Plant Archives Vol. 18 No. 2, 2018 pp. 2252-2256 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

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 1×10^6 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 leishmamial 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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et al., 2010, Craigie, 2011) as well It has likewise been used as an animal feed supplement because of antioxidant properties and amelioration of stress tolerance and immune function in animals (Fike *et al*., 2001) and also as a human nutritional supplement (Rayorath *et al*., 2009). *A. nodosum* contains a few bioactive components, for example, polysaccharides (e.g., fucans, laminarin and alginic acid), vitamins, polyunsaturated unsaturated fatty acids (PUFA), peptides, secondary metabolites (e.g., phlorotannins), antioxidants etc., (Kim and Wijesekara, 2010, Wijesekara *et al*., 2010). These components have a beneficial properties such as anti-bacterial, antioxidant, cell-mediated immunomodulation (Allen *et al*., 2001, Turner *et al*., 2002) and anti-inflammatory activities (Dell'Antonio *et al*., 2002). A few studies have announced that, a rich in polysaccharides and polyphenols extracts from the marine brown alga *Ascophyllum nodosum* have different useful biological properties including antiviral (Trinchero *et al*., 2009) anti-inflammatory (Mizuno *et al*., 2009), antitumoral (Choi *et al*., 2005), anticoagulant and antithrombotic (Mourao, 2004) and antioxidant effects (Abu *et al*., 2013).

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 1×10^6 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 $1x^{10}$ 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 1ml 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 ìl 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: (Soflaei *et al*., 2014).

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

Chronic toxicity test in mice

Study on acute toxicities of concentrations of *Ascophyllum nodosum* extract were carried out in mice. It was administered orally (0.1ml/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 1×10^6 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 (15min, 30min and 1hrs) 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

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

Treatment	Time		
	15min	30min	1hr
*Control	98.1%	96.3%	95.87%
Extract 25%	50.30%	35.30%	0
Extract 50%	35.62%	16.51%	0
Extract 75%	2.08%	0	0
Extract 100%	0	0	0
*Pentostam	88.00%	80.3%	65.00%

*$P < 0.05$

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(0.1ml\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 exhiited 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 leishmamial parasites showed haemorrhage, infiltration of lymphocytes, proliferative for kupffer cell as a result of the presence of parasites, compared with control group

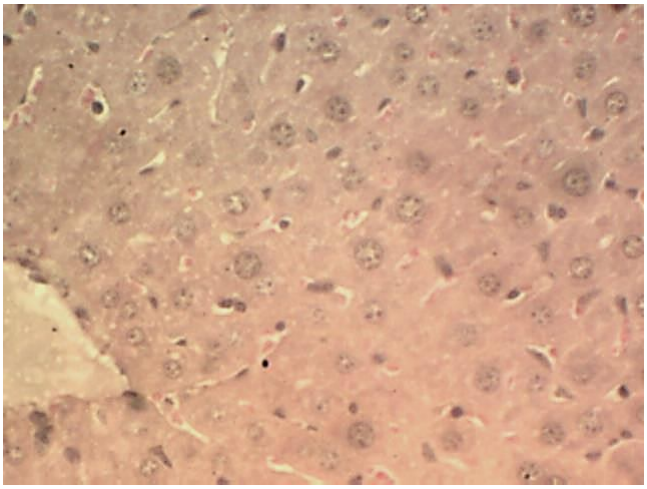

Fig. 1: Liver section shows the normal liver structure for non-infected mice. (H & E.20X)

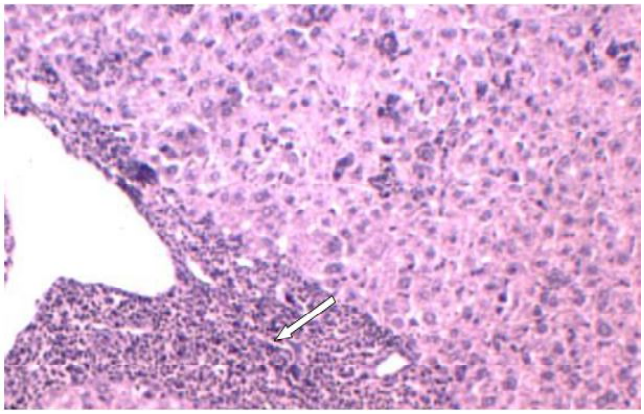

Fig. 2: Liver section of mice infected with *leishmania donovani* after 21 days shows infilteration of lymphocytes and proliferative of kupffer cells. H&E (40x)

liver showing normal structure. fig. 1 and fig. 2.

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

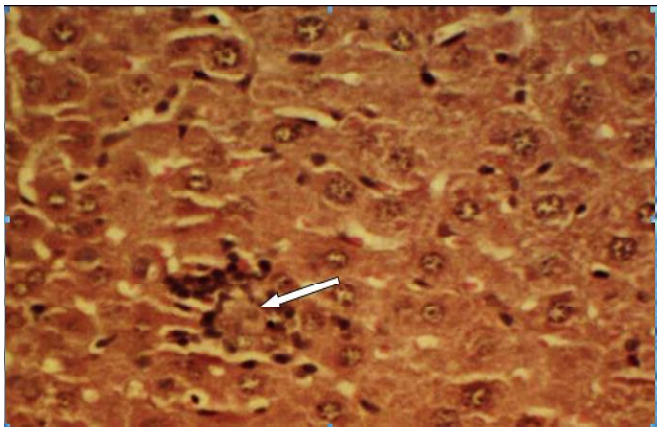

Fig. 3: Liver section of mice treated with seaweed extract after 21day shows aggregation of lymphocytes and hydropic degeneration. (H & E.20x).

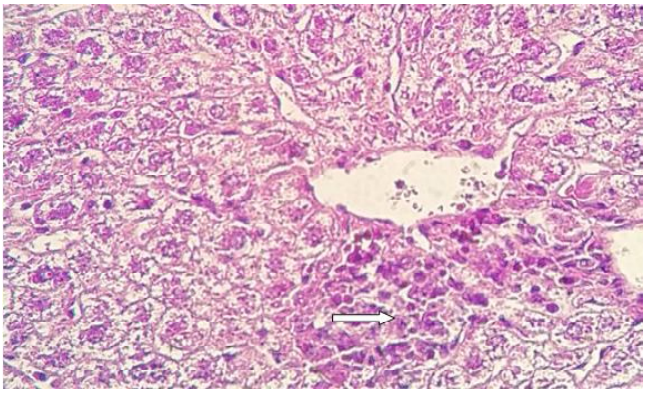

Fig. 4: Liver section of mice treated with pentostam after 21days shows infiltration of lymphocytes and mild activation of kupffer cells.(H&E.40x).

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 dendritis 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

Abu, R., Z. Jiang, M. Ueno, T. Okimura, K. Yamaguchi *et al.* (2013). *In vitro* antioxidant activities of sulfated polysaccharide ascophyllan isolated from *Ascophyllum nodosum*. *Int. J. Biol. Macromol.,* **59**: 305–312.

Aliança, S.S., K.F.L. Anjos, T.N.V. Reis, M.M. Higino, Castro, M.C.A.D.

Allen, V.G.; K.R. Pond, K.E. Saker, J.P. Fontenot, C.P. Bagley, R.L. Ivy, R.R. Evans, R.E. Schmidt, J.H. Fike, X. Zhang *et al*. (2001). Tasco: Influence of a brown seaweed on antioxidants in forages and livestock—A review. *J. Anim. Sci.* ; **79:** E21–E31.

Alvar, J., I.D. Velez, C. Bern, M. Herrero, P. Desjeux, J. Cano, J. Jannin and M. den Boer (2012). Leishmaniasis worldwide and global estimates of its incidence. PLoS One. **7**:e35671.

Baneth, G., I. Aroch (2008). Canine leishmaniasis: A diagnostic and clinical challenge.*Vet. J.*, **175**: 14–15.

Bates, P.A. (2007). Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. *Int J Parasitol.,* **37**: 1097–106.

Bianco, E.M.B. and R.C.Q. Figueiredo (2014). The *in Vitro* Biological Activity of the Brazilian Brown Seaweed Dictyota mertensii against Leishmania amazonensis. *Molecules,* **19**: 14052-14065.

Chappuis, F., S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar, M. Boelaert (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control. *Nat. Rev. Microbiol.* **5**: 873–882.

Choi, E.M., A.J. Kim, Y.O. Kim and J.K. Hwang (2005). Immunomodulating activity of arabinogalactan and fucoidan *in vitro*.. *J. Med. Food.*, **8**: 446–453.

Craigie, J.S. (2011). Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.,* **23**: 371–393.

Dell'Antonio, G., A. Quattrini, E. Dal-Cin, A. Fugenzi, M.E. Ferrero (2002). Relief of inflammatory pain in rats by local use of the selective P2X7 ATP receptor inhibitor, oxidized ATP. *Arthritis Rheum*, **46**: 3378–3385.

Fike, J.H.; Allen, V.G.; Schmidt, R.E.; Zhang, X.; Fontenot, J.P.; Bagley, C.P.; Ivy, R.L.; Evans, R.R.; Coelho, R.W.; Wester, D.B. Tasco-Forage (2001): Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. *J.*

Anim. Sci., **79**: 1011–1021.

Freile-Pelegrin, Y., D. Robledo, M.J. Chan-Bacab, and B.O. Ortega- Morales (2008). Antileishmanial properties of tropical marine algae extracts. *Fitoterapia,* **79**: 374-377.

Haldar, A.K, P. Sen and S. Roy (2011).Use of antimony in the treatment of leishmaniasis:Current status and future directions. *Mol. Bio. Int.,* 23-28.

Karatzia, M., E. Christaki, E. Bonos, C. Karatzia and P. Florou-Paneri (2012). The Influence of dietry *Ascophyllum nodosum* on hematological parameters of dairy cows. *Ital J. Anim. Sci.,* **11**: 169-173.

Kato, K.C., E. Morais-Teixeira, P.J. Reis, N.M. Silva-Barcellos, P. Salaün, P.P. Ampos, J.D. Corrêa-Junior, A. Rabello, C. Demicheli and F. Frézarda (2014). Hepatotoxicity of pentavalent antimonial drug: possible role of residual Sb(III) and protective effect of ascorbic acid. *Antimicrobial Agents and Chemotherapy,* **58(1)**: 481–488.

Khan, W., U.P. Rayirath, S. Subramanian, M.N. Jithesh, P. Rayorath, D.M. Hodges, A.T. Critchley, J.S. Craigie, J. Norrie, B. Prithiviraj (2009). Seaweed extracts as biostimulants of plant growth and development (Review). *J. Plant. Growth Regul.,* 28: 386–399.

Kim, S.K.; I. Wijesekara (2010). Development and biological activities of marine derived bioactive peptides: A review. *J. Funct. Foods,* **2**: 1–9.

León-Deniz , L.V.L., E. Dumonteil, R. Moo-Puc1 and Y. Freile-Pelegrin (2009). Antitrypanosomal *in vitro* activity of tropical marine algae extracts. *Pharmaceutical Biology,* **47(9)**: 864–871.

Loeuillet, C., A.L. Bañuls and M. Hide (2016). Study of *Leishmania* pathogenesis in mice:experimental considerations. *Parasites and vectors,* **9(1)**: 144.

Mauricio. I.L., J.R. Stothard and M.A. Miles (2000). The strange case of Leishmania chagasi. *Parasitology Today,* **16**: 188–9.

Mizuno, M., Y. Nishitani, T. Hashimoto and K. Kanazawa (2009). Different suppressive effects of fucoidan and lentinan on IL-8 mRNA expression in in vitro gut inflammation. *Biosci Biotechnol Biochem.* **73**: 2324–2325.

Mourao, P.A. (2004). Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. *Curr. Pharm. Des.,* **10**: 967–981.

Pérez , M.J., E. Falqué and H. Domínguez (2016). Antimicrobial Action of Compounds from Marine Seaweed. *Mar. Drugs,* **14(3).**

Rayorath, P., B. Benkel, D.M. Hodges, P. Allan-Wojtas, S. Mackinnon, A.T. Critchley *et al*. (2009). Lipophilic components of the brown seaweed, Ascophyllum nodosum, enhance freezing tolerance in Arabidopsis Thaliana. *Planta,* **230**: 135–147.

Sangha, J.S., D. Hobson, D. Hiltz, A.T. Critchley and B. Prithiviraj (2010). The use of commercial seaweed extracts as a means to alleviate abiotic stress in land plants: A review. *Algal Resour.,* **3**: 153–168.

Sénécheau, C.V., M. Kaiser, I. Devambez, A. Vastel, I. Mussio and A.N. Rusig (2011). Antiprotozoal Activities of Organic Extracts from French Marine Seaweeds. *Mar. Drugs,* **9**: 922-933.

Soflaei, S., A. Dalimi, A. Abdoli, M. Kamali, V. Nasiri, M. Shakibaie and M. Tat (2014). Anti-leishmanial activities of selenium nanoparticles and selenium dioxide on *Leishmania infantum*. *Comparative Clinical Pathology,* **23(1)**: 15-20.

Torresa,F.A.E., T.G. Passalacquaa, A.M.A. Velásqueza, R.A. Souzab, P. Colepicolod and M.A.S. Graminha (2014). New drugs with antiprotozoal activity from marine algae review, *Rev. Bras. Farmacogn,* **24**: 265-276.

Trinchero, J., N.M. Ponce, O.L. Cordoba, M.L. Flores, S. Pampuro *et al.* (2009). Antiretroviral activity of fucoidans extracted from the brown seaweed *Adenocystis utricularis*. *Phytother Res.,* **23**: 707–712.

Turner, J.L., S.S. Dritz, J.J. Higgins and J.E. Minton (2002). Effects of *Ascophyllum nodosum* extract on growth performance and immune function of young pigs challenged with *Salmonella Typhimurium*. *J. Anim. Sci.,* **80**: 1947–1953.

Ugarte, R., J.S. Craigie and A.T. Critchley (2010). Fucoid Flora of the Rocky Intertidal of the Canadian Maritimes: Implications for the Future with Rapid Climate Change. In *Seaweeds and Their Role in Globally Changing Environments*; Israel, A., Einav, R., Seckbach, *J., Eds.; Springer: Berlin,* Germany.

Wijesekara, I., N.Y. Yoon and S.K. Kim (2010). Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. *Biofactors,* **36**: 408–414.