“ANTHELMENTIC ACTIVITY OF LEAVES AND STEM OF ARGYREIA CUNEATA”

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ABSTRACT

Approximately 3 million people are infected with helminthes worldwide. Helminthes infections are commonly found in villages of developing countries and are being recognized as cause of much acute as well as chronic illness among the human beings as well as cattle’s. Hence, the treatment for helmintic infection is of almost need. The high cost of modern anthelmintics has limited the effective control of these parasites. The Methanol and aqueous extract of Argyreia Cuneata was evaluated for invitro anthelminthic activity. In present study different concentration of Extract 50 mg/ml, 100 mg/ml, 200 mg/ml were investigated for its anthelmintic activity. The result indicates that the 200 mg/ml Extract posses significant anthelmintic activity. Dose dependent effects were observed with 50 mg/ml, 100 mg/ml, and 200 mg/ml. The paralysis time [P] and death time [D] is comparable with standard Piperazine citrate 100 mg/ml.

KEY WORDS: Helminthes infection, chronic illness, in vitro anthelminthic effect, paralysis time, death time.

INTRODUCTION

Helminthiasis is a disease in which a part of the body is infected with worms such as pinworm, roundworm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs. They produce harmful effect on host by depriving him of food, causing blood loss and by secreting toxins. In India, infections with these parasites are regarded as amongst the most common public health problems,
particularly in rural areas and urban slums. Anthelmintics from natural sources could play a key role in the treatment of this parasite.\textsuperscript{[1,2,3]}

Anthelmintics act either locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminthes or developmental forms that invade organs or tissues. Currently used synthetic anthelmintics such as benzimidazoles, piperazine, Diethylcarbamazine citrate, Ivermectin, Levamisole, etc., are suffering from a variety of adverse effects including anorexia, nausea, vomiting, dizziness, diarrhea, occasional fever, rashes etc.\textsuperscript{[4]}

**Epidemiology of Helminth\textsuperscript{[1]}**

Areas with the highest prevalence of helmenthiasis are tropical and subtropical areas including sub-Saharan Africa, central and east Asia, and the America.

Neglected tropical diseases: Some types of helminthiasis are classified as neglected tropical diseases. They include.

2. Roundworm infection such as lymphatic filariases, dracunculiasis, and onchocerciasis.
3. Trematods infection such as schistosomiasis, and food-borne trematodiases including Faescioliasis, clonorchiasis, opisthorchiasis, and paragonimiasis.
4. Tapeworm infection such as cysticerosis, taeniasis, and echinococcisis.

**Geographical distribution**

Soil transmitted helminth infections are widely distributed in tropical and subtropical areas and, since they are linked to a lack of sanitation, occur wherever there is poverty. Latest estimates indicate that more than 880 million children are in need of treatment for these parasites. Helminths larvae is transmitted through contaminated soil in areas where sanitation is poor. WHO control interventions are based on the periodic administration of anthelmintics to groups of people at risk, supported by the need for improvement in sanitation and health education. WHO recommends annual treatment in areas where prevalence rate of soil – transmitted helminthiasis is between 20% and 50% and a biannual treatment in areas with prevalence rates of over 50%.

Soil transmitted helminthiasis and schistosomiasis are the most important helminthiasis, and are among the neglected tropical diseases.\textsuperscript{[1]} This group of helminthiasis have been targeted under the joint action of the world leading pharmaceutical companies and non-governmental
organizations through project launched in 2012 called the London Declaration on Neglected Tropical Diseases, which aims to control or eradicate certain neglected tropical diseases by 2020.[2] Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socioeconomic development, and poverty.[3] Chronic illness, malnutrition and anemia are further examples of secondary effects. Soil transmitted helminthiasis are responsible for parasitic infection in a much as a quarter of the human population worldwide.[5] One well–known example of soil –transmitted helminthiasis is ascariasis.

Classification of Helminths[6]

Two main type of helminh.
1) Platyhelminths (Flatworms).
   a) Cestodes
   b) Trematodes
2) Phylum (Nemathelminthes).
   a) Nematodes ((Roundworms).

Table No. 1 Available Treatments and Side Effects[7]

<table>
<thead>
<tr>
<th>Worm</th>
<th>First choice of drugs</th>
<th>Alternative drugs</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round worm</td>
<td>Mebendazole, albendazole</td>
<td>Piperazine, levamisole</td>
<td>Diarrhoea, nausea, abdominal pain</td>
</tr>
<tr>
<td>Hook worm</td>
<td>Pyrantel, mebendazole.</td>
<td>Levamisole, ivermectin</td>
<td>Dizziness, headache, fever, alopecia, jaundice and neutropenia.</td>
</tr>
<tr>
<td>Filarial</td>
<td>Diethyl-carbamazine, ivermectin</td>
<td>Albendazole.</td>
<td>Headche, Dizziness.</td>
</tr>
<tr>
<td>Tape worm</td>
<td>Praziquantle, niclosamide, praziquantel.</td>
<td>Niclosamide, albendazole</td>
<td>Nausea, abdominal discomfort, urticaria.</td>
</tr>
<tr>
<td>Hydatid disease</td>
<td>Albendazole</td>
<td>Mebendazole.</td>
<td>Loss of appetite, Weakness, pruritus, rash.</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Drugs and Plant Material

The plant material of *Argyreia cuneata* was collected from Karad region of Maharashtra, India. The herbarium was authenticated by Department of Botany, Krishna Mahavidyalaya, Rethare, Karad. The collected fresh leaves and stem were dried in hot air oven below 50ºC.
and when properly dried leaves and stem were powdered by using grinder. The powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature. [8]

**Preparation of Plant Extract**

The powdered plant material was extracted with methanol and aqueous in a Soxhlet apparatus. After complete extraction, the extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary vacuum flask evaporator. A greenish semi-solid extract was obtained & was kept under refrigerator for further use. The methanolic extract and aqueous extract of *Argyreia cuneata* is taken as test drug and used for the evaluation of anthelmintic activity. [8]

**ANTHELMINTIC ACTIVITY**

The entire plant extract of *Argyreia cuneata* were evaluated for anthelmintic activity in *Pheretima posthuma* (earth worm) of nearly equal size. *Pheretima posthuma* is used due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human begins. Because of easy availability of earthworms, they have been used widely for the initial evaluation of the anthelmintic compounds. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into five groups of three earth worms in each and placed in eight petri dishes containing the extract solutions or the reference drugs as mentioned below.

**Group-1**: Received normal saline solution (control)

**Group-2**: Received Piperazine citrate 100 mg/ml (standard)

**Group-3**: Received Methanolic extract in different doses such as,
- Received Methanolic extract at a dose of 50mg/ml
- Received Methanolic extract at a dose of 100mg/ml
- Received Methanolic extract at a dose of 200mg/ml

**Group-4**: Received Aqueous extract in different doses such as,
- Received Aqueous extract at a dose of 50mg/ml
- Received Aqueous extract at a dose of 100mg/ml
- Received Aqueous extract at a dose of 200mg/ml

All Petri dishes were kept under room temperature. The living or viable worms were kept under close observation. Observations were made for time taken to complete paralysis (PT) and death (DT) for individual worms. Each worm was frequently applied with external
stimuli which stimulates and induce movement in earthworms, if alive. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading of the body colour. The motionless worms were then transferred at 40º C to confirm that they were dead.\textsuperscript{[9,10,11,12]}

**OBSERVATION**

1. Earthworm treated with standard Piperazine citrate 100mg/ml (fig.1).

![Figure 1: Anthelmintic activity of 100mg/ml of Piperazine citrate on earthworm.](image1)

2. Earthworm is treated with 50mg/ml, 100mg/ml and 200mg/ml methanolic extract of *Argyreia cuneata* respectively (fig.2).

![Figure 2: Anthelmintic activity of 50mg/ml, 100mg/ml and 200mg/ml of methanolic extract of *Argyreia cuneata* on earthworm respectively.](image2)

3. Earthworm is treated with 50mg/ml, 100mg/ml and 200mg/ml aqueous extract of *Argyreia cuneata* respectively (fig.3).
Figure 3: Anthelmintic activity of 50mg/ml, 100mg/ml and 200mg/ml of aqueous extract of *Argyreia cuneata* on earthworm respectively.

**RESULT**

![Graphical representation for the anthelmintic activity of *Argyreia cuneata* methanolic extract compared to standard drug (Paralysis).](image1)

Figure 4: Graphical representation for the anthelmintic activity of *Argyreia cuneata* methanolic extract compared to standard drug (Paralysis).

![Graphical representation for the anthelmintic activity of *Argyreia cuneata* aqueous extract compared to standard drug (Paralysis).](image2)

Figure 5: Graphical representation for the anthelmintic activity of *Argyreia cuneata* aqueous extract compared to standard drug (Paralysis).
Figure 6: Graphical representation for the anthelmintic activity of *Argyreia cuneata* methanolic extract compared to standard drug (Death).

Figure 7: Graphical representation for the anthelmintic activity of *Argyreia cuneata* aqueous extract compared to standard drug (Death).

Table 2: Anthelmintic Activity of *Argyreia cuneata* entire plant extract in earth worms

<table>
<thead>
<tr>
<th>Name of the group.</th>
<th>Name of the extract.</th>
<th>Concentration. (mg/ml)</th>
<th>Time taken for paralysis. (min)</th>
<th>Time taken for death. (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline solution</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Standard</td>
<td>Piperazine citrate</td>
<td>20</td>
<td>22.18 ± 0.51</td>
<td>59.2 ± 0.72</td>
</tr>
<tr>
<td>Test sample-1</td>
<td>Methanolic extract.</td>
<td>50</td>
<td>27.42 ± 0.5</td>
<td>29.30 ± 0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>9.23 ± 0.8</td>
<td>12.30 ± 0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>5.5 ± 0.32</td>
<td>7.00 ± 0.71</td>
</tr>
<tr>
<td>Test sample -2</td>
<td>Aqueous extract.</td>
<td>50</td>
<td>32.33 ± 0.16</td>
<td>39.22 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>15.50 ± 0.45</td>
<td>25.49 ± 0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>6.52 ± 0.40</td>
<td>10.45 ± 0.46</td>
</tr>
</tbody>
</table>
The anthelmintic activity of entire plant extract of *Argyreia Cuneata* was carried out in earthworm. Different concentrations of the methanolic extracts and aqueous water extract were used for the studies. The time taken for paralysis and death of earthworms were recorded in observation table (Table 2). The methanolic extract at the concentration of 200 mg/ml showed both paralysis and death time in 5.5 and 7 minutes respectively. The effect increased with concentration. The extract caused paralysis followed by death of the worms at all tested dose levels. It was observed that the methanolic extract of *Argyreia cuneata* is more potent than the aqueous extract. The potency of the extract was found inversely proportional to the time taken for paralysis and death of worms.

**DISCUSSION**

Anthelmintic activity was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings.[13] Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic activity of compounds *in-vitro* are available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic compounds was advocated.[1] The predominant effect of Piperazine citrate on the worms is to cause paralysis by increasing chloride ion conductance of worm muscle membrane that produces hyperpolarisation which reduces excitability and flaccid paralysis.[14] The leaves and stem extracts of *Argyreia cuneata* were not only demonstrated paralysis, but also caused death worms. The anthelmintic activity may be attributed to the phytoconsituents present in the plant.

**CONCLUSION**

Methanolic and aqueous extract of *Argyreia cuneata* shows anthelmintic activity on Indian *Pheretima posthuma* (earthworm). But Methanolic extract of *Argyreia cuneata* has more significant *in vitro* anthelmintic activity than the aqueous extract of *Argyreia cuneata*. Hence *Argyreia cuneata* can be used as an anthelmintic purpose.

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