Evaluation of anthelmintic activity of ethanolic extracts of *Carica papaya* leaves using *Paramphistomum cervi* and *Haemonchus contortus*

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The aim of this present study is to evaluate the anthelmintic activity of leaves extracts of *Carica papaya* using *Paramphistomum cervi* and *Haemonchus contortus* as the test worms. A range of concentrations (100, 50 and 25%) of ethanolic extracts of *C. papaya* were tested to assay the procedure. This is mainly applied for the determination of time of death (D) and time of paralysis (P) of the tested worms. After the analysis, it was shown that for the *H. contortus* at 100% concentration, the paralysis occurred within the shortest time (P=24.5 min) and death came at the lowest possible time (D=56 min). The time of death and paralysis increased at 50% (D=64 min and P=28 min) and 25% concentration (D=74 min and P=34 min), respectively compared to the Piperazine citrate (P= 24 min and D= 54) at concentration of 10 mg/ml. Here, distilled water is as a control solution. The results of this study revealed that the ethanolic extracts of the leaves of the *C. papaya* expressed a demonstrated paralysis significantly, and also responsible for the death of *P. cervi* and *H. contortus* especially at the higher concentration (100%) compared to the standard reference of Piperazine citrate. Therefore, from the results it is declared that the ethanolic extracts of the leaves of *C. papaya* showed a great anthelmintic activity against *P. cervi* and *H. contortus* worms. Hence, the present research work signifies that the leaf of *C. papaya* has a major anthelmintic activity and also can be used as a potent drug for its low cost and availability.

**Key words:** *Paramphistomum cervi* and *Haemonchus contortus*, anthelmintic, *Carica papaya* leaves, Albendazole.

**INTRODUCTION**

The word “anthelmintic” is from the Greek word, “anti” which means “against” and “helminis” means “worm” which means “to kill or wipe out worms or parasites”. Anthelmintics are drugs that either kill (vermicide) or
expel (vermifuge infesting helminthes. Parasites have been of concern to the medicinal field for centuries.

Helminthiasis or helminth infection is a parasitic disease of humans and other animals in which a part of the body is infected with parasitic worms (Paramphistomum cervi and Haemonchus contortus). They often live in the gastrointestinal (GIT) of their hosts, but may also stay in other organs. Scientific studies have shown that many plants that are used in the human ethnomedicinal practice showed huge pharmacological activities and can be helpful in ethno-veterinary practice. In human body, GIT is the abode of many helminths, but some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving them of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins (Tripathi, 2003).

Anthelmintics drugs can either expel (vermifuge) or kill (vermicide) the infesting helminths. Vaccinations play a role also in the lungworms. On the other hand, some others problems have come up with the use of the anthelmintics. Resistance also occurred in the helminths (Dhar et al., 1965) to a variety of anthelmintic drugs and classes, and chemical residue and toxicity problems also occurred (Kanthal et al., 2012). Studies have shown that the plant kingdom is widely known to be rich sources of botanical antibacterial, anthelmintics, and insecticides activities (Hounzangbe-Adote et al., 2005). Also, a lot number of medicinal plants have been applied to treat some parasitic infections in animals and man (Stepek et al., 2006).

Carica papaya Linn (caricaceae) is a large, stemmed, singled, herbaceous perennial tree that is cultivated worldwide; it contains huge biologically active compounds, for example: chymopapain and papain. The concentration of these compounds can vary in the latex, fruit, roots and leaves. Additionally, the quantity of the phytochemicals compounds also differs from female and male trees. For example, phenolic compounds are normally found higher in male trees than female trees.

MATERIALS AND METHODS

Test material

Ethanol extract of C. papaya leaves

Test pathogen (Helminth): H. contortus and P. cervi

Leaves collection: The leaves of C. papaya (family Caricaceae) were collected from Savar (Dist., Dhaka, Bangladesh) in January 2019.

Test pathogen collection

The live parasite (H. contortus and P. cervi) was collected from freshly killed cattle at the local abattoirs. After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na2HPO4 and 0.27 g KH2PO4 in 1 L of distilled water at 37±1°C.

Preparation of sample

To prepare the suspension of extract at concentrations of 25, 50 and 100 mg/ml; 0.25, 0.5 and 1 g of extracts were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent. Final volume was made to 10 ml for respective concentration with PBS.

For the preparation of standard albendazole at concentrations of 15 mg/ml; 150 mg of albendazole powder were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration with PBS.

Study design

The anthelmintic activity of ethanolic leaves extract of C. papaya was examined on the live parasites (H. contortus and P. cervi). The anthelmintic assay procedure was carried out by Mali and Manoj method with some minor modifications (Manoj et al., 2009; Mali, 2007). The Indian adult earthworms (H. contortus and P. cervi) of 5 cm in length and 0.3 cm in width were used here. Three different concentrations of 100, 50 and 25% in distilled water (each of 50 ml preparation) were prepared. Also, the standard solution (Albendazole) was poured in the different Petri dishes. The two earthworms were then washed in the normal saline solution. The time period of paralysis was then recorded whilst no movement was seen unless shaken strongly. After that, the death time period was recorded after making sure that the parasite did not move when shaken forcefully, dipped in the warm water (50°C), or set to the external stimuli. The anthelmintic activity was presented as the time required for the paralysis and the death of parasites in contrast to control.

RESULTS AND DISCUSSION

The suspensions of ethanolic extracts of C. papaya were made in Normal Saline using the Tween80 to get 25, 50 and 100% concentration of the solution. The similar concentration of the solution of the reference standard (albendazole drug) was made in normal saline as well. 2 ml of each solution of various ethanolic extracts and standard drug solution were then diluted to 10 ml with normal saline separately and poured in different Petri dishes.

The Petri dishes were then divided into ten separate groups. Group 1 consists of normal saline and group 2 consists of the standard drug (albendazole). Groups 3-10 consist of 8 extracts. Each group contains 25, 50 and 100% concentrations. After that, 2 equal sizes of adult earthworms were released in each concentration. Times were then recorded also at the time of releasing these earthworms in the solution.

The time that is taken in minutes for death and paralysis of these earthworms were then recorded. The anthelmintic activity of the C. papaya was evaluated on the adult earthworm P. cervi and H. contortus due to the
Table 1. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Paralysis time (Min)</th>
<th>Death time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>13.41±0.24*</td>
<td>18.59±0.35*</td>
</tr>
<tr>
<td>Test-I (25 mg/ml)</td>
<td>71.35±0.53*</td>
<td>92.53±0.51*</td>
</tr>
<tr>
<td>Test-II (50 mg/ml)</td>
<td>40.98±1.06*</td>
<td>66.71±0.63*</td>
</tr>
<tr>
<td>Test-III (100 mg/ml)</td>
<td>21.59±0.61*</td>
<td>31.88±0.41*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean (n = 6)
* indicates P<0.05 when compared with control.

Figure 1. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

Anthelmintic activity against *H. contortus*

The data revealed that the ethanolic extract of *C. papaya* leaves showed dose dependent anthelmintic activity. The effect was maximum at a concentration of 100 mg/ml, whereas the other test concentrations showed marked degree of anthelmintic activity (Table 1 and Figure 1).

Anthelmintic activity against *P. cervi*

The data revealed that the ethanolic extract of *C. papaya* leaves showed dose dependent anthelmintic activity.
Table 2. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Paralysis time (Min)</th>
<th>Death time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Standard</td>
<td>7.79±0.31*</td>
<td>13.8±0.46*</td>
</tr>
<tr>
<td>Test-I (25 mg/ml)</td>
<td>27.45±0.65*</td>
<td>38.64±0.61*</td>
</tr>
<tr>
<td>Test-II (50 mg/ml)</td>
<td>17.14±0.71*</td>
<td>25.26±0.62*</td>
</tr>
<tr>
<td>Test-III (100 mg/ml)</td>
<td>12.11±0.58*</td>
<td>17.11±0.69*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean (n = 6).

* indicates P<0.05 when compared with control.

Figure 2. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

against *P. cervi*. The effect was highest at a concentration of 100 mg/ml, whereas the other test concentrations showed marked degree of anthelmintic activity (Table 2 and Figures 1 and 2).

**Conclusion**

From the end result, it is said that the leaves of *C. papaya* give a clear idea about major anthelmintic activity compared with standard anthelmintic drug (Albendazole). This drug should be explored further for phytochemical profile to recognize the active constituents responsible for the anthelmintic activity.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

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