Plant Archives Vol. 20 No. 1, 2020 pp. 395-398 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDY OF THE ACTIVITY OF *ANABAENA VARIABILIS* ALGA ON SOME IMMUNE INDICATORS IN MALE MICE

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Abstract

High nutritional content of Microalgae and many others advantages make it an important source for immunomodulating studies. *Anabaena variabilis* is a filamentous microalga with a heterocyst and it is capable of both photoautotrophic and heterotrophic growth. This study was done to investigate the activity of *A. variabilis* aqueous extract on some immunological, cytological and haematological parameters. Mice were divided into four groups, the control and the rest were received different doses from algal extract (40, 1200, 2400 mg/kg). ELISA (Enzyme-Linked Immunosorbent Assay) kit was used to measure interleukins (2, 4, 12) and gamma interferon. The results revealed that the aqueous extract increased the production of interleukins (2, 12) and gamma interferon while the production of interleukin 4 was decreased. The significant differences were not observed in each mitotic and haematological parametres, except in lymphocytes percentage. This aqueous extract this could be used as a therapeutic way for prevention of (Th2) cytokine-dominant disease.

Key words: Algae, interleukins, *Anabaena variabilis*, lymphocytes

Introduction

Anabaena is one of the prevalent microalgae in food supplements and nutraceuticals (Kim, 2015). *Anabaena variabilis* is a filamentous microalga with a heterocyst and it is capable of photoautotrophic growth in the light and true heterotrophic growth in the dark using fructose as both carbon and energy source (Thiel *et al*., 2014). This genus is found in water and soil and has antibiotic effect (Cynthiaricini *et al*., 2013) and it has a various species which synthesize a variety of primary and secondary metabolites (Pant *et al*., 2012). Innate immunity and acquired immunity are the two major immune system components which protect the body from the invasion of foreign substances. Innate immunity is the first line of defense (nonspecific defense), while the acquired immunity (adaptive immunity) is specific immune response (Whitlock, 2009). Cytokines play a very important role in immunity and inflammation. The interactions between immune and inflammatory cells are mediated in large part by proteins, termed interleukins (IL) (Mizel, 1989). Leukocytes are an important component of the host defense system, responsible for protection against bacteria, fungi, viruses, and invading parasites**.** (Stock and Hoffman, 2000). These cells are classified into two main groups: Granulocytes and non-granulocytes. Granulocytes: include neutrophils, eosinophils and basophils, while non-granulocytes include lymphocyte and monocyte (Hedrich, 2012). Algae have been at the origin of only few studies focused on immunological applications. The biological properties of immunological interest have been demonstrated in 140 species of algae, and they have been found to have useful applications in human health, particularly in the fields of oncology and immunology (Courtois *et. al*., 2008). (Hégaret *et al*., 2011) refer that some harmful algae act as immunostimulants, whereas others are immunosuppressive by causing a suppression of immune functions, like the decrease in phagocytosis, and cause an increase in the percentage of dead haemocytes, which could be attributable to the action of chemical toxins .The aim of the current study was to examine the immunological effects of *A. variabilis* extract in three different doses.

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Materials and methods

Preparation of aqueous extracts of *A. variabilis*.

Algal extract was done by mixing 1 g dry powder with 20 ml distilled water and shaken for 24 h at 20°C in the dark. The mixture was separated by high speed centrifugation and the extraction procedure repeated

twice. The combined aqueous extracts were evaporated to dryness and the residue re-dissolved in distilled water. **(**H.M. Khairy and H.Y. El-Kassas 2010**).**

Animals of experiment

Albino male mice in age 8-10 weeks and weight 23-25g were divided into four groups; each group was kept in a separate plastic cage. Mice were maintained at a temperature of 23-25^{0}C, and they had free excess to food (standard pellets) and water. Mice were injected intra-peritoneal with crude aqueous extract in three doses (40, 1200 and 2400 mg/kg) and for the control group mice

Table 1: Immunological effect of aqueous extract of *Anabaena variabilis.*

Parameters Doses	Interleukin- 2	Interleukin -4	Interleukins -12	IFN-ϒ
Control	14.700±0.723b	22.067±1.271a	25.167±1.590c	303.000±3.512c
Aqueous extract / 40 mg/kg	17.267±1.680b	24.033±1.819a	27.167±1.590bc	321.000±4.359c
1200 mg/kg	34.200±2.921a	15.250±0.388b	46.717±2.038a	404.667±5.548a
2400 mg/kg	31.200±0.814a	15.000±1.501b	32.300±1.480b	360.000±12.124b
LSD P ≤ 0.05	5.774	4.414	5.505	23.579

Small letters indicate to comparison in columns , similar letters are non-significantly differences between means at ($p \leq 0.05$), Using (LSD test).

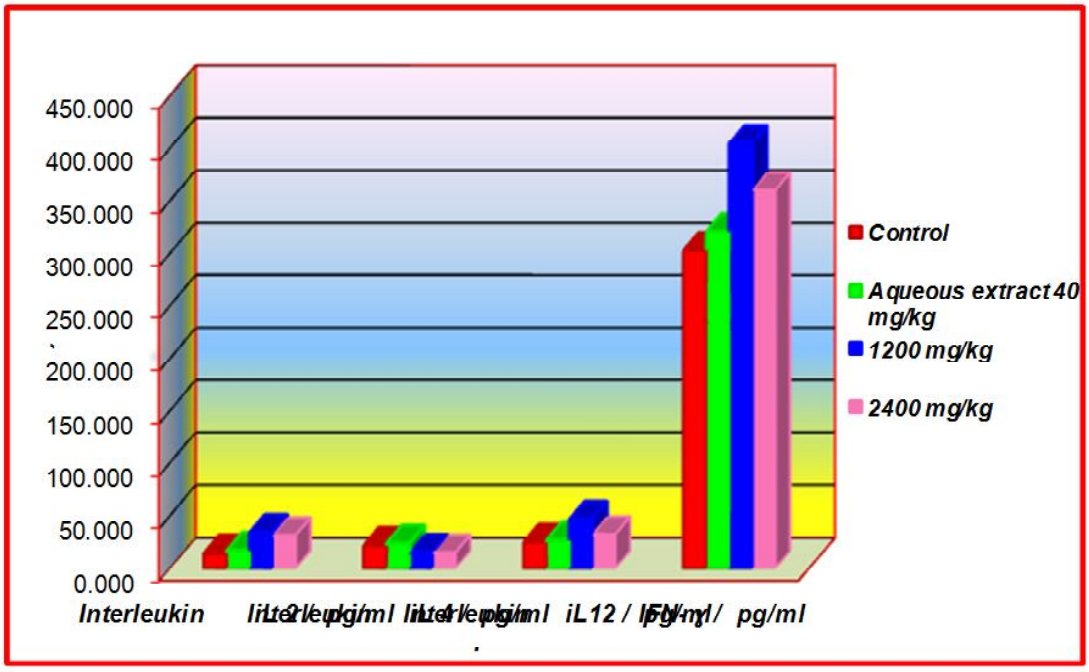

Fig. 1: Effect of different doses from *Anabaena variabilis* aqueous extract on the production of some cytokines in mice.

Table 2: Hematological and cytological effect of aqueous extract of *Anabaena variabilis.*

Parameters Doses	Phagocytic index	Mitotic index	Blast index	Total W.B.C.	Lymphocytes	Neutrophil
Control	82.730 ab	16.970 a	36.567 a	7.700 a	73.833 b	10.333 ab
	±	±	±	±	±	±
	0.869	2.344	3.045	1.387	2.092	1.453
Aqueous extract 40mg/kg	85.070 a	16.620 a	36.517 a	9.800 a	83.000 a	9.767 ab
	±	±	±	±	±	±
	1.396	2.334	2.021	2.836	1.609	0.491
1200 mg/kg	81.067 b	12.947 a	46.090 a	9.233 a	85.533 a	8.567 b
	±	±	±	±	±	±
	1.099	0.379	3.199	0.371	2.356	0.260
2400 mg/kg	81.243 b	12.303 a	41.103 a	13.400 a	67.033 c	11.733 a
	±	±	±	±	±	±
	0.380	1.166	4.297	3.355	1.819	0.837
LSD P ≤ 0.05	3.283	5.752	10.574	7.536	6.486	2.881

were injected with distilled water.

Determination of interleukins in mice blood samples

Blood samples from control groups and treated mice were taken by using insulin syringe (1 ml), they were put in eppendrof microfuge tube and left for 15 min. The samples were centrifuged in a micro centrifuge to get the serum. The samples of serum were kept in a deep freeze until to be analyzed for IL-2, IL-4, IL-12 and interferon gamma (IFN γ). The current study followed the instructions which illustrated in ABCAM'S, which is *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit for the quantitative measurement of IL-2 and IL-4 in mouse serum, as well as to the ADI's Mouse IFG ELISA for measuring Mouse IFG in serum.

Phagocytic Index (PI)

The procedure of (Salum, 2001) was followed to evaluate the *In vitro* phagocytosis which was carried out on blood samples obtained from treated mice with algal extracts.

Hematological and Cytological Parameters

Blood was collected from control and treated mice groups using insulin syringe, and stored in labeled EDTA tubes .The total and differential count of white blood cells for the collected blood samples were determined using an auto-hematology analyzer. Metaphase index was determined for cells obtained from bone marrow of treated mice with certain concentration of algal extract following the procedure of (Allen *et al.*, 1977).

Statistical analysis

The results of a different parameters for the treatments in a current study, were analyzed by using the analysis of variance (ANOVA), F-test and T-test, carried out in complete randomized design (CRD). Differences between means of treatments were analyzed using least significant differences (LSD) at ($P \leq 0.05$), and expressed as (mean ± Sd). Programming excel application and SPSS program (2010) was used to find the results and draw the figures with some effects to explain the statistical difference.

Results and Discussion

Table 1 shows that *A. varabilis* aqueous extract has the ability to increase the production of IL-2 and the significant differences appeared in the two doses (1200 and 2400 mg/kg). This study suggested that this extract could stimulate the immune response by stimulating T-helper zero (Th0). The results also revealed that the IL-4 which is a key cytokine that drives Th-2 immune responses decreased by increasing the dose of extract. On the other hand, the production of IL-12 increased and the significant differences appeared in the doses (1200 and 2400 mg/kg). This interleukin is a central cytokine in immune response which produced early during the response to infectious agents or to other antigens and induces production of IFN-γ first primarily by natural killer cells and then by T cells (Trinchieri, 1994). The result

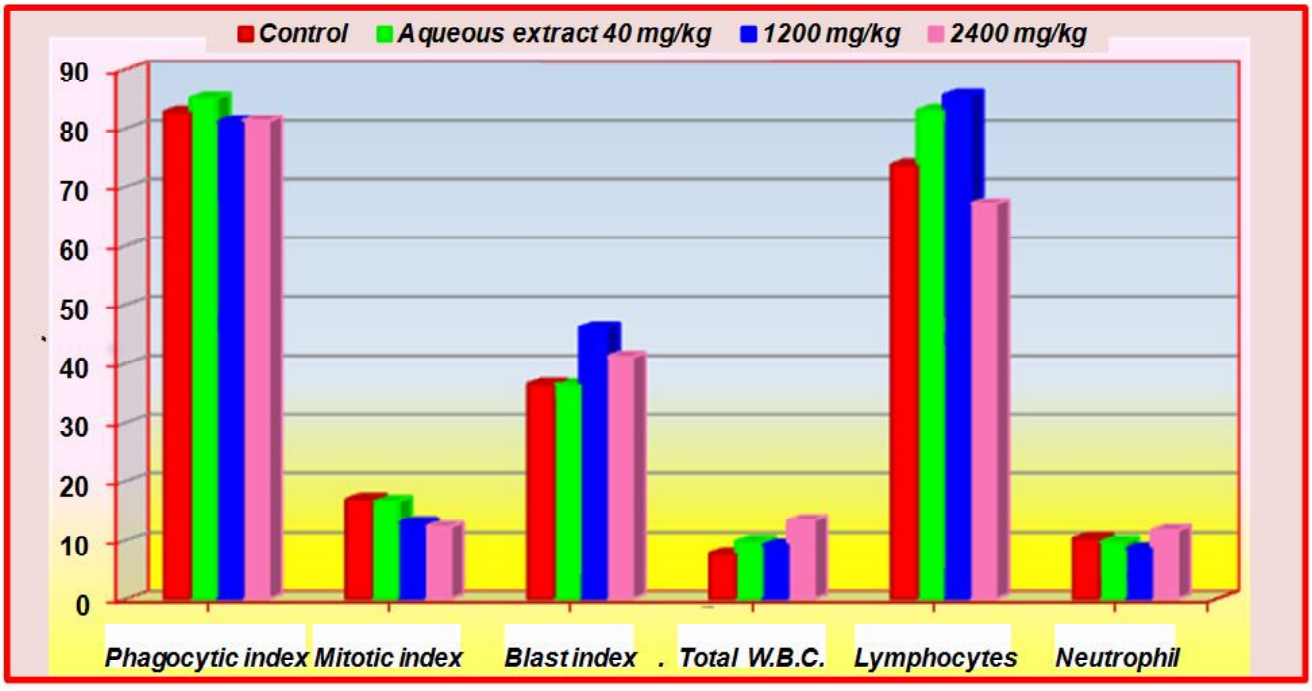

Fig. 1: Effect of different doses from *Anabaena variabilis* aqueous extract on some hematological and cytological parameters in mice.

also show that there is a significant differences in the production of IFN-γ for the doses (1200 and 2400 mg/kg) as compared with control. These results agree with the suggestion of (Gharb *et al*., 2016) which refer that the algal extract could stimulate the cellular immunity (Cytotoxic T-cell) and humeral immune response by increasing IFN-γ and reducing the production of IL-4. (Fig. 1).

Table 2 shows that significant differences was not appeared in all the studied parameters except in percentage of lymphocytes. On the other hand, the percentage of neutrophil shows significant differences between the last two doses and in phagocytic index the difference appeared in first dose (40 mg/kg). (Fig. 2).

Conclusion

In conclusion, *A. variabilis* aqueous extract with selected doses decrease IL4 and increase interferon-γ which is a (Th1) cytokine that promotes cell-mediated immunity and suppresses Th2 immune responses, this extract could be used as a therapeutic way for prevention of (Th2) cytokine-dominant disease. On the other hand, more investigation needs to study the histopathological effect of this extract.

Reference

Allen, J.M., C.V. Shuler, R.W. Mendes and S.A. Latt (1977). A simplified technique for *in vivo* analysis of sister chromatid exchanges 5-bomo-deoxy uridine tablets.*Cytogentics and cell Genetics,* **18:** 231-237.

Courtois, A., C. Simon-Colin, C. Boisset, C. Berthou, E. Deslandes, J. Guézennec and A. Bordron (2008). Floridoside Extracted from the Red Alga *Mastocarpus stellatus* Is a Potent Activator of the Classical Complement Pathway. *Mar. Drugs.,* **6:** 407-417.

Cynthiaricini, S., B.A. Sampath, M.R. Babu, K. Sujatha, R.S.J. Singh and B.D. Rao (2013). Beneficial Effect of Cyanobacteria Anabaena variabilis on Quantitative Traits of Eri Silkworm. *Asian Journal of Agricultural Sciences,* **5(3):** 36-39.

Gharb, L.A., A.L.M. Jawad and M.S. Shawkat (2016). Evaluation of Immunity enhancing activity of *Cladophora glomerata* crude extracts and phytol in male albino mice. *World J. Exp. Biosci.,* **4:** 118-122.

Hégaret, H., P.M. da Silva, G.H. Wikfors, H. Haberkorn, S.E. Shumway and P. Soudant (2011). In vitro interactions between several species of harmful algae and haemocytes of bivalve mollusks. Cell Biol Toxicol.

Khairy, H.M. and H.Y. El-Kassas (2010). Active substance from some blue green algal species used as antimicrobial agents. *African Journal of Biotechnology,* **9(19):** pp. 2789-2800.

Kim S.K. (2015). Handbook of marine microalgae: Biotechnology Advances. Academic Press. 604 pp.

Pant, G., G. Kumar, L. Karthik, R.G. Prasuna and K.V. B. Rao (2012). Effect of electric treatment on total phenolic content and antioxidant activity of *Anabaena variabilis*. *International Journal of Pharmacy and Pharmaceutical Sciences,* **4(4):**.

Salum, D.F. (2001). Comparative study of some immunological factors between typical and a typical pneumonia .MSC. Thesis. University of Baghdad. College of science.

Thiel, T. and B.S. Pratte (2014). Regulation of Three Nitrogenase Gene Clusters in the Cyanobacterium Anabaena variabilis ATCC 29413. *Academic Journal,* **4(4):** p944.

Trinchieri, G. (1994). Interleukin-12: A Cytokine Produced by Antigen-Presenting Cells With Immunoregulatory Functions in the Generation of T-Helper Cells Type 1 and Cytotoxic Lymphocytes. *Blood,* **84(12):** 4008-4027.