STUDY OF SOME BIOLOGICAL ASPECTS OF MICE INFECTED WITH HYDATID CYST OF Echinococcus granulosus PARASITE AND THE THERAPEUTIC CAPACITY OF ETHANOLIC EXTRACT OF Cladophora glomerate ALGAE

Hadeel Abdulatif Majeed1*, Amal K. Abbas2, and Shatha K. Abbas1

1. Department of Biology, College of Sciences, Mustansiriyah University, Baghdad, Iraq
2. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

*Corresponding author: Dr.hadeel7227@uomustansiriyah.edu.iq (Majeed)

ABSTRACT

This study aimed to find the effect of Cladophoraglomerata extract on some Biological, aspects of hydatid cyst of Echinococcus granulosus in mice infected with cystic Echinococcosis and evaluating the efficiency of algae extracts in treating the disease. Forty mice were used, and were divided to 4 equal groups; three groups of mice were injected into the peritoneal cavity with protoscolices, while the fourth group was left as a negative control group. After 4 months of infection the second and third groups were given the extraction of Cladophoraglomerata at concentration 128mg/ml and 256mg/ml respectively, while the first group considered control positive. Six months after the start of the infection, mice were dissected, the organs examined. The levels of some liver enzymes, the concentration of some metals and some immunological parameter were measured, the study found that the algae extract in two concentrations decrease the activity of ALP (p≤0.05) in treated group, a significant increase was observed in the concentrations of Ca and Na in the treated groups (p≤0.05), The algae extract also had a significant reduction in the level of IL2 and IL4(p≤0.05), It can be concluded that the ethanol extract of Cladophoraglomerata contains effective substances that can be used to treat the disease caused by Echinococcus granulosus

Keywords: Echinococcus granulosus, Cladophoraglomerata, ethanolic extract.

INTRODUCTION

Cystic Echinococcosis (CE) is one of the most important zoonotic helminthic diseases throughout the world (1). The larval stages of Echinococcus granulosus develop to a fluid-filled cyst (hydatid cyst) enclosed by peri-parasitic granuloma and fibrosis, in the infected organ (2). The primary hosts of Echinococcus are several carnivores, the common being the dog. All mammals (more often being sheepand cattle) are secondary hosts (3). The
clinical symptoms of CE are often non-specific and many patients may be asymptomatic, the symptoms depend on the size and site of the lesion\(^4\). CE is still a major economic and public health problem in Iraq because there is no national control program\(^5\). According to WHO 2017, more than 1 million people are affected with echinococcosis at any one time. *Cladophora glomerata* is a filamentous green alga widely spread throughout freshwaters of the world, the classification of *Cl. Glomerata* is difficult because the high morphological plasticity of species *glomerata*. Microscopically, its appear as thalli are consist of joined cylindrical cells, with lengths of 6-20 μm, widths of 4-10 μm and with dichotomously branching filaments. Usually it tends to stay on one spot, which makes it easy to remove\(^6\).

**MATERIALS AND METHODS**

**The source of parasite**
The hydatid cysts were collected from infected patients attending Al-Kadmiyah Teaching Hospital by surgical removal, the sex, age, size and the numbers of cysts for each patient were recorded. Then the samples had been carried in sterile containers to the labs of Collage of Sciences / Baghdad University.

**Isolation of Protoscolices of hydatid cysts**
The process of isolation and configuration was done according to the method\(^7\) the precipitated hydatid cysts were kept in Ringer solution which prepared according to method\(^8\).

**Examination the viability of Protoscolices**
Protoscolices were examined for 90% viability by 0.1% eosin staining\(^9\).

**Collection and Diagnosis of Algae samples**
The samples were collected according to\(^10\) from the bottom of the Al-Najaf sea zone 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 used.

**Preparation of extract**
The method of\(^11\) was depended on the preparation of the extracts of *Cladophora glomerata*.

**Mice treatment**
In this study, 40 males of the laboratory animal's white mice *Mus musculus Balb/C*, age between (6-8) weeks, weight ranged between (20-40) gm, the animals were divided to 4 equal groups, the first, second and third groups, were injected into the peritoneal cavity with 2000 protoscolices for each mouse, while the fourth group was left as a negative control group, injected with normal saline. After 4 months of infection the second and third groups were given the algae extract at 128mg/ml and 256mg/ml respectively, one time every day for 2 weeks while the first group considered control positive given the normal saline. Six months after the start of the infection, mice were dissected, the organs examined, and the site of hydatid cysts had been determined in them, the weight of these organ had been measured and kept in formalin 10%, the histological sections prepared and stained with hematoxylin and eosin stains as the method of\(^12\).

**Collection of serum**
The blood collected from the mice that have been anesthetized with ether by heart puncture\(^13\), serum kept at -20°C until used to estimate the liver enzymes ALP, GOT and GPT as method in\(^14\) used solutions.
supplemented from Randox British company with units' IU/ l, the minerals: Mg, Ca, Na, K and the interleukins, IL2 and IL4.

The statistics
The result analysis used the ANOVA method, the analysis least significance difference (LSD) used in comparative, the analysis of variance used to detect the significant difference between two concentrations.

RESULTS

The results of this study which described in the table (1) showed that significant difference (p≤0.05) between the level of the liver enzyme ALP, GOT and GPT in the positive control group compared with negative control group and treated groups, the results showed that three enzymes significantly lowered level in mice treated with algae extract (p≤0.05).

Table 1: The effect of algae extract of the concentration of ALP, GOT and GPT in mice

<table>
<thead>
<tr>
<th>Mice Group</th>
<th>Liver enzyme (unit/L)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALP</td>
<td>GOT</td>
<td>GPT</td>
<td></td>
</tr>
<tr>
<td>Control negative</td>
<td>61.16±2.56</td>
<td>9.04±0.19</td>
<td>8.08±0.16</td>
<td></td>
</tr>
<tr>
<td>Control positive</td>
<td>130.93±8.5</td>
<td>15.35±0.44</td>
<td>17.75±0.45</td>
<td></td>
</tr>
<tr>
<td>Treated mice with 128mg/ml of algae extract</td>
<td>109.31±10.05</td>
<td>13.95±0.44</td>
<td>16.3±0.48</td>
<td></td>
</tr>
<tr>
<td>Treated mice with 256mg/ml of algae extract</td>
<td>97.45±6.31</td>
<td>12.9±0.62</td>
<td>14.6±0.65</td>
<td></td>
</tr>
</tbody>
</table>

The value (Mean±SD)

Table (2), showed that the concentration of Mg and K in positive control group is low difference compared with negative control group and treated groups while the concentration of Ca and Na in positive control group is significantly lower (p≤0.05) compared with other groups.

Table 2: The effect of algae extract of the concentration of (Mg, Ca, K, Na) in mice

<table>
<thead>
<tr>
<th>Mice Group</th>
<th>Minerals (mmol/L)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg</td>
<td>Ca</td>
<td>K</td>
<td>Na</td>
</tr>
<tr>
<td>Control negative</td>
<td>51.94±0.35</td>
<td>95.50±0.22</td>
<td>4.35±0.55</td>
<td>175.75±0.34</td>
</tr>
<tr>
<td>Control positive</td>
<td>50.12±0.17</td>
<td>85.5±1.25</td>
<td>4.22±0.22</td>
<td>160.3±0.08</td>
</tr>
<tr>
<td>Treated mice with 128mg/ml of algae extract</td>
<td>50.80±0.35</td>
<td>91.45±0.56</td>
<td>4.66±0.44</td>
<td>164.53±4.11</td>
</tr>
<tr>
<td>treated mice with 256mg/ml of algae extract</td>
<td>50.35±0.35</td>
<td>92.49±0.70</td>
<td>4.55±0.4</td>
<td>170.94±4.2</td>
</tr>
</tbody>
</table>
Table (3), showed that the concentration of IL2 and IL4 in positive control group is significantly increased \((p\leq 0.05)\) compared with negative control group while in treated group with 256mg/ml of algae extract is significantly decreased compared with positive control group.

### Table 3: The effect of algae extract of the concentration of (IL2, IL4) in mice

<table>
<thead>
<tr>
<th>Mice Group</th>
<th>Interleukins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL2</td>
</tr>
<tr>
<td>Control negative</td>
<td>2.27±0.07</td>
</tr>
<tr>
<td>Control positive</td>
<td>6.81±0.21</td>
</tr>
<tr>
<td>Treated mice with 128mg/ml of algae extract</td>
<td>5.8±0.23</td>
</tr>
<tr>
<td>Treated mice with 256mg/ml of algae extract</td>
<td>3.62±0.14</td>
</tr>
</tbody>
</table>

Figure (1) showed section of liver of negative control group, the positive control group showed histological changes as degeneration and necrosis figure (2), while the results of histological examination of liver cells in the treated group showed the proliferation of Kupffer cells with few mononuclear cells in sinusoids fig (3).

Figure 1: A section of negative control liver of mice showed normal structure (200x) (H&E).
The histological examination of the intestine of negative control group showed normal appearance of the mucosa and sub mucosa layers fig. (4). Fig. (5) showed the intestine of positive control group which appeared abnormal structure and infiltration of inflammatory cells, fig (6) showed the appearance of intestine after treating with algae extract which seem as normal appearance.
Figure 4: A section of control negative intestine of mice showed the normal structure (200x) (H&E).

Figure 5: A section of control positive intestine of mice showing the abnormal structure. (200x) (H&E).

Figure 6: A section of mice intestine treated with Algae extract (200x) (H&E)
The histological examination for kidney of negative control groups showed normal appearance of glomeruli, nephrods, renal tubules proximal and distal convoluted tubules (Fig 7). Fig (8) showed an increase in the activity of cell division as a result of infection, aggregate of inflammatory cells between renal tubules with hemorrhage. Fig (9) showed a section of kidney approximately appeared as normal structure.

Figure 7: A section of control negative kidney of mice showing normal structure (200x) (H&E)

Figure 8: A section of positive control Kidney of mice showing abnormal structure (200x) (H&E)
DISCUSSION

The hydatid cyst disease remains a significant public health hazard in endemic areas such as Iraq\(^{(15)}\). Ringer solution was adopted to maintain the protoscolices, it is a complex solution that contains different materials commensurate with the need of protoscolices of nutrients \(^{(16)}\). The serum ALP concentration had increased significantly in the positive control group, this may be due to the effect of the infection of the liver which led to hepatocyte destruction and enzyme release\(^{(17)}\), and started to belong to the normal range in treated mice and this is may be due to the effects of algae extract which consider rich source of bioactive compounds. Screening test of this extract revealed many constituents among which are Alkaloids, Tannins, Phenols and Flavonoids \(^{(6)}\) which have pharmacological activities including anticancer, antimicrobial, antiviral, anti-inflammatory and antioxidant \(^{(18)}\). The level of GOT enzyme increases during liver damage, GPT is an enzyme increases dramatically in acute liver damage and in infected host tissue with echinococcosis\(^{(19)}\), so the higher values of concentration of GOT and GPT were recorded in positive control group in this study, these results are consistent with many studies indicated that the damage of liver caused a rise in concentration of liver enzymes\(^{(20)}\). It was observed that the groups treated with algae extract recorded a significant decrease of those enzymes and this is probably due to the effects of ingredients of algae extract such as alkaloids where these compounds overlap with the chain of metabolic reactions of proteins necessary for the continued vitality of the protoscolices leading to destruct the cell wall of parasite \(^{(21)}\). Trace elements are essential components of biological structures, a previous study reported high values of Ca, K, Na in hydatid cyst fluid which play important role as a reductant these conclusions may explain the lower values of some elements in the serum of positive control group of mice as a result of their consumption by the parasite, on the other hand, the Mg element is essential for intercellular ionic bridging changes at the cell surface and shape of the cell during cleavage of parasite\(^{(22)}\), may be for this reason the concentration of Mg decreased in serum of positive control group and started to return to normal value in treated group of mice. IL2 product of Th1, so Th1 response for inactive the hydatid cyst formation, the cellular immunity and Th1 cytokines increase to an attempt to control parasite growth in some individuals and to limit the lesions size in the patients\(^{(23)}\) and this might be due to...
higher significant of IL-2 level in current study of positive control group compared with negative control group, on the other hand the IL-4 which product of Th2 recorded highly significant level of positive control group, this indicate that there was a shift in immune response from Th1 into Th2 (23), in treated group the level of IL-2 and IL-4 decreased significantly as compared with positive control group, this might be due to the treated effects of algae extract which contents many of constituents such as Tannins, where it is characterized by penetrate the cell membrane and block the active sites of some enzymes inside the cell that may be necessary for the growth and reproduction of organism (24). Regarding the results of the histological study, in the infected liver it has been observed that neutrophil, lymphocyte, and macrophage infiltration to the site of the infection is due to the release of toxic substances in the liver by the parasite where the macrophage play important role in removal the debris of host tissues, the atrophy and degeneration of hepatic cells and lack of coordinate pattern (16), the result in fig. (2) agreed with others where the infected with hydatid cyst lead to changes in the tissue of the liver (16,18), when the algae extract had been used especially at the 256 mg/ml lead to hyperplasia, infiltration of inflammatory cells, extensive hyalinization around the hepatocytes fig(3), and degeneration of some hepatic cells simultaneously with formation of new cells (18) the treated role of the algae extract might be due to the components such as phenols which destructed the essential compound in *E. granulosus* (25). The effect of hydatid cyst of intestine and kidney showed in fig (5,8) it is noticed the changes in structure of intestine and infiltration of inflammatory cells to it, while the appearance of kidney represented by collection of lymphocytes between renal tubules formed inflammatory spots, hemorrhage and degeneration of renal tubules, the effect of algae extract on treated groups seen in Fig (6,9) as noticed that the appearance of intestine and kidney gradually returned to normal structure and this is might be due to the role of Phenolic compounds which known as antioxidants not only because of their ability to donate hydrogen atoms or electrons but also because of their stable radical intermediates which prevent the oxidation of various ingredients, particularly fatty acids and oils (26).

**CONCLUSION**

The ethanol extract of Cladophora glomerata contains effective substances that can be used to treat the disease caused by Echinococcus granulosus.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**FUNDING**: Self-funding

**REFERENCES**


8. Kadir M, Rasheed S and Tahir S. Comparison between the efficacy of some chemical drugs and medical herbs on hydatid cysts. Abstracts of the 11th Science Cong Faculty of Veterinary Medicine, Assiut University, Egypt 2004: 372-382


19. Mero WMS and Abdullah Arshad M. Comparative estimation of total protein content and enzymatic activities of hydatid cyst of *Echinococcus granulosus* isolated from sheep and goats in Duhok province, Kurdistan region of Iraq. 2nd international conference on ecological, environmental and biological sciences. Bali Indonesia 2012: 14-17


25. Khalaf AKH, Al-Mayah SH and Athbi AM. In vitro activity of alkaloids extracted from chlorophylla and cyanophylla against the hydatid disease compared with albendazole. Thi-Qar Medical Journal 2011;5(3):56-70