

STUDY AT THE DIFFERENT CONCENTRATION OF GRAPES FRUITS JUICE SHOW ANTIAMOEBIIC PROPERTIES IN CASE OF AMOEBIASIS IN NIH MEDIA

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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 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. 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 populations have been reported from various developing countries.

KEYWORDS: Trophozoite, amoebiasis, parasite,

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INTRODUCTION

Parasite enters the body through contaminated food or water. The infection is common in areas with poor sanitation of living conditions. This parasite can live in the intestine without causing symptoms or it can produce severe symptoms it is a very common problem in India²¹. People with amoebiasis pass *Entamoeba histolytica* parasites in their feces. The infection spreads when infected people do not dispose of their faces in a sanitary manner or do not wash their hands properly after going to the toilet. Contaminated hands can be spread the parasites to food that may be eaten by other people and surfaces that may be touched by other people hands can also become contaminated when changing the nappies of an infected infants¹. The doctor will prescribe medication such as metronidazole, diloxanide furoate, dihydroemetine, emetine or paromomycin. The doctor may prescribe more than one drug. If medication is stopped when the symptoms are gone but before the parasite is eliminated from the body, the infection may

return. An anti-diarrhoeal medication may also be prescribed. Metronidazole can produced a metallic taste in the mouth and may give rise to nausea. Alcoholic drinks must be avoided while taking metronidazole². The responses of a strain of *Amoeba proteus* to conditions which could give rise to induced increases in resistance to streptomycin have been investigated. An indirect selection method based on survival time in high concentrations of streptomycin was devised²². In this study assessing the kinetics of drug release from antibiotic fibrin seal compounds and the antibacterial efficacy of the delivered drug has been performed. Antibiotic sensitivity and the amount of drug released were measured by means of agar diffusion test. Standard and experimental curves were established for each antibiotic and each bacterial test in order to evaluate the quantities of the drug released during each 24 hrs. period. The evaluation of the kinetics of elution of the antibiotics from the fibrin clots showed that all of the antibiotics had been almost completely released by 96 hrs. The delivered

amount of each drug was enough to maintain the MIC until the 4th day of culture tool the most of antibiotics, resulting in a prolonged release of the drug²³.

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

METHODS

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 aseptical 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 grapes 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.

Present study showed the observation table:

Table 1 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. showed growth, and no growth at 72 hrs.

Table 2 showed the different growth of *Entamoeba histolytica* at different time on 24 hrs., 48 hrs., 72 hrs., after 24 hrs. showed heavy growth, while after 48 hrs. showed low growth, and after 72 hrs. did not show growth.

Table 3 showed the different growth of *Entamoeba histolytica* at different time on 24 hrs., 48 hrs., 72 hrs. they showed No growth in medium.

REFERENCE

1. Abd-Alla MD & Ravdin JI. Diagnosis of amoebic colitis by antigen capture ELISA in patients presenting with acute diarrhoea in Cairo, Egypt. Trop. Med. Int. Health 2002;7: 365-370.

2. Abd-Alla MD, TG Jackson, and JI Ravdin. Serum IgM antibody response to the galactose-inhibitable adherence lectin of *Entamoeba histolytica*. *Am. J. Trop. Med. Hyg.* 1998; 59: 431-434.
3. Abd-Alla MD, TF Jackson, S Reddy and JI Ravdin. Diagnosis of invasive amoebiasis by enzyme-linked immunosorbent assay of saliva to detect amebic lectin antigen and anti-lectin immunoglobulin G antibodies. *J.clin Microbiol.* 2000 ; 38:2344-2347.
4. Abe N, Kimata I & Iseki M. [Usefulness of multiplex-PCR for identification of *Entamoeba histolytica* and *Entamoeba dispar* (in Japanese)]. *Kansenshogaku Zasshi* 2002; 76: 921-927.
5. Ackers JP. The diagnostic implications of the separation of *Entamoeba histolytica* and *Entamoeba dispar*. *J. Biosci.* 2002; 27: 573-578.
6. Adagu IS, Nolder D, Warhurst DC & Rossignol JF. In vitro activity of nitazoxanide and related compounds against isolates of *Giardia intestinalis*, *Entamoeba histolytica* and *Trichomonas vaginalis*. *J. Antimicrob. Chemother.* 2002; 49: 103-111.
7. Agha Rodina AI & Teoderescu I. Prevalence of intestinal parasites in three localities in Gaza Governorates - Palestine. *Arch. Publ. Hlth.*, 2002; 60: 363-370.
8. Ahsan T, Jehangir MU, Mahmood T, Ahmed N, Saleem M, Shahid M, Shaheer A & Anwer A. Amoebic versus pyogenic liver abscess. *J. Pak. Med. Assoc.* 2002; 52: 497-501.
9. Al-Bayatti SM. Etiology of chronic diarrhea. *Saudi Med. J.* 2002; 23: 675-679.
10. Amin OM. Seasonal prevalence of intestinal parasites in the United States during 2000. *Am. J. Trop. Med. Hyg.* 2002; 66: 799-803.
11. Ankri S. Strategies of the protozoan parasite *Entamoeba histolytica* to evade the innate immune responses of intestinal epithelial cells. *J. Biosci.* 2002; 27: 609-614.
12. Arisue N, Hashimoto T & Hasegawa M. Early evolution of eukaryotes inferred from genome data. *International Congress Series* 2002; 1246: 209-215.
13. Arteaga-Nieto P, Lopez-Romero E, Teran-Figueroa Y, Cano-Canchola C, Luna Arias JP, Flores-Carreón A & Calvo-Mendez C. *Entamoeba histolytica* : purification and characterization of ornithine decarboxylase. *Exp. Parasitol.* 2002; 101: 215-222.
14. Avila EE, Martinez-Alcaraz ER, Barbosa-Sabanero G, Rivera-Baron EI, Arias-Negrete S & Zazueta-Sandova IR. Subcellular localization of the NAD⁺-dependent alcohol dehydrogenase in *Entamoeba histolytica* trophozoites. *J. Parasitol.* 2002; 88: 217-222.
15. Ayeh-Kumi PF & Petri WA. Diagnosis and management of amoebiasis. *Infect. Med.* 2002; 19: 375-382.
16. Balci NC & Sirvanci M. MR imaging of infective liver lesions. *Magn. Reson. Imaging Clin. N. Am.* 2002; 10: 121-135
17. Banerjee S, Das S & Lohia A. Eukaryotic checkpoints are absent in the cell division cycle of *Entamoeba histolytica*. *J. Biosci.* 2002; 27: 567-572.
18. Bansal D, Sehgal R, Chawala Y, Mahajan RC, Malla N. In vitro activity of antiamoebic drugs against clinical isolates of *Entamoeba dispar*, *Ann Clin Microbiol Antimicrob* 2004; 3: 21-33.
19. Banuelos C, Orozco E, Gomez c, Gonzalez A, Medel O, Mendoza L & Perez DG. Cellular location and function of the P-glycoproteins (EhPgps) in *Entamoeba histolytica* multidrug resistant trophozoites. *Microb. Drug Resist.* 2002; 8: 291-300.
20. Barwick RS, Uzicanin A, Lareau S, Malakmadze N, Imnadze P, Iosava M, Ninashvili N, Wilson M, Hightower AW, Johnston S,

Bishop H, Petri Jr WA & Juranek DD. Outbreak of amoebiasis in Tbilisi, Republic of Georgia, 1998; 67: 223-227

Table 1: Concentration of grapes fruit juice when dissolve in 1.7 ml

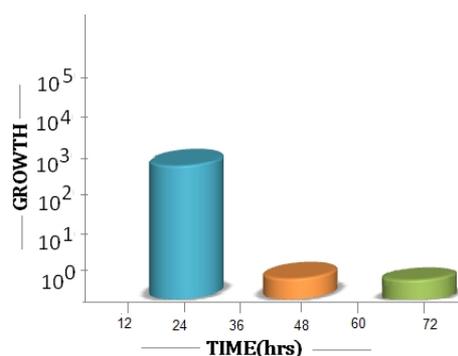
Time [hrs.]	Growth of <i>Entamoeba histolytica</i>	
24	++++	High growth
48	++	growth
72	++	growth

Table 2: Concentration of grapes fruit juice when dissolve in 1.8 ml

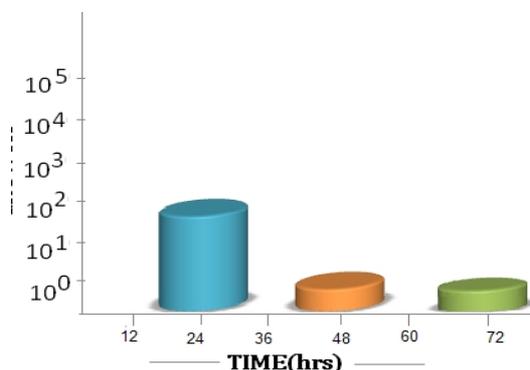
Time [hrs.]	Growth of <i>Entamoeba histolytica</i>	
24	+++	High growth
48	+	Low growth
72	—	No growth

Table 3: Concentration of grapes fruit juice sat 2 ml

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



Graph 1: Concentration of grapes fruit juice at 1.7 ml



Graph 2: Concentration of grapes fruit juice when 1.8 ml dissolve

