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STUDY THE EFFECT OF CLADOPHORA GLOMERATE ALGAE EXTRACT ON THE PARASITE OF ENTAMOEBA HISTOLYTICA

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ABSTRACT

Was isolated parasitic amoeba fabric *E.histolytica* in samples bloody diarrhea mucosa of patients infected with Wales a hot amoebic Amoebic dysentery and has its own development in the center of Luc-eggs (LE) axis to add protein pro complex) GALNER) instead of white emulsion, the results showed the success of the implant in the middle, accompanied bacteria, growth continued for two weeks, included this term secondary farm to perpetuate the amoeba.

The effectiveness of selected two concentrations chloroform extract *cladophora glomerat* first concentration 128 mg / ml and second concentration 256 mg / ml. The results showed the effect of two concentrations against amoeba condition of the fabric in the glass. the effectiveness of the extract with the increase mixture focus, where it came from homicide rate of 80% when using the extract 128 mg / ml and 90% when using concentration 256 mg / ml, has influence came to extract a higher content of alkaloids, phenols and Alsabonyat and Alkleikosadat.

In-vitro was, VFD was measuring enzyme AIP in the positive control 0.28± 28.6 K.A.U / 100ml and dosed Group by concentration 128 mg / ml was 11.48± 0.6 K.A.U/ 100ml and7.42± 0.055 K.A.U/ 100ml in the second concentration as compared with the negative control group5.23± 0.072 K.A.U/ 100ml treated falagel 7.21± 0.080 K.A.U/ 100ml.

enzyme LDH results were in the range of positive control 68.82± 0.3425 U/ L dosed group by concentration 128mg/ ml was50.28± 0.1398 U/ L. As the second concentration 36.36± 0.3425 U/ L. As compared to negative control Group25.28± 0.3425 U/ L group dosed by flagel 51.11± 0.16 U/ L. The cholesterol was in the positive control group and the group141.3± 10.1 U/ L dosed group 128mg/ ml was 156± 0.3422.9 U/ L the second concentration 174.17± 4.40 U/ L as compared to the negative control group184.53± 0.26 U/ L by filagel treatment group 180.5.± 0.221 U/ L, Keriaten was within the normal boundaries (1.2-0.5) mg/dl.

A concentration of glucose in the positive control group was 92.28± 5.55 U/ L and the dosed group by low concentration was 86.6± 0.34211 U/ L. The high concentration was 95.5± 5.11 U/ L compared to the negative control group 99.32± 3.41 U/ L and the treatment group95.5± 5.11 U/ L. The total protein concentration, was positive in the control group 6.65± 0.33 U/ L and the dosed by the low first concentration 7.71± 2.84 U/ L and the higher 6.53± 0.561 U/ L as compared with the negative control control 7.21± 0.802 U/ L and treatment by filagel 6.25± 0.31 U/ L.

Key Words: Cladophora glomerata *Cladophora glomerata*, *Entamoeba histolytica*

INTRODUCTION

The amoeba causes to the condition of *E. histolytica*, amebiasis, or amoebic dysentery. This disease is one of the most common parasitic diseases in the world, spreading among the poor, and may be due to malnutrition or unhealthy conditions, spread throughout the world, especially the tropical region Tropies and subtropical subtro-pics (Ouattara, *et al.*, 2010)

WHO reports that amoeba parasites are responsible for deaths of more than 100,000 people per year, which ranks second after malaria in the high mortality rate of primary parasites?(Bhanu , Vandara *et al.* 2011)

Amoeba is a primary a condition of parasite. It needs to be addressed to doctors and spread to other organs, especially the liver, causing the amoebic liver abscess (Samnel et al., 2001).

In this study, there is a kind of algae called Cladophora glomerata, which lives in fresh and salty water, is attached to the Thalose by sub-roots, which is much forked, that includes many nuclei and plastid green plaques containing on many starch production centers. It has branchy albino filaments, and has many transverse walls.

Intracellular and extracellular cytotoxic extracts have showed some activity against bacteria, fungi, and Parasites(Takin, et al., 2007, Raga et al., 2008)

MATERIALS AND METHODS

Sources of access to parasite: The parasite was obtained from the stool samples of the patients who visited Al- Kadhimiya Teaching Hospital in, after verifying their parasitic infection with the laboratory diagnosis of the samples. The mucosal diarrheal samples were tested as an ideal source for getting many numbers of Trophozoite parasites and infected red blood cells.

Diagnosis: The color and strength of the samples, the existence or the absence of odor, either microscopy was examined by force of the great (40x) using dyed wet strips and iodized wet strips,

the first helps in the detection of activities, and the second helps in the detection of Cysts.

Assessment of parasite' Vitality: Using 1% of water Eosine solution, was used as the dye of the Eosine stains all contents except the living protoplasm.

Culture Media that used: By Using the media of Locke diphasic, and the media was introduced in accordance with the **Brand** method(Brand, Rees *et al.* 1945) with some modifications. After the distribution of the Pro Complex (Gainer) protein emulsion that was added 6 ml and placed in a boiling water bath for 15-20 minutes. The tubes were then cooled and 6 mL of the loque solution was added and the two-stage sterility was stabilized 15 minutes. The following substances were added to each tube 1 ml of serum inhibitor and 0.2 mL of antibiotic solution, like amplin or streomycin and nastalin.

Collection and diagnosis of Algae samples: The samples were collected from the bottom of the AL Najaf Sea zone on 1/3/2014 by a plastic container, size 5 liters, washed with tap water to remove dirt and left to dry at room temperature, then grind with an electric mill and pressed in dry packaging. Then grinded and placed in the refrigerator at a temperature of 4 ° C. Until they will be used.

Preparation of Extracts: The method of Desmuk and Borle (Beshmuk and Borle 1975) was depended on in the preparation of the extracts of green algae Cladophora glomerata, where the weight of 20 g of vegetable powder in the Thimble cobbler closed the sides, the sample was placed in the Soxhlet and added chloroform at a concentration of 99%). The extraction process was then carried out in the extraction apparatus for 4-5 hours until a colorless filter was obtained at a temperature of 60-50 ° C.

The extract and filter was taken with the filter paper (Whatman No.1). After that drain, the residual leachate in the incubator at 37 ° C for 48 hours to obtain the dry powder and store it in the refrigerator until use. A number of qualitative studies were conducted to identify the chemical compound of the extract, including the detection of alkaloids, phenols and terpenes(Al-Abid 1985), sapphones1g of dry extractor in 2 ml of alcohol to obtain 500mg / ml and infertility by filtration paper in 0.22 micrrons, which was considered the primary measurement solution, and was attended by 128mg / ml and 256mg /ml.

The biochemical tests of the rat serum were measured using a special kit and equipped with Roch Germany 2016. Alkaline phosphate was determined using a special test kit equipped with Biomerienx (No. 61511). In addition, Lhydate dehydrogena (LDH) using a special kit and equipped by Randox Inc. (N. 1529).

For determining the numerical and biological density of the amypas grown in glass, the blood cell count and the 1% Eosine dye were used, depending on the non-permeability of the eosin layer to the live parasite cell. Amoeba was used extensively (400,000 amoeba / liter) and 95% vitality. The test was carried out by the addition of 1 milliliter of the amoeba farm to the seedling medium (LE) to each test tube.

The first group was treated with 0.1 mg of concentration of 128mg / ml, the second group of 0.1mg of concentration of 256mg / ml, and the third group of amoeba was left untreated as a control group. Pour the tubes gently to distribute the two extractors evenly into the amoebae medium, then incubate in the incubator at 37 ° C for a period of couple weeks. The piping was calculated and the percentage of the maima killed was estimated according to the following law:

Percentage of dead amoeba = (number of amyosin dyed amoeba) / (number of total amoeba) x 100

- The process was repeated several times.

Design of Experience: 20 mice of both sexes, white mice of type Mu mussculus mice/c of both sexes, ranging in weight between 25-23 g, mice were given 1 mL of suspension containing 10^3 Cyst / ml, using Stomach syringe was sterile 1 ml and tested rats were tested for 10 days to confirm the infection.

Moreover, was randomly divided after being injected with *E. histolytica* parasites. After the infection was confirmed, five rats were divided into each group. In the group, 1 mL of the extract of 256mg / ml was injected and the second group was 1 ml 128 mg / ml, and the third group was injected with 1 mg ml with 30 mg / ml, and the fourth group represented the parasite control group.

Statistical analysis: The results were statistically analyzed by depending and using the least significant difference (L.S.D) at a significant level 0.05

RESULTS

Table 1: Compare between group show ALP and LDH

	Unit / L LDH mean ± SD	Armstrong / 100 ml AIP mean ± SD
Control⁻	25.28 ± 0.34254	5.32 ± 0.072
Control⁺	68.82 ± 0.3425	28.6 ± 0.28
Flagel	51.11 ± 0.1602	7.21 ± 0.802
Low Concentration $128mg/ml$	50.28 ± 0.1398	11.48 ± 5.66
High Concentration $256mg/ml$	36.37 ± 0.1702	7.42 ± 0.055

In this table, we note that the basal phosphatase enzyme (AIP) was increased in the positive control group, as it was 28.6 ± 0.28 KAU / 100ml and decreased in the group with a concentration of 128mg / ml was 11.48 ± 5.66 KAU / 100ml, while the concentration was 256mg / ml It was 7.42 ± 0.055 KAU / 100ml compared with the negative control group 5.32 ± 0.072 KAU / 100ml, and the m TNA group was 7.21 ± 0.802 KAU / 100ml.

In the control group (LDH), the positive control group was 68.82 ± 0.3425 KAU / 100ml and decreased in the group with a concentration of 128mg / ml where it was 50.28 ± 0.1398 U / L. In the group, which dose 256mg / ml was 36.37 ± 0.1702 U / L compared to the negative control group 25.28 ± 0.3425U / L and the mRNA group 51.11 ± 0.1602U / L.

Table 2: Compare between different groups show cholesterol and creatine

	Creatine mg/dl mean ± SD	***Cholestrol mg/dl mean ± SD***
Control⁻	0.17 ± 0.0087	184.53 ± 0.261
Control⁺	0.141 ± 0.087	141.3 ± 10.1
Flagel	0.122 ± 0.0071	180.5 ± 0.221
Low Concentration $128mg/ml$	0.1422 ± 0.01297	156 ± 12.9
High Concentration $256mg/ml$	0.152 ± 0.00788	174.17 ± 4.40

In Table 2, we note that the concentration of creatine in serum mice infected with a significant decrease slightly at the abstract level ($p < 0.05$), where it was 0.141 ± 0.087 mg / dl, and was 0.1422 ± 0.01297 mg / dl in handling Group with 128mg / ml, a rise not significant with a positive control, and was up significantly with Handling Group with 256mg / ml, where it was 0.152 ± 0.00788 mg / dl as compared to the negative control 0.171 ± 0.0087 mg / dl, but the Group handling with metronidazole 0.122 ± 0.0071 mg / dl, and you This increase is within the normal limits of concentration in the serum (1.2-0.5 mg / Dalton).

Table 3: Compare between different groups show glucose and total protein

	Total protein g/l mean ± SD	Glucose mg/dl mean ± SD
Control⁻	7.21 ± 0.802	99.32 ± 3.41
Control⁺	6.65 ± 0.33	92.28 ± 5.55
Flagel	6.25 ± 0.31	96.4 ± 5.11
Low Concentration $128mg/ml$	4.71 ± 2.84	86.6 ± 11.43
High Concentration $256mg/ml$	6.53 ± 0.561	95.5 ± 5.11

In Table 3, we observed that the protein in the positive control group decreased significantly as, 6.65 ± 0.33 g / l, and in the 128mg / ml group was 4.71 ± 2.84 g / l and increased in the 256 mg / ml group was 6.53 ± 0.561 g / l compared to the negative control group 7.21 ± 0.82 g / l and the batch group in the flagel 6.25 ± 0.31 g / l. The concentration of glucose was significantly reduced in the infected group: 92.28 ± 5.55 mg / dl and the total group was 86.6 ± 11.43 mg / dl and the 256 mg / ml group was

95.5± 5.11 mg / dl compared to the negative control group 99.32 ± 3.41 mg / 96.4 ± 5.11 mg / dl.

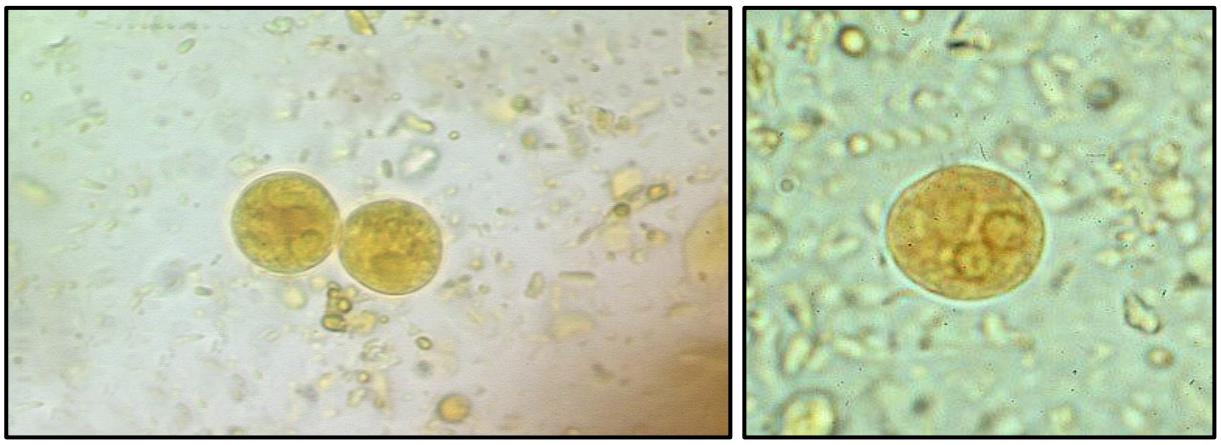

FIGURE 1: THE CYST PHASE IS SHOWN IN *ENTAMOEBA HISTOLYTICA*

In Vitro: The results of the tests for the extract of algae and metronidazole in the amoeba kill showed the condition of the tissue. It was calculated at the concentration of 128mg / ml which killed 320,000 amoeba / ml by 80%. The concentration of 256mg / ml was 320,000 amoeba / ml and 90% Any losses mentioned in the control group are not treated by any factor.

DISCUSSION

The increase in the value of the enzyme (AIP) and (LDH) is due to the destructive effect caused by the parasite in the liver cells, as this damage leaks enzymes from the damaged liver cells into the bloodstream, and therefore high serum level (Mason 2004), enzymes means reduced damage to liver cells, liver cells return, and liver tissue gradually returns to normal(Abdel, Hekmat et al. 2014)

The protein parasites need to build their amino acids, and therefore rely on the intestines to multiply the turquoise (Ermawati and Wibisono 2017; Jain 2017; Klaewklad, *et al.* 2017), as well as protein to break the wall of the bag.

LEM (Al-Hussuny, et al., 2016) 80% and 90% of the concentration of Algae extract may be explainned by effective chemical compounds. Salsalic acid monohydrogen (a phenolic acid with beta hydroxyl is characterized as organic acids in the form of crystalline in the extract of alfalfa 16.09%, while belongs to 6-Octadecenoie acid, which is hydrocarbons and it is an antioxidant. Dodecanoic acid 10.18% and 10-Hepladcenoic acid 8.68% and Tetradecanoic acid 8.29%, all the polyunsaturated fatty acids are antibacterial.

Turbines are antiviral, bacteriological, fungicide, and primary compounds (Cohen, *et al.*, 2001). The relative efficacy of cladophora glomerata was studied for fungi exposed to plants. The inhibitory activity is due to the nature of the material contained in the algae.

The increase in creatine is within its normal limits. Creatine is the least nitrogenous substance in the blood, and its examination is one of the important diagnostic signs for the examination of kidney function, because it is a useless metabolic product thrown out by the kidneys and any height beyond its natural limits reflects the condition Functional function of the kidney (Ou, Zhang *et al.*, 2012) Cholesterol and low cholesterol may be due to parasite feeding on cholesterol, which is important for the formation of the cell membrane and obtained from the intestines(Yousif 2014)

Conclusion:

The results showed the effect of two concentrations against amoeba condition of the fabric in the glass. the effectiveness of the extract with the increase mixture focus, where it came from homicide rate of 80% when using the extract 128 mg / ml and 90% when using concentration 256 mg / ml, has influence came to extract a higher content of alkaloids, phenols and Alsabonyat and Alkleikosadat.

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