

STUDY AT DIFFERENT CONCENTRATION OF POMGRANATE SHOWED ANTIAMOEBIIC PROPERTIES INCASE OF AMOEBIASIS IN NIH MEDIUM

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ABSTRACT

Amoebiasis 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 it's definitive host is human beings. It's Infective stage is quadric nucleated 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. Mucous secreted 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 has been reported from various developing countries. A 1998 study in Africa suggests that 2 tablespoons per week of papaya seeds may have some antiamoebic action and aid in prevention of amoebiasis, but this remains unconfirmed. Papaya fruit and seeds are often considered beneficial to digestion in areas where this plant is common.

KEYWORDS: Trophozoite, Amoebiasis, Parasite, *Entamoeba histolytica*

INTRODUCTION

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. Amebic liver abscess is the most common manifestation of invasive amoebiasis, but The diagnosis of amebic liver abscess (ALA) may be difficult since direct demonstration of trophozoites of *Entamoeba histolytica* other organs can also be involved including pleuropulmonary, cardiac, cerebral, renal, genitourinary and coetaneous sites. The trophozoites can penetrate and invade the colonic mucosal barrier, leading to tissue destruction, secretary 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 children³⁶.

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 samples³².

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.

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 used³⁰. 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 submucosal vessels are also included³⁴. A patient, who developed superior vena cova syndrome

due to a pulmonary amoebic abscess without liver involvement. The pulmonary amoebiasis occurs haematogenous spread from a primary site, but the liver is not involved in this type infection³⁸. In the traditional system of medicine in India, the formulation has been prescribed for intestinal disorders. Study based on the five medicinal herbs, like *Boerharia diffusa*, *Berberis aristata*, *Tinospora cordifolia*, *Terminalla chebula* and *Zingiber officinale*. The dried and pulverized plants were extracted in ethanol together and individually. In Mexico, it is common to use herbal tinctures of *chaparro amargo*. 30 drops are taken in a small glass of water first thing in the morning, and 30 drops before the last meal of the day, for seven days straight. After taking a seven day break from the treatment, it is resumed for seven days. Some mild cramping may be felt, it is claimed this means that the amoebas are dying and will be expelled from the body. Many Mexicans use the *chaparro amargo* treatment regularly, three times a year. The efficacy of such treatments has not been scientifically proven. A 1998 study in Africa suggests that 2 tablespoons per week of papaya seeds may have some antiamoebic action and aid in prevention of amoebiasis, but this remains unconfirmed. Papaya fruit and seeds are often considered beneficial to digestion in areas where this plant is common.

MATERIALS AND METHODS

MATERIALS

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 (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. Beat 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 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 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/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 37^oC 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 & RESULTS

We showed our work by the observation table

Table 1 showed the heavy growth of culture at 0.1 ml up to 0.9 ml concentration of pomegranate juice at 24, 48 & 72 hrs. **Figure.1**

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.

Table 2 showed the different growth of culture at 0.3 ml & 0.4 ml concentration of Pomegranate juice at 24, 48 & 72 hrs. After 24 hrs. Some growth observed, while in 48hrs. Showed low growth in a culture medium and no growth observed at 72 hrs. **Figure.2**

Table 3,4&5 showed the different growth of culture at 0.5 ml up to 1.5ml concentration of Pomegranate juice at 24, 48 & 72 hrs. Low growth observed at 24 hrs. While no growth observed at 48 & 72 hrs. **Figure.2**

Table 6 showed, No growth of culture at different time in culture media.

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Table 1 Pomegranate juices used in 0.1 ml up to 0.2 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+++	Heavy Growth
48hrs.	+++	Heavy Growth
72hrs.	+++	Heavy Growth

Table 2 Pomegranate juices used in 0.3 ml up to 0.4 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	++	Growth
48hrs.	+	Low Growth
72hrs.	-	No Growth

Table 3 Pomegranate juices used in 0.5 ml up to 0.9 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+	Low Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

Table 4 Pomegranate juices used in 1 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+	Low Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

Table 5 Pomegranate juices used in 1.1 ml up to 1.5 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	+	Heavy Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

Table 6 Pomegranate juices used in 1.6 ml for *Entamoeba histolytica*

Time (Hours)	Growth of <i>Entamoeba histolytica</i>	
24hrs.	-	No Growth
48hrs.	-	No Growth
72hrs.	-	No Growth

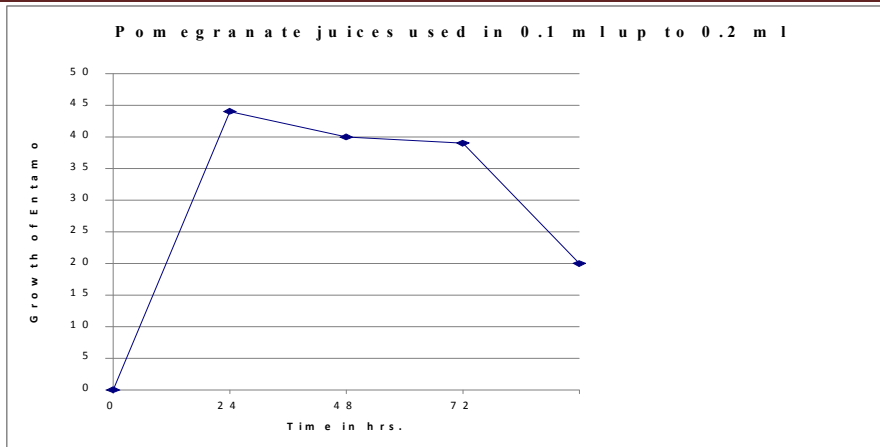


Figure 1 (Graph plotted between Cell growth and Time)

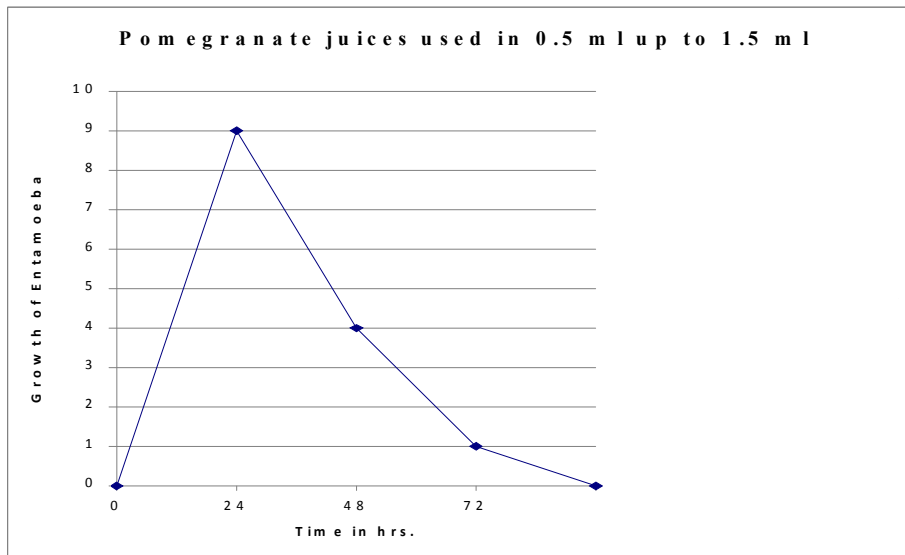


Figure 2 (Graph plotted between Cell growth and Time)

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