

## FUCOIDAN, AFA, HABERLEA RHODOPENSIS, PROPOLIS INDUCED CHANGES IN IMMUNOGLOBULINS LEVELS IN RATS

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### ABSTRACT

Immunoglobulins are part of the humoral immunity response against foreign antigens. Their blood levels could be influenced by different agents, including food supplements as Aphanizomenon flos-aquae (AFA), Propolis, substances as Fucoïdan and herbal extracts. **Purpose.** To evaluate whether Fucoïdan, AFA, a total leaf extract of *Haberlea rhodopensis* and Propolis augment the IgG and IgM levels into the blood stream of rats. **Methods.** Sixty male Wistar rats were divided into six groups (n=10) and treated as follows: 1<sup>st</sup> group – Aqua destillata p.o.; 2<sup>nd</sup> group – 0.9% NaCl i.p.; 3<sup>rd</sup> group – AFA in a dose 500 mg/kg bw p.o.; 4<sup>th</sup> group – Fucoïdan in a dose 100 mg/kg bw i.p.; 5<sup>th</sup> group – *Haberlea rhodopensis* in a dose 50 mg/kg bw i.p. and 6<sup>th</sup> group – Propolis in a dose 100 mg/kg bw i.p. Two hours after single administration of the substances blood samples were collected and analyzed. **Results.** AFA increased IgG level in peripheral blood of rats in comparison to controls. Fucoïdan, *H. rhodopensis* and Propolis induced nonsignificant decrease in serum IgG level. We registered a slight increase in serum IgM level after treatment with Fucoïdan, AFA and Propolis. *H. rhodopensis* has no influence on this type immunoglobulins in serum. **Conclusion.** Fucoïdan, AFA and Propolis may be used as possible immunomodulators and immuno-enhancers based on the registered elevated serum levels of immunoglobulins in rats. A single dose of *H. rhodopensis* leaf extract has negative to no effect on the immune response.

**Key words:** *Fucoïdan, AFA, Haberlea rhodopensis, IgG, IgM*

### Introduction

Immunoglobulins are part of the immune response against foreign antigens. These glycoproteins are produced by white blood cells and their main function is recognizing and binding of specific antigens (originated from viruses or bacteria). In human there are five isotypes of immunoglobulins (Ig) – IgA, IgD, IgE, IgG and IgM. B-lymphocytes, after first impact with an antigen, differentiate into plasma cells, which can produce immunoglobulins.

Most important role in the humoral immune response is played by IgG. Once synthesized by B-lymphocytes, it can remain in blood circulation for a long time. Elevated levels of this immunoglobulin may be used as marker for a previous infection.

IgM, in contrast to IgG, is the first immunoglobulin, released by B-cells after first contact with a specific antigen. The prime function of IgM is neutralizing this specific antigen. Its level in serum is elevating in the early phase of immune response. This isotype could be found in serum, as well on B-lymphocytes surface as antigen receptor.

Fucoïdan, sulfated polysaccharides, derived from brown seaweed, leads to elevated levels of circulating hematopoietic progenitor stem cells (HPSC) and white blood cells (WBC) after intravenous application in mice [11]. Antibacterial and antiviral activity of this substance is reported by Bilan MI and Zvyagintseva TN [1, 16]. Clinical trial, including ingestion of Fucoïdan, registered

a slight decrease in the lymphocytes and leucocytes after 12-day treatment. Nevertheless, these authors found a small increase in the CD34+ cells [6].

StemEnhance is a food supplement based on *Aphanizomenon flos-aquae* (AFA), a specific cyanobacteria isolated from Klamath lake, USA. Oral administration of the supplement is associated with rapid mobilization of HPSC in peripheral blood of humans [7].

*Haberlea rhodopensis* is a resurrection plant, endemic to Europe's Balkan area [5]. DelAqua et al. [2] reported antioxidant properties, increased skin elasticity after treatment with leaf extract of *H. rhodopensis*.

Propolis is a substance, produced by honeybees from plants and its chemical composition differ based on the geographic region. Propolis has been found to exert antimicrobial [15], immune enhancing [8, 4], wound healing and anti-inflammatory effect [3, 14]. There is evidence that water-soluble derivatives of propolis stimulates the hematopoiesis in mice and may be used for treatment of cytopenias [9].

### **Materials and methods**

#### *Animals*

Sixty male Wistar rats (body weight 180 – 200g) were divided into six groups (n = 10). They were treated as follows: 1<sup>st</sup> group (control I) – Aqua destillata in presence of 5% glycerin, p.o; 2<sup>nd</sup> group (control II) – 0.9% NaCl, i.p.; 3<sup>rd</sup> group – AFA (500 mg/kg bw), p.o.; 4<sup>th</sup> group – Fucoidan (100 mg/kg bw), i.p.; 5<sup>th</sup> group – *Haberlea rhodopensis* (50 mg/kg bw), i.p. and 6<sup>th</sup> group – Propolis (100 mg/kg bw), i.p.

Rats were kept under standard laboratory conditions (temperature 22 ±1°C, humidity 45% and 12-h light cycle). The rodents received food and water ad libitum.

#### *Drugs*

Suspension of AFA (StemEnhance, STEM Tech Health Inc., Klamath Falls, OR) in distilled water, containing 5% glycerin was prepared 30 minutes before oral administration.

Fucoidan from *Fucus vesiculosus* (Sigma - Aldrich Co; St Louis, MO) was dissolved in 0.9% NaCl.

Total leaf extract of *H. rhodopensis* was dissolved in 0.9% NaCl before the intraperitoneal injection.

Propolin<sup>®</sup> (Psych-LP, Bulgaria), a 16% ethanol Propolis extract from the Rhodopi mountains (Bulgaria), was diluted with 0.9% NaCl for intraperitoneal administration.

#### *Experimental procedures*

Two hours after the first administration of the tested substances, blood samples were collected from the tail vein of the animals. Serum was collected in tubes without anticoagulant. The rats were sacrificed and the samples were coded and send for analysis immediately.

Ig G и Ig M were measured by clinical chemistry analyzer (Konelab 60 i).

#### *Statistical analysis*

All results are shown as percentage ± SEM. The mean of the tested parameter in the control group was taken for 100%. The parameters of each group were compared to these of the control group with the same application route.

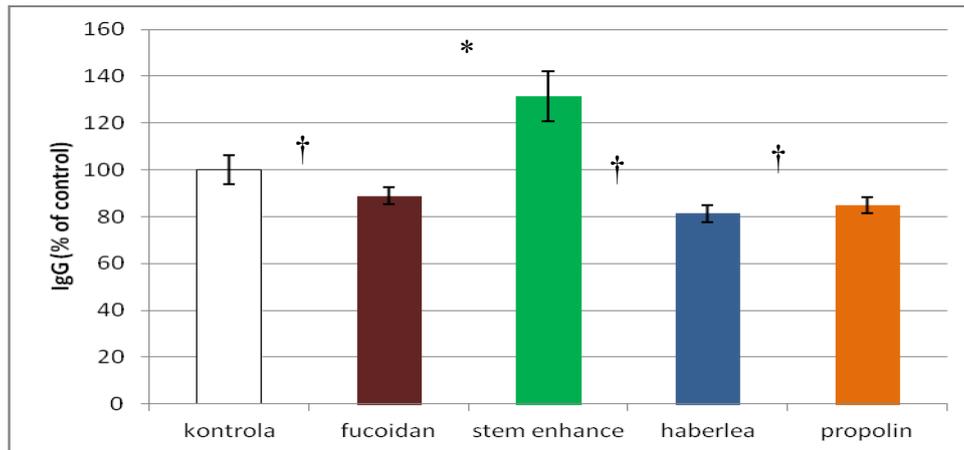
Data were analyzed using SPSS 19.0. One sample Kolmogorov-Smirnov test was performed to study the normal distribution. One way ANOVA with Tuckey post hoc test was used in case of normal distribution; non-parametric Wilcoxon signed rank test and Mann Whitney test were conducted in the other case.

Results were considered significant at p<0.05.

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All experiments were approved by the Animal Health and Welfare Directorate at Bulgarian Food Safety Agency with permit No 86/09.01.2014 and and Medical University – Plovdiv Research Ethics Committee (meeting №3/26.06.2014).

**Results and discussion**

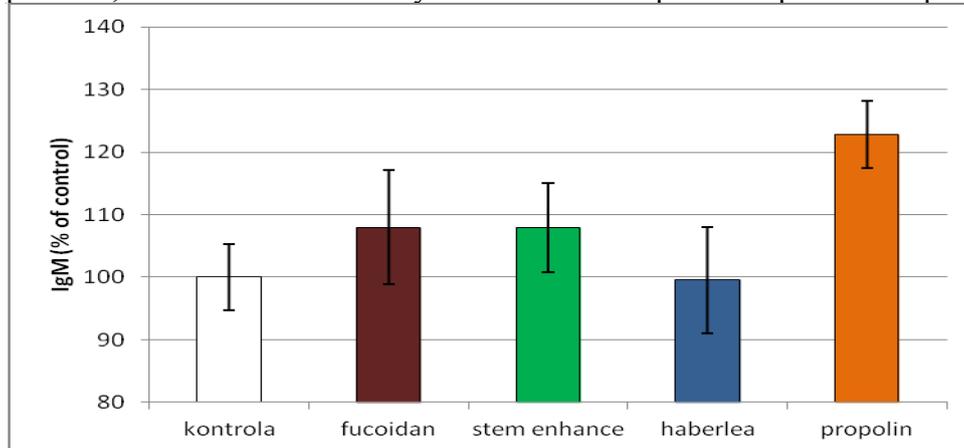


**Figure 1.** Effect of Fucoidan, AFA, *H. rhodopensis* and Propolis on IgG level in peripheral blood of rats. \* -  $p < 0.05$  compared with controls; † -  $p < 0.05$  - compared with AFA.

AFA significantly increased IgG levels in rats ( $131.48\% \pm 10.74$ ) compared to controls ( $p < 0.05$ ). We observed a slight increase in WBC and lymphocytes after single ingestion of this food supplement in rats but without significant difference to control rats (unpublished data). The increased levels of IgG in this case may be related to increased levels of B-lymphocytes.

Fucoidan ( $88.85\% \pm 3.60$ ), *H. rhodopensis* ( $81.36\% \pm 3.66$ ) and Propolis ( $84.94\% \pm 3.51$ ) induced significant decrease of this parameter in comparison to rats, treated with AFA ( $131.48\% \pm 10.74$ ). IgG levels in rats, injected with Fucoidan, *H. rhodopensis* and Propolis were lower than corresponding levels in control rats, but the difference was nonsignificant.

Decreased IgG levels after treatment with *H. rhodopensis* may be due to lower WBC and lymphocyte count in peripheral blood (unpublished data). In contrast to our data, Popov B. [10] reported significant increase in IgG levels in rabbits after long term treatment with *H. rhodopensis* extract. It is possible, the immunostimulatory effect of the compound requires multiple application.



**Figure 2.** Effect of Fucoidan, AFA, *H. rhodopensis* and Propolis on IgM level in peripheral blood of rats.

We found no significant change in the levels of IgM in serum of rats, treated with vehicle and rats, treated with tested compounds. A slight increase was registered after treatment with AFA ( $107.93\% \pm 7.19$ ) and Fucoidan ( $107.99\% \pm 9.06$ ).

AFA increased the plasma levels of IgG and IgM as a result of elevated levels of WBC and lymphocytes. Based on this result we can conclude AFA augments the differentiation of WBC, activating their differentiation into B-lymphocytes.

Increased levels of circulating WBC after treatment with Fucoidan are reported by Sweeney [11]. Elevated levels of WBC provide an explanation of registered increase in IgM levels. WBC,

more exactly B-lymphocytes, produce IgM after differentiation in plasmatic cells. Other authors [13] also reported increase in serum immunoglobulin levels after treatment with Fucoidan.

IgM levels after treatment with *H. rhodopensis* (99.54%±8.50) were similar to control rats.

After injection of Propolis (122.83%±5.40) a well defined tendency of increase was observed, but didn't reached the statistical significance threshold ( $p>0.05$ ). Regarding IgG and IgM levels, Takagi Y et al. [12] reported similar results. They found significant increase in IgM and significant decrease in IgG in comparison to controls. IgM could be released by circulating B-lymphocytes or by B-lymphocytes, residing in secondary lymphoid organs and bone marrow. As a result, Propolis may not influence the circulating B-lymphocytes, but it may still lead to stimulated differentiation of WBC into B-lymphocytes, providing an explanation of registered higher IgM levels in peripheral blood. The immuno-enhancing effect of Propolis, reported by other authors [8] may be due to the higher IgM levels in circulating blood.

### Conclusion

Elevated level of immunoglobulins in peripheral blood after treatment of rats with Fucoidan, AFA and Propolis reveal the potential of these substances as possible immunomodulators and immuno-enhancers. A single dose of *H. rhodopensis* leaf extract has negative to no effect on the immune response.

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