

Antiparasitic activity of Natural Plant *Carica papaya* Seed Extract against Gastrointestinal Parasite *Entamoeba histolytica*

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ABSTRACT: The principal objective for our study was to evaluate the efficacy of *Carica papaya* seeds against *Entamoeba histolytica* infection compared with metronidazole by using mice models. A single oral dose of *Caricapapaya* seeds water extracts of up to (0.1ml /mice/day) reduced parasite appearance in feces and disappear completely at the 8th day compared with metronidazole the cysts parasite continued till 10th day. The histomorphological study findings showed in the group which was given *Carica papaya* seeds the majority of tissue intestine return to normal shape. These findings indicated that *Carica papaya* seeds may be effective in treating *Entamoeba histolytica*.

KEYWORDS: *Entamoeba histolytica*, *Carica papaya*, seeds water extract, metronidazole, intestinal tissue.

1 INTRODUCTION

Amoebiasis is caused by *Entamoeba histolytica*, a protozoan parasite of humans and the causative agent of intestinal amoebiasis. This disease is a major health problem in developing countries [1]. Although it is asymptomatic in 90% of cases, about 50 million people are estimated to suffer from symptoms associated with amoebiasis, such as hemorrhagic colitis and amoebic liver abscess [2]. Several means of transmitting *E. histolytica* are known, ingestion of the infective cyst occur in contaminated food and water and it may also be transferred via homosexual men [3].

In developing countries, medicinal plants are popular because their products are safe and widely available at low cost. Some compounds extracted from medicinal plants already play an important role against infectious disease [4]. *Carica papaya* Linn. (family: Caricaceae) is a tropical tree which is native to the tropics of the Americas but now widely cultivated in other tropical regions of the world [5]. Previous studies on biological activities of *Carica papaya* parts, extracts and isolated compounds showed that the latex and root extracts inhibited *Candida albicans* while extracts of pulp and seeds showed bacteriostatic properties against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Entamoeba histolytica* [6], its root aqueous extract has equally been shown to have purgative effect [7]. Also these parts are known to be analgesic, cardiogenic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifugic [8].

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 [9]. The most frequently reported side effects of metronidazole are vaginal discharge, symptomatic candidiasis, and vulvovaginal irritation; following oral metronidazole therapy, the side effects are gastrointestinal disorders, nausea, and metallic taste [10]. This study aimed to compare the therapeutic effects of *C.papaya* seeds extract with metronidazole on *E. histolytica*.

2 MATERIALS AND METHODS

2.1 FECES SAMPLES

This study included the collection of 60 stool samples from people suffering from diarrhea and samples were collected from various areas of civil laboratories in Baghdad. Has slides were prepared by direct smear and after confirming the presence of *E. histolytic* at the samples behaved. The refrigerated and transported to the laboratory at university of AL-Mustansiriya.

2.2 PARASITIC PURIFICATION

Robert and Thombson [11] method was used to isolate the Parasite then (cysts & Trophozoites) were suspended in phosphate buffer saline (PBS-7.2) and the final concentration was attended by rate 1×10^3 celles/0.1 ml.

2.3 PREPARATION OF THE C.PAPAYA SEEDS EXTRACT

The seeds were collected freshly from the market of Maskat / Oman, Fig(1). Washed with clean water to remove dirt. The seeds were sundried and later grinded into powdery forms. The *C.papaya* seed powders were weighed (50g), and blended into liquefaction in 120ml of distilled water, the mixture was then centrifuged at 1500rpm. The supernatant was filtered through sterile filter papers into conical flask as the study extract. One milliliter of the filtrate is expected to contain 0.3g (300mg/ml).



Fig. 1. Dried seeds of *C.papaya* collected freshly from the market of Maskat / Oman

2.4 ANIMALS

In this experimental used male Swiss albino mouse about 14-15 weeks old, weighing 15-20g were obtained from the Animal house in college of medicine Baghdad University were housed under standard condition. Has feces of mice were examined to ensure that they are free from parasitic infection before starting the experiment. Then the mice were immunosuppressed by which injection with (0.01 ml/ mice/ day) with dexamethasone according to Regh [12].

2.5 EXPERIMENTAL DESIGN

Thirty mouse was inoculated orally with (0.1ml) contain 1×10^3 cell from *E. histolytic*, day after dosing each mice were examined prepare direct smear and after confirmation of infection get infected into four groups and each group of (10) mice was inoculated as follow:

Group 1: Given orally 0.1 from papay extraction once a day [13].

Group 2: Given (0.1 ml) of metronidazole (30mg/kg/day) orally at a single dose per day.

Group 3: Given only (0.1 ml) of normal saline and promised as appositive group.

Group 4: This group intact mice and non-infected with parasite and were given orally (0.1 ml) of PBS pH 7.2 and promised as a negative control group.

2.6 ENUMERATION OF *E.HISTOLYTICA*

Cysts & trophozoite in faces were enumerated as Shukla *et al* [14]. Briefly mice feces were collected first three groups daily from each mouse, one gram of fecal sample was dissolved in 10 ml of normal saline, homogenized then counted every day by suing hemocytometer.

2.7 HISTOPATHOLOGICAL STUDY

After the end of experimental period the mice in [G1; G2 G3 and G4] were sacrificed and small intestine was removed fixed in 10% buffered formalin processed stained with hematoxylin and eosin for study histopathological changes.

2.8 STATISTICAL ANALYSIS

Data are reported as mean \pm standard deviation and the inter group variation performed by t-test.

3 RESULT AND DISCUSSION

In this study we compare the efficacy of the *Carica papaya* seed extract with metronidazole in mice with acute cause by the results in fig(2) . Showed that the numbers of *Entamoeba histolytica* in G1 was reach to 2.66 cyst/gm at first day ,not like in positive control G3 3.66 cyst/gm while reach to 5.33 and 4 cyst/gm at second and third day respectively but at the 5th day the number of parasite was similar to the first day then start to decrease in 5th and 6th days till reach zero in the 8th day ,in compare with G3 that reach to 14.33 cyst/gm in 7th day and decrease to 12 cyst /gm in 14th day ,moreover when we compare the results G2 we saw increasing number of parasite on days (2,3,4,5) then start decrease in days (6,7,8) till reach zero at 10th day.

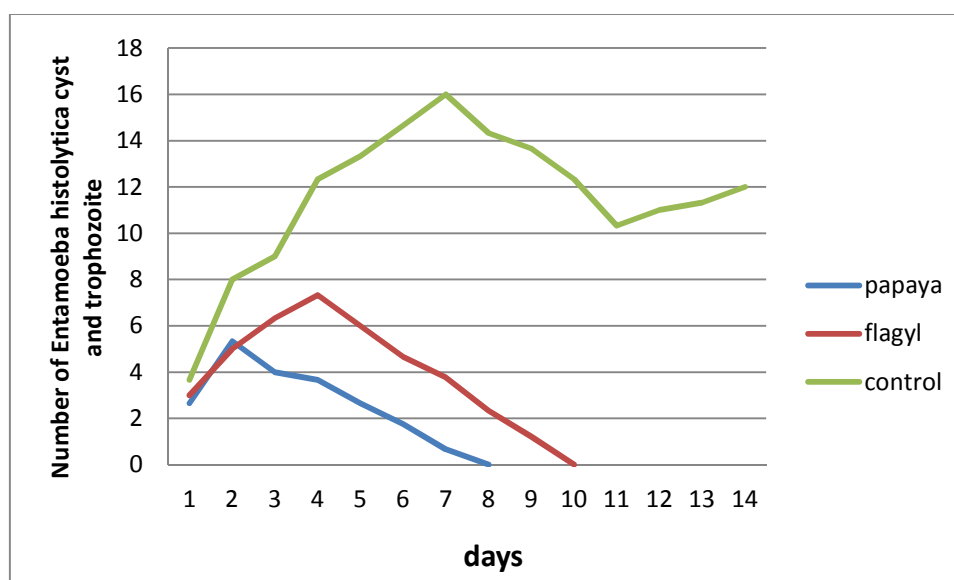


Fig. 2. Number of *Entamoeba histolytica* parasites in treatment and control groups.

The histological study showed that *C.papaya* plant was able to re-structure of intestinal tissue Fig (5) has been able to bring along the length of the villa to normal when compared with control mice Fig (3), after it suffered from the palace because of parasite which lead to causes necrosis in tissue intestine Fig (4).

Also the metronidazole drug reappeared the tissue but not completely still some of some necrosis and infiltration of lymphocytes fig (6).

The aqueous extracts administered in this study caused significant reduction the parasite burden of the mice. the reduction of parasite loads observed with aqueous extracts of *C.papaya* may be attributed to the presence of papain in capable of digesting bacteria and parasitic cells, hence its use an anthelmintic and antibiotics[15]. Adu *et al* [16] reported that *C. papaya* has certain chemical components that are of high anthelmintic attributes in poultry with satisfactory efficacy at the dosage of 1200mg / bird.

Sarker *et al* [17] reported that the mature seeds of *C. papaya* have antiamebic effect but less pronounced than metronidazole. The mechanism of action of the efficacious plant cysteine proteinases (papaya) is similar and probably identical involving digesting and removal of the cuticle.

Our results support previous studies [18] suggesting that papaya may have potential as an anthelmintic against nematode parasites and too define the mechanisms of its antiparasitic action.

Hiramoto *et al* [19] reported that the oral administration of the fermented papaya preparation to mice may have a therapeutic potential for the prevention of contact hypersensitive immuno-response, also Melissa *et al* [20] found no morphological alteration in the integrity of the intestine mucosa after 3months administrated for Wister rats.

The use of plant material for the management of nematode population is apparently effective and environmentally friendly compared to synthetic nematicides. The tropical fruit *Carica papaya* and its seeds have proven antihelminthic and anti-amoebic activities. To determine the effectiveness of air-dried *C. papaya* seeds on human intestinal parasitosis, 60 asymptomatic Nigerian children with stool microscopic evidence of intestinal parasites received immediate doses (20 mL) of either an elixir composed with air-dried *C. papaya* seeds and honey (CPH) or honey alone (placebo) in two randomized treatment groups, there were no harmful effects and the stool clearance rate for the various types of parasites encountered was between 71.4% and 100% following CPH elixir treatment compared with 0-15.4% with honey. Thus, air-dried *C. papaya* seeds are efficacious in treating human intestinal parasites and without significant side effects. Their consumption offers a cheap, natural, harmless, readily available mono therapy and preventive strategy against intestinal parasitosis, especially in tropical communities. Further and large-scale intervention studies to compare *C. papaya* with standard antiparasitic preparation are desirable [21].Sujit *et al* [22] suggests that the mature seeds of *C. papays* have antiamebic effect but less pronounced than meteronidazole.*C papay* contain many biologically active compounds in its different parts, a many the compound carpaine and benzylisothiocyanate found mainly in seeds, antiamebic activity's of papay a seeds may be occurred due to these compounds but further study is required for validation form the study.

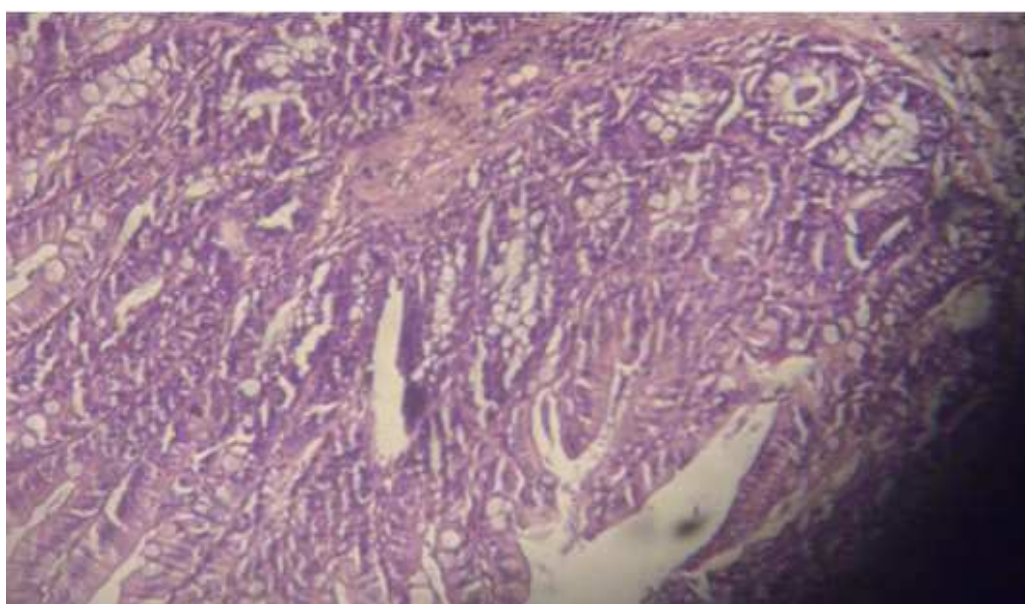


Fig. 3. Normal tissue structure for control mice intestine.4x

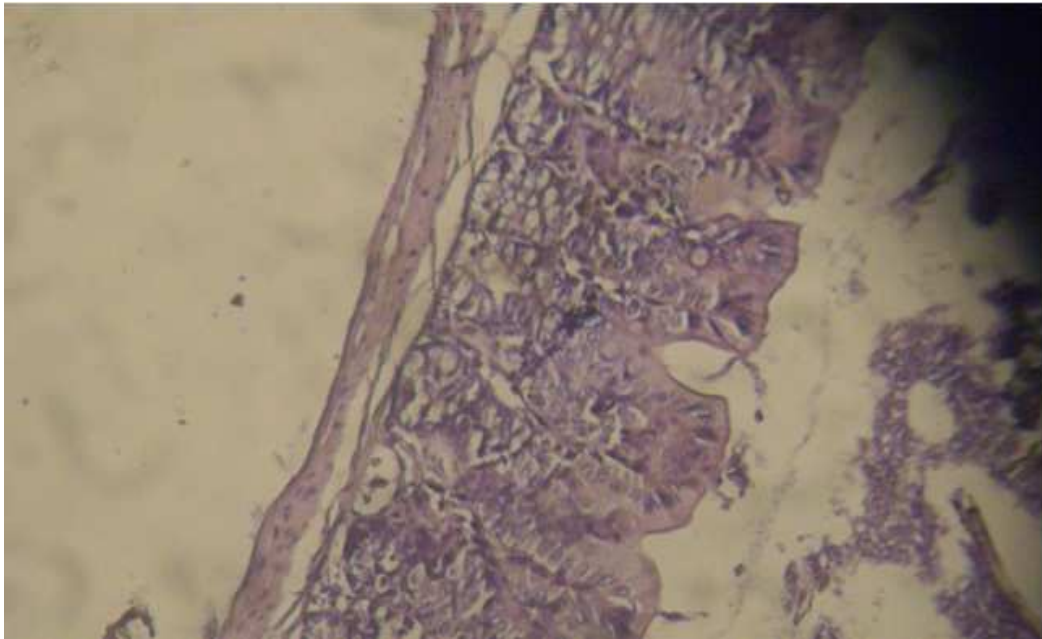


Fig. 4. *Necrosis accrue in tissue intestine which a results of parasites infection (control positive).4x*

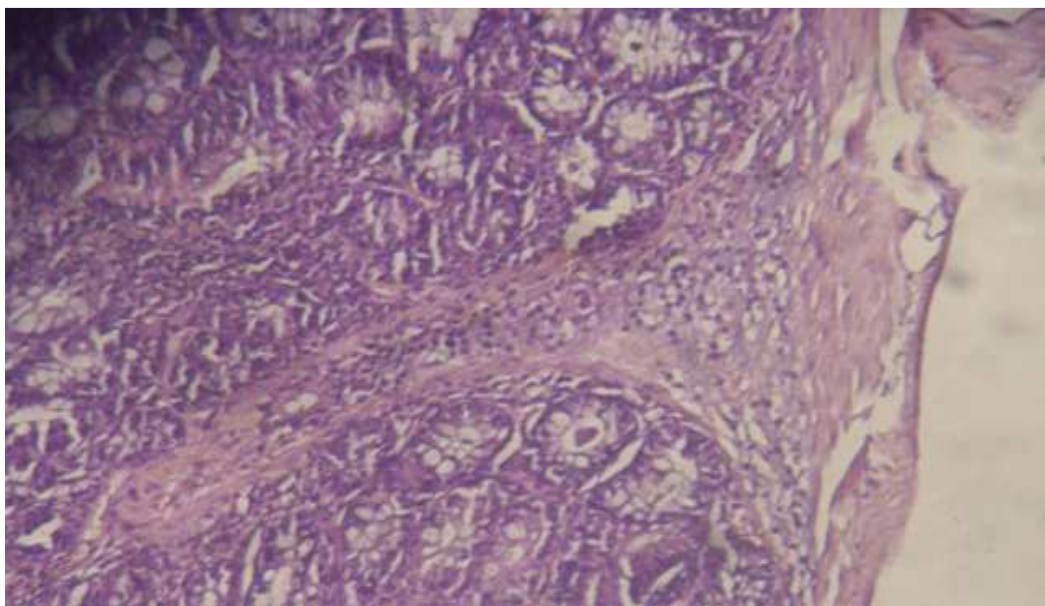


Fig. 5. *Retune the intestinal tissue to normal structure after treated with C.papaya.4x*

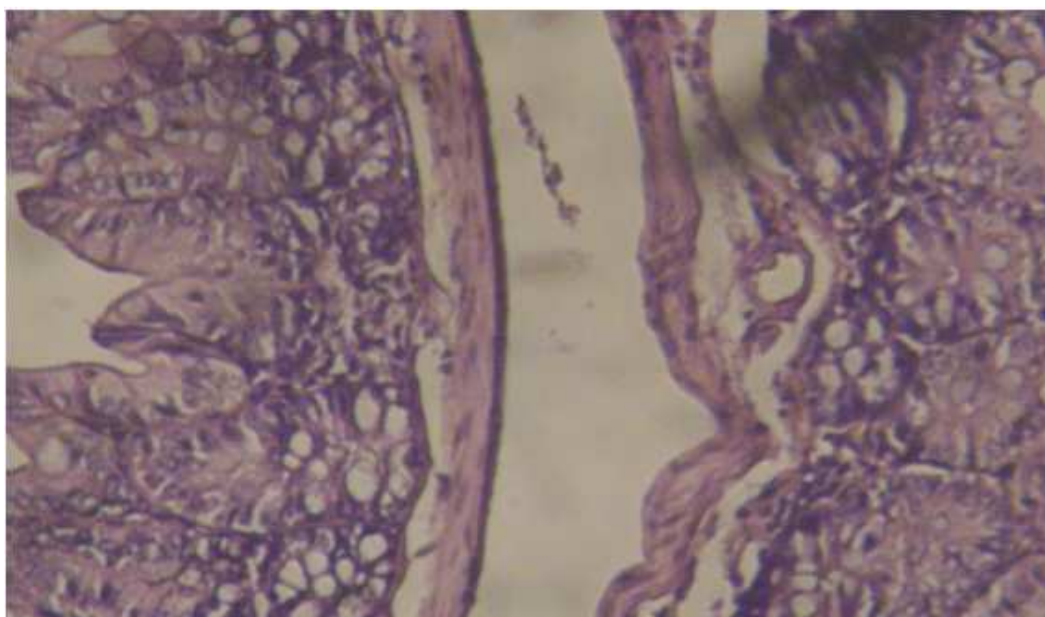


Fig. 6. Still of some necrosis and infiltration of lymphocytes in tissue intestine of mice treated with metronidazole. 4x

4 CONCLUSIONS

We conclude that *C. papaya* seeds could be beneficial in the treatment and plausibly prevention of intestinal parasites, more so being readily available, harmless, and cheap. We therefore recommend their consumption for not only for their nutritive but their medicinal value.

5 RECOMMENDATION

- 1-Study the effect of *C. papaya* seeds on *E.histolytica* In vitro .
- 2- Study the effect of immature *C. papaya* seeds on the parasites.
- 3-Try to extract active materials and use it.
- 4-study the effect of fruit,leaf,and roots for *C. papaya* .

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