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IN-VITRO STUDY OF *ENTAMOEBA HISTOLYTICA* WITH COCONUT JUICE ACT AS AN ANTIAMOEBIC AGENT AT DIFFERENT CONCENTRATION IN NIH MEDIUM

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ABSTRACT
Amoebiasis is a parasitic infection caused by *Entamoeba histolytica*. It is usually contracted by ingesting water or food contaminated with amoebic cysts. Amoebiasis is an intestinal infection that may or may not be symptomatic. When symptoms are present it is generally known as invasive amoebiasis. It is anaerobic parasitic protozoan, motile, commonly found in human intestine and it is also found in animals example like cat and goat but its definitive host is human beings. Its Infective stage is quadrinucleated cyst is called trophozoite. Invasive intestinal amebiasis is initiated with attachment of trophozite to the colonic mucous layer and it starts the mucous disruption and depletion. Mucous secret by fecal. Infection spread mainly by soiled hands, contaminated water and food or direct contact with carrier containing cysts of the protozoa. Man who has sex with man can also become infected. Approximately 50 million people have invasive disease resulting in 1, 00,000 death/year. After malaria it is the second severe disease because the parasite has a worldwide distribution so it is called worldwide disease. More than 10% of the population have been reported from various developing countries.
KEYWORDS: Trophozoite, Amoebiasis, Parasite

INTRODUCTION

Amebiasis is an intestinal illness caused by a microscopic parasite called *Entamoeba histolytica*, to trace the cause of the disease it is necessary to know what you ate and drank in the previous weeks, and/or where you traveled before you became ill. *Entamoeba histolytica* parasites are only found in humans. After infection, it may take from a few days up to two to four weeks before becoming ill. Some people with amebiasis may carry the parasite for weeks to years, often without symptoms. Amoebiasis can progress to amoebic dysentery in the wide, lower part of the intestine and then spread to cause severe damage to the intestine. Although rarely, amoebiasis can cause abscesses in the liver, lungs, and brain or even elsewhere in the body. Amoebiasis occurs when *Entamoeba histolytica* parasites are taken in by mouth, eaten or swallowed something infected with such parasite, however the most common way this happens is by person-to-person spread. People with amoebiasis have *Entamoeba histolytica* parasites in their feces, and their contaminated hands can spread the parasites to surfaces and objects which will be touched by other people. Under certain circumstances, this disease may also spread sexually by oral-anal contact. In your household, the risk to spread amoebiasis can be reduced if people infected or suffering gastroenteritis do not prepare or handle food to be eaten by other people and that no one shares their towel or wash cloths. Food handlers, child care workers and health care workers with amoebiasis must not work until symptoms have stopped. Amebic liver abscess in the most common manifestation of invasive amebiasis, but other organs can also be involved including pleura pulmonary, cardiac, cerebral, renal, genitourinary and coetaneous sites[10]. The trophozoites can penetrate and invade the colonic mucosal barrier, leading to tissue destruction, secretory bloody diarrhea and colitis resembling inflammatory bowel disease. In addition, the trophozoites can spread hematogenously via the portal circulation to the liver or even to more distant organs[15]. Amebic liver abscess is 7-12 times more common in man than in women, with predominance among men aged 18-50 years. The reason for this sexual disparity is unknown, although hormonal effects may be implicated, as the prevalence of amebic liver abscess is also increased among postmenopausal women. The sexual distribution is equal in children [25].

The detection of an *Entamoeba histolytica* antigen using an
1) Enzyme - linked Immunosorbent assay (ELISA)
2) The use of the polymerase chain reaction (PCR) &
3) The culture of stool samples[28].

We used the culture of stool sample by the help of microscope. Many antibiotics are using for this disease now a days like Paromomycin, Furamide, Metronidazole. It is commonly used. Other antibiotic are like Imidazole, Idoquinone are also used for this purpose. Paromomycin, Furamide are not commercially available in U.S.A. or Canada only being available from the centers for disease control and prevention.

E. histolytica infections occur in both the intestine and (in people with symptoms) in tissue of the intestine and/or liver. As a result two different sorts of drugs are needed to rid the body of the infection, one for each location. Metronidazole, or a related drug such a tinidazole, is used to destroy amoebae that have invaded tissue. It is rapidly absorbed into the bloodstream and transported to the site of infection. Because it is rapidly absorbed there is almost none remaining in the intestine. Since most of the amoebae remain in the intestine when tissue invasion occurs, it is important to get rid of those also or the patient will be at risk of developing another case of invasive disease. Several drugs are available for treating intestinal infections, the most effective of which has been shown to be Paromomycin (also known as Humatin); diloxanide furoate is used in the US.

A review of the nearly four decades worth of published literature on metronidazole use in pregnant women indicates that it is not teratogenic, regardless of the trimester in which it is used[23. A] 15 year old captive female Dama Wallaby (Macropus eugenii) died with the numerous *Entamoeba histolytica* infection diseased periods was 3 month which is related to weight loss, anorexia and diarrhea. In this infection trophozoites within the gastric mucosa and less frequently, gastric sub mucosa and sub mucosal vessels are also included[29]. In the traditional system of medicine in India. The formulation has been prescribed for intestinal disorders. This study based on the five medicinal harbs, like *Boerharia diffusa, Berbesis aristata, Tinospora cordifolla, Terminalla chebula* and *Zingiber officinale*. The dried and pulverized plants were extracted in ethanol together and individually.

MATERIALS AND METHODS

MATERIALS

a) Direct microscopy for intestinal amoebiasis (from stool sample)
1. Stool sample
2. Centrifuge
3. Formal saline

4. Ether
5. Iodine
6. Distilled water

Methods

The stool sample was taken and mixed thoroughly take 2 ml stool and dilutes it in 10 ml distilled water centrifuge and mix for 5 minutes at 300 rpm. Discarded the supernatant and take the pellet. Apart of pellet was use for acid fast staining in remaining pellet acid 5 ml 10% formal solution in pellet followed by 3ml of ether. Centrifuge at 300 rpm for 5 minute discarded supernatant and take the pellet and mix and make a slide and see it under microscope.

Cultivation method of *E. histolytica* by NIH method. The Preparation of NIH media and ringer's solution are as

Fresh egg fluid 270 ml.

Ringer's solution 70 ml mix thoroughly, distribute 5-6 amount coagulate ringer's solution.

1. sodium chloride (NaCl) 8g/l
2. Calcium chloride ($CaCl_2$) 2g/l
3. Potassium chloride (KCl) 0.2 g/ml
4. Distilled water 1000 ml

The egg brake aseptically and collect the fluid in sterile 500ml flask containing glass beads. Bead the fluid mix yolk, albumin, filter through gauze and measure add the required amount for ringer solution and mix again now distribute 5 to 7 ml amount in screw cap bottle, inspissations in 850 g and coagulate in slanting position. Cool and overlay the silent with lock solution and then autoclave at 15 Ibs. presser for 15 min all the work must be done with aseptic condition.

Lock's solution formula

Sodium chloride, (NaCl) 8.00g
Calcium chloride ($CaCl_2$) 0.2g
Potassium chloride (KCI) 0.2 g
Disodium hydrogen phosphate 2.0 g
Magnesium chloride ($MgCl_2$) 0.01 g
Sodium bicarbonate ($NaC0_3$) 0.4g
Potassium di hydrogen phosphate 0.3g
Distilled water 1000 ml
pH range 7.1

Dissolve, autoclave at 15 lbs for 15 minutes than wile using adjust the reaction at pH 7.1 with N/l0 HCl

Culture

About owe the inoculums from a rich culture showing 40-50 amoebae low pressure filled of microscope is put in the fresh medium bottles. A loopful sterile rich starch is also put in addition to penicillin (1000 per unit) of ever and addition of antiseptic culture bottle is incubated at 37^0C and observed 24 hour subculture is done after an hrs inoculation.

Identification Method: These are following

1. Slide Method
2. Microscopic Examination
3. Hanging drop method
4. Sub-Culturing NIH Media

OBSERVATION

Present study showed my work by the observation table

Table 1 showed the different growth of *Entamoeba histolytica* at different time on 24, 48 & 72 hrs, after 24 hrs. Show low growth, after 48 hrs. Show very low growth, after 72 hrs. No growth observed.

Table 2 showed the different growth of *Entamoeba histolytica* at different time on 24, 48 & 72 hrs, after 24 hrs. Showed low growth, after 48 hrs. Show no growth, after 72 hrs. No growth observed.

Table 3 showed the different growth of *Entamoeba histolytica* at different time on 24, 48 & 72 hrs, after 24 hrs. Showed no growth, after 48 hrs. No growth, after 72 hrs, also no growth observed.

RESULT AND DISCUSSION

Amoebiasis is the second major health problem of world which is caused by *Entamoeba histolytica*. It is the disease of large intestine or liver. Infection spread mainly by soiled hands, contaminated water and food or direct contact with carrier containing cyst of the protozoa. Man who has sex with man can also become infected. Amoebic liver abscess is 7-12 times more common in man than in women, with predominance among men aged 18-50 years. The reason for this sexual disparity is unknown, although hormonal effects may be implicated, as the prevalence of amoebic liver abscess is also increased among postmenopausal women. The sexual distribution is equal in children.

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Table 1: 20 mg concentration of Coconut water

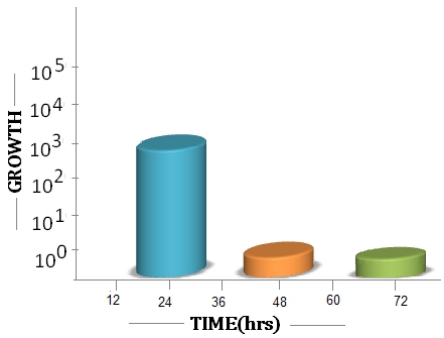
Time [hrs.]	Growth of *Entamoeba histolytica*	
24	+ + + + +	High growth
48	+ +	Low growth
72	__	No growth

Table 2: 30 mg concentration of Coconut water

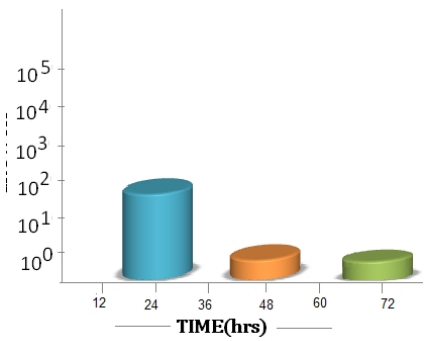
Time [hrs.]	Growth of *Entamoeba histolytica*	
24	+ + +	High growth
48	+	Low growth
72	__	No growth

Table 3: 40 mg concentration of Coconut water

Time [hrs.]	Growth of *Entamoeba histolytica*	
24	-	No growth
48	-	No growth
72	-	No growth



Graph 20 mg Concentration of Coconut water (Graph plotted between Cell growth and Time)



Graph 30 mg Concentration of Coconut water Graph plotted between cell growth and time (hrs)

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