Vol. 13(12), pp. 146-150, 8 July, 2019
DOI: 10.5897/AJPP2019.5017
Article Number: FFB0C3F61356
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

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

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Received 19 February, 2019; Accepted 27 May, 2019

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)

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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

Ethanolic 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 Na_2HPO_4 and 0.27 g KH_2PO_4 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 (albandazole). 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.*

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

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

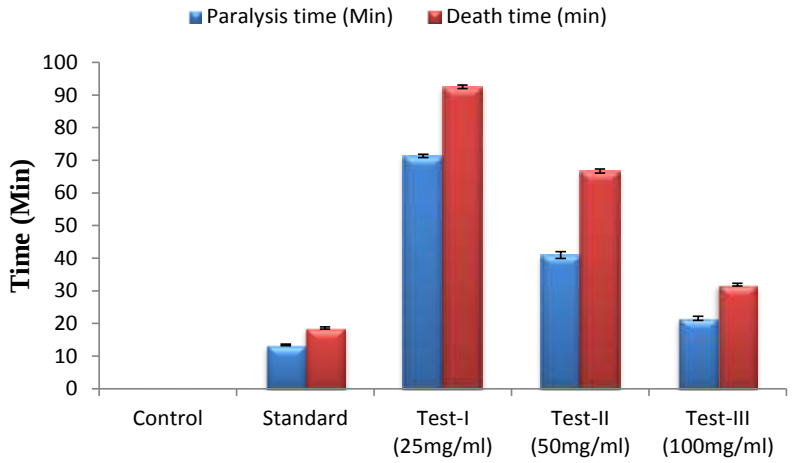

Figure 1. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus.*

resemblance of its physiological and anatomical characteristics with human beings. Paralysis occurred while the worm did not exist in normal saline. Therefore, death was recorded when the worms lost their mobility followed by the fading of body color. As seen in Table 1, the ethanolic extracts of *C. papaya* exhibits the anthelmintic activity in the very dose dependent manner in taking the shortest time for the paralysis (P) and death (D) with highest concentration (100%). Hence, the ethanolic extracts of *C. papaya* exhibited different percentage of anthelmintic activities at different concentrations. It shows the shortest time of paralysis (P=21.59 min) and death (D= 31.88 min) in 100% concentration against *H. contortus*, while the time increased for 50% of concentration (P= 40.98 min and D= 66.71 min) and for 25% of concentration (P= 71.35 min and D=92.53 min) respectively. The standard solution (albendazole) took the very short time (P= 13.41 min and D= 18.59). Anthelmintic activity of ethanolic extract of *C. papaya* leaves against *P. cervi* is also similar to the effect against *H. contortus.* For 100% solution, the effect is highest {(P=12.11 min) and death (D= 17.11 min)} and for 50% solution the effect is comparatively slow {(P= 17.14 min) and death (D= 25.26 min)}. At the 25% solution the effect is very low {(P= 27.45 min) and death (D= 38.64 min)} as compared to the standard solution {(P= 7.79 min) and death (D= 13.8 min)}. The effect of albendazole on the worm is due to the flaccid paralysis in expulsion of the worm by peristalsis. Thus, ethanolic extact of *C. papaya* showed a significant anthelmintic activity as compared to the standard reference (albendazole) and control (distilled water).

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.*

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

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

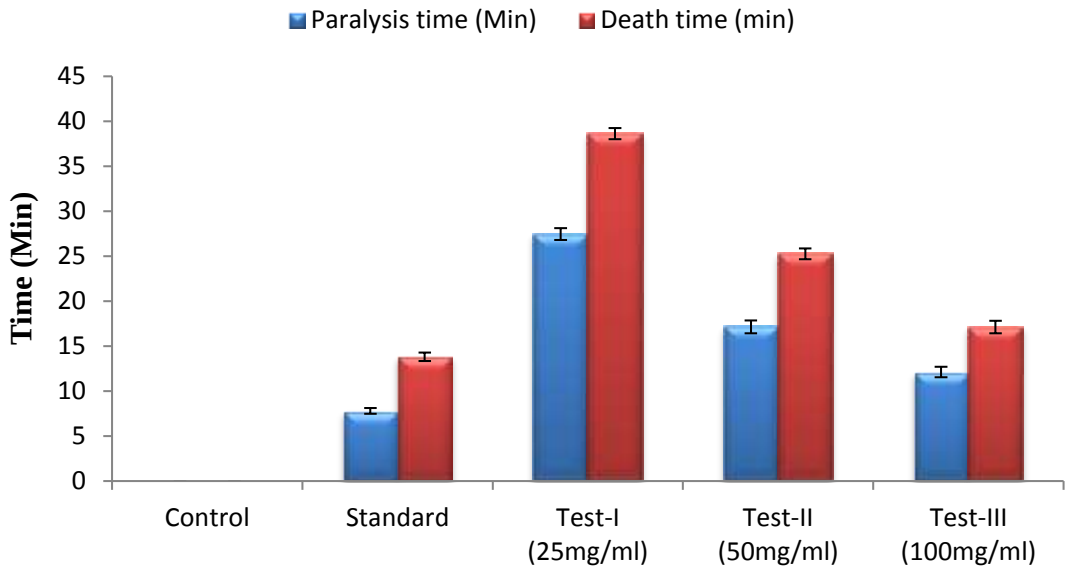

Figure 2. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus.*

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

The authors are really thankful to head of Pharmacy Department of University of Asia Pacific, Dhaka, Bangladesh, for providing the superb facility to carry out this research work. The authors are also grateful to Dr, Anisur Rahman, Associate Professor, Khulna university of Pharmacy discipline, for his great guidance as an expert to do this work.

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