Efficacy of different concentrations of seed extract of Carica papaya against Clinical Isolates of *Entamoeba histolytica*

Alisha Ahmed
ID: 2006-1-70-001
Department of Pharmacy
East West University

December 2009

Efficacy of different concentrations of seed extract of Carica papaya against Clinical Isolates of *Entamoeba histolytica*

A Thesis paper submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy

A Collaborative study between Department of Pharmacy, East West University and Parasitology Laboratory, International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B)

Certificate

This is to certify that the thesis ''Efficacy of different concentrations of seed extract of Carica papaya against clinical Isolates of *Entamoeba histolytica*'' submitted to the Department of Pharmacy,EastWest University,Mohakhali,Dhaka in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy(B.Pharm) was carried out by Alisha Ahmed (ID:2006-1-70-001) under our guidance and supervision and that no part of our thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of this connection is duly acknowledged.

Sufia Islam 23.12.2009

SUFIA ISLAM, Ph D
Supervisor
Associate Professor
Dept. of Pharmacy
East West University
Mohakhali,Dhaka

Farhana Rizwan 24.12.09

FARHANA RIZWAN
Co-supervisor
Lecturer
Dept. of Pharmacy
East West University
Mohakhali,Dhaka

Dr. CHOWDHURY FAIZ HOSSAIN
Chairperson
Department of Pharmacy
East West University
Mohakhali,Dhaka

List of Tables

Table 1..15

Table 2.. 21

Table 3..23

Table 4..32

List of figures

Figure 1……………………………………………………………6

Figure 2……………………………………………………………13

Figure 3……………………………………………………………30

Figure 4……………………………………………………………51

Figure 5……………………………………………………………51

Figure 6……………………………………………………………53

Figure 7……………………………………………………………54

Figure 8……………………………………………………………55

Figure 9……………………………………………………………56

Figure 10…………………………………………………………..57

Figure 11…………………………………………………………..58

List of flow charts

Flow chart 1..**7**

Flow chart 2..**8**

Flow chart 3..**12**

LIST OF CONTENTS

LIST OF TABLES..i

LIST OF FIGURES..ii

LIST OF FLOW CHARTS..iii

ACKNOWLEDGEMENTS...1

ABSTRACT...2

CHAPTER 1: INTRODUCTION

1.1. Amoebiasis..5
1.1.1Diagnosis..6
1.1.2. Symptoms of Amoebiasis..9
1.1.3. Nature of the disease...10
1.1.4. Food analysis...11
1.2. *E. histolytica*..12
1.2.1. Geographic distribution..12
1.2.2. Infection rate..13
1.2.3. Classification..14
1.2.4. Pathogenesis..16
1.2.5. Multiplication and life cycle...18
1.2.6. Epidermology...18
1.3. Treatment of Amebiasis...18
1.3.1. Treatment with plant extract...19
1.3.2. Carica Papaya..19
1.3.3. Composition..20
1.3.4. Classification...21
1.3.5. The plant and fruit structure... 22
1.3.6. Nutritional value..22
1.3.7. Medicinal value..23
1.3.8. Uses...24
1.3.9. Other uses of carica papaya..26

1.3.9.1. Nutritional supplement...26

1.3.9.2. Antimicrobial properties...27
1.3.9.3. Sperm concentration...27
1.3.9.4. Nematode infection...27
1.3.9.5. Abortifacient and contraceptive properties.......................................27
1.3.9.6. Topical use...28
1.3.9.7. Healing properties...28
1.3.9.8. Antifungal properties...28
1.3.9.9. Anti-asthma...29
1.3.9.10. Antiamoebic...29
1.3.9.11. Digestive properties...29
1.3.9.12. Molluscicidal activity...29
1.3.9.13. Protective effect...29
1.3.9.14. Tocolytic activity...30
1.3.9.15. Anti-inflammatory...30
1.3.9.16. Modulation of jejuna contraction.......................................30
1.3.10. Parts of the papaya tree with different medicinal value.......................30
1.3.10.1. Fruit...31
1.3.10.2. Skin...31
1.3.10.3. Root...31
1.3.10.4. Flowers...31
1.3.10.5. Leaf...31
1.3.10.6. Seeds...32
1.3.10.7. Latex...32

CHAPTER 2

2.1Aim of the Study……………………………………………………………..….34

2.2 Significance of the Study……………………………………….…………35

CHAPTER 3: MATERIALS AND METHODS

3.1. Materials

3.1.1. Research design……………………………………………………………37

3.1.2. Preparation of seed extract of Carica papaya………………………………37

3.1.3. Collection of Clinical isolates………………………………………………37

3.2. Methods…………………………………………………………………………38

3.2.1. Xenic Culture Media…………………………………………………………38

3.2.2. Preparation of rice starch……………………………………………………39

3.2.3. Diphasic media…………………………………………………………….…39

3.2.4. Axenic Culture Media…………………………………………………………41

3.2.5. Establishment of cultures……………………………………………………42

3.2.6. Elimination of unwanted organisms……………………………………...43

3.2.7. Principles of inducing encystment of *E. histolytica*……………………....*47*

3.2.8. Rexenization of axenically cultivated *E. histolytica*……….……………*48*

3.2.9. Maintenance of cultures………………………………………………….49

3.3. Measurement of amoebicidal activity………………………………………50

CHAPTER 4: RESULTS 52

CHAPTER 5: DISCUSSION...62

CHAPTER 6: CONCLUSION...65

REFERENCES

Acknowledgement

This thesis arose in part out of research that has been done since last year. By that time, I have worked with a great number of people whose contribution in assorted ways to the research and the making of the thesis deserved special mention. It is a pleasure to convey my gratitude to them all in my humble acknowledgment.

I gratefully acknowledge Dr. Sufia Islam for her advice, supervision, and crucial contribution, which made her a backbone of this research and so to this thesis.

I would also like to pay my sincere gratitude to Ms Farhana Rizwan, Sr lecturer, Department of pharmacy. Her involvement with her originality has triggered and nourished my intellectual maturity that I will benefit from, for a long time to come.

I would also like to express my gratitude to Dr. Rashidul Haque, Scientist and Head, Parasitology Laboratory at, International Centre for Diarrhoeal Disease research,Bangladesh (ICDDR, B), for giving me the opportunity to work in his laboratory and for his overall support. I convey special acknowledgement to Md. Abdullah Siddique, for his friendly assistance in the ICDDR, B laboratory during this research project.

My special thanks go to Dr.Chowdhury Faiz hossain, Chairperson and Professor, Department of Pharmacy; for his inspiration in my study.

I am gratefully acknowledged to Prof. Dr. Muniruddin Ahmed as he has made available his support in every possible ways.

My parents, sibilings and my dear friends deserve special mention for their inseparable support and prayers. Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

THIS THESIS PAPER IS DEDICATED TO MY MOTHER, RASHEADA NASSER WASSAY

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

Abstract

Amoebiasis is a major public health problem throughout the world. Almost Ten percent people of the world suffer from amoebiasis. The causative protozoan parasite, *Entamoeba histolytica*, is a potent pathogen, responsible for substantial morbidity and mortality in developing countries. It occurs mostly in tropics and subtropics. Poverty, ignorance, overcrowding, poor sanitation and malnutrition favor transmission and increased disease burden. Prevalence varies country to country and within a country.

The present study was carried out to find out the efficacy of Carica papaya seed extract against clinical isolates of *Entamoeba histolytica*.

The clinical isolates of *Entamoeba histolytica* was collected from the stool and cultured in culture medium. The concentrations of Carica papya seed extract were 3.954mg/ml, 1.972mg/ml, 0.986mg/ml, 0.493mg/ml, 0.216mg/ml, 0.108mg/ml and 0.054mg/ml. The incubation periods were 24 hours and 48 hours with different concentrations of clinical isolates of *Entamoeba histolytica.* The presence of viable and non viable *Entamoeba histolytica* was counted in each concentration of Carica papaya seed extract after incubation.

The results show that the Carica papaya seed extract of different concentrations (3.95mg/ml, 1.97mg/ml and 0.98mg/ml) are highly sensitive to the clinical isolates of *Entamoeba histolytica*. The rate of inhibition of *Entamoeba histolytica* was 100% after 48 hours of incubation.

It can be concluded that seed extract of Carica papaya in different concentrations (3.95mg/ml, 1.97mg/ml and 0.98mg/ml) can inhibit the clinical isolates of *Entamoeba histolytica*.

CHAPTER-1
INTRODUCTION

INTRODUCTION

Amebiasis is defined as an infection with *Entamoeba histolytica*, regardless of associated symptomatology. It is the second leading cause of death from parasitic disease worldwide. The causative protozoan parasite, *Entamoeba histolytica*, is a potent pathogen. Secreting proteinases that dissolve host tissues, killing host cells on contact, and engulfing red blood cells, *E. histolytica* trophozoites invade the intestinal mucosa, causing amoebic colitis. In some cases amoebas breach the mucosal barrier and travel through the portal circulation to the liver, where they cause abscesses consisting of a few *E. histolytica* trophozoites surrounding dead and dying hepatocytes and liquefied cellular debris. New models of disease have linked *E. histolytica* induction of intestinal inflammation and hepatocyte programmed cell death to the pathogenesis of amoebic colitis and amoebic liver abscess. (Stanley, 2003).

In resource-rich nations, this parasitic protozoan is seen primarily in travelers to and emigrants from endemic areas. Infections range from asymptomatic colonization to amebic colitis and life-threatening abscesses. Importantly, disease may occur months to years after exposure. Although *E. histolytica* was previously thought to infect ten percent of the world's population, 2 morphologically identical but genetically distinct and apparently nonpathogenic *Entamoeba* species are now recognized as causing most asymptomatic cases. To avoid unnecessary and possibly harmful therapies, clinicians should follow the diagnostic and treatment guidelines of the World Health Organization (Pritt et.al, 2008).

Amoebiasis is caused by *Entamoeba histolytica* and is a problem of public world health. The most frequent clinical presentation is the dysentery and the amebic liver abscess. Fifty millions of cases and more than 100.000 deaths for this disease are reported annually worldwide. The life cycle of *E. histolytica* has two phases: trophozoite and cyst. Trophozoites are the causal agent of disease (J.Vargas-Villareal et.al, 2009).

Currently amoebiasis is a major public health problem in tropical and subtropical countries. Although a number of antiamoebic agents are used for its treatment, yet the

susceptibility data on clinical isolates of *Entamoeba histolytica* and *Entamoeba dispar* are not available (Bansal et.al, 2004).

Infection with *Entamoeba histolytica*, the protozoan parasite that causes amoebic colitis and liver abscess, results in 34 million to 50 million symptomatic cases of amoebiasis (all illnesses caused by *E. histolytica*, including amoebic dysentery) worldwide each year, causing 40 thousand to 100 thousand deaths annually. As a result of accruing biochemical, genetic and immunological data, *E. histolytica* was re-defined in 1993 to recognise the existence of two morphologically identical but genetically distinct human parasites: *E. histolytica*, the aetiological agent of invasive intestinal and extraintestinal amoebiasis, and *Entamoeba dispar*, a non-pathogenic intestinal parasite. (Huston et.al, 1999).

1.1. Amoebiasis: Amoebiasis is the infection of human gastrointestinal tract by *Entamoeba histolytica* (*E.histolytica*), a protozoan parasite capable of invading the intestinal mucosa and that may spread to other organs, mainly the liver which usually leads to amoebic liver abscess. This infection remains a significant cause of morbidity and mortality world-wide. (Flore et.al, 2005).It is an infection caused by a single celled organism (protozoa) called an amoeba which infects the large bowel.Amoebae are passed in the stool from where they can contaminate water or food causing infection in others. Therefore the provision of toilets and a safe

East West University
LIBRARY
43, Mohakhali, C/A

water supply are important to prevent amoebiasis. (Woods, July 2007).

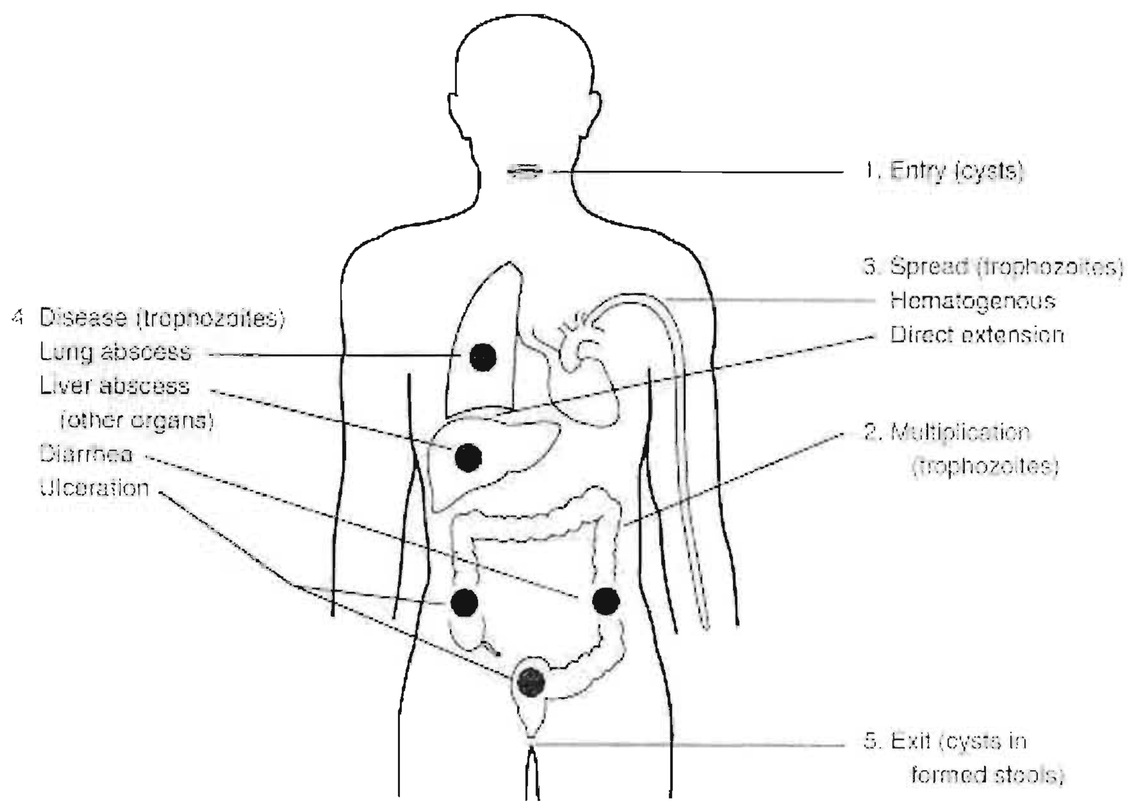
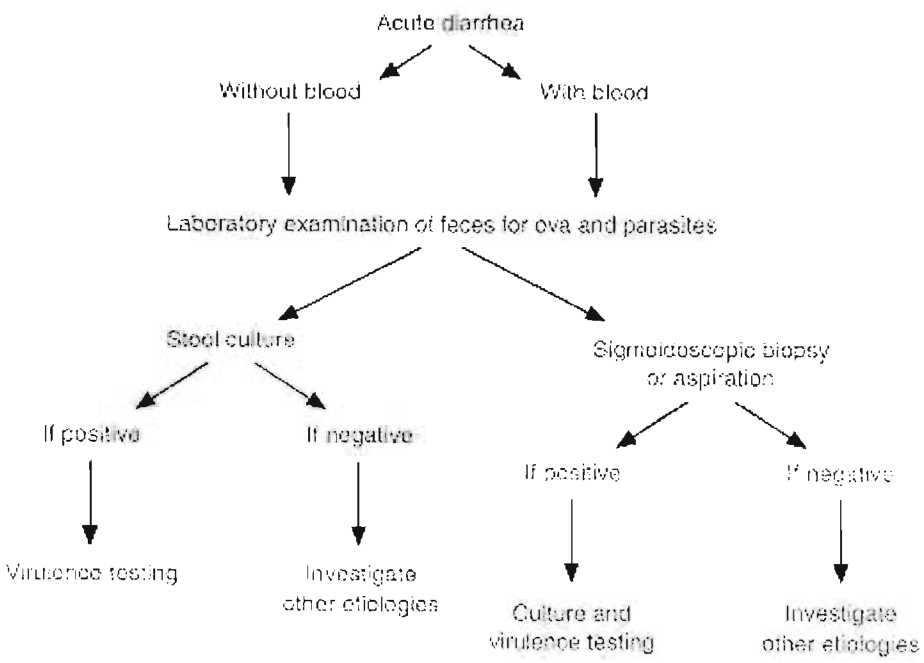


Figure 1: Pathogenesis of *E. histolytica* infection (Medical microbiology,Samuel baron,4rth edition)

1.1.1. **Diagnosis:** Diagnosis may be made by the microscopic identification of cysts in solid feces or trophozoites in diarrheic stool. The parasite may be detected in aspirated material of liver abscess. Serologic tests, such as IFA, ELISA, for specific antibodies to *E. histolytica* are very helpful in diagnosis of invasive amebiasis. It is difficult to definitively diagnose acute amebiasis, particularly when this condition is superimposed on inflammatory bowel disease. *(*Ozin et.al, 2009*)*

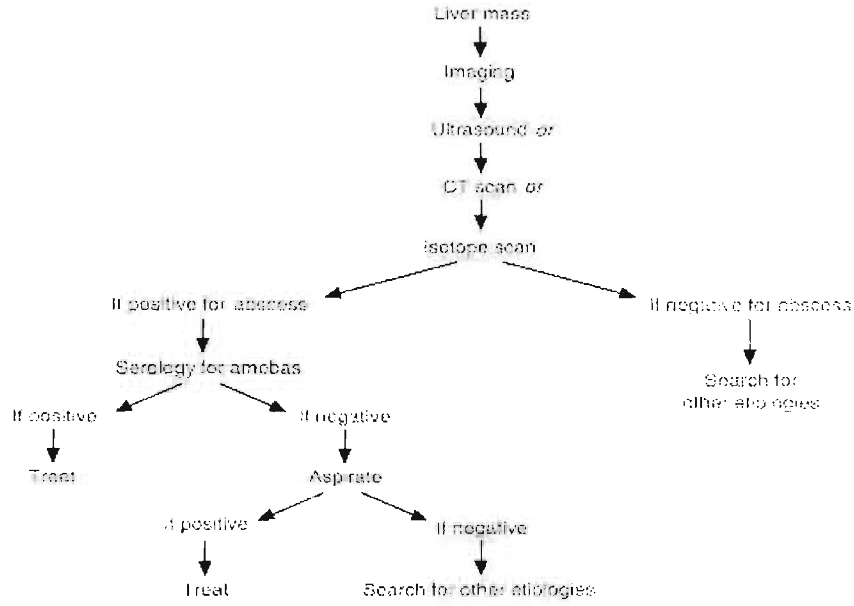
Amebic infections are diagnosed definitively by identifying the ameba in stool or exudate



Flow chart 1: Evaluation of suspected cases of intestinal amebiasis(Medical microbiology,Samuel baron,4rth edition)

Under some circumstances, however, the physician must settle for a presumptive diagnosis based on serologic or clinical evidence alone. Diagnosis may be difficult if few organisms are shed in the stool. Effective methods exist for concentrating cysts but not trophozoites in stool specimens. Fortunately, a direct relationship is usually seen (although there are exceptions) between the severity of disease and the number of amebas shed in the stool; hence, the more severe the infection the easier the diagnosis. Unfortunately, a number of substances that may be administered to the patient in the course of diagnosis or therapy can impair the ability to make a direct diagnosis. These compounds can suppress the shedding of amebas into the stool but may not interfere with the course of invasion infection. Such compounds include barium, bismuth, kaolin, soapsuds (as enemas), and antimicrobials that can reach the intestinal lumen. The suppression of shedding may be short-lived (soapsuds enema), or may last weeks or months (broad-spectrum antibiotics). These compounds render timely direct diagnosis

unreliable and often impossible. Amebas may be identified in direct smears, but specific diagnosis usually depends upon obtaining a fixed stained preparation. Trophozoites deteriorate rapidly in stool specimens, and therefore preservatives, either polyvinyl alcohol or the merthiolate-iodine-formaldehyde (MIF) combination, are important diagnostic aids. Finally, it is unrewarding to search for trophozoites in formed stool because most trophozoites encyst as the stool desiccates.Trophozoites can be found in diarrhea. Most infections in formed stool specimens will be detected by examining three specimens passed over a 7- to 10-day period. A negative examination of single stool specimen does not rule out infection. Trophozoites may be obtained by administering a purgative agent or by scraping suspicious lesions at the time of sigmoidoscopy. Amebas are difficult to demonstrate in aspirates from extraintestinal abscesses unless special precautions are taken. The contents of most amebic abscesses are relatively free of the organism. Instead, the organisms concentrate adjacent to the wall of the abscess cavity. If care is taken during aspiration to separate serial aliquots of aspirate, amebas may be found in the last syringe that empties the cavity. Cysts or trophozoites are only found in approximately one-half of all patients with amebic liver abscess. (Baron, 4rth edition)



Flow chart 2: Evaluation of suspected cases of hepatic amebiasis(Medical microbiology,Samuel baron,4rth edition)

Testing with monoclonal antibodies demonstrates ameba in the stool, and, if the galactose adhesion epitopes are tested for, pathogenicity may be determined as well. Broad scale application is proposed.A number of nonpathogenic amebas that can inhabit the human intestinal tract may confuse direct diagnosis. These include *Entamoeba hartmanni, Entamoeba gingivalis, Entamoeba coli, Endolimax nana*, and *Iodamoeba butschlii.* Although these parasites do not cause illness, they indicate that the patient has ingested feces-contaminated food or water, so their presence may prompt careful study of additional specimens. (Baron, 4rth edition)

1.1.2. **Symptoms of Amoebiasis:** The clinical manifestations vary with the extent of involvement. Mucosal erosion causes diarrhea, which increases in severity with increasing area and depth of involvement. Symptoms are also affected by the site of the infection. The more distal the lesion in the colon, the greater the likelihood and severity of symptoms; thus small rectal lesions are more likely to be symptomatic than larger cecal lesions. Rectal bleeding is only slightly less common than diarrhea and is usually, but not invariably, associated with diarrhea. Such bleeding may be grossly apparent or may be occult and demonstrable only by chemical testing for blood. Urgency, tenesmus, cramping abdominal pain and tenderness may be present. The intestinal syndromes caused by *E. histolytica* form a continuum ranging in severity from mild diarrhea to hemorrhagic dysentery. The span from mild to severe diarrhea is classified as non-dysentery colitis. Amebic dysentery has a dramatically different clinical presentation. The diarrhea is replaced by dysenteric stools consisting largely of pus and blood without feces. There is evidence of systemic toxicity with fever, dehydration, and electrolyte abnormalities. Tenesmus and abdominal tenderness are regular features. This fulminant presentation may occur suddenly or evolve from less severe, pre-existing disease.Occasionally, and for no apparent reason, colonic infection with *E. histolytica* will evoke a proliferative granulomatous response at an ulcer site. This infectious pseudotumor, called an *ameboma*, may become the leading point of an intussusception or may cause intestinal obstruction. This complication is uncommon. Peritonitis as a result of perforation has been reported

in connection with severe amebic colitis and, much less often, in patients with few or no symptoms. Other complications of intestinal amebiasis include colocutaneous fistula, perianal ulceration, urogenital infection, colonic stricture, intussusception, and hemorrhage. Most of these complications are uncommon and therefore may prove difficult to diagnose. The term post-amebic colitis is used for nonspecific colitis following a bout of severe acute amebic colitis. In such cases, the colon is free of parasites and the clinical findings resemble those of chronic ulcerative colitis. Extraintestinal amebiasis begins with hepatic involvement. Many patients with acute intestinal infection also have hepatomegaly, but in these cases amebas are not demonstrable in the liver and the pathogenesis of this hepatomegaly is not clear. A focal amebic abscess in the liver represents metastasis from intestinal infection. Symptomatic intestinal infection need not be present. The abscess appears as a slowly enlarging liver mass. Often the patient will have right upper quadrant pain, which may be referred to the right shoulder. If the abscess is located in a palpable portion of the liver, the area will be tender. Occasionally the enlarging abscess presses on the common bile duct and causes jaundice. If located under the dome of the diaphragm, the abscess may cause elevation of the dome of the diaphragm which presses on the right lung base, causing atelectasis and physical findings of consolidation. As the abscess nears the diaphragm the inflammation may stimulate pleural effusion.Pleural, pulmonary, and pericardial infection occurs as a result of direct extension from the liver. Lung involvement is far more common than pericardial infection. Infection metastatic from the liver can involve other viscera or can give rise to a brain abscess. However, these complications are uncommon. (Baron, 4rth edition).

1.1.3. Nature of the disease

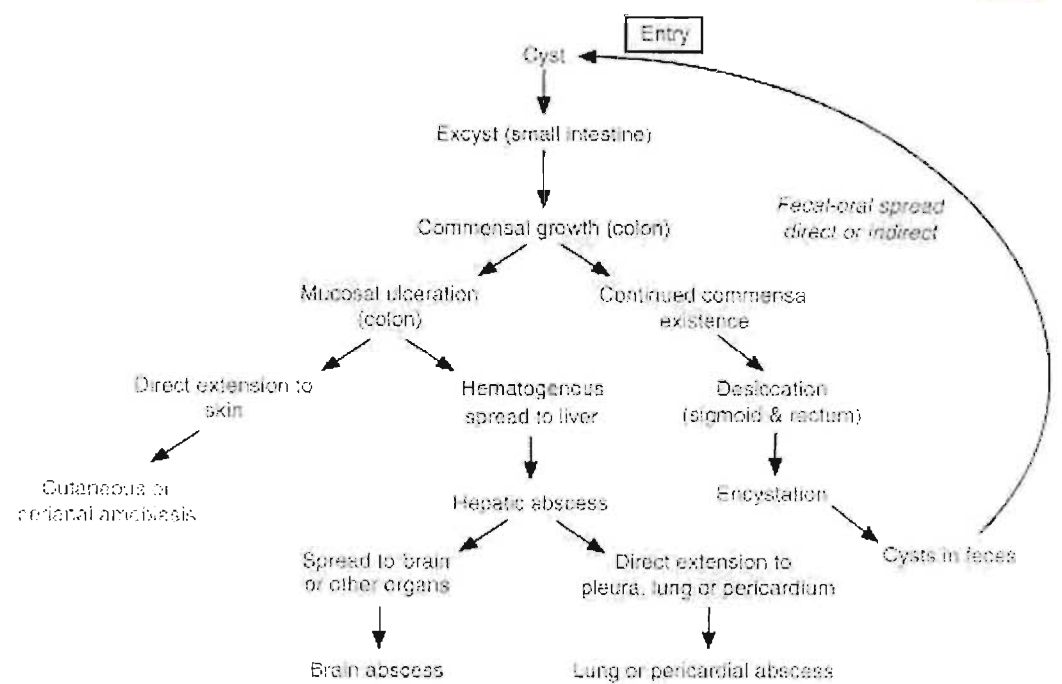
Most infected people, perhaps 90 percent, are asymptomatic, but this disease has the potential to make the sufferer dangerously ill. It is estimated by the World Health Organization that about 70,000 people die due to amoebiasis annually worldwide.Infections can sometimes last for years. Symptoms take from a few days to a

few weeks to develop and manifest themselves, but usually it is about two to four weeks. Symptoms can range from mild diarrhoea to dysentery with blood and mucus. The blood comes from amoebae invading the lining of the intestine. In about 10% of invasive cases the amoebae enter the bloodstream and may travel to other organs in the body. Most commonly this means the liver, as this is where blood from the intestine reaches first, but they can end up almost anywhere.Onset time is highly variable and the average asymptomatic infection persists for over a year. It is theorised that the absence of symptoms or their intensity may vary with such factors as strain of amoeba, immune response of the host, and perhaps associated bacteria and viruses.In asymptomatic infections the amoeba lives by eating and digesting bacteria and food particles in the gut, a part of the gastrointestinal tract.It does not usually come in contact with the intestine itself due to the protective layer of mucus that lines the gut. Disease occurs when amoeba comes in contact with the cells lining the intestine. It then secretes the same substances it uses to digest bacteria, which include enzymes that destroy cell membranes and proteins. This process can lead to penetration and digestion of human tissues, resulting first in flask-shaped ulcers in the intestine. *Entamoeba histolytica* ingests the destroyed cells by phagocytosis and is often seen with red blood cells inside when viewed in stool samples. Especially in America, a granulomatous mass (known as an amoeboma) may form in the wall of the ascending colon or rectum due to long-lasting immunological cellular response, and is sometimes confused with cancer. (Day, David W et.al 2003).

1.1.4. **Food analysis**

E. histolytica cysts may be recovered from contaminated food by methods similar to those used for recovering *Giardia lamblia* cysts from feces. Filtration is probably the most practical method for recovery from drinking water and liquid foods. *E. histolytica* cysts must be distinguished from cysts of other parasitic (but nonpathogenic) protozoa and from cysts of free-living protozoa as discussed above. Recovery procedures are not very accurate; cysts are easily lost or damaged beyond recognition, which leads to many falsely negative result s in recovery tests. (Manual, Retrieved 2008-03-26)

1.2.E. histolytica:



Flow chart 3: Multiplication and life cycle of *E histolytica(*Medical microbiology,Samuel baron,4rth edition*)*

Diseases: amebiasis, amebic dysentery, amebic hepatitis

1.2.1. Geographic distribution. Worldwide in tropical and temperate zone

Entamoeba histolytica, is the etiological agent of amoebic dysentery and amoebic liver abscess (ALA). Worldwide, 40–50 million symptomatic cases of amoebiasis occur annually and 70,000 to 100,000 deaths due to this infection.There are two distinct, but morphologically identical species of pathogenic.Morphologically identical species of *Entamoeba*: *Entamoeba histolytica*, is pathogenic. *E. histolytica*, has the capacity to invade intestinal mucosa resulting in intestinal amoebiasis and cause extra intestinal amoebiasis .(Bhansal,2004)

1.2.2.* Infection rate*: The actual incidence of amebiasis in the worldwide remains unknown. Survey indicates that the infection rates vary from 0.2 to 50%, being directly correlated with sanitary conditions.

Amebae						
	Entamoeba histolytica	*Entamoeba hartmanni*	*Entamoeba coli*	*Entamoeba polecki**	*Endolimax nana*	*Iodamoeba bütschlii*
Trophozoite						
Cyst						

*Rare, probably of animal origin

Figure 2: Amebas found in stool specimens of humans (Samuel Baron,4rth edition)

Many infections with *E. histolytica* occur without evidence of invasion of the intestinal lining. Virulence in the ameba—the ability to produce intestinal invasion or extraintestinal disease—is a heritable characteristic. Morphologically identical amebas may be identified as pathogenic or non-pathogenic on the basis of size, cultural characteristics, virulence in a rat model or in tissue culture, selective agglutination by lectins, reaction with monoclonal antibodies, or isoenzyme patterns. A pathogen-specific galactose adhesion epitope is described. Ribosomal RNA sequence analysis and restriction fragment length polymorphism analysis also can separate pathogenic from non-pathogenic strains.A number of non-pathogenic but apparently genuine *E. histolytic* strains have been isolated from human carriers. These amebas can be cultured at room

temperature as well at 37° C and will grow in hypotonic media, whereas pathogenic amebas require isotonic media and 37° C for growth. These low-temperature strains have isoenzyme patterns identical with the sewage- associated, non-pathogenic *Entamoeba moshkovskii*. Two classic tests to identify pathogenic strains are the ability to cause cecal ulceration in weanling rats and agglutination by the lectin concanavalin A. These tests of virulence have been supplanted by isoenzyme analysis and the use of monoclonal antibodies to identify pathogenic strains of *E histolytica*, but the clinical applicability of this technique is pending.lsoenzyme patterns are known for four amebic enzymes: glucose phosphate isomerase (GPl), hexokinase (HK), malate:NADP+ oxidoreductase (ME), and phosphoglucomutase (PGM). The isoenzyme patterns of three of these, GPI, HK, and PGM, can be used to define 20 zymodemes of *E histolytica*. The enzyme markers associated with pathogenicity are the presence of a b band and the absence of an a band for PGM.Zymodemes II, VI, VII, XI, XII, XIII, XIV, XIX, and XX are pathogenic. Zymodemes II and Xl are responsible for liver abscesses. There have been several reports of cultured amebas undergoing a change in zymodeme pattern after manipulation of associated bacterial flora. Attempts to reproduce these observations have not been successful. Zymodeme patterns are of epidemiologic and research interest but their limited availability makes them less useful clinically. It is possible to distinguish with monoclonal antibodies the galactose-specific adhesions from pathogenic and non-pathogenic ameba. This offers the possibility of simplified laboratory determination of pathogenicity. (Samuel baron, 4rth edition)

1.2.3. Classification:

Many infections with *E. histolytica* occur without evidence of invasion of the intestinal lining. Virulence in the ameba—the ability to produce intestinal invasion or extraintestinal disease—is a heritable characteristic. Morphologically identical amebas may be identified as pathogenic or non-pathogenic on the basis of size, cultural characteristics, virulence in a rat model or in tissue culture, selective agglutination by lectins, reaction with monoclonal antibodies, or isoenzyme patterns. A pathogen-specific galactose adhesion epitope is described. Ribosomal RNA sequence analysis and restriction fragment length polymorphism analysis also can separate pathogenic from

non-pathogenic strains.A number of non-pathogenic but apparently genuine *E. histolytic* strains have been isolated from human carriers. These amebas can be cultured at room temperature as well at 37° C and will grow in hypotonic media, whereas pathogenic amebas require isotonic media and 37° C for growth. These low-temperature strains have isoenzyme patterns identical with the sewage- associated, non-pathogenic *Entamoeba moshkovskii*. Two classic tests to identify pathogenic strains are the ability to cause cecal ulceration in weanling rats and agglutination by the lectin concanavalin A. These tests of virulence have been supplanted by isoenzyme analysis and the use of monoclonal antibodies to identify pathogenic strains of *E histolytica*, but the clinical applicability of this technique is pending. (Medical microbiology,Samuel baron,4rth edition)

Classification of Amebiasis

WHO Clinical Classification of Amebiasis Infection (Modified)	Pathophysiologic Mechanisms
Asymptomatic infection	Colonization without tissue invasion
Symptomatic infection	Invasive infection
Intestinal amebiasis	
A. Amebic dysentery	Fulminant ulcerative intentional disease
B. Nondysentery gastroenteritis	Ulcerative intestinal disease
C. Ameboma	Proliferative intestinal disease
D. Complicated intestinal amebiasis	Perforation, hemorrhage, fistula
E. Post-amebic colitis	Mechanism unknown
Extraintestinal amebiasis	
A. Nonspecific hepatomegaly	Intestinal infection with no demonstrable invasion
B. Acute nonspecific infection	Amebas in liver but without abscess
C. Amebic abscess	Focal structural lesion
D. Amebic abscess, complicated	Direct extension to pleura, lung, peritoneum, or pericardium
E. Amebiasis cutis	Direct extension to skin
F. Visceral amebiasis	Metastatic infection of lung, spleen, or brain

Table 1: Classification of amoebiasis (Medical microbiology, Samuel baron, 4rth edition)

Isoenzyme patterns are known for four amebic enzymes: glucose phosphate isomerase (GPI), hexokinase (HK), malate:NADP+ oxidoreductase (ME), and phosphoglucomutase (PGM). The isoenzyme patterns of three of these, GPI, HK, and PGM, can be used to define 20 zymodemes of *E histolytica*. The enzyme markers associated with pathogenicity are the presence of a b band and the absence of an a band for PGM.Zymodemes II, VI, VII, XI, XII, XIII, XIV, XIX, and XX are pathogenic. Zymodemes II and XI are responsible for liver abscesses. There have been several reports

of cultured amebas undergoing a change in zymodeme pattern after manipulation of associated bacterial flora. Attempts to reproduce these observations have not been successful. Zymodeme patterns are of epidemiologic and research interest but their limited availability makes them less useful clinically. A number of other factors, primarily environmental, that affect virulence are discussed below.It is possible to distinguish with monoclonal antibodies the galactose-specific adhesions from pathogenic and non-pathogenic ameba. This offers the possibility of simplified laboratory determination of pathogenicity. (Medical microbiology, Samuel baron, 4rth edition)

1.2.4. Pathogenesis

The fecal-oral transmission of the ameba usually involves contaminated food or water. The parasite can also be transmitted directly by ano-genital or oro-anal sexual contact. Latent infections can become invasive in a setting of impaired host immunity.Ingested cysts of *E. histolytica* excyst in the small intestine. Trophozoites are carried to the colon, where they mature and reproduce. The parasite may lead a commensal existence on the mucosal surface and in the crypts of the colon. Successful colonization depends on factors such as inoculum size, intestinal motility, transit time, the presence or absence of specific intestinal flora, the host's diet and the ability of the ameba to adhere to the colonic mucosal cells. The ameba adherence molecule has been identified as a lectin which can bind to either of two common carbohydrate components of cell membrane, galactose and *N*-acetyl glactoseamine. Binding to colonic mucins blocks adherence to mucosal cells. Depletion of mucus results in binding to the mucosa, an essential step in the development of the disease. If amebas pass down the colon they encyst under the stimulus of desiccation, and then are evacuated with the stool. The factors that lead to tissue invasion by *E. histolytica* are poorly understood. The genetic virulence factors mentioned above play a major role but several environmental factors are also important. Although the mechanisms of action are not clear, both changes in the intestinal flora and the nature of the host's diet have been implicated. All virulence factors come to a final common path where the ameba attacks and kills the host cell. Binding involving the galactose adherence lectin is essential for the cytolytic effect. Blocking adherence blocks the killing. This cytolytic event is a result of incorporation in the host cell membrane of

an ameba-produced, pore-forming protein, amoebapore. This protein forms ion channels in lipid cell membranes and results in cell death within minutes of cell contact with the ameba. Amoebapore has been isolated, synthesized and well characterized. Non-pathogenic strains of *E. histolytica* can also produce amoebapore but are much less efficient at its production and the molecule is not exactly similar to that produced by virulent strains.The initial lesion is in colonic mucosa, most often in the cecum or sigmoid colon. The slow transit of the intestinal contents in these two locations seems an important factor in invasion of the mucosa, both because it affords the ameba greater mucosal contact time and because it permits changes in the intestinal milieu that may facilitate invasion. The initial superficial ulcer may deepen into the submucosa and muscularis to become the characteristic flask-shaped, chronic amebic ulcer. Spread may occur by direct extension, by undermining of the surrounding mucosa until it sloughs, or by penetration that can lead to perforation or fistulous communication to other organs or the skin. If the amebas gain access to the vascular or lymphatic circulation, metastases may occur first to the liver and then by direct extension or further metastasis to other organs, including the brain. Virulent *E. histolytica* strains are capable of penetrating intact intestinal mucosa. The infection is not opportunistic and does not require pre-existing mucosal damage. Numerous proteases have been isolated in *E. histolytica*; however, the mechanism of penetration remains unclear. Metastatic foci present as abscesses with a central zone of lytic necrosis surrounded by a zone of inflammatory cell infiltration. Metastatic abscesses behave as space-occupying lesions unless they become secondarily infected or rupture. The clinical presentation of intestinal infections depends on the extent and anatomic location of the ulceration and mucosal damage. Small, sparse ulcerations may be asymptomatic. As the involved area of the mucosa increase in size and/or in depth, motility disturbances occur, primarily diarrhea with cramping pain. Exudation from the denuded mucosa adds to intestinal content. When the mucosal involvement becomes extensive, diarrhea is replaced by dysentery, with the passage of exudate, blood and mucus. Toxic megacolon and perforation are rare complications of extensive involvement. Systemic signs of infection include fever, rigor, and polymorphonuclear leukocytosis. (Medical microbiology,Samuel baron,4rth edition)

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

1.2.5. Multiplication and Life Cycle

Amebas multiply in the host by simple binary fission. Most multiplication occurs in the host, and survival outside the host depends on the desiccation-resistant cyst form. Encystment occurs apparently in response to desiccation as the ameba is carried through the colon. After encystment, the nucleus divides twice to produce a quadrinucleate mature cyst. Excystment occurs after ingestion and is followed by rapid cell division to produce four amebas which undergo a second division. Each cyst thus yields eight tiny amebas. (Medical microbiology,Samuel baron,4rth edition)

1.2.6. Epidemiology

Fecal-oral transmission occurs when food preparation is not sanitary or when drinking water is contaminated. Contamination may come directly from infected food handlers or indirectly from faulty sewage disposal. Endemic or epidemic disease may result. The prevalence of amebiasis in underdeveloped countries reflects the lack of adequate sanitary systems. Amebas are found in all climates, arctic to tropical. Symptomatic infections (amebic disease) are far more prevalent in certain geographic foci, and this uneven prevalence of disease, as opposed to infection, is now explained by the variable geographic predominance of pathogenic zymodemes. Similar environments thus are likely to have a comparable infection rate but may have widely different disease prevalence. (Medical microbiology,Samuel baron,4rth edition)

1.3. Treatment of Amebiasis:

Drugs of choice for invasive amoebiasis are tissue active agents, like metronidazole, tinidazole and chloroquine or the more toxic emetine derivatives, including dehydroemetine. Metronidazole and tinidazole are derived from 5-nitroimdazole which kill the trophozoites by alterations in the protoplasmic organelles of the amoeba, but are ineffective in the treatment of cyst passers. Chloroquine is derived from 4-aminoquinolines, which acts on the vegetative forms of the parasite and kills it by

inhibiting DNA synthesis. Emetine, a plant alkaloid, kills the trophozoites of *E. histolytica* mainly by inhibiting protein synthesis. (Devendra Bansal et.al, 2004)

1.3.1. Treatment with plant extract:

Clinical isolation of *Entamoeba histolytica* can be treated with different types of medicinal plants such as papaya extract with methanol and petrolium ether.

1.3.2. Carica Papaya:

The papaya (sometimes called papaw) and the passionflowers are closely relatedSome botanists place them all in one family even though the papaya is an erect plant and the passion-flowers are tendril-bearing vines; but recent botanists separate them into the Caricaceae (or Papayaceae) and Passifloraceae. In botanical structure, the fruits are very similar, and they are related not distantly to the Cucurbitaceae (pumpkins and melons). (Manual of Tropical and Subtropical Fruits", by Wilson Popenoe, 1920)

Papaya, *Carica papaya* L., is one of the major fruit crops cultivated in tropical and sub-tropical zones. Worldwide over 6.8 million tones. Papaya is a fast-growing, semi-woody tropical herb. The stem is single, straight and hollow and contains prominent leaf scars. Papaya exhibits strong apical dominance rarely branching unless the apical meristem is removed, or damaged. Palmately-lobed leaves, usually large, are arranged spirally and clustered at the crown, although some differences in the structure and arrangement of leaves have been reported with Malaysian cultivars (Chan and Theo 2000). Generally, Papaya cultivars are differentiated by the number of leaf main veins, the number of lobes at the leaf margins, leaf shape, stomata type, and wax structures on the leaf surface, as well as the colour of the leaf petiole.The fruit is melon-like, oval to nearly round, somewhat pyriform, or elongated club-shaped, 15-50 cm long and 10- 20 cm thick and weighing up to 9 kg (Morton 1987). Semiwild (naturalized) plants bear small fruits 2.5-15 cm in length. The skin is waxy and thin but fairly tough. When the fruit is immature, it is rich in white latex and the skin isgreen and hard. As ripening progresses, papaya fruits develop a light- or deep- yellow-orange coloured skin while the thick wall of succulent flesh becomes aromatic, yelloworange or various shades of salmon or red. It is then juicy, sweetish and somewhat like a cantaloupe in flavor but some types are quite musky

(Morton 1987). Mature fruits contain numerous grey- black ovoid seeds attached lightly to the flesh by soft, white, fibrous tissue. These corrugated, peppery seeds of about 5 mm in length are each coated with a transparent, gelatinous aril. 'Sunset Solo', 'Kapoho Solo', 'Sunrise Solo', 'Cavity Special', 'Sinta' and 'Red Lady' are commonly known Philippine varieties (Silva et.al 2007)

1.3.3. Composition:

C. papaya contains many biologically active compounds. Two important compounds are chymopapain and papain which are widely known as being useful for digestive disorders and disturbances of the gastrointestinal tract. Huet *et al.* (2006) showed that papaya-derived papain, caricain, chymopapain, and glycine endopeptidase can survive acidic pH conditions and pepsin degradation. However, at low pH, a conformational transition that instantaneously converts their native forms into molten globules that are quite unstable and rapidly degraded by pepsin. Thus, they may need to be protected against both acid denaturation and proteolysis for them to be effective in the gut after oral administration for the control of gastrointestinal nematodes. Apart from papain and chymopapain, *C. papaya* contains many biologically active compounds. *C. papaya* lipase, or CPL, a hydrolase, is tightly bonded to the water-insoluble fraction of crude papain and is thus considered as a "naturally immobilized" biocatalyst. Domínguez de María *et al.* (2006) reviewed several applications of CPL: (i) fats and oils modification, derived from the sn-3 selectivity of CPL as well as from its preference for short-chain fatty .Nutrient content of ripe papaya.

Constituent Appropriate value :Water 89 % Calcium 24 mg Sodium 3 mg ,Calories 39 kcal Iron 0.1 mg Niacin 0.34 mg,Protein 0.61 g Phosphorous 5 mg Pantothenic acid 0.22 mg, Fat 0.14 g Potassium 257 mg Vitamin A 1094 IU, Carbohydrate 9.8 g Magnesium 10 g Vitamin E 0.73 mg, Source: USDA Nutrient Database for Standard Reference, Release 18 (2005).55acids; esterification and inter-esterification reactions inorganic media, accepting a wide range of acids and alcohols as substrates; and more recently, the asymmetric resolution of different non-steroidal anti-inflammatory drugs (NSAIDs), 2-

(chlorophenoxy)propionic acids, and nonnatural amino acids.(Tree and Forestry Science and Biotechnology, 2007)

Strain	Total Solids	Ash	Acids	Protein	Total Sugars	Fat	Fiber
	%	%	%	%	%	%	%
Trinidad. .	12.14	.53	.06	.43	9.72	.06	.78
South Africa	13.00	.54	.09	.68	10.73	.07	.81
Honolulu. .	12.20	.56	.07	.50	10.29	.05	.66
Barbados	11.72	.48	.06	.46	8.05	.06	.76
Panama. .	14.41	.90	.14	.50	11.12	.25	1.09

Table 2: Composition of the Papaya (Manual of Tropical and Subtropical Fruits", by Wilson Popenoe, 1920)

1.3.4. **Classification:**

The classification of papaya has undergone many changes over the years. The genus *Carica* was previously classified under various plant families, including Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae. However it is presently placed under Caricaceae, a plant family incorporating 35 latex-containing species in four genera, *Carica*, *Cylicomorpha*, *Jarilla* and *Jacaratia* (Kumar and Srinivasan 1944). It is widely believed that papaya originated from the Caribbean coast of Central America, ranging from Argentina and Chile to southern Mexico (Manshardt 1992) through natural hybridization between *Carica peltata* and another wild species (Purseglove 1968). *Carica* consists of 22 species and is the only member of the Caricaceae that is cultivated as a fruit tree while the other three genera are grown primarily as ornamentals (Burkill 1966). *Cylicomorpha* is the only member of the Caricaceae that is indigenous to Africa, and consists of two species. *Jacaratia*, found in tropical America, consists of six species. *Jarilla*, from central Mexico consists of only one species. The mountain papaya (*C.*

candamarcencis Hook. f.), is native to Andean regions from Venezuela to Chile at altitudes between 1,800- 3,000 m (Morton 1987). The 'babaco', or 'chamburo' (*C. pentagona* Heilborn), is commonly cultivated in mountain valleys of Ecuador; plants are slender, up to 3 m high, and pentagonal fruits reach 30 cm in length (Morton 1987). Compared to the well known tropical papaya, *C. papaya*, fruits of the mountain papayas tend to be smaller in size and less succulent. Recently, another taxonomic revision was proposed and supported by molecular evidence that genetic distances were found between papaya and other related species (Jobin-Décor *et al.* 1996; Badillo 2002; Kim *et al.* 2002). Some species that were formerly assigned to *Carica* were classified in the genus *Vasconcella* (Badillo 2002). Accordingly, the classification of Caricaceae has been revised to comprise Cylicomorpha, Carica, Jacaratia, Jarilla, Horovitzia and Vasconcella), with Carica papaya the only species within the genus Carica.(Silva et.al 2007)

1.3.5. The plant and fruit :

Fruits from female trees are round whereas fruits from hermaphrodite trees are elongated. The fruit is a berry that can range from 5 cm in iameter and 50 g in weight to 50 cm or longer, weighing 10 kg or more (Storey 1969). Papaya fruits are covered with a smooth thin green skin that turns to yellow or red when ripe. The flesh is succulent, varying in texture and colour ranging from yellow to orange to red. Papaya is a major fruit crop worldwide that is primarily consumed as fresh fruit.(Tree and Forestry Science and Biotechnology, 2007)

1.3.6. Nutritional value

Papaya fruits consist mostly of water and carbohydrate, low in calories and rich in natural Vitamins and minerals, particularly in vitamins A and C, ascorbic acid and potassium .One hundred g of papaya contains: 55 calories, 0.61 g pro- tein, 9.8 g carbohydrates, 1.8 g dietary fiber, 89% water, 283 IU vitamin A, 62 mg vitamin C, 38 mg folate and 257 mg potassium.(Silva et.al 2007)

Mineral composition of unripe pulp of *Carica papaya*	
Sodium	4.0
Calcium	24.86
Iron	2.56
Phosphorus	12.56
Potassium	223.0
Zinc	0.056
Copper	0.001
Magnesium	23.54
Manganese	0.008
Cobalt	ND*
Cadmium	ND*
Lead	ND*

ND* = not detected.

Table 3: Mineral composition of unripe pulp of carica papaya (Oloyede, 2005)

1.3.7. Medicinal value: The ripe Papaya fruit has a protein content of 4-6grams per kilo which is not very high.Howerver the protein is highly digestible and papain in papaya can digest 35times more protein than its own weight.(Harald W. Tietze, Page:34-37)

Analyses of the ripe fruit show it to be fair source of iron and calcium. Hermano reports that it is a good source of vitamins A and B and is an excellent source of vitamin C. Hermano and Sepulveda say that the green fruit is good source of vitamin B. Wehmer records that the leaves, fruits, stem and roots contain a proteolytic enzyme, papain (papayotin), mallic acid, and calcium mallate. The leaves contain a glucoside, carposide, and an alkaloid, carpaine. The fruit contains saccharose 0.85 per cent, dextrose 2.6 per cent, levulose 2.1 per cent, mallic acid, pectin, papain, and citrates.The roots are official in the Mexican Pharmacopoeia; the leaves in the Mexican and Venezuelan Pharmacopoeias; the fruit, in the Venezuelan Pharmacopoeia; and the latex and seeds in the Mexican Wurtz, the earliest investigator on the action of papain, found the enzyme active in acid, alkaline, and neutral media. Chittenden, Mendel and McDormottmade an exhaustive study of papain and reported that it is true, soluble, digestive ferment or a mixture of ferments of vegetable origin; its proteolytic action is marked in acid, alkaline, and neutral solutions and also in the presence of many chemicals, antiseptics, and

therapeutic agents; it has a peculiar softening and disintegrating actions in proteids; and its general proteolytic action is that of a genuine digestive ferment, similar to that of the ferments of animal origin. It has some amylolytic action. It acts in the way rennet does upon milk, and has a pronounced digestive power at a wide range of temperatures. (Lohiya, 2002)

1.3.8. Uses:

Papaya plants are also produced for papain and chymopapain, two industrially important proteolytic enzymes found in the milky white latex exuded by fruits. In general, female fruits tend to exude more papain than hermaphrodite fruits (Madrigal *et al.* 1980). and is used in cosmetic products (Singh and Sirohi 1977; Knight 1980), the light industry and food processing. Papaya latex is often used as a cheap and affordable substitute for protease in high school DNA extraction experiments (Teixeira da Silva, unpublished results). Green fruits are generally better sources, containing more papain than ripe fruits. Benzyl isothiocyanate and the corresponding glucosinolate (benzyl glucosinolate, glucotropaeolin) can be found in papaya. Some of the highland papayas, whose center of origin lies in Ecuador, have latex of unripe fruit has activity 15-fold higher than *C. papaya* (Scheldeman *et al.* 2002). Nakamura *et al.* (2007) separated papaya seed and edible pulp and then quantified the amounts of benzyl isothiocyanate and glucosinolate in both. The papaya seed (with myrosinase inactivation) contained >1 mmol of benzyl glucosinolate in 100 g of fresh weight which is equivalent to quantities found in Karami daikon (the hottest Japanese white radish) and cress.In Australia it is believed in some quarters that several cancer diseases can improve after drinking papaya leaf extract. Papaya is used in tropical folk medicine.. Packages of dried, pulverized leaves are sold by "health food" stores for making tea, despite the fact that the leaf decoction is administered as a purgative for horses in Ghana and in the Ivory Coast it is a treatment for genito-urinary ailments. The dried leaf infusion is taken for stomach troubles in Ghana and it is used as a purgative. In addition, allergies to papaya fruit,latex, papain and papaya flower pollen exist among sensitive individuals (Blanco *et al.* 1998). IgE-mediated reactions induced by the ingestion of papaya and papain have been reported (Mansfield *et al.* 1985; Sagona *et al.* 1985; Castillo *et al.* 1996).Moreover, occupational IgE-mediated asthma

induced by the inhalation of papain has been described (Tarlo *et al.* 1978; Baur and Fruhmann 1979; Novey *et al.* 1979; Baur *et al.* 1982). Externally the latex is an irritant, dermatogenic, and vescicant. Internally it causes severe gastritis. The acrid fresh latex can cause severe conjunctivitis and vesication. Anaphylaxis is reported in about 1% of cases of chymopapain injections. Generally papaya wood has very little application. It has long been used in the manufacture of rope but it was recenty shown that papaya bark can be used as a new biosorbent of heavy metals and has potential application to the treatment of waste water.

Saeed *et al.* (2006) demonstrated that 97.8, 94.9 and 66.8% of 10 mg/L copper (II), cadmium (II) and zinc (II) solutions, respectively were removed with 5 g/L papaya wood during a shake flask contact time of 60 minutes.(Silva et.al, 2007)

Papaya is not teratogenic and will not cause miscarriage in small, ripe amounts. The stem and the bark are also used in rope production.The black seeds are edible and have a sharp, spicy taste. They are sometimes ground up and used as a substitute for black pepper. In some parts of Asia the young leaves of papaya are steamed and eaten like spinach. In parts of the world papaya leaves are made into tea as a preventative for Malaria, though there is no real scientific evidence for the effectiveness of this treatment. (Bhande, and S. Panneerdoss, March 2002).

The papaya fruit is susceptible to the Papaya Fruit Fly. This wasp-like fly lays its eggs in young fruit. In cultivation it grows rapidly fruiting within 3 years, however it is highly frost sensitive. (Mishra et.al, 2002).

In the 1990s, two varieties of papaya, SunUp and Rainbow, that had been genetically-modified to be resistant to the papaya ring spot virus, were introduced into Hawaii.By 2004, non-genetically modified and organic papayas throughout Hawaii had experienced widespread contamination from the genetically-modified varieties.(Lohiya, N. K et.al,March 2002).

1.3.9. Other uses of carica papaya: Many parts of the plant are employed in the treatment of several ailments; for example the seed is used for expelling worms, and the seed and the roots are also used as abortifacient agent. The leaves (especially fallen ones) are used variously for the treatment of fever, pyrexia, diabetes, gonorrhoea, syphilis, inflammation and as dressing for foul wounds (Gill, 1992).

Some of the scientifically validated uses of *C. papaya* include the abortifacient activity of the seeds (Oderinde et al., 2002), the fruit juice for lowering blood pressure (Eno et al., 2000), the wound healing effects of the leaves (Starley et al., 1999; Mikhal'chik et al., 2004), and several other studies.

1.3.9.1. Nutritional supplement: Carica papaya seed extract is currently being marketed as a nutritional supplement with purported ability "to rejuvenate the body condition and to increase energy". The product, Carica R _ Seed Extract, is made from the seeds and the skin of Philippine wild papaya and is claimed to be a rich source of anti-oxidants like bioflavonoids and superoxide dismutase [Carica, The Gift of Health, Carica Herbal Health Products, Inc].

Bioflavonoids are said to strengthen the immune system and have both protective and curative effects against diseases associated with oxidative stress (A.C. Emeruwa et.al,1961).

The product is suggested for use in ailments such as cancer, leukemia, cysts, goiter, amoebiasis, pneumonia, emphysema, and intestinal parasitism. Several studies have demonstrated the antimicrobial and anti-helminthic properties of *Carica papaya* fruit and seeds, the producers of the Carica R _ seed extract allude to hundreds of testimonials from its users regarding the immunostimulatory properties of *Carica papaya.* However, the commercially available extracts have not been scientifically analyzed and no reference has been found to date detailing the possible immunomodulatory actions of Carica seed extract. Ripe and unripe Carica papaya fruits (epicarp, endocarp, seeds and leaves) were extracted separately and purified. All the extracts except that of leaves produced very significant antibacterial activity on Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Shigella flexneri. (Emeruwa AC, 1982.)

1.3.9.2. Antimicrobial properties: The roots of *C. papaya.* have been claimed by herbalists to possess antimicrobial properties against microorganisms like gonorrhea, and so forth. In West Africa, the plant is mainly used as diuretic (root and leaves), antihelmintic (leaves and seeds), and to treat bilious conditions (fruit) (Dalziel, 1937).
Ripe and unripe fruits (epicarp, endocarp, and seeds) showed very significant antibacterial activity with *Staphylococcus aureus.*, *Bacillus cereus.*, *Escherichia coli.*, *Pseudomonas aeruginosa.*, and *Shigella flexneri.* (Georges & Pandelai, 1949).
Literature searches revealed that few scientific investigations have been carried out on the pharmacodynamic properties of *C. papaya..* However, Nigerian traditional medical practitioners use the seed extract for treatment of various types of ailments without considering adverse side effects. Fruit and seed extracts have pronounced bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella flexneri* Emeruwa (1982) reported that extracts from fruits showed effective anti-microbial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* sp. and *Shigella.* The Dutch and Malays use leaves and young fruit extracts to eradicate intestinal worms and to treat boils (Burkill 1966) while young shoots and male flowers are consumed as a vegetable dish in the Malay Peninsula. (Silva et.al, 2007)

1.3.9.3. Sprem concentration: Papaya seeds (Carica papaya Linn) have been found to have a significant effect on sperm characteristics in some mammals, including humans and dogs. (An Ortega-Pacheco1, 2009).

1.3.9.4. Nematode infection: Treating chickens against nematode infections with papaya latex result in reduction in worm burdens and hence higher weight gains.(Chota et.al, 2009)

1.3.9.5. Abortifacient and contraceptive properties: Phytochemicals in papaya may suppress the effects of progesterone. Women in India, Pakistan, Sri Lanka, and other parts of the world have long used green papaya as a folk remedy for contraception and abortion. Enslaved women in the West Indies are also noted for consuming papaya to

prevent pregnancies and thus preventing their children from being born into slavery. Medical research in animals has confirmed the contraceptive and abortifacient capability of papaya, and also found that papaya seeds have contraceptive effects in adult male langur monkeys, possibly in adult male humans as well. In India, unripe and semi- ripe papaya fruits are ingested or applied on the uterus to cause abortion. Recently a study with rats at different stages of gestation showed that the consumption of unripe and semi-ripe papaya fruits could be unsafe during pregnancy given the high levels of latex in the fruits at these stages of maturity. But consumption of ripe fruits during prenancy causes no risk (Bhande,and S. Panneerdoss,March 2002).

The seeds of papaya has effects on germinal epithelium of the seminiferous tubules (Uche-Nwachi et al., 2001),Extracts of papaya seeds could be used as a contraceptive in rats, specifically two principal compounds, MCP I and ECP I (the code names of the major purified compounds of methanol and ethyl acetate subfractions of the benzene chromatographic fraction of the chloroform extract of the seeds of *C. papaya*, respectively; the methanol sub-fraction or MSF of the seeds of *C. papaya*, a putative male contraceptive,could be safely used in rats as a male anti-fertility agent.(Lohiya *et al.* 2005)

1.3.9.6. <u>Topical use:</u> Papain(a cysteine protease hydrolase enzyme present in regular papaya ,*Carica papaya* and mountain papaya) is also popular (in countries where it grows) as a topical application in the treatment of cuts, rashes, stings and burns. Papain ointment is commonly made from fermented papaya flesh, and is applied as a gel-like paste. Unripe papaya is especially effective in large amounts or high doses. (Bhande,and S. Panneerdoss,March 2002)

1.3.9.7. <u>Healing properties:</u> The latex serves as an excellent meat tenderizer, for treatments of gangrenous wounds or burns (Starley 1999; Hewitt *et al* 2000), According to Reed (1976), papaya latex is very much useful for curing dyspepsia and is externally applied to burns and scalds (Silva et.al,2007).

1.3.9.8. <u>Antifungal properties:</u> Papaya milk latex shows anti-bacterial properties, inhibits fungal growth, especially that of *Candida albicans* (Giordani and Siepai 1991),

and thus would be useful in the treatment of skin eczema caused by this fungus. (Silva et.al, 2007)

1.3.9.9. Anti-asthma: In Mauritius, the smoke from dried papaya leaves relieves asthma attacks.(Silva et.al ,2007)

3.2.10. Anti-ulcerant: According to Reed (1976), papaya latex is very much useful for curing dyspepsia and is externally applied to burns and scalds. (Silva et.al,2007)

1.3.9.10. Antiamoebic: Carica papaya fruit and seeds have antihelminthic and anti-amoebic activities. Okeniyi *et al.* (2007)

1.3.9.11. Digestive properties: In intestinal indigestion due to insufficient production of pancreatic juice and consequent imperfect digestion of fats and formation of peptones, papäin is useful in a high degree. (A Practical Treatise on Materia Medica and Therapeutics" book, Roberts Bartholow, 1908).

1.3.9.12. Molluscicidal activity: C. papaya may be used as potent molluscicides since the concentrations used to kill the snails are not toxic for the fish Colisa fasciatus which shares the same habitat with the snail L. acuminata.(Veterinary parasitology,April 2008).

1.3.9.13. Protective effect: A single dose (500 mg/kg) of Carica Papaya Leaf(CPL) aqueous extract is able to protect the rat gastric mucosa against haemorrhagic lesions produced by alcohol. The CPL aqueous extract offered some protection against alcohol induced oxidative damage to the gastric mucosa. The antioxidant system present in CPL might play a protective role against the production of reactive oxygen species and lipid peroxidation by-products. CPL extract is a promising candidate for the development of phytomedicine against gastric ulcer, and further studies are needed in this direction. (Indran M et.al, 2008)

1.3.9.14. Tocolytic activity:

High concentration Carica papaya L. seeds extracted with 80% ethanol is capable of causing irreversible uterine tocolysis probably due to the damaging effect of BITC (its chief phytochemical) on the myometrium. (Adebiyi A et.al, 2003)

1.3.9.15. Anti-inflammatory:

Papaya extracts significantly reduces paw oedema in the carrageenan test. Likewise the extract produces significant reduction in the amount of granuloma formed. (V Bamidele, 2008)

1.3.9.16. Modulation of jejuna contraction: *Carica papaya* L. (papaya) seed preparations are used in traditional medicine to expel intestinal worms in human and ruminants papaya seed extract and benzyl isothiocyanate, its principal bioactive constituent are capable of weakening the contractile capability of rabbit isolated jejunum. (Adaikan, 2005)

1.3.10. Parts of the papaya tree with different medicinal value:

All parts of the tree have medicinal value. The fruit flesh, the flowers, the leaves. the seeds, the stems, the latex, the barks and even the roots.

Figure 3: Papaya tree (Technical manual on small-scale processing of fruits and vegetables by Gaetano Paltrinieri,chapter 4)

1.3.10.1. <u>Fruit:</u> The ripe fruits are rich in vitamins. They contain vitamin A,C and vitamin B complex,amino acids,calcium,iron,enzymes and so on. The protein in papaya is highly digestable.Papaya is of great value to people with insufficient digestion or unhealthy diets.And too much indigestible protein.It is also benefit for people with fast food diet.Papaya is not only highly digestible protein itself but it also helps to break up hard to digest proteins.The green fruit has even higher nutritional properties than the fully ripe fruit.The green fruit has 1/3 of the calories of the ripe fruit but has approximately twice as much of highly digestible protein as the ripe fruit.The green fruit has only 2/3 of the carbohydrates.The fruit flesh of the green fruit has less beta carotene than the ripe fruit but more in the skin.

1.3.10.2. <u>Skin:</u>The skin of the unripe fruit and also from the ripe fruit is treasured by many as the most potent part of the plant.The skin of the fruit should never be disregarded.One can make it tasty without decreasing the healing properties.

1.3.10.3. <u>Root:</u> The root is used in many countries for healing.There is not much known about the components. These roots are cooked as a tea for the elimination of intestinal parasites, Jaundice, Kidney colic and for bleeding.

1.3.10.4. <u>Flowers:</u> The flowers, cooked as a tea and sweetened with sugar can be used for jaundice, bronchitis and other illness.

1.3.10.5. <u>Leaf:</u> The leaf of the tree is probably the most valuable part of the plant.Fresh leaves are of higher value than the dried leaves as some beta carotene is lost during the drying process due to oxidation.The leaves are richer in protein with approximately 15times more than ripe fruits. The fully ripened fruit has nearly no papain however on the other hand the leaves are rich in papain.

	Fruit	Leaf
Niacin	33	93
Iron	38	48
Fibre	75000	145000
Fat	22000	136000
Carbohydrate	991000	556000
Beta carotene	123	514
Ascorbic acid	5700	6200
Protein	57000	326000

Table 4: Nutritional value of papaya leaves (Papaya the Medicine Tree by Harald W. Tietze, page-35)

1.3.10.6. Seeds: The seeds richest in highly digestible proteins containing over 24%.They contain 32% carbohydrates and 25% oils including some essential oils. Similar to the super enzyme papain the enzyme Myrosin is also present as well as the alkaloid carpaine.Carpaine has similar action as Foxglove (Digitalis) with a calming effect to the heart, bronchus and muscles. However if injected overdose amounts it can lead to cardiac arrest.If possible one uses fresh seeds but the seeds may also be dried and stored in airtight containers for 2-3years in a cool place. If needed the seeds are ground or pulped nad used as medicine.

Bark: The bark and the inner bark can be used as a remedy for tooth ache.

1.3.10.7. Latex: The latex (as the milk of the leaves, young shoots and unripe fruit is called), is after the fruits, the second best money earner in the papaya industry.Latex contain 5.3% papain.Fresh latex is used for variety of skin problems.(Papaya the Medicine Tree By Harald W. Tietze, Page:34-37)

The Latex Collected From Young Papaya Fruits Or Other Species Of The Genus, Carica Is Purified To Liberate The Papain Of Commerce, Which Being A Rich Source Of Proteolytic Enzymes Finds Utilization In A Number Of Big Or Small Industries. (Production Technology of Papaya Latex by B.C. Mazumdar)

CHAPTER 2
AIM AND SIGNIFICANCE OF THE STUDY

Aim of the Study

Amebiasis, also known as amebic dysentery, is one of the most common parasitic diseases occurring in humans and human beings are the only known host of the amebiasis organism, and all groups of people, regardless of age or sex, can become affected. There are number of synthetic drugs to treat this disease but the number of herbal agents is few. Treatment of amoebiasis with plant extract is rare. Therefore the present study was aimed to asses the in vitro susceptibility of clinical isolates of *E.histolytica* to seed extract of Carica papaya and to quantify the efficacy of different concentrations of seed extract of Carica papaya against Clinical Isolates of *Entamoeba histolytica.*Determination of the amoebicidal impact of Carica papaya seed extract on viable and non viable counts of *Entamoeba histolytica* is also a major objective of this study.

Significance of the study

Amebiasis, also known as amebic dysentery, a type of gastroenteritis caused by a tiny parasite, *Entamoeba histolytica*, which infects the bowel, is one of the most common parasitic diseases occurring in humans, with an estimated 500 million new cases each year. It has a very high incidence in tropical countries; mostly in Bangladesh the risk of infection is very high.

About 90 percent of infections are asymptomatic and the remaining ten percent produces a spectrum varying from dysentery to amoebic liver abscess. In Bangladesh there are some pharmaceutical companies making antiamoebic drugs. So a number of synthetic drugs present but the incidence of side effect is common and the demand for drugs obtained from other than synthetic source is high.

Some of the synthetic drugs have lower efficacy, which can result the patients uncured or resistant to the drug forever. Indiscriminate use of drugs and different efficacy of a single drug treated with a patient at different times may lead to the physiological problems.

Extract of Carica papaya seed is a potential antiamoebic preparation obtained from natural source. From some study it is observed that by using the seed extract the incidence of amoebic dysentery reduces effectively. It is found that the seed extract of Carica papaya is safer and less toxic than synthetic drugs in many ways because it is obtained from plant source.

So the result of the study may show the efficacy of the seed extract of Carica papaya at different concentrations on the rate of inhibition of clinical isolates of *Entamoeba histolytica*.

CHAPTER 3: MATERIALS AND METHODS

Materials and methods

3.1. Materials

3.1.1. <u>Research design:</u> Efficacy of antiamoebic seed extract of Carica papaya against clinical isolates of *Entamoeba histolytica*

3.1.2. Preparation of seed extract of Carica papaya:

The plant materials used in this study were collected from an authentic source and identified it in Botany department of Dhaka University, whether it belonged to Carica papaya plant or not.

Then the dried seed (200g) was grounded and extracted with a Methanol: Petrol (50:50) by maceration for 3days.The solvent was evaporated using rotary evaporator.The residue of extract was stored at 4^0 c.

Finally, the mixture was filtered by folding clothes through funnel. The resulting filtrate was concentrated by rotary evaporator in 40^0C under vacuum until it became dry. Polar compounds in the solution were mostly eluted with methanol.

3.1.3. Collection of Clinical isolates

Clinical isolates from patients attending the Out Patient Departments of ICDDR, B hospital, attached to the Parasitology Laboratory, ICDDRB, Dhaka, Bangladesh, identified earlier as either *Entamoeba histolytica* by Techlab ELISA were used in the present study. These have been cultured in Robinson's medium (Robinson GL, 1968).

Instruments

Name of the instruments

- Analytical balance
- Mortar and Pestle
- Vortex machine
- Micropipettes
- Eppendrof (1ml & 2ml)

- Microtiter plate (NUNC)
- Microscope (Olympus)
- Haemocytometer.
- Microtips

East West University LIBRARY 43, Mohakhali, C/A

3.2. Methods

Preparation of culture media

Clinical isolates are cultured in first xenic media then it is axenically process to axenic or pure culture. Mentioned below these are the processes:

3.2.1. Xenic Culture Media

Xenic cultures will be for identification of the species of *Entamoeba* present in the sample, for example by isoenzyme analysis. Several intestinal species of *Entamoeba* can be found in humans who are sufficiently similar to cause diagnostic confusion and all can grow in the same media. Because *E. histolytica* is the only species that causes invasive disease, differentiation from the closely related, more common, and morphologically identical species *E. dispar* in particular is desirable, in order to prevent unnecessary chemoprophylaxis. Until less laborious methods become widely tested and implemented, isoenzyme analysis will remain the standard for separation of these two parasites.

The most common source of material will be stool samples and this is what is assumed below. In rare instances rectal biopsies or liver abscess aspirates have been the starting point for cultures. In the latter case, since the abscess is sterile, addition of a bacterial flora is necessary before inoculation of amebae into xenic culture. Such material has also been used for the direct establishment of monoxenic cultures. Unless the stool sample is from a patient with dysentery the amebae will be in the encysted form. This allows for several approaches to the establishment of cultures.

Preparation of culture bottle

First large beaker was taken. Then 1 liter distilled was taken, 7 gm of sodium chloride was added. Then heat and 15 gm of BactoTM agar media was added. Then again heat,

after the heat the solution must be cool and taken into a small glass bottle by a syringe (agar bottle). Then autoclave, after completed the autoclave, the liquid solution bottle taken into a box and the box must be settled by an angle state because the angle bottle (egg shape) can be suitably for parasite.

3.2.2. Preparation of rice starch

Purified rice starch is important for growth of *E.histolytica* in all the following media. To prepare (Diamond, L. S. 1983), 500 mg of powdered rice starch is placed into each of several culture tubes (16 by 125 mm) and is heated at 150°C, with loose caps, in a dry oven for about 3 hours. Sterilization of the rice starch prevents alteration of the bacterial flora when it is added to the culture and is thus recommended.

After cooling, the caps are tighten and are stored at room temperature. To prepare for use, 9.5 ml of sterile distilled water or phosphate-buffered saline (PBS) is added to one tube. The tubes are vortexed for resuspension. 1 ml of the resuspended starch is distributed to each of 10 tubes containing 9 ml of sterile water or PBS, and they are refrigerated.

The final concentration of diluted rice starch is 5 mg/ml. Before use, the rice is resuspended by vortexing or vigorous shaking. The desired volume is taken into culture tubes with medium, making sure that the stock rice stays in suspension. Different isolates require various amounts of rice starch, but 0.2 ml (1 mg) is often a suitable amount to add per culture tube. *Entamoeba* will not ingest all forms of rice. Most important is the size of the rice particle, as it must be within the ameba's ability to phagocytize it.

3.2.3. Diphasic media

Robinson's medium. Robinson's medium (Robinson, G. L. 1968) is a complex medium that has nevertheless found widespread use for the isolation of enteric amebae. Robinson's medium is prepared with the six following stock solutions.

(a) 0.5% erythromycin 0.5% erythromycin is prepared in distilled water and filtered and sterilized. Then it is refrigerated.

(b) 20% Bacto Peptone. 20% Bacto Peptone is prepared in distilled water. Then is autoclaved and refrigerated.

(c) 10Xphthalate solution stock. 10 X phthalate solution stocks are prepared by mixing 102 g of potassium hydrogen phthalate and 50 ml of 40% sodium hydroxide. The solution is made to 1 liter at pH is adjusted to 6.3. Then the solution is autoclaved for 15 min at 121°C under a pressure of 15 lb/in2. The solution is stored at room temperature. It is diluted 1:10 with sterile water before use. A stock of phthalate-Bacto Peptone can be made by adding 1.25 ml of 20% Bacto Peptone per 100 ml of 1 X phthalate solution. The solution is refrigerated.

(d) 10X R medium stock. 10 X R medium stocks, is prepared by dissolving the following in distilled water: 25.0 g of sodium chloride; 10.0 g of citric acid; 25.0 g of potassium phosphate, monobasic; 5.0 g of ammonium sulfate; 0.25 g of magnesium sulfate. 7H2O and 20 ml of 85% lactic acid solution. It is made to 500 ml. Stock is diluted to 1:10, pH is adjusted to 7.0. The solution is autoclaved for 15min at 121°C under a pressure of 15 lb/in^2 in 20-ml amounts.

(e) BR medium. To prepare BR medium, inoculate 1×R medium with a standard *E. coli* strain such as O111. Incubate at 37°C for 48 h and store at room temperature (good for several months).

(f) BRS medium. To prepare BRS medium, add an equal volume of heat-inactivated bovine serum to BR medium and incubate at 37°C for 24 h. Store at room temperature (good for several months).

To prepare agar slants, many people use screw-cap glass bijou bottles (total volume, 7 ml), but we have also used standard culture tubes with good success. Autoclave a solution of 1.5% Noble agar in 0.7% sodium chloride–distilled water for 15 min at 121°C under a pressure of 15 lb/in2. Dispense in 5-ml (tube) or 3-ml (bottle) amounts, reautoclave, and slant until cool and set. For slants in tubes, use an angle that produces a 12- to 15-mm (ca. 0.5-in.) butt. When cool, tighten lids and store at room temperature or refrigerated.

To one tube or bottle add the following: 3 ml of 1×phthalate-Bacto Peptone, 1 ml of BRS medium, and 50μl of erythromycin. This must be done on the same day as inoculation. Note that although erythromycin is added to Robinson's medium at every subculture, this does not lead to a monoxenic culture as occasionally stated. Additional antibiotic treatment would be needed for this to occur.

3.2.4. Axenic Culture Media

One constant problem facing those who rely on axenic cultures is the fastidiousness of these organisms. Although the others are also affected to a significant degree, this is especially true of *E. histolytica*. Lot-to-lot variations in several components of the axenic culture media in particular can have profound effects on the ability of a medium to support growth of the organisms; some lots may even be toxic. Trypticase (casein digest peptone), yeast extract, and serum are the medium components most commonly affected, but the quality of the distilled water and even the type of glass used in making the culture tubes can cause problems (Diamond, L. S. 1983) (screw-cap borosilicate glass tubes should be used when possible). For this reason, we highly recommend that those wishing to undertake axenic cultivation of these organisms test the ability of each new lot of reagent to support growth before starting to use it.

***E. histolytica.* LYI-S-2:** In the course of developing YI-S, several combinations of liver digest and yeast extract were studied. One of these, designated LYI-S-2 (containing liver digest, yeast extract, iron, and serum), was found to result in growth equal to that in TYI S-33. Intent on producing a medium with as few biological ingredients as possible, the medium containing only yeast extract, YI-S, was extensively tested and published. No difference in the ability of YI-S and LYI-S-2 to support growth of *E. histolytica* was

observed (unpublished results). After publication of YI-S, further testing within our laboratory and by others disclosed the fact that some lots of yeast extract would not support any growth of the ameba while with others growth was very poor. In the case of the latter it was found that substitution of a small amount of liver digest for an equal amount of yeast extract enhanced growth considerably. LYI-S-2 is recommended when a given lot of yeast extract will support some growth, though poorly, of *E. histolytica*. LYI-S-2 is identical to YI-S except that weight for weight it contains 0.5% neutralized liver digest and only 2.5% yeast extract. It has been used in the long-term cultivation of several isolates of *E. histolytica* and a number of other *Entamoeba* species, with yields similar to those observed with the more widely used TYI-S-33 and YI-S (unpublished data).

3.2.5. Establishment of cultures

It is very important to remember that a negative culture result does not mean that the patient is uninfected. None of the organisms being considered here produce cultures 100% of the time from microscopy-positive samples, and in the case of *E. histolytica* the success rate appears to be between 50 and 70% in most laboratories, based on personal communications. It is also important to remember that what grows in culture is not necessarily the organism seen by microscopy.

General considerations

E. histolytica needs to be established in xenic culture. The most-common source of material will be stool samples, and this is what is assumed below. In rare instances rectal biopsy specimens or liver abscess aspirates have been the starting point for cultures. In the latter case, since the abscess is sterile, addition of a bacterial flora is necessary before inoculation of amebae into xenic culture. Such material has also been used for the direct establishment of *E. histolytica* into monoxenic cultures with either a bacterium (Freedman, 1958) or a trypanosomatid (Wang, 1973) as the associate. Unless a stool sample is from a patient with dysentery, it is likely that the amebae will be in the encysted form. This allows for several approaches to the establishment of cultures.

3.2.6. Elimination of unwanted organisms:

One of the banes of xenic cultivation is the likelihood of unwanted organisms overgrowing the desired ameba. The most-frequent source of this problem is *B. hominis*, which may be the most-common parasitic infection of humans. This organism is often missed on stool examination but grows luxuriantly in all the media used to cultivate xenic *Entamoeba*. Some authors control the growth of *B.hominis* with acriflavin as first described by Dobell and Laidlaw (Dobell C, 1926), but this also has an adverse effect on the bacterial flora and, directly or indirectly, on the ameba of interest. We have successfully used two methods to eliminate *B. hominis* from *Entamoeba* cultures. The first method also was described by Dobell and Laidlaw in 1926 (Dobell C, 1926). In this method, cysts are treated with 0.1 N hydrochloric acid at room temperature for 10 min, washed thoroughly with distilled water, and reinoculated into culture medium to which a suitable bacterial flora has been added. The acid kills the bacteria, any fungi, *B. hominis*, intestinal trichomonads, and any nonencysted amebae while leaving the cysts intact and viable. We have found that it is not necessary in most cases for the cysts to be mature. We do not know whether the cysts complete their maturation upon inoculation or whether immature cysts respond to the stimulus and excyst directly. The cysts used can be either from stool or cultures; *Entamoeba* cultures in LE medium in particular frequently produce small numbers of cysts spontaneously. The bacterial flora used in the above method is separated from another xenic culture by inoculating into culture medium, without rice starch, a small amount of supernatant from an established culture, subculturing twice, and refrigerating the flora for 48 h. The successful separation of the flora can be checked by inoculating a substantial volume into fresh medium with rice starch and checking for amebal growth. The flora can be stored at 4°C indefinitely. The second method is that of Smedley (Smedley, S. R. 1956) and is used when *B. hominis* appears in cultures after inoculation. It does not rely on cysts being present and so has advantages over the method of Dobell and Laidlaw in that respect. However, the method may need to be repeated a couple of times before the *B. hominis* is completely eliminated. Cultures are pelleted, and the pellet, which contains a mixture of all the organisms present, is resuspended in distilled water at room temperature for 15 min. The material is then repelleted and inoculated into fresh culture medium. Perhaps surprisingly, many

Entamoeba trophozoites survive this treatment while *B. hominis* generally does not. A few cells or cysts of *B. hominis* may survive and start to grow, and the procedure will then need to be repeated. The advantages of Smedley's method are its simplicity and the fact that no separate bacterial flora is needed. Other unwanted organisms such as fungi and trichomonads will usually disappear from xenic cultures after several passages. However, occasional instances of balanced mixed cultures are known.

Isolation:LE medium has proven to be the best medium for primary isolation of *Entamoeba* species from stool, although we have limited experience with Robinson's medium, which is widely used by others for this purpose. TYSGM-9 can also be used for isolation, but its primary utility is in generating large numbers of amebae from established cultures. The numbers of amebae obtained from the two diphasic media are generally low in comparison with TYSGM-9, but their success in primary isolation of amebae from microscopically positive stool is higher (Sehgal, 1995). In all cases, rice starch is added to the medium before inoculation, as are the antibiotics when needed. Material for inoculation of xenic cultures can be prepared in several ways (Diamond, L. S. 1983). Most commonly, stool samples are emulsified in saline and passed through a mesh to remove most of the larger particulates from the material before addition to the culture medium. However, small-pea-size pieces of fecal material can be added directly to the medium. It is always a good idea to include portions of the stool that appear mucoid or bloody if these are present. Stool fractionation by flotation in zinc sulfate (Ash, R. L, 1987) or sucrose (Walderich, B, 1997) is also used, as this reduces the amount of debris while concentrating the cysts present in the sample. However, zinc sulfate shrinks the cysts and may damage them, resulting in lower isolation rates. Cyst purification on Percoll gradients (Avron, B, 1983) is very successful when using culture material as a starting point and could well be adapted to isolation from stool. We routinely use more than one medium, if available, and set up duplicate cultures in which one has antibiotics added and the other does not. Penicillin streptomycin and erythromycin are the antibiotics of choice, as they appear to have little direct effect on the amebae. However, the widespread occurrence of antibiotic resistance in bacteria makes it impossible to generalize about the amount and type of antibiotics necessary to control the growth and rice-splitting activity of human bacterial flora. Culture tubes, containing

medium and rice starch, to which stool-derived material has been added, are incubated vertically at 35.5°C for 48 h before examination (Figure:5). A drop of sediment can be extracted from the tube for examination on a microscope slide. Alternatively, cultures can be examined in situ by slanting the tubes and using an inverted microscope. Amebae can be observed adhering to the walls of the glass culture tubes above the fecal material and above the slant in diphasic media. In situ examination is much easier in monophasic medium due to its relative clarity. If no growth is observed at 48 h, a blind passage should be made. Most of the liquid overlying the sediment is discarded to leave less than 1 ml in the tube. The sediment is resuspended in the remaining fluid and transferred to a fresh culture tube with medium and rice starch (and antibiotic if appropriate). After incubation for an additional 48 h the culture is reexamined as described above. If no amebae are seen further 48-h incubation is warranted, and this is followed by reexamination. If there are still no amebae seen, the culture is discarded as negative. If cultures are positive for amebae, it is usually helpful to centrifuge the cultures in a swinging-bucket rotor (275 × *g*, 3 min) and divide the pellet among the recipient tubes. This can be done by chilling the culture tubes for 5 min in an ice-water bath, inverting several times to detach adherent amebae, and transferring the liquid phase to an empty culture tube before centrifugation. Cultures in LE medium can also be pelleted in situ, but in our experience the agar slant of Robinson's medium is not as conducive to this approach. As growth improves, centrifugation is no longer necessary as measured inocula (<2 ml) can be transferred to the fresh medium.

Axenization:Axenization of *E. histolytica* is a long and laborious procedure involving gradual adaptation of the parasite to a new way of life. A brief overview of the method is given here. A more complete description can be found in the works of Diamond (Diamond, L. S. 1968, Diamond, L. S. 1983). The first step is to grow the organisms in monoxenic culture and is achieved by washing the xenic amebae in PBS to remove as many bacteria as possible and then placing the trophozoites in a rich medium with their new food organism and antibiotics. The medium can be a specialized monoxenic culture medium as described previously (Diamond, L. S. 1968), but we have also had success initiating such cultures using one of the axenic media such as TYI-S-33. The monoxenic associate we have used most frequently is *C. fasciculate* ReF-1: PRR (Diamond, L. S.

1968).Improved method for the monoxenic cultivation) (ATCC 50083). This insect flagellate is grown as a stock culture at room temperature and added to the monoxenic culture of amebae at each subculture, as *Crithidia* does not grow at the incubation temperature of the amebae; the amount added varies. *T. cruzi* Culbertson (ATCC 30013) has also been used successfully as the associated organism but is not recommended due to the potential for infection, even though this strain is of very low virulence. The antibiotics added vary both in type and amount depending on the sensitivities of the flora in which the amebae were growing. We have used a cocktail of rifampin, amikacin, oxytetracycline, and cefotaxime with good success. Except for the first agent, these have little effect on the amebae. The initial concentration is often as high as 0.1 mg/ml of culture medium. After 24 h, the cells are pelleted by centrifugation and the medium is replaced. As the ameba cell numbers increase, the cell pellet can be divided between two tubes. By reducing the antibiotic concentration gradually in one of a pair of tubes to test for bacterial growth, sterility can be achieved gradually while at the same time the numbers of amebae are increasing. At least two subcultures in the absence of antibiotics should be performed before the cultures can be considered free of bacteria. This can be verified using standard aerobic and anaerobic testing procedures for bacteria, including mycoplasmas, and fungi (Diamond, L. S. 1983). Established monoxenic cultures, those in which growth is reproducible and bacteria are absent, are then used to initiate axenic cultures. This uses the same medium but with no *Crithidia* added. After a few subcultures the flagellates disappear as a result of dilution and ingestion. It is often helpful, although not always necessary, to add a small amount of Noble agar to the tubes at each subculture (0.01%, wt/vol; for example, adding 0.25 ml of a melted 0.5% stock agar solution to 13 ml of medium in a tube). It appears to form a substrate for the amebae. In addition, the tubes should be incubated vertically rather than at 5° to the horizontal, as this appears less "stressful" to the cells. Often the culture will flourish initially and then numbers will crash. It is at this crisis point that the cultures are most vulnerable. As long as a few live cells persist, it is worth continuing to replace the medium every few days. With luck, the numbers will gradually start to increase again, and eventually addition of the agar will no longer be needed. When established, the axenic cultures can be incubated at 5° to the

horizontal and eventually should reach cell concentrations of 150,000 to 300,000 per ml on a twice-per-week subculture schedule.

3.2.7. Principles of inducing encystment of *E. histolytica*

The methods of inducing encystment of *E. histolytica* are based on Dobell and Laidlaw's discovery that "cyst production may sometimes be temporarily increased by cultivating the amoebae in starch-free media for one or two generations, and then transferring them to media containing this substance: but the results are uncertain, and the number of cysts produced in any culture cannot be predicted" (Dobell C, 1926). Each point these authors make holds true to this day, and anyone attempting to induce encystment must bear these points in mind at all times. To date, cysts of this species have been induced only in xenic culture. No one as far as we can determine has published a detailed account of the process of inducing encystation. Here we present a protocol used for many years in the NIH Laboratory of Parasitic Diseases but never previously published in detail. Three things are of special concern in obtaining cysts: the media, bacterial flora, and rice starch. Some media are better than others for this purpose.

The accompanying bacterial flora present in a xenic culture plays an important role in the process of encystment. Here again some are better than others. It is good practice for those requiring a steady source of large numbers of cysts to isolate and maintain the bacterial flora of a xenic culture in which cysts regularly form spontaneously. A technique for doing this is presented above (under "Elimination of unwanted organisms").

(i) Protocol: encystment

(a) Day 1. Begin the process with three amebae-rich 48-h cultures in LE medium. Harvest them by chilling the culture tubes for 5 min in an ice water bath, invert the tubes 10 times to mix contents and free amebae adhering to the glass and egg slant, and centrifuge for 3 min at 275 × *g*. Remove and discard all but 1 ml of the spent overlay. Resuspend pelleted amebae, pool, and transfer equal amounts to six tubes of LE medium without rice. Incubate the cultures in an upright position for 72 h.

(b) Day 4. Harvest each of the six cultures: Chill, remove and discard all but 1 ml of overlay. Mix remaining overlay of each culture and transfer equal amounts to two tubes of LE medium without rice. There will now be 12 cultures. Incubate 48 h.

(c) Day 6. Harvest the 12 cultures and subculture as on day 4. Incubate the 24 cultures for 48 h.

(d) Day 8. Carefully remove the overlay from each culture, leaving only enough to cover the sediment at the interface of the egg slant and overlay. Collect the sediments from three cultures and transfer to one tube of medium to which rice has been added. Repeat with the remaining cultures. Incubate the resulting eight cultures for 48 h.

(e) Day 10. Remove a small drop of sediment from each culture, stain with Lugol's iodine solution, and search for presence of quadrinucleate cysts. If found, harvest cultures as on day 1. Remove overlay, leaving only the sediment. Pool sediments and wash two times with distilled water. Cysts will remain viable from 10 to 14 days when stored at 4°C. If cysts are not found, incubate an additional 24 h.

(ii) Protocol: excystment of cysts induced in vitro

Inducing *E. histolytica* to excyst is relatively easy compared to getting the ameba to encyst. How this is accomplished depends on the goal. If the goal is to propagate the amebae in a xenic environment, then the medium in which the cysts were induced is used, in this case LE medium. If the goal is to excyst them in a bacteria-free environment, any of the monophasic liquid media devised for axenic culture can be used. In the latter case freshly prepared medium must be used. While the amebae will excyst in the axenic media, no one, as yet, has been able get them to encyst in this environment. Best results are obtained when at least 50% of the cysts produced are in the quadrinucleate stage. Usually no more than 25% of the cysts can be expected to excyst. To induce excystment, the cysts are first treated to remove unwanted organisms as recommended above. They are then placed in a tube of LE medium inoculated with a suitable bacterial flora for xenic growth or in a medium capable of sustaining axenic growth. Upon incubation most of the cysts capable of undergoing excystation will have done so by the end of 6 h.

3.2.8. Rexenization of axenically cultivated *E. histolytica*

Occasions will arise when it is desirable to return axenized amebae to the xenic state. The following protocol has worked well in our hands.

(i). Inoculate three tubes of LE medium with a bacterial flora known to support xenic growth.

(ii). Chill a 72-h culture of axenically cultivated amebae in an ice-water bath for 5 min. Invert culture tube 10 times to dislodge amebae from glass surfaces. Centrifuge 3 min at 275 × *g*. Remove supernatant and discard.

(iii). Resuspend amebae in 1 ml of fresh medium for axenic culture, count cells, and inoculate the tubes of LE medium with 1 ×105, 2 ×105, and 4 ×105 amebae, respectively.

(iv). Incubate 48 h. Remove all but approximately 1 ml of overlay. Resuspend the sediment located at the interface of the slant and overlay. Examine a drop with a microscope. The majority of inoculated amebae will have died. Select the best of the three cultures and subculture.

(v). Subculture. The number of amebae transferred can be determined only by trial and error. In the early stages of establishing the culture, transfer one-half of the material from the old culture to each of two tubes of fresh medium (do not add additional bacteria after the initial inoculation of the medium). Later, as amebic growth improves, transfer smaller portions, e.g., one-third to one-fourth. Do not reduce the inoculum further. If only a few amebae are present, transfer all the sediment to a fresh tube of medium. Subculture three times per week as noted under "Maintenance of Cultures."

3.2.9. Maintenance of cultures

Established cultures of all parasites are handled in essentially the same way. Xenic cultures of *E. histolytica* are routinely passaged at 48- to 72-h intervals; usually a Sunday-Tuesday-Thursday schedule is convenient. Occasionally cultures of these organisms will be found that do better with twice-weekly subculture. The inoculum size for the longer incubation period should be smaller than that for shorter incubations. However, variation among isolates and flora means that no generalities can be made regarding the size of inocula or the amount of rice and antibiotics to be added to the medium for optimal growth. It is very much a case of trial and error combined with experience in evaluating growth of cultures that leads to successful establishment of these parasites in xenic culture. It is recommended that xenic cultures be passaged using two or more inoculum sizes to ensure a successful subculture. A significant threshold effect can sometimes be encountered, in which a certain inoculum size gives rise to a healthy culture but an inoculum smaller by as little as 50 μl may result in no growth.

Established axenic cultures of *E. histolytica* are passaged at 72- and 96-h intervals, with a Sunday-Thursday schedule being convenient. Visual inspection of every culture before subculture is recommended, since what appears to be a heavy culture may in fact contain many lysed cells, indicating that the inoculum previously used was too large. An increased inoculum volume may be warranted for the subsequent subculture to compensate for the dead amebae. Likewise, parallel duplicate cultures are recommended in case of inadvertent contamination or tube breakage. The unused culture can be kept at 33°C as a backup in case of problems.

The method for subculturing many types of cultures is essentially the same. Cultures are chilled in an ice-water bath for 5 min (xenic cultures and axenic *E. histolytica*) to release trophozoites attached to the glass culture tube. Tubes are inverted several times to disperse the cells and a measured inoculum is passed aseptically to a culture tube containing fresh medium. The tubes are capped tightly and incubated at 36 to 37°C, either vertically at 5° to the horizontal (established axenic cultures of *E. histolytica*).

3.3. Measurement of amoebicidal activity:

The extract of the seeds of Carica papaya has a good amoebicidal activity according to the study.The clinical isolates of *Entamoeba histolytica* were treated with the seed extract of Carica papaya at different concentrations (3.95mg/ml, 1.97mg/ml,0.98mg/ml,0.21mg/ml/0.11mg/ml and 0.05mg/ml). A control group was made to measure the change in the viable counts and was put into the elisa plate.Each well of an elisa plate now contained different concentration of extract and same amounts of *Entamoeba histolytica (*100micro liters*)*.After that the preparation was incubated for a definite period of time (24,48 hours).Finally the viable and non-viable counts of *Entamoeba histolytica* was counted and recorded in a table or graphs which demonstrated that seed extract of Carica papaya has good sensitivity against clinical isolates of *Entamoeba histolytica.*

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

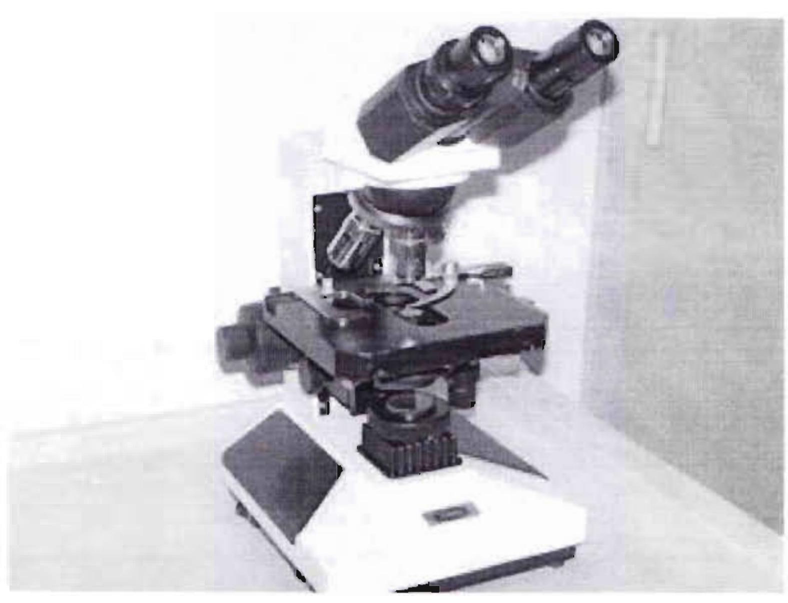


Figure 4: Microscope

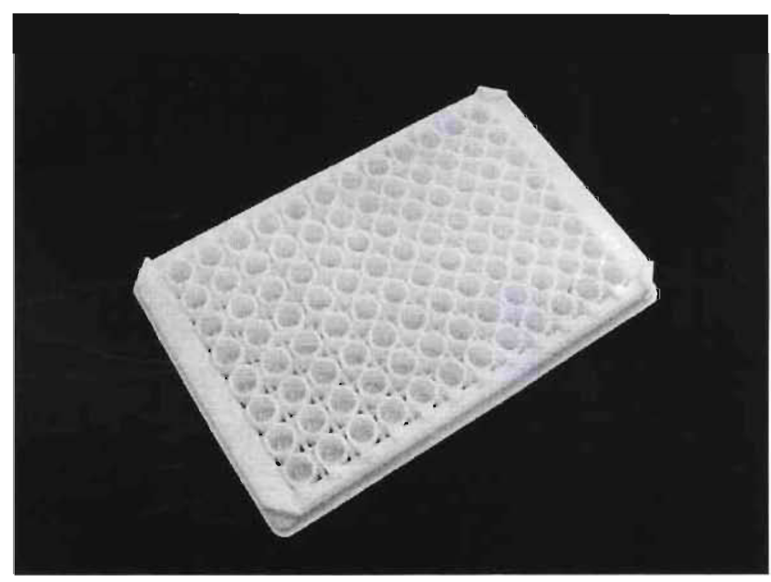


Figure 5: ELISA plate

CHAPTER 4
RESULTS

Table: 4.1.Percentage of viable count of *Entamoeba histolytica* after 48 hours of incubation with different concentrations of seed extract of Carica papaya.

.

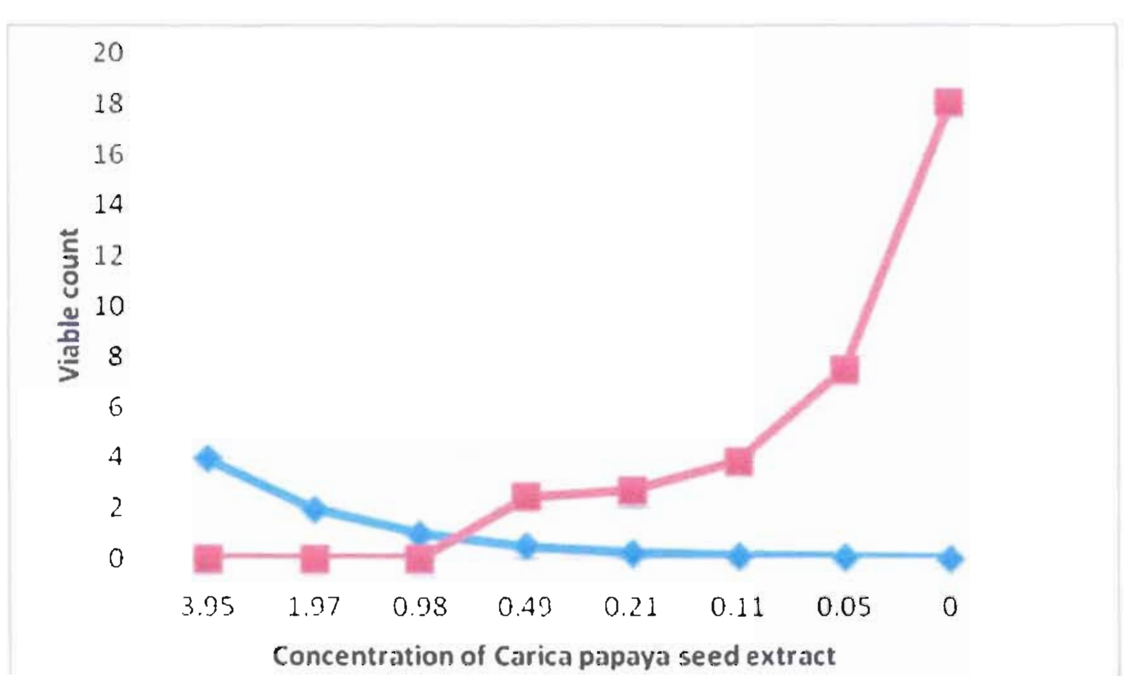


Figure 6: Percentage of viable counts of *Entamoeba histolytica* after 48 hours of incubation with different concentrations of seed extract of Carica papaya.

Table 4.1 shows the viable count of *Entamoeba histolytica* after treating with different concentrations of seed extract of Carica papaya for 48 hours. The initial count of *Entamoeba histolytica* was $1.94*10^6$ mg/ml. After 48 hours the viable count was 0% in 3.95, 1.97 and 0.98 mg/ml of concentrated seed extract of Carica papaya papaya.Viable count of *Entamoeba histolytica* was increased to 2.448% when the concentration of seed extract of Carica papaya was decreased to 0.49 mg/ml and the viable counts increased as the concentration of Carica papaya seed extract decreased. In 0.21mg/ml,0.11mg/ml and 0.05mg/ml concentrations of Carica papaya seed extract the percentage of viable count of *Entamoeba histolytica*(per ml) was 2.706%,3.865% and 7.469% .

Table: 4.2.Percentage of viable count of *Entamoeba histolytica* after 24 hours of incubation with different concentrations of seed extract of Carica papaya.

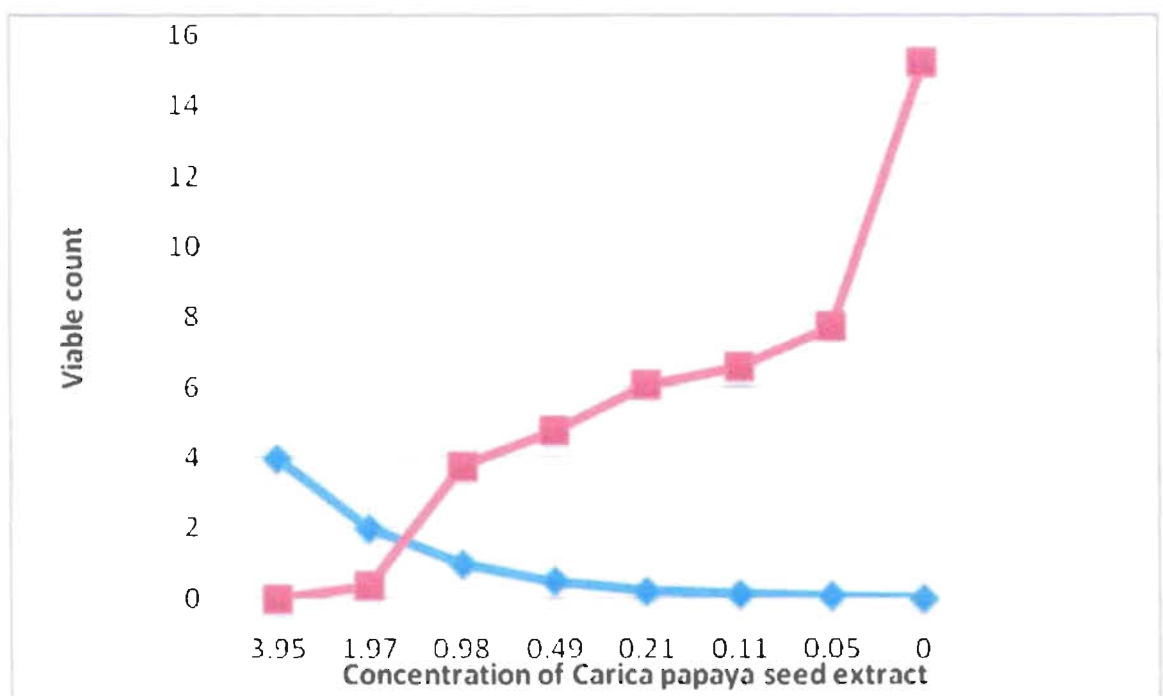


Figure7: Percentage of viable counts of *Entamoeba histolytica* after 24 hours of incubation with different concentrations of seed extract of Carica papaya.

Table 4.2 shows the viable count of *Entamoeba histolytica* after treating with different concentrations of Carica papaya seed extract for 24 hours. The initial count of *Entamoeba histolytica* was $1.94*10^6$ mg/ml. After 24 hours the viable count was 0% in 3.95 mg/ml of concentrated Carica papaya seed extract. Viable count of *Entamoeba histolytica* was increased to 0.36% when the concentration of Carica papaya seed extract decreased to 1.97mg/ml and the viable counts increased as the concentration of seed extract of Carica papaya decreased. In 0.98mg/ml,0.49mg/ml and 0.21mg/ml,0.11mg/ml and 0.05mg/ml concentrations of Carica papaya seed extract the viable count of *Entamoeba histolytica*(per ml) was 3.737%, 4.768%,6.056%,6.572%,7.731% and 16.206%.

Table: 4.3.Percentage of viable count of *Entamoeba histolytica* after 48 hours of incubation with different concentrations of seed extract of Carica papaya.

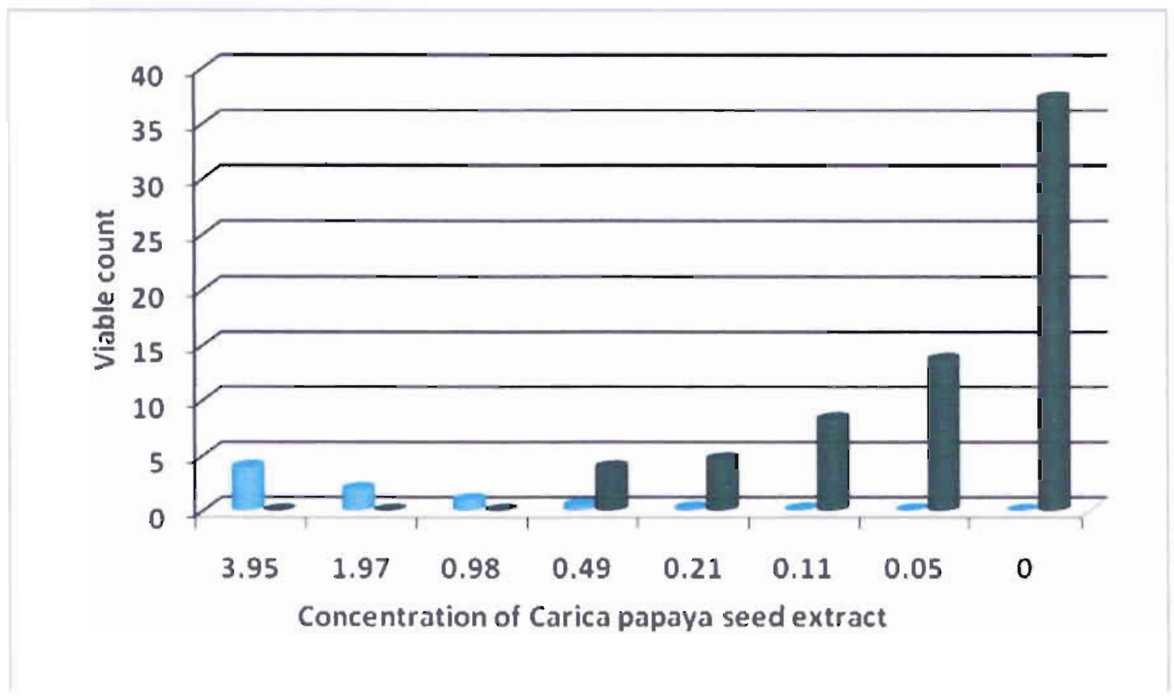


Figure 8: Percentage of viable counts of *Entamoeba histolytica* after 48 hours of incubation with different concentrations of seed extract of Carica papaya.

Table 4.3 shows the viable count of *Entamoeba histolytica* after treating with different concentration of seed extract of Carica papaya for 48 hours. The initial count of *Entamoeba histolytica* was $1.025*10^6$ mg/ml. After 48 hours the viable count was 0% in 3.95,1.97 and 0.98 mg/ml concentrated seed extract of Carica papaya.Viable count of *Entamoeba histolytica* was increased to 3.902% when the concentration of seed extract of Carica papaya was decreased to 0.49 mg/ml and the viable counts increased as the concentration of seed extract of Carica papaya decreased. In 0.21mg/ml,0.11mg/ml and 0.05mg/ml concentrations of Carica papaya seed extract the viable counts of *Entamoeba histolytica*(per ml) were 4.634%,8.292% and 13.658%.

Table: 4.4.Percentage of viable count of *Entamoeba histolytica* after 24 hours of incubation with different concentrations of seed extract of Carica papaya.

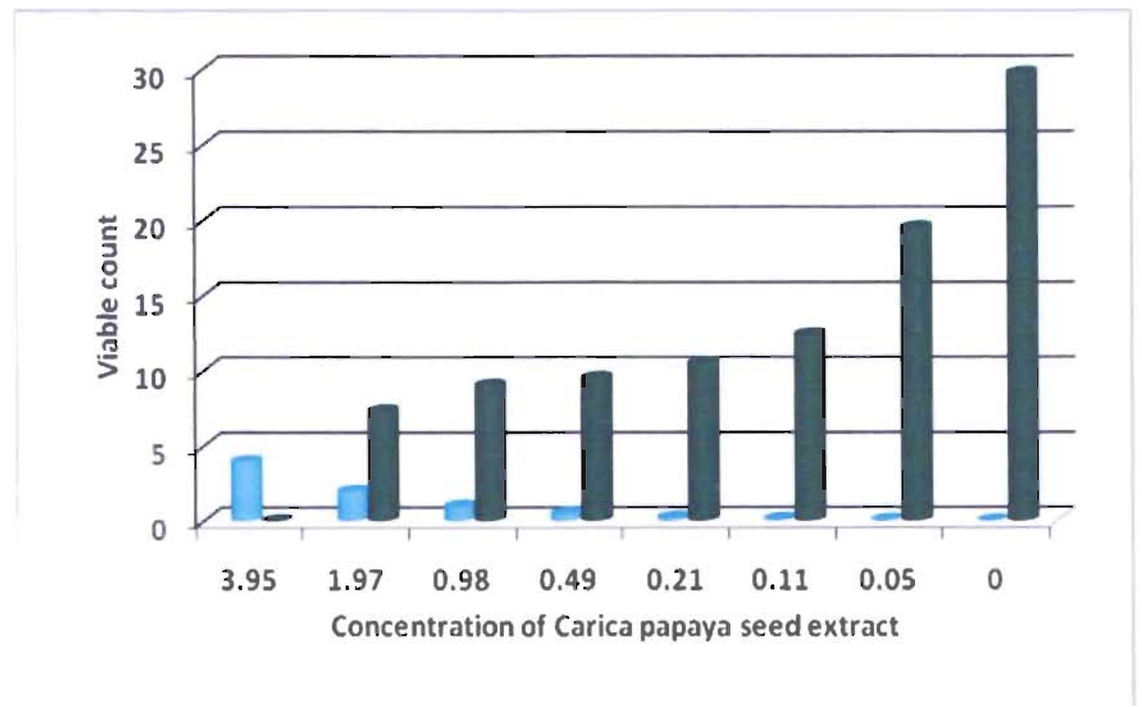


Figure 9: Percentage of viable counts of *Entamoeba histolytica* after 24 hours of incubation with different concentrations of seed extract of Carica papaya.

Table 4.4 shows the viable count of *Entamoeba histolytica* after treating with different concentrations of seed extract of Carica papaya for 24 hours. The initial count of *Entamoeba histolytica* was $1.025*10^6$ mg/ml. After 24 hours the viable count was 0% in 3.95 mg/ml concentrated seed extract of Carica papaya.Viable count of *Entamoeba histolytica* was increased to 7.317% when the concentration of Carica papaya seed extract decreased to 1.97mg/ml. The viable counts increased as the concentration of Carica papaya decreased. In 0.98mg/ml,0.49mg/ml and 0.21mg/ml,0.11mg/ml and 0.05mg/ml concentrations of Carica papaya seed extract the viable count of *Entamoeba histolytica*(per ml) was 9.024%,9.512%,10.487%,12.439% and 19.512%.

Table: 4.5.Percentage of non-viable count of *Entamoeba histolytica* after 48 hours of incubation with different concentration of seed extract of Carica papaya.

Concentration of papaya extract	Initial count of parasite(per ml)	Percentage of viable count of *Entamoeba histolytica*	Percentage of non-viable count of *Entamoeba histolytica*
3.95	1450000	0%	100%
1.97	1450000	0%	100%
0.98	1450000	0%	100%
0.49	1450000	1.724%	98.276%
0.21	1450000	3.103%	96.897%
0.11	1450000	4.482%	95.518%
0.05	1450000	10.137%	89.863%
0	1450000	14.655%	85.345%

Figure10: Percentage of non-viable count of *Entamoeba histolytica* after 48 hours of incubation with different concentration of seed extract of Carica papaya.

Table: 4.5 shows the non viable count of *Entamoeba histolytica* after 48 hours of incubation with different concentrations of seed extract of Carica papaya but the initial concentration of the *Entamoeba histolytica* was same as $1.45*10^6$ per ml. Non viable count of parasite was increasing with the increasing concentration of the seed extract of Carica papaya and when the concentration reached to 0.98 mg/ml then it becomes constant (100%).

The lowest number of non viable count of *Entamoeba histolytica* was found in the control and highest in the, 3.95 mg/ml, 1.97 mg/ml, and 0.98 mg/ml of extract of Carica papaya seed. The non-viable count in 0.49mg/ml,0.21mg/ml,0.11mg/ml and 0.05mg/ml were 98.276%, 96.897%, 95.518% and 89.863%.The non-viable count in the control group was 85.345%.

Table: 4.6. Percentage of non-viable count of parasite after 24 hours of incubation with different concentrations of seed extract of Carica papaya.

Concentration of papaya extract	Initial count of parasite(per ml)	Percentage of viable count of *Entamoeba histolytica*	Percentage of Non-viable count of *Entamoeba histolytica*
3.95	1450000	0%	100%
1.97	1450000	6.034%	93.966%
0.98	1450000	7.068%	92.932%
0.49	1450000	8.448%	91.552%
0.21	1450000	9.482%	90.518%
0.11	1450000	10.172%	89.828%
0.05	1450000	12.413%	87.587%
0	1450000	21.034%	78.966%

Figure1: Percentage of non viable count of parasite after 24 hours incubation with different concentration of seed extract of Carica papaya.

Table: 4.6 shows the non viable count of *Entamoeba histolytica* after 48 hours of incubation with different concentrations of seed extract of Carica papaya but the initial concentration of the *Entamoeba histolytica* was same as $1.45*10^6$ per ml. Non viable count of parasite was increasing with the increasing concentration of the seed extract of Carica papaya and when the concentration reached to 0.98 mg/ml then it becomes constant (100%).

The lowest number of non viable count of *Entamoeba histolytica* was found in the control and highest (100%) in the, 3.95 mg/ml of extract of Carica papaya seed. The non-viable count in 1.97mg/ml,0.98mg/ml,0.49mg/ml,0.21mg/ml,0.11mg/ml and 0.05mg/ml were 93.966%, 92.932%, 91.552%, 90.518%89.828% and 87.587% respectively.The non-viable count in the control group was 78.966%.

CHAPTER 5: DISCUSSION

Discussion

The results obtained from this study show potential therapeutic efficacy of extract of Carica papaya seed used against *Entamoeba histolytica* associated amoebiasis treatment.

Carica papaya has always been a medicinal plant as a source of drugs based mainly on herbal remedies used in traditional medicine.

In Ayurverdic system of traditional medicine, 0.5–1 g of powdered papaya seeds is prescribed for medicinal use (Kapoor, 1990). In Cuba, up to 4.5 g of papaya seeds are recommended per day as vermifugal agent (Roig y Mesa, 1974). Nevertheless, no investigation has been undertaken with a view to determine the effect of antiamoebic potential of seed extract of Carica papaya.

Under suitable physiological conditions, isolated *Entamoeba histolytica* are capable to respond to seed extract of Carica papaya and the incidence of amoebic dysentery is reduced in a similar fashion to synthetic drugs but the concentration needed for seed extract of Carica papaya to inhibit susceptibility of *Entamoeba histolytica* is greater in comparison to synthetic drugs.

It is observed that extract of Carica papaya seed has high efficacy on inhibition of clinical isolates of *Entamoeba histolytica* which is the organism responsible for amoebiasis. It can effectively inhibit the disease causing parasite trophozoite.

Different concentrations of seed extract of Carica papaya (3.95 mg/ml, 1.97mg/ml, 0.98mg/ml, 0.49mg/ml, 0.21mg/ml, 0.11mg/ml, 0.05mg/ml) caused inhibition of *Entamoeba histolytica* in different manner. In 3.95mg/ml,1.97mg/ml and 0.98mg/ml concentration for an incubation period of 48 hours the amoebicidal activity was highest and the viable count of *Entamobea histolytica* was 0%.Viable count of *Entamoeba histolytica* was varied with the decreasing concentrations of seed extract of Carica

papaya. For an incubation period of 24 hours the count was increasing in similar manner with the decreasing concentrations of seed extract of Carica papaya. For non viable counts of *Entamoeba histolytica* the count was directly proportional to the concentration of the Carica papaya seed extract which implies that the non-viable count of *Entamoeba histolytica* was decreased with the decreasing concentrations of seed extract of Carica papaya. At concentrations 3.95mg/ml,1.97mg/ml and 0.98mg/ml the non-viable count was 100% for an incubation period of 48 hours and it decreased gradually with the decreasing concentration of Carica papaya seed extract. For 24 hours the result was found in similar manner. A control group was made to measure the change in the viable counts of *Entamoeba histolytica.*

Though extract of Carica papaya seed is comparatively new or innovative treatment option against amoebiasis but its sensitivity towards *Entamoeba histolytica* is high according to our study.

CHAPTER 6:
CONCLUSION

Conclusion

Amoebiasis is a common infection of the human gastrointestinal tract. It has a world wide distribution. It is probable that invasive amoebiasis, accounts annually for 40,000 to 110,000 deaths in the world. *Entamoeba histolytica* is the third leading cause of morbidity and mortality due to parasitic disease in humans (after malaria and schistosomiasis) and is estimated to be responsible for between fifty thousand and one lac deaths worldwide every year.

Extract of Carica papaya seed is a new natural medicine of *Caricaceae* family used to treat amoebiasis. This extract was taken to study to see the efficacy on clinical isolates of *Entamoeba. histolytica*.

Different concentrations of the seed extract of Carica papaya was treated with same amount of *Entamoeba histolytica* (100 mircoiliters). The concentrations are 3.954mg/ml, 1.972mg/ml, 0.986mg/ml, 0.493mg/ml, 0.216mg/ml, 0.108mg/ml and 0.054mg/ml. We ran all the concentrations and measured their capability of inhibiting the clinical isolates of *Entamoeba histolytica* and a control was used to count the viable parasite without any extract which was useful in comparison. The rate of inhibition of the clinical isolates increased with the increasing concentration of the extract. The maximum concentration of seed extract of Carica papaya was 3.95mg/ml which resulted 100% inhibition of the clinical isolates of *Entamoeba histolytica* for incubation periods 48 and 24hours respectively.

From this result, we found that the extract of Carica papaya seed has a very good efficacy. No chance of substandard therapeutic efficacy as it is obtained from natural source. The solvent (DMSO) in which the extract was dissolved has no lethal effect on clinical isolates of *Entamoeba histolytica.* So, it can be said that the solvent did not interpret of getting the accurate result.

From above discussion it can be concluded that extract of Carica papaya seed has high sensitivity against clinical isolates of *Entamoeba histolyticas.*

References:

A Practical Treatise On Materia Medica And Therapeutics book, Roberts Bartholow, 1908.

A Prospective Study of Delirium in Hospitalized Elderly

Joseph Francis, MD, MPH; David Martin, MD; Wishwa N. Kapoor, MD, MPH

JAMA. 1990;263(8):1097-1101.

Ann Clin Microbiol Antimicrob. 2004 Dec 21; 3:27. *In vitro activity of antiamoebic drugs against clinical isolates of Entamoeba histolytica and Entamoeba dispar.*Devendra Bansal, Rakesh Sehgal, Yogesh Chawla, Ramesh Chander Mahajan and Nancy Malla

Asian Journal of Andrology 4: 17–26- Bhande,and S. Panneerdoss,March 2002,P.K.Mishra,N.Pathak, 2002,Lohiya, N. K et.al,March 2002,Gill, 1992.

Medicina (B Aires). 2009; 69(3):318-20.*Sensibility of Entamoeba histolytica trophozoites to ivermectin*

Carica, The Gift of Health, Carica Herbal Health Products, Inc

Child health care,Prof. David Woods,July 2007

Dobell, C., and P. P. Laidlaw. 1926. On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. Parasitology 18:283–318.

Diamond, L. S. 1968. *Techniques of axenic cultivation of Entamoeba histolytica* Schaudinn, 1903 and E. histolytica-like amebae. J. Parasitol. 54:1047–1056.

Diamond, L. S. 1968. *Improved method for the monoxenic cultivation of Entamoeba histolytica* Schaudinn, 1903 and E. histolytica-like amebae with trypanosomatids. J. Parasitol. 54:715–719.

Eur J Intern Med. 2009 Sep,Day, David W.; Basil C. Morson, Jeremy R. Jass, Geraint Williams, Ashley B. Price (2003). *Morson and Dawson's Gastrointestinal Pathology*. John Wiley & Sons, Inc... ISBN 9780632042043

Expert Reviews in Molecular Medicine (1999), 1:9:1-11 Cambridge University Press

Molecular-based diagnosis of *Entamoeba histolytica* infection,Christopher D. Huston , Rashidul Haque and William A. Petri Jr

FDA Bacteriological Analytical Manual, Retrieved 2008-03-26

Inflammopharmacology 16 (2008) 168–173, Inflammopharmacology Research Article,*Anti-inflammatory activities of ethanolic extract of Carica papaya Leaves,*Bamidele V. Owoyele, Olubori M. Adebukola, Adeoye A. Funmilayo and Ayodele O. Soladoye

In Vitro Cultivation of Protozoan Parasites by LS Diamond, JB Jensen - 1983 - CRC Press

J Med Food. 2007 Mar; 10(1):194-6,*Effectiveness of dried Carica papaya seeds against human intestinal parasitosis: a pilot study.*Okeniyi JA, Ogunlesi TA, Oyelami OA, Adeyemi LA.

J. Nat. Prod. 45 (1982), Indian J. Med. Sci., 1961; Indian J. Pharmacology, 1965; J. Ethnopharmacol. (1995).

Life Sci. 2003 Dec 19; 74(5):581-92. *Tocolytic and toxic activity of papaya seed extract on isolated rat uterus.* Adebiyi A, Ganesan Adaikan P, Prasad RN.

Medical microbiology,Samuel baron,4rth edition

Modulation of Jejunal Contractions by Extract of Carica papaya L. Seeds, Adebowale Adebiyi, P. Ganesan Adaikan ,Phytotherapy Research,Volume 19 Issue 7, Pages 628 - 632

Multiplication and life cycle of *E histolytica(*Medical microbiology,Samuel baron,4rth edition

Manual Of Tropical And Subtropical Fruits", by Wilson Popenoe, 1920

On the cultivation of Entamoeba histolytica and some other entozoic by C Dobell, PP Laidlaw - 1926

Peninsula Tree and Forestry Science and Biotechnology, 2007 .

Robinson, G. L. 1968. *The laboratory diagnosis of human parasitic amoebae.* Trans. R. Soc. Trop. Med. Hyg. 62:285–294.

Techniques of axenic cultivation of Entamoeba histolytica Schaudinn, 1903 and Entamoeba histolytica.
LS Diamond - The Journal of Parasitology, 1968

Tree and Forestry Science and Biotechnology, 2007

Tocolytic and toxic activity of papaya seed extract on isolated rat uterus,

The Lancet, Volume 361, Issue 9362, Pages 1025-1034,S.Stanley, Jr

Tree and Forestry Science and Biotechnology, 2007

Trop Anim Health Prod,*A comparative study of the efficacy of piperazine and Carica papaya for the control of helminth parasites in village chickens in Zambia ,Amos Chota & Chummy Sikalizyo* Sikasunge & Andrew M. Phiri & Martha N. Musukwa & Felix Haazele & Isaac K. Phiri

Veterinary parasitology,Volume 152, Issues 3-4, 15 April 2008, Pages 264-270

West Indian Med J. 2008 Sep; 57(4):323-6.*Protective effect of Carica papaya L leaf extract against alcohol induced acute gastric damage and blood oxidative stress in rats.*