

IN VITRO STUDY OF ENTAMOEBA HISTOLYTICA WITH MANGO JUICE ACT AS AN ANTIAMOEBIIC AGENT AT DIFFERENT CONCENTRATION IN NIH MEDIA

Shrivastava Bhanu^{1*}, Khare R.K², Bhatnagar Vandana³

¹IASCA, ITM Universe, Gwalior, MP, India

²Professor of Botany & Coordinator of Microbiology, Department of Botany & Microbiology, Govt. SMS Science College Gwalior M.P, India

³Faculty of life Sciences in Microbial biotech Research and Training centre, Gwalior M.P., India

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ABSTRACT

Amebic infection of man is one of the most frequently occurring parasite diseases in the tropics and is nearly exclusively acquired in the tropical & subtropical zones of the earth, Recently however amoebiasis is found to occur increasingly in Europe. 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 populations have been reported from various developing countries. Amebiasis is the disease of large intestine or liver caused by *Entamoeba histolytica*. 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 amoebiasis is initiated with attachment of trophozoite to the colonic mucous layer and it starts the mucous disruption and depletion.

KEYWORDS: Trophozoite, amebiasis, parasite

***Corresponding Author**

Bhanu Shrivastava, Research Scholar in Department of Microbiology, Jodhpur National University, Assistant Professor in IASCA, ITM Universe, Gwalior M.P, India Email: abs_micro@yahoo.com

INTRODUCTION

Amebic liver abscess is the most common manifestation of invasive amoebiasis, but other organs can also be involved including pleuropulmonary, cardiac, cerebral, renal, genitourinary and cutaneous sites. 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. 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 children¹⁷. The detection of an *Entamoeba histolytica* antigen using an

- Enzyme - linked immunosorbent assay (ELISA)
- The use of the polymerase chain reaction (PCR) &
- The culture of stool samples.

During this study 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.

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 used¹⁰. 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

submucosa and submucosal vessels are also included¹². A patient, who developed superior vena cava syndrome due to a pulmonary amoebic abscess without liver involvement. The pulmonary amoebiasis occurs haematogenous spread from a primary site. The colon, but the liver is not involved in this type infection⁸. In the traditional system of medicine in India. The formulation has been prescribed for intestinal disorders. This study based on the five medicinal herbs, like *Boerhavia diffusa*, *Berberis aristata*, *Tinospora cordifolia*, *Terminalla chebula* and *Zingiber officinale*. The dried and pulverized plants were extracted in ethanol together and individually.

MATERIAL AND METHODS

Direct microscopy for intestinal amoebiasis (from stool sample)

Stool sample

Centrifuge

Formal saline

Ether

Iodine

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 of *E. histolytica* or culture method or NIH methods use the NIH media and ringer's solution as the material for (NIH media).

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 (CaCl₂) 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, inspissate in 850 g and coagulate in slanting position. Cool and overlay the silent with lock solution and then autoclave at 15 lbs. Presser for 15 min all the work must be done with aseptic condition.

Lock's solution formula

Sodium chloride, (NaCl) 8.00g

Calcium chloride (CaCl₂) 0.2g

Potassium chloride (KCl) 0.2 g

Disodium hydrogen phosphate 2.0 g

Magnesium chloride (MgCl₂) 0.01 g

Sodium bicarbonate (NaCO₃) 0.4g

Potassium dihydrogen 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/10 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 37⁰C and observed 24 hour subculture is done after an hrs. inoculation.

Identification Method

Slide Method

Microscopic Examination

Hanging drop method

Sub-Culturing NIH Media

OBSERVATION

Fruit juices observation showed by table 1, table2, and table3.

Concentration of mango fruit juice when 1 gm dissolve in 1 ml distill water:

RESULTS 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.

Study showed observation table:

Table 1 show the different growth of *Entamoeba histolytica* at different time on 24 hrs., 48 hrs., 72 hrs., after 24 hrs. Showed heavy growth, after 48 hrs. showed growth, after 72 hrs. showed low growth.

Table 2 showed the different growth of *Entamoeba histolytica* at different time on 24 hrs., 48 hrs., 72 hrs.,

after 24 hrs. Showed growth, after 48 hrs. and 72 hrs. Showed No growth,

Table 3 showed the different growth of *Entamoeba histolytica* at different time on 24 hrs., 48 hrs., 72 hrs., after 24 hrs. Showed low growth, while after 48 hrs. and 72 hrs. Showed No growth

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Table 1: 0.2 ml concentration of mango juice

Time [hrs.]	Growth of <i>Entamoeba histolytica</i>	
24	++++	High growth
48	+++	Growth
72	++	Low Growth

Table 2: 0.3 ml concentration of mango juice

Time [hrs.]	Growth of <i>Entamoeba histolytica</i>	
24	+++	Growth
48	+	Low growth
72	-	No Growth

Table 3: 0.4 ml concentration of Mango juice

Time [hrs.]	Growth of <i>Entamoeba histolytica</i>	
24	+	Low growth
48	-	No Growth
72	-	No Growth

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