A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2010; 5: 45-47**
Journal homepage: www.banglajol.info; www.bdjpharmacol.com
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded and Social Sciences Citation Index
ISSN: 1991-007X (Print); 1991-0088 (Online); DOI: 10.3329/bjp.v5i1.5003

In vitro study of antiamoebic effect of methanol extract of mature seeds of *Carica papaya* on trophozoites of *Entamoeba histolytica*

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Article Info

Received: 11 May 2010
Accepted: 12 June 2010
Available Online: 28 June 2010
Keywords:
Amoebiasis
Entamoeba histolytica
Carica papaya
Number of Figure: 1
Number of Refs: 8
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Abstract

Antiamoebic activity of methanol extract of mature seeds of *Carica papaya* was tested *in vitro* on axenic culture of *Entamoeba histolytica* using metronidazole as a reference amoebicidal agent. The MIC of seed extract was > 62.5 µg/mL as compared to < 0.8 µg/mL for metronidazole. The present study suggests that the mature seeds of *C. papaya* have antiamoebic effect but less pronounced than metronidazole.

Introduction

A large number of populations in Bangladesh are suffered from amoebiasis caused by *Entamoeba histolytica*. Only limited numbers of drugs are available for the treatment of amoebiasis. Among them metronidazole is used for many years. But indiscriminate use may cause drug resistance in future (Bansal et al., 2006). To overcome this problem we have to find out more antiamoebic drugs. Plants may be a good source. The antiamoebic effect of *Carica papaya* (seed), *Mangifera indica* (stem bark) and marine sponge *Haliclona exigua* have been reported elsewhere (Tona et al., 1998; Calzada et al., 2006; Lakshmi et al., 2009). The minimum inhibitory concentration (MIC) values of *C. papaya* were conflicting. Therefore the present study was conducted to evaluate the effectiveness of mature seeds of papaya against *E. histolytica*, using metronidazole as a reference standard.

Materials and Methods

Plant materials: Dried mature seeds of *C. papaya* were collected from Manikgonj in November, 2006. The plants were identified at Bangladesh National Herbarium, Dhaka where a voucher specimen has been deposited (DACB accession number was 31374) for this collection.

Preparation of extract: Dried papaya seeds were first ground to a coarse powder and then methanol extract was prepared by macerating powered seeds in methanol for a week. Then the slurry was first filtered and solvent was evaporated under reduced pressure by using a rotary evaporator. Then the extract thus obtained was stored until *in vitro* antiamoebic test.

Culture of E. histolytica: DS 4 868 strain of *E. histolytica* was used in the present study. Here, established axenic culture of *E. histolytica* and LYI-S-2 culture media was

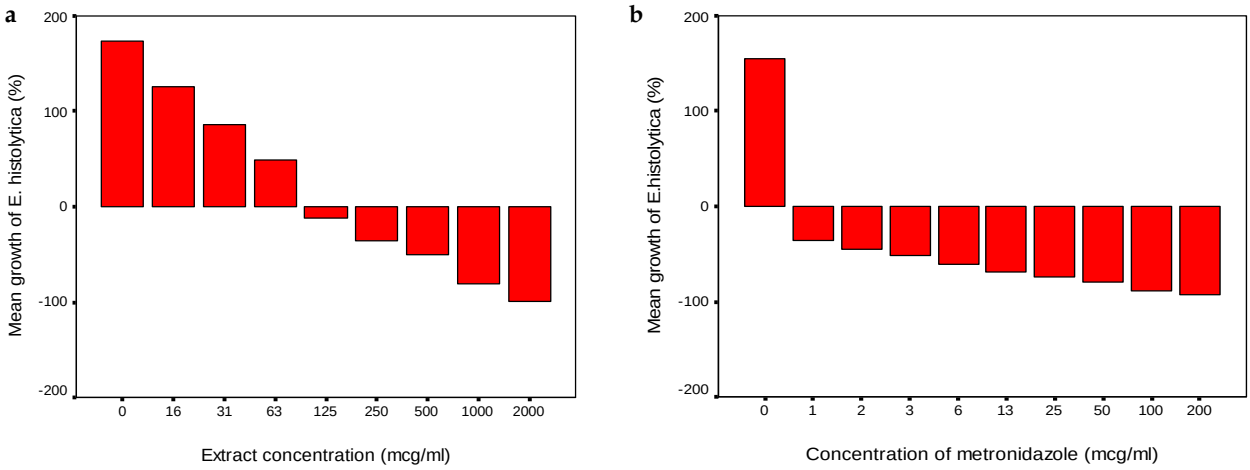

Figure 1: Mean growth of *E. histolytica* in presence of extract of *C. papaya* (a) and metronidazole (b)

kindly provided by Dr. Rashidul Haque, Head of Parasitology Laboratory, ICDDR,B Dhaka.

Serial dilutions: Serial dilutions of stock solution in culture medium were conducted in 24 well culture plates. For the extract, serial dilutions were conducted to get 2000 to 15.63 µg/mL and for metronidazole solutions were made from 200 to 0.8 µg/mL.

Trials of the study: Several trials were conducted for the study. Each trial included blank (only culture medium), control (culture medium and *E. histolytica*) and test (extract or metronidazole, culture medium and *E. histolytica*).

Count of E. histolytica: After incubation for 48 hours at 37^0C viable trophozoites of *E. histolytica* were counted with the aid of Neubauer's cell of Hemocytometer.

Results

Antiamoebic activity of mature seeds of *C. papaya* was investigated under different experimental conditions for determination of MIC. The MIC is considered as the gold standard for determining the antiamoebic test *in vitro* (Andrew, 2001). In this study MIC was defined as the lowest concentration of seed extract and metronidazole that inhibited the growth of *E. histolytica* after 48 hours incubation.

All the trials of *C. papaya* suggested that the methanol extract of mature seeds of *C. papaya* had antiamoebic effect on trophozoites of *E. histolytica* and the MIC value was >62.5 µg/mL ($p< 0.001$). Figure 1a shows the statistical analyses of the extract study. It shows the mean growth of *E. histolytica*. In this study concentrations of extract were 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.63 µg/mL. It was evident that the growth of organism occurred at the extract concentration of 0 to 63 µg/ml where 0 µg/mL was the control and growth was inhibited at the extract concentration of 125 to 2,000 µg/mL. Therefore, the MIC was > 62.5 but < 125 mg/mL.

Figure 1b shows the statistical analyses of the metronidazole study. It shows the mean growth of *E. histolytica*. In this study concentrations of metronidazole were 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 µg/mL. It was evident that growth of organism was inhibited at all concentrations, except 0 µg/mL which was the control. In control there was increased count of organism compared with initial count. But growth of organism was inhibited by all the concentrations of metronidazole, which was also compared with initial count. Therefore, the MIC was < 0.8 µg/mL.

Discussion

In this study, the methanol extract of mature seeds of *C. papaya* was tested for its antiamoebic activity. Axenic culture of *E. histolytica* by LYI-S-2 medium is a newer procedure, which involving gradual adaptation of the parasite to a new way of life (Clark and Diamond, 2002). During the study several trials were conducted but only few trials were accepted. Others failed due to contamination of axenic culture. For this study only non -contaminated axenic culture was used.

Previous study reported the MIC value of aqueous extract of mature seeds of *C. papaya* was <7.81 µg/mL and that of immature seeds was 62.5 µg/mL and MIC value of metronidazole was <2.5 µg/mL (Tona et al., 1998) and the IC_{50} value of *C. papaya* seeds was 153 µg/ml, while that of metronidazole was 0.04 µg/mL (Calzada et al., 2006).

The result of the present study is consistent with the findings observed by previous study but difference in MIC value which may be due to difference in variety of papaya seeds, full maturity of seeds, time of seed

collection, extraction procedure, Strain of *E. histolytica*, culture media or incubation period. *C. papaya* contains many biologically active compounds in its different parts. Among the compound carpaine and benzyliso-thiocyanate found mainly in seeds (Krishna et al., 2008). Antiamoebic actvity of papaya seeds may be occured due to these compounds but further study is required for validation. From the study it was also observed that metronidazole is still sensitive to *E. histolytica* and exhibits greater amoebicidal activity as compared with the papaya seeds. This may be due to crude nature of the test extract. Drug resistance against metronidazole is still not established but it may develop near future so continuous study is required for herbal extracts and metronidazole.

In conclusion, the present study suggests that metronidazole is more potent than the methanol extract of C. *papaya*.

Acknowledgement

The authors are grateful to Dr. Rashidul Haque, Head of Parasitology Laboratory, ICDDR,B Dhaka for providing lab facilities to carry out the research.

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