EFFICIENCY OF SEAWEED EXTRACT OF BROWN ALGAE
ASCOPHYLLUM NODOSUM AGAINST LEISHMANIA DONOVANI
PARASITE

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Abstract

The screening study to search for new natural antiprotozoals, we screened the commercial product seaweed extract of brown
algae Ascophyllum nodosum and tested them in vitro and in vivo against Leishmania donovani parasite the responsible for
one of the major endemic parasitic diseases ,the viability rate after treated with seaweed extract was decreased with increasing
of concentration at 50% was (35.62%,16.51% and 0 ) after (15min, 30min and 1hrs) respectively ,but in concentration 25% the
viability became (50.30%,35.30% and 0) after the same incubation period compared with control treatment and drug treatment
(pentostam) . The histological changes of liver tissue for treating males white mice orally with seaweed extract for three weeks
after injecting with 1×10⁶ cell/ml intraperitonial by Leishmania donovani showed As observed a clear improvement in liver
hyperplasia in kupffer cell , and hydropic degeneration but noticed reduce presence of parasites and approximately mild
reaper occur beside the group of mice treated with drug pentostam , compared with the positive control ( mice inoculated
orally 0. 1ml/day) normal saline . The histological section of liver for infected mice with leishmanial parasites showed
haemorrhage, infiltration of lymphocytes, proliferative for kupffer cell as a result of the presence of parasites.

Key word: Leishmania donovani, Ascophyllum nodosum, liver, MTT

Introduction

Leishmaniasis is a vector-borne disease, obligate intracellular protozoa of the genus Leishmania are
responsible for this disease. Leishmaniasis is still one of the world’s most dismissed diseases until a few years
ago, nearly twelve million people are infected with a species of Leishmania at any given time point. It is
predominant in no less than 88 nations over the tropical and subtropical districts of Africa, Asia, South and Central
Americas, the Mediterranean and Southern Europe (Alvar et al., 2012). It is transmitted to human by 30 by
phlebotomine spp infected sand flies vector (Bates, 2007). There are at least, three main clinical forms of
leishmaniasis, the visceral leishaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis
(MCL), the most severe form of the disease is VL (Bates. et al., 2000 ). Visceral leishmaniasis caused by the
protozoan parasite, Leishmania donovani, is deadly when left untreated. Nearly 90% of VL infections are
found in five countries, India, Nepal, Brazil, Bangladesh, and Sudan (Alvar et al., 2012). In the Old World, the
etiological agent of human VL is represented by two parasite species belonging to L. donovani complex: L.
infantum which diffuse as a zoonosis with domestic dogs and wild canids as the essential reservoirs (Baneth. and
Aroch, 2008), and L. donovani, which is accepted to be anthroponotic and mostly transmitted among humans
(Chappuis et al., 2007).

Ascophyllum nodosum, is an important edible seaweed marine ingredient, belonging to the brown algae
(Phaeophyceae) (Karatzia et al., 2012). It is generally known as “Rockweed”, and it is found vulnerable waters
of the North Atlantic Ocean (Ugarte, R. et al., 2010 ). A. nodosum is widely utilized as a part of agricultural and
horticultural manufactures (Khan, et al., 2009, Sangha

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Efficiency of Seaweed extract of brown algae *Ascophyllum nodosum* against *Leishmania donovani* parasite

**Materials and Methods**

**Leishmania strain**

It was obtained from the department of biology science from biotechnology center/AL- Naharin University, then maintained *in vitro* by serial passage in NNN medium. Then washed three times with phosphate buffered saline (PBS) and adjusted to concentration $1 \times 10^6$ promastigote/ml.

**Preparation the *Ascophyllum nodosum* extract concentration**

Seaweed extract of brown algae *Ascophyllum nodosum* is obtained as a commercial foreign product from College of Agriculture, Baghdad university. Sterilized dislled water was used to prepare different concentrations of *Ascophyllum nodosum* extract: 25, 50, 75, 100%.

**Ascophyllum nodosum extract Activity Against Promastigote viability in vitro**

Haemocytometer slide prepared $1 \times 10^6$ cell/ml then added one milliliter from this number in tubes which divided into five groups each one had 6 repeater. Then added to first group 1 ml of pentostam drug, second, third, fourth and fifth groups added 1 ml of *Ascophyllum nodosum* extract (25, 50, 75 and 100%) respectively, and left sixth group without addition consider as control ,all tubes incubated at 26 C° then after (15 min, 30min) put 100 µl of microplates well from each tube, then 10 il of the MTT solution was added to each well . Incubated for 4 hr at 25 C°. The media was removed, then added 100 µl of the solubilization solution into each well. Checked for complete solubilization of the purple formazan crystals then measured the absorbance of the samples by using a microplate (ELISA) reader, at wavelength was 600-570 nm. The percentage of viability was calculated from OD readings according to the following formula: (Soflaci et al., 2014).

$$\text{Viable cells} = \frac{\text{absorbance of treated cells}}{\text{absorbance of control cells}} \times 100$$

**Chronic toxicity test in mice**

Study on acute toxicities of concentrations of *Ascophyllum nodosum* extract were carried out in mice. It was administered orally (0.1 ml/day) in six mice for 21 days, showed no death occurred in the mice following the oral administration of the maximum dose (100%).

**Ascophyllum nodosum extract (ANE) Activity Against parasite in vivo**

**Animals**

Twenty-Four male albino mice aged 12-13 weeks, were obtained from the animal house in college of Medicine Baghdad University were housed under standard condition. Then, 18 mice were infected by injecting with $1 \times 10^6$ cell/ml intraperitonial. After 14 days, the infected mice were divided into 4 groups, each group contains 6 mice, the last 6 non infected mice remain as negative control. Then each group inoculated as a follow:

1. Group 1: inoculated orally by stomach tube (0. 1ml/day) from ANE every day.
2. Group 2: injected intraperitonial with (0. 1ml/day) pentostam each day.
3. Group 3: inoculated orally (0. 1ml/day) normal saline considers as positive control.
4. Group four (none infected): inoculated orally (0. 1ml/day) normal saline considers as negative control.

After 21 days post inoculation all the mice were scarified, liver was removed then hematoxylin and eosin stained liver sections from mice of all groups were examined microscopically for histopathological changes.

**Results and discussion**

**Effect of extract on promastigote viability in vitro**

Table (1) summarizes the results obtained showed the seaweed extract resulted in promastigote of *Leishmania* parasites with extract 100% lead to decrease in viability rate reached zero after only 15 min, while when exposed the parasite to (75%) the viability reached...
to zero after (30 min) and the viability rate after treated with seaweed extract (50%) was (35.62%, 16.51% and 0) after (15 min, 30 min, and 1 hrs) respectively, but in (25%) the viability also decrease and became (50.30%, 35.30% and 0) after same incubation period. Comparison with a pentostam significant decrease occurs in a parasite viability rate reached to (88.00%, 80.3% and 65.00%) respectively but the extract more effective than pentostam.

Untreated parasites (control) the viability percentage of parasite remain very high was (98.1%, 96.3% and 95.87%) respectively, that a statistically significant difference (P<0.05) compared with pentostam and control so our study has shown the effects of extract in the viability of the parasite in vivo, also this reduction increasing with increase extract concentration.

The reduction in the viability percentage using by seaweed extract compared with negative control and positive control (pentosam) may be due to the metaolitites active of seaweed such as polysaccharides, fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids and halogenated compounds aiding in the protection against different environmental stresses. These compounds show antiviral, antiprotozoal, antifungal, and antibacterial properties (Pérez, M.J. et al., 2016). Few studies have reported antiprotozoal screening of marine algae, in a study of (Sénécheau C.V. et al., 2011, Aliança S.S. et al., 2014) revealed high activity of brown seaweed extract against L. donovani because these seaweeds have high potential to yield new and structurally unique natural products with biological activity against leishmaniasis.

**Toxity test of seaweed extract on mice**

There have no deaths occur in mice over a period of 21 days after inoculating with (0.1 ml/day) from seaweed extract daily. This means that it is no have toxicity in animals. The dministered seaweed extract may have negative side-effects on the health condition of the mice. These findings may support the idea that the oral route is an effective and safe route for ascophyllan in order to attain better antitoxicity effect this study was agreed with who exhibited the normal characteristic features of radiating hepatic cells around a normal central vein with narrow sinusoid, with no sinusoidal congestion and mild cellular swelling In rats administrated orally seaweed extract brown algae *Sargassum polycystum*.

**Histological study**

The histological section of liver for infected mice with leishmanial parasites showed haemorrhage, infiltration of lymphocytes, proliferative for kupffer cell as a result of the presence of parasites, compared with control group

![Fig. 1](http://example.com/fig1.jpg)

**Fig. 1:** Liver section shows the normal liver structure for non-infected mice. (H & E 20X)

![Fig. 2](http://example.com/fig2.jpg)

**Fig. 2:** Liver section of mice infected with *leishmania donovani* after 21 days shows infiltration of lymphocytes and proliferative of kupffer cells. H&E (40x)

A living section of treatment infected mice with seaweed extract for three weeks appeared hyperplasia in kupffer cell, and hydropic degeneration but noticed the reduced presence of parasites and approximately mild reaper occur fig. 3.

While the liver section for mice treated with pentostam drug showed repair occurred in parenchymal

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**Table 1:** Compare between extract and Pentostam effect in promasitgote viability percentage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>15min</th>
<th>30min</th>
<th>1hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Control</td>
<td></td>
<td>98.1%</td>
<td>96.3%</td>
<td>95.87%</td>
</tr>
<tr>
<td>Extract 25%</td>
<td></td>
<td>50.30%</td>
<td>35.30%</td>
<td>0</td>
</tr>
<tr>
<td>Extract 50%</td>
<td></td>
<td>35.62%</td>
<td>16.51%</td>
<td>0</td>
</tr>
<tr>
<td>Extract 75%</td>
<td></td>
<td>2.08%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Extract 100%</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*Pentostam</td>
<td></td>
<td>88.00%</td>
<td>80.3%</td>
<td>65.00%</td>
</tr>
</tbody>
</table>

*P < 0.05
liver with mild infiltration of lymphocytes and mild activation of kupffer cells, fig. 4.

The ability of seaweed extract to heal liver tissue after treating with leishmania donovani belongs to the macroalgal chemical compounds which has a wide spectrum in pharmaceutical studies, resulting in new drugs with leishmanicidal (Torresa et al., 2014) and trypanocidal activity (León-Deniz et al., 2009). Although the study and use of algal compounds against parasites are recent, many reports have already been published describing isolated compounds from several algae with strong antiprotozoal activity and low toxicity. Therefore, the discovery of novel molecules with a high therapeutic potential for marine macroalgae is very welcome (Freile-Pelegrin et al., 2008).

After injection parasite, the promastigote invade (and defect) the resident macrophage, kupffer cell and dendritics cell where they become amasitgote and replicate during the first week (Loeuillet et al., 2016), pentostam effect may be eradication and elimination parasite before increase loaded in liver and spleen during 7 days by directly killing parasite via inhibits DNA (Haldar et al., 2011). Kota et al. (2014) showed the hepatotoxicity of activity pentavalent compound in a murine model of visceral leishmaniasis after end day of treatment (21 days) included an increase swollen and vacuolization of hepatocyte that could be attributed to alteration in water homeostasis that may be caused by free radical during reduction pentavalent antimonial [Sb(V)] to the trivalent form [Sb(III)], while did not show significant histological change in the spleen upon treatment with antimonial drug.

References


