in triplicate and the time of transfer was recorded.

Observation were made with test drug at an interval of 30 minutes till the mortality of worms occurred. During observation the time required for paralysis of worm and complete cessation of motility was recorded.

The worms were gently pressed at either end with a blunt glass rod to confirm complete cessation of motility. At the end of trial, the worms were transferred to tyrode solution at 40°C to check the motility if any and those worms showing no motility were considered as dead worms[12]. The findings were confirmed by taking number of observation. All the experiments were conducted at room temperature varying between 97°F to 104°F[13].

**Statistical Analysis**

All the values in the test are presented as Means ± SEM. Statistical differences between the means of the various groups were evaluated using FRBD. A ‘P’ value of less than 5% was considered to be statistically significant (P < 0.05). The data generated will be analysed statistically by standard statistical procedure[14].

**RESULT AND DISCUSSION**

The anthelmintic activity of *Phyllanthus niruri* was screened against *Paramphistomes* at the different concentration of extract i.e 5 and 10 mg/ml. The anthelmintic activity was compare with fenbendazole @ 5 mg/ml as referral standard. The time taken for complete cessation of motility (paralysis) and mortality in hrs in different concentration are presented in Table-1 and 2 (expressed as a mean ± SEM in hours of 10 worms in each group) respectively.

The table reveals that the activity observed was dose dependent. As the concentration was increased the death and paralysis occurred was earlier than the small amount of dose used. The results were significantly observed and were significant at 5 % level of significance.

**Table 1: Time required for paralysis and death in hours for Paramphistomes**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paralysis</th>
<th>Death</th>
<th>Average</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.46±0.083</td>
<td>13.36±0.128</td>
<td>10.91±2.45</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>2.44±0.185</td>
<td>4.41±0.028</td>
<td>3.425±0.985</td>
<td>**</td>
</tr>
<tr>
<td>Extract 5mg/ml</td>
<td>2.15±0.051</td>
<td>4.35±0.055</td>
<td>3.25±1.1</td>
<td>*</td>
</tr>
<tr>
<td>Extract 10mg/ml</td>
<td>1.45±0.107</td>
<td>4.15±0.168</td>
<td>2.8±1.35</td>
<td>**</td>
</tr>
</tbody>
</table>

(P< 0.05) **Significant at 5 % L.S.

**In Vitro Anthelmintic activity of hot aqueous whole plant extract of Phyllanthus niruri against Paramphistomes**

**CONCLUSION**

Aqueous whole plant extract of *Phyllanthus niruri* exhibited significant in vitro anthelmintic activity in a dose dependant manner.

In conclusion, the preliminary phytochemical screening of the extracts revealed the presence of flavonoids, alkaloids, tannins and saponins. It has been well established that fenbendazole by increasing chloride ion conductance of parasite muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis[15,16]. Thus, our drug may have the similar profile of mechanism of action. Further, it has been reported that tannins which are polyphenolic compounds produce anthelmintic activity by binding to glycoprotein on the cuticle of the parasite and thus leads to death of the worm[16,17]. Therefore, standardization of each extracts and isolation of phyto-constituents in each extracts for anthelmintic activity is required in the future. Furthermore, the pharmacological studies for anthelmintic activity should be undertaken in other parasites to mimic the exact human helminthesis.

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**REFERENCES**