The Biological Activity of Extraction of Protein Compound From Metapenaeus on Giardia Lamblia

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Abstract

The study includes the extraction of whole bodies of local shrimp from Shatt Al-Arab during Jun 2012 on Giardia lamblia which collected (30) sample from Ibn Al-Balady hospital in Baghdad from patients clinic. The result of study the extract caused all effect was killed of parasite on sixth day followed by 100 mg/ml shrimp extract and then 70 mg/ml shrimp extract and cyst continue to decline until it reached zero in seventh day and (WBC) in 100 mg/ml extract was $547 \pm 0.20 \times 10^3$ mm$^3$ and $7.1 \pm 0.01 \times 10^3$ mm$^3$ in 70 mg/ml extract compare with control group $0.4423 \pm 8.62 \times 10^3$ mm$^3$ and Flagel group $(5 - 8 \pm 0.11) \times 10^3$ mm$^3$, and the result found MLC in 70 mg/ml killed the parasite in seventh day.

Keywords: Giardia lamblia, Metapenaeus, Red blood cells, White blood cells, Hemoglobin, Packed cell volume.

Introduction

Giardia lamblia parasite is flagellated protozoan of the small intestine and stomach of Mammals [1-3].

With Giardia, the nutritional status of the host is very important in young children with poor nutrition who was suffering failure to thrive [4].

Life cycle of G. lamblia consisting of two stages, the infective stage cyst is resistant to environmental factors and responsible for the transmission of G. lamblia [5], and trophozoite is a pear-shaped cited by Espelage [6].

The specific mechanism of Giardia pathogenesis lead to diarrhea, causing symptoms there are associated with diarrhea including dehydration vomiting nausea, malabsorption and malnutrition [7]. The drug metronidazole more efficient for the infection with Giardia lamblia [8, 9] has pointed to the evolution of resistance some strains of the parasite Giardia lamblia against drug metronidazole.

In this study used extraction of shrimp metapenaeus on Giardia lamblia, the Ocean creatures produce certain chemicals that helps her to kill pathogenic microorganisms, those chemicals have been used lately as anti-inflammatory agents and to cure cancer.

M. Affinis considered one of the Ocean creatures that live in deep Ocean that can be used for this purpose [10].

M. Affinis considered from crustacean composed from protein which peptides binds to each other, these peptides most are small peptides less than 10 kd are positive and amphipathic and gives fast and immediate effect when invade microorganisms [11].

Aim of this study is to found substance efficient for the infection of Giardia lamblia.

Materials and Methods

The study includes the extraction of whole bodies of eight local shrimp from Shatt Al-Arab during Jun 2016. The fisher was preserved in deep freezer (-1°C) till was used.

Preparation of Shrimp Extract

M. Affinis was washed with tap water to remove any debris then washed with distilled
water, 50% contraction of 98% acetic acid and mixed for five minutes.

The mixture was left 24hrs in (26-23) °C and PH = 5.

The mixture was then centrifuged 200 cycle/ mins for 5 mins in 26°C.

To get rid of salt in the filtrate the dialysis tubing, 4°C for 48hrs was use.

The distilled water surrounded the dialysis tubing was changed every 6hrs.

At the end of dialysis, the solution and stored in (23-26) °C when used for the study.

**Direct Stool Smear Examination**

Conducted this examination for each sample of stool samples it was prepared to clean and dry glass slide and put a drop of normal saline and one of Lu gols Iodine on another and of glass slide, then took a small amount of stool from different places of the sample by wood sticks and mixing well drop normal saline and drop of Lu gols Iodine, after that covered with cover slide and examined under microscope with power magnification 40x [12].

**Purification of Parasite**

Method was to isolate the parasite the cysts and trophozoites were suspended in phosphate buffer saline (PBS 7.2).

The final concentration was attended by rate 1x10^5 cell/0.1 ml [13].

Stool samples are culture after prepared (HSP -1) media. 0.1 of stock that have 1x10^5 cell of cyst and (5) trophozoite in one field take to the culture media and add to this media (3 ml) of human serum. And put mg from (70, 100, 200, 300, 400, 500 and 1000) to standard the minimal inhibitory concentration (MLC) [14]. And used of normal saline for the detection of the parasite (cyst and trophozoite). The Iodine kills the parasite so motion will no longer be seen [15], and distinguish it forms for other types of parasite and these dyes.

**Preparation of Laboratory Animals**

Using 80 mouse from white swiss mice (males and females) were obtained from national center for research and Brug control and an average age between (5-12) weeks and weight (16-22) gm. 80 mice given 1x10^3 cell/0.1 ml after (4-8) hrs, the stool of all mice examined and after sure infected by Giardia divided into four groups, each group contain 20 mice, then inoculated as follows:

- **Group 1:** 0.1 ml from metronidazole orally at a single dose per day [16].
- **Group 2:** 0.1 ml from 70 mg/ml from shrimp extract.
- **Group 3:** 0.1 ml from 100 mg/ml from shrimp extract.
- **Group 4:** 0.1 ml from normal saline and consider as positive control.

**Hematological Studies**

The blood collected from mice heart puncture [17], and put tub cloning with Heparinized for hematological studies in white blood cells (WBC) and red blood cells (RBC), blood hemoglobin (Hb), PCV packed cell volume [17].

**In Vitro**

Used (HSP -1) media and put 0.1 of stock that have 1x10^3 cell of cyst and (5) trophozoite and add to this media 3 ml of human serum and put (70, 100, 200, 300, 400, 500, 1000)mg and standard minimal inhibitory concentration (MIC) and incubate this plates in 37°C for 24hrs and determine (less concentration for extract inhibited parasite).

| Table 1: The numbers of cyst raised in mice with positive group |
|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Group               | 1              | 2              | 3              | 4              | 5              | 6              |
| Control +v          | 42.0 ± 14.1    | 41.80 ± 0.25   | 40.10 ± 0.41   | 40.00 ± 0.32   | 38.60 ± 0.37   | 38.10 ± 0.23   |
| Flagel              | 15.00 ± 1.25   | 14.5 ± 1.26    | 7.10 ± 0.67    | 3.0 ± 0.35     | 2.5 ± 0.31     | 0.00 ± 0.00    |
| 70mg/ml             | 33.7 ± 0.31    | 29.00 ± 0.23   | 28.8 ± 0.42    | 16.60 ± 0.47   | 9.10 ± 0.31    | 3.10 ± 0.33    |
| 100mg/ml            | 29.00 ± 0.23   | 28.8 ± 0.42    | 22.3 ± 0.52    | 14.40 ± 0.54   | 5.70 ± 0.36    | 2.40 ± 0.16    |

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Table 2: Shown RBC, WBC, PCV, HB in different groups

<table>
<thead>
<tr>
<th></th>
<th>Hb g/dl</th>
<th>PCV %</th>
<th>RBC x10⁶/m³/m</th>
<th>WBC x10⁶/m³/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control +v</td>
<td>10.61 ± 0.07</td>
<td>34.3 ± 0.99</td>
<td>6.8 ± 0.01</td>
<td>0.4423 ± 0.63</td>
</tr>
<tr>
<td>Flagel</td>
<td>12.2 ± 0.68</td>
<td>40.51 ± 1.75</td>
<td>9.0 ± 0.02</td>
<td>5.8 ± 0.11</td>
</tr>
<tr>
<td>70mg/ml</td>
<td>11.08 ± 0.113</td>
<td>33.97 ± 2.26</td>
<td>7.0 ± 0.04</td>
<td>7.1 ± 0.01</td>
</tr>
<tr>
<td>100mg/ml</td>
<td>12.45 ± 0.0849</td>
<td>38.7 ± 3.12</td>
<td>8.5 ± 0.02</td>
<td>5.47 ± 0.205</td>
</tr>
</tbody>
</table>

Result and Discussion

The result of present study in table (1) shows that therapeutic substances caused all effect in the treatment. And shown in above table that the best effect was killed of parasite on the sixth day followed by (700mg/ml) shrimp extract, and then (70mg/ml) shrimp extract cysts continue to decline until it reached Zero on the seventh day.

The result of present study in table (2) shows that the (WBC) in (100mg/ml) shrimp extract was \(5.47 \pm 0.205 \times 10^6 / \text{mm}^3\), and \(7.1 \pm 0.01 \times 10^6 / \text{mm}^3\) in (70mg/ml) shrimp extract compare with control group \(0.4423 \pm 8.63 \times 10^6 / \text{mm}^3\), and Flagel group \(5.8 \pm 0.11 \times 10^6 / \text{mm}^3\).

The mean increases in control group, because leukocytes from a protective that helps defend body against damage by parasites, bacteria and tumor cell [18].

But the concentration of extracts 70mg/ml decrease to \(7.1 \pm 0.01 \times 10^6 / \text{mm}^3\). In (RBC) decrease in control group \(6.8 \pm 0.01 \times 10^6 / \text{mm}^3\) compare with Flagel group \(9.0 \pm 0.02 \times 10^6 / \text{mm}^3\) and increased in group 70mg/ml extract \(7.0 \pm 0.04\), \(8.5 \pm 0.02\) \(10^6 / \text{mm}^3\).

Red blood cells serve as a carrier of hemoglobin. Which react with oxygen carried in the blood to form ox hemoglobin and carbon dioxide in the body during respiration. Thus, a reduced red blood count implies a reduction in the level of oxygen that would be carried to the tissue as well as level of carbon dioxide returned to the lungs [19, 20].

Hb lower in parasite group 10.61 ± 0.07 g/dl compare with Flagel group 12.2 ± 0.68 g/dl, but increased in treat group with 70mg/ml11.08 ± 0.1135 g/dl and 12.45 ± 0.084 g/dl [21]. But PCV was 34.3% in control group, compare with Flagler group 40.5%, but in the treat group with 70mg/ml 33.9%, and 38.76% with 100mg/ml.

The shrimp from crustacean which rich with protein and comosited from chain peptides most are small peptides less than 10 Kd are positive and amhipathic and gives fast and immediate effect when invade microorganism this peptides created in red blood cells and release in plasma after responded immunity and contact on the epidermis which (penaeid ins) in cuticle which action against organism [14, 21, 22].The result found (MLC) in 70mg/ml killed the parasite in seventh day.

References

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