Immunomodulatory of Cordia myxa (L.) Aqueous Extract Fruit in Immunized Mice with Hydatid Cyst Fluid

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Abstract

Human cystic echinococcosis caused by Echinococcus granulosus is one of most important and widespread parasitic zoonoses in the world. The present study was aimed to identify the immunomodulatory activity of aqueous extract of Cordia myxa fruit since this plant considers one of medically important plants, which is widely used for treatment of numerous diseases, that correlate with the effectiveness of immunized by hydatid cyst fluid antigen HCFAg. Forty Balb/c mice were divided into equal groups, first group was immunized with HCFAg, the second group was treated with aqueous extract of C. myxa fruit, the third group was immunized and treated, the fourth group was as a control. Delayed type hypersensitivity (DTH), Mitotic index (MI) and histopathological change in spleen in all groups were studied. A higher increase of thickness was showed in immunized mice and treated after 10 days of treatment with aqueous extract of C.myxa fruit after 10 days(3rd group), that reached 1.23± after 3 hrs. The MI of bone marrow and spleen cells was significantly increased as a post immunized and treatment mice (88.57±1.40), (94.7±0.76) respectively in comparison with the other groups. Histopathological examination of spleen showed marked hyperplasia of lymphoid corpuscles, confused some times to form large follicle. The successive aqueous extract was found to stimulate cell mediated and immune responses in mice/

Keywords: HCF, C. myxa, DTH, MI

1. Introduction

Cystic echinococcosis is a silent cyclozoanotic infection of humans and domestic animals caused by larvae of Echinococcus granulosus. It is one of the important helminthic diseases in the Middle East including Iraq (Echert, et al., 2001; AL-Shammary, 2002; Zhang et al., 2003; Echert and Deplazes,2004). Hydatid cyst fluid antigens HCFAg was the source of immunization, which stimulate the production of Th1 response known to induce a protective immunity against secondary hydatidosis (Al-Quad et al., 2008). Current treatment of hydatidosis is mainly surgery, and medical treatment using benzimidazole compounds (Gangopadhyay et al., 2009). Recently there are trends for using plants in therapy as a reside of side effect, plant products still remain the principle source of pharmaceutical agents used in traditional medicine (Jawaid et al., 2011).

Cordia myxa fruit locally known as "Bumber" is one of the largest genera in the family Boragiacaeas, about 300 species have been identified worldwide mostly in warmer region. C. myxa is a sweete fruit because it contain the maximum amount of sucrose, glucose, fructose and high total dietary fiber, which plays one important role in deceasing risk of many diseases, C. myxa fruits are a rich source of proteins, carbohydrates, ash, fat and essential minerals (Aberoumand, 2011; Aberoumand and Deokule, 2010; Pandey et al., 2014).

Quantitative assay for the presence of plant phytochemical analysis of C. myxa indicated the presence of restively high levels of glycosides, flavonoids, sterols, saponins, trepenoids, alkaloids, phenolic acids, gums and mucilage (Afzal et al., 2004; Abdallah et al., 2011; Malik and Ahmad, 2015). The fruit of C. myxa is used for treatment of chest, urinary infection, disease of the lung and spleen and against liver fibrosis when measured level of hepatic enzymes ALT, ALP, AST (Afzal et al., 2009). Antiradical activity was measured, it contain 40/100g of scorbic acid and antioxidant of C. myxa which may be due phenols, scorbic acid and lycopeine (Kachhwaha and Gehlot, 2015). C. myxa is used in popular folk medicine as abortive in tropical region of the world Analgesic and as inflammatory agents in both acute and chronic phase (Silva et al., 2004; Ranjbar et al., 2013).

The fruit of C. myxa is popularly used for the treatment of chest, urinary tract infections, diseases of the lung and spleen analgesic, anti-inflammatory cytotoxic, antiviral, antiulcer, anticancer, antihelmintic and anti-HIV-1(Al-Awady et al., 2001; Silva et al., 2003; Afzal et al., 2007, Costa et al., 2008; Aberoumand and Deokule, 2010; Abdullah, 2011; Moghimipour et al., 2012; Moustafa et al., 2014; Rashed et al., 2014).

Mucilage extract of C. myxa was shown to be active against promastigotes of Leishmania major and L. infantum (Saki et al., 2015).

Hence the present investigation was undertaken to determine the immunomodulatory activity of aqueous extract of C. myxa fruit with immunized mice with HCFAg.

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2 Materials and methods

1- Preparation of the antigen:
Crude sheep HCFAg was prepared by a septic condition according to (Moosa and Abdel-Hafez1994). Protein concentration was measured according to (Bradford, 1976). Results was 3mg/ml for final amount (60 ml) fluid obtained by dialysis, HCFAg was stored at -20°C until use.

2- Laboratory animals: BALB/c mice (4 to 5 weeks old, body weight 20±2g) were used in the experiment. They were divided into four groups, and each group was kept in a separate plastic cage under regular laboratory conditions. The mice were maintained at a temperature of 23-25°C and they had free excess to food (standard pellets) and water.

3- Immunization: Immunization was performed to (Al-Qaoud etal.,2008), with some modification the procedure as follow: 0.2 ml of 150 µg/mouse/dose of HCFAg were mixed with equal volume of Freund's adjuvant as first injection and followed by two boosters of antigen in incomplete Fruend's adjuvant within seven days intervals.

4- Fruit extract preparation: Aqueous extract of C. myxa fruits were prepared according to (Sato etal., 2000).

5- Experimental design: Forty mice were randomly divided into four groups (10 each) included, first group: immunized mice with HCFAg, second group: treated mice processed with aqueous extract of C. myxa fruits for 10 days, third group: immunized mice and treated with plant extract for 10 days and the forth group used as the control.

6- The immunological study
A- Delayed type hypersensitivity test (DTH): Cellular immune response (DTH) was detected according to (Ali-Khan, 1978).

B- Methods of mitotic index (MI): Bone marrow and spleen cells prepared using direct method (Allen etal., 1977) with some modification; the percentage of MI was recorded by using the following equation:

\[ MI = \frac{\text{Number of the dividing cells}}{\text{Total number cells}} \times 100 \]

7- Histopathological study: Investigation of spleen and lymph node sections from immunized and treated mice prepared according to (Godkar and Godkar, 2003).

8- Statistical analysis: Data are expressed as mean ±S.E. and were analyzed with one-way ANOVA test was used according (SAS, 2012).

Results and Discussion

HCFAg might be a good candidate for immunization induces inflammatory response represented by infiltration of lymphocytes, neutrophils and macrophages to the site of injury. HCFAg stimulates cellular immune response, CMI and antibody production, the antigens stimulate complex immune response, these include polarized Th2 responses balanced with Th1 responses (AL-Qaoud and Abdel-Hafez, 2008; Hashemi Tabar etal., 2008; Youssifi etal.,2010). When injected HCFAg are converted to lymphoblasts and secrete lymphokines which attract more scavenger cells to the site of reaction. The infiltration cells thus immobilized to promote defensive inflammatory reaction (Jagzap etal., 2012).

Medical plants are very important in our daily life as these are used for the treatment of many diseases. C. myxa contained various compounds which are responsible for protection against pathogenesis (Farnsworth etal., 1985; Rahimi-Esboei etal. 2014). Recently, there are trends for using plants in therapy (back to the nature) as a result of side effects and complications of chemotherapies.

The results of DTH test showed a higher significant (p<0.05) increase in the means of foot pad thickness of immunized groups as compared with the control. There was difference in the thickness after seven day past immunizes which reached 0.95±0.13mm and 0.37±0.55mm at 3 and 24 hrs. A higher increase of thickness was show in immunized group after 21 day post immunization that reached 1.02±0.78mm. A higher increase of thickness was shown in immunized and treated group after 10 days with aqueous extract of C. myxa fruit after 10 days which reached 1.23±0.13mm after 3 hrs. and 0.53±0.44mm after 24 hrs. (Table1). Test was used when small quantities of antigen HCFAg were injected subcutaneously, obvious marked cellular immune response was elicited represented by thickness and swelling into the site of lesion within 3 to 24 hrs.
Table (1): skin thickness (mm) at 7, 14 and 21 days post immunization of HCFAg and 10 days post treated with aqueous fruit extract of *C. myxa* (mean ±S.E.).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Skin test after</th>
<th>Skin test after immunized and treated</th>
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<tbody>
<tr>
<td>Day post immunization</td>
<td>immunization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 hrs.</td>
<td>24 hrs.</td>
</tr>
<tr>
<td>7</td>
<td>0.95±0.13</td>
<td>0.3±0.55</td>
</tr>
<tr>
<td>14</td>
<td>1.27±0.10</td>
<td>0.42±0.06</td>
</tr>
<tr>
<td>21</td>
<td>1.02±0.15</td>
<td>0.47±0.07</td>
</tr>
<tr>
<td>Immunized and treated</td>
<td>1.23±0.13</td>
<td>0.53±0.44</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
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The findings suggest the presence of CMI exemplified by swelling in the footpad caused by inflammatory edema and infiltrate referred to the subset of T-helper cells responsible for DTH responses (Dematteis et al., 2001) referred (Dematteis et al., 2001). The parameter of DTH is caused by the various cytokines secreted by mononuclear cell series espically macrophage and T cells which are activated when they can across the insulation Ags. With time, depletion in these T cell occurs which is associated with a decrease in footpad thickness, due to the low Ag induced lymphocyte transformation indices. Immunized with treated aqueous *C. myxa* fruit provides long-live source of the protein and prolonged expression of Ag as effective means for elicited both CMI and Abs.

**MI test:** MI of bone marrow and spleen cells in mice immunized with HCF Ag showed significant increase (P< 0.05) in MI (47.96±2.08 and 49.90±3.88) respectively compared with the negative control, and such MI was significantly increased when mice were treated with aqueous extract of *C. myxa* after 10 days. The MI of bone marrow and spleen cells was high significantly increased as a post immunized +treatment mice (88.57±1.40 and 94.7±0.76) respectively compared to the other groups (Table 2).

**Table (2): MI of experimental mice (mean ±S.E.).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone marrow</td>
</tr>
<tr>
<td>Immunized with HCFAg</td>
<td>47.96±2.08</td>
</tr>
<tr>
<td>Treated with aqueous extract of <em>C. myxa</em></td>
<td>88.57±1.40</td>
</tr>
<tr>
<td>Immunized and Treated</td>
<td>93.16±1.00</td>
</tr>
<tr>
<td>Control</td>
<td>27.14±2.08</td>
</tr>
</tbody>
</table>

MI is simple measurement to determine the percentage of cells undergoing mitosis, and may be elevated during necessary processes to life such as the normal growth of plant or animals. The activity of immune system and the effect of different agents and it depend on the ability of lymphoid organs depend on dividing cells of bone marrow because it is the source of all blood cells (Hughes, 2001).

**Histopathological examination:** The histopathological sections in immunized mice HCFAg showed the proliferation of lymphoid follicles in spleen with diffuse infiltration of mononuclear cells mainly lymphocytes, macrophages and plasma cells (Fig1), while in the treated group there were mild to moderate lymphoid tissue proliferation which extended to red pulp and medulla. In red pulp of spleen there were congestion of blood vessels sinuses, dilated filled with blood and contained lymphocytic cells (Fig2).
Figure 1: Microphotograph of spleen in immunized mice showed infiltration of mononuclear cells in red pulp (H&E stain, 400X).

Figure 2: Microphotograph in red pulp of spleen in treated mice showed congested and dilated B.V. sinus filled with blood and contained mononuclear cells. (H&E stain, 100X).

In the immunized and treated group the sections revealed marked hyperplasia of lymphoid corpuscles confusion to form large follicle (Fig3). Congestion of blood vessel sinuses some of them dialed filled with blood and contained lymphatic cells also diffuse infiltration of mononuclear cells (Fig4and5).
Figure 3: Microphotograph of confused lymphoid follicles in white pulp of spleen of immunized and treated mice. (H&E stain, 400X).

Figure 4: Microphotograph of spleen in red pulp of immunized and treated mice with diffuse lymphocytes infiltration and within congested blood vessel sinuses (H&E stain, 400X).
Figure 5: Microphotograph of red pulp in spleen of treated and immunized mice showing focal aggregation and diffuse infiltration of mononuclear cells (H&E stain, 400X).

The pathological results of the present work coincide with the results of the immunological tests. In immunized mice showed proliferation of lymphoid follicles in spleen with diffuse infiltration of mononuclear cells mainly lymphocytes, macrophages and plasma cells associated with elevation in the rates of mitotic index in the spleen and bone marrow. *C. myxa* has anti-inflammatory and highly immunogenic effect to protect the soft organs like the liver and the spleen from the effect of different types of harm full agents (Afzal *et al.*, 2007; Ranjbar *et al.*, 2013). Many workers attributed that action alkaloid were found to have immune stimulatory effect, both humoral and CMI which exhibit efficiency against *E. granulosus in vitro* (Xingming *et al.*, 2004), saponin also induces strong cytotoxic CD8⁺ lymphocytes response (Fenwich *et al.*, 1991), *C. myxa* presence of polyphenols was found to have antibacterial, anti-inflammatory, anti-allergic, antiviral and antineoplastic activities (Alan and Miller, 1996; Thirupathi *et al.*, 2008). This may explain the minor inflammatory infiltration in spleen of immunized and treated mice, congestion of blood vessels sinuses and contained lymphocytes cells and mononuclear cells.

**Conclusion**: It is concluded that the aqueous extract is a drug with immunity effect for cell mediated immunity, which has a great potential as a source for natural health products.

**References**


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