ORIGINAL ARTICLE

EFFECT OF FUCOIDAN, *HABERLEA RHODOPENSIS* AND PROPOLIS ON MOBILIZATION OF THE CD34+ STEM CELLS IN RATS

ELISAVETA G. APOSTOLOVA[1]*, VESELA KOKOVA[1], ZHIVKO PEYCHEV[2], STELA PEYCHEVA[3], APOSTOL APOSTOLOV[4]

[1]*Department of Pharmacology and Drug Toxicology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria*
[2]*Department of Medical Informatics, Biostatistics and Interactive Education, Faculty of Public Health, Medical University, Plovdiv, Bulgaria*
[3]*Department of Paediatric Dentistry, Faculty of Dental Medicine, Medical University, Plovdiv, Bulgaria*
[4]*Department of Anaesthesiology and Intensive Care, "St. George" University Hospital, Plovdiv, Bulgaria*

**corresponding author: apostolova1212@gmail.com*

Manuscript received: February 2016

Abstract

We evaluated the influence of fucoidan, *Haberlea rhodopensis* (*H. rhodopensis*) and propolis on the hematopoietic stem cells (CD34+) levels into the blood stream of rats. Four groups of male Wistar rats (n = 10) were treated intraperitoneally as follows: 1st group – 0.9% NaCl; 2nd group – fucoidan (100 mg/kg b.w.); 3rd group – *H. rhodopensis* (50 mg/kg b.w.) and 4th group – propolis (100 mg/kg b.w.). Two hours after a single administration of the substances, blood samples were collected and analysed. We found a significant increase in the number of CD34+ cells in the blood stream after the administration of the tested substances. Fucoidan also caused a significant increase in the number of leukocytes compared to the control group. These results show that the tested substances could mobilize stem cells in the peripheral blood of rats. Fucoidan may increase the count of circulating white blood cells (WBC), *H. rhodopensis* decreases this value, while propolis does not influence leukocytes level in peripheral blood.

Rezumat

A fost evaluată influența fucoidanului, *Haberlea rhodopensis (H. rhodopensis*) și a propolisului asupra nivelelor sangvine de celule stem hematopoietice (CD34+) la șobolani. Patru grupuri de șobolani masculi Wistar (n = 10) au fost tratați intraperitoneal după cum urmează: grupul I (control) – ser fiziologic; grupul II – fucoidan (100 mg/kg corp); grupul III - *H. rhodopensis* (50 mg/kg corp) și grupul IV - propolis (100 mg/kg corp). Animalele au fost supuse unei singure administrări a substanțelor, iar la 2 ore după aceasta, probele de sânge au fost colectate și analizate. S-a înregistrat o creștere semnificativă a numărului de celule CD34+ din sânge, după administrarea substanțelor testate. Fucoidanul a determinat, de asemenea, o creștere semnificativă a numărului de leucocite, comparativ cu grupul control. Aceste rezultate arată că substanțele testate ar putea mobiliza celule stem din sângele periferic al șobolanilor. Fucoidanul poate crește numărul de leucocite circulante, *H. rhodopensis* scade acest număr, în timp ce propolisul nu influențează nivelul leucocitelor din sângele periferic.

Keywords: CD34+ cells, fucoidan, *Haberlea rhodopensis*, propolis

Introduction

CD34+ cells are hematopoietic stem and progenitor cells (HPSC), derived from the bone marrow (BM). HPSC are capable of trafficking between BM and blood compartments. They also can differentiate into hematopoietic lineages including leukocytes (white blood cells, WBC), erythrocytes (red blood cells, RBC) and platelets (PLT), maintaining the normal haematopoiesis. The progenitor cells reside in specific niches in BM and their count in the peripheral blood is usually low [7, 11, 12].

Recently, the mechanisms of mobilization are studied intensively. Granulocyte colony-stimulating factor acts as a direct activator of granulocytes and leads to their expansion. One of the disadvantages of the therapy with this cytokine is the variations in the number of HPSC, mobilized by the patients. Moreover, some of them could not mobilize the amount of stem cells, required for autologous transplantation [17].

Over the past few years, the interest in natural substances which can mobilize HPSC in peripheral blood is increasing. Latest studies are focused on fucoidan, a composition of sulfated polysaccharides, derived from brown algae species [5]. This natural product not only shows important pharmacological activities (e.g. antioxidant, antitumor effect) but also induces rapid stem cells mobilization [3, 5].

H. rhodopensis, as a resurrection plant, was subjected to extensive research recently [14, 16]. Extracts of the plant are found to show antioxidant properties, increase skin elasticity and can be used as anti-aging cosmetic products. They also showed antibacterial and cytotoxic activities against human cancer cells [16].

Propolis, a substance produced by honeybees from plants has been found to exert antimicrobial, cytostatic and wound healing effect [2, 9]. Orsolic *et al.* reported that water-soluble derivatives of propolis can be used for the treatment of cytopenia due to their stimulating effect on the haematopoiesis in mice [13]. These authors studied the influence of propolis on the formation of different types of spleen colonies. However, the influence of *H. rhodopensis* extracts and propolis on the CD34 positive cells and mature blood cells remained unclear.

The aim of this study was to evaluate the effect of fucoidan, *H. rhodopensis* and propolis on the number of circulating CD34+ cells in peripheral blood of rats. We performed complete blood count (CBC) in order to determine the eventual influence on blood cell maturation.

Materials and Methods

Animals

The experiment protocol was approved by the Animal Health and Welfare Directorate at Bulgarian Food Safety Agency (permit No 86/09.01.2014) and Medical University – Plovdiv Research Ethics Committee (№3/26.06.2014).

Forty male Wistar rats (weight between 180 - 200 g) were divided into four groups (n = 10). The animals received intraperitoneal injection with the following solutions: 1st group – 0.9% NaCl; 2nd group – fucoidan (100 mg/kg b.w.); 3rd group – *H. rhodopensis* (50 mg/kg b.w.) and 4th group – propolis (100 mg/kg b.w.).

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*.

Reagents

Fucoidan from *Fucus vesiculosus* (Sigma-Aldrich Co, St Louis, MO) was purchased and stored according to the instructions.

The total leaf extract of *H. rhodopensis* was prepared as follows: 48 hour maceration in 70% ethanol, followed by percolation. The obtained liquid extract was concentrated in a *vacuum* rotation evaporator (BUCHI, Rotavapor R II). The herb-to-extract ratio at the end of the procedure was 3:1.

Propolin® (Peych-LP, Bulgaria) (16% ethanolic propolis extract from the *Rhodopi Mountains*, Bulgaria) was purchased one day before its application.

Fucoidan, propolis, and *H. rhodopensis* extract were dissolved or diluted in 0.9% NaCl for intraperitoneal administration (0.1 mL/kg b.w.) on the day of the experiment.

Experimental

Two hours after single dose administration of the tested substances, venous blood samples were collected in tubes containing EDTA from the tail vein of the animals using a previously described method [1]. The samples were coded and send for analysis immediately. CD34+ cells level was measured by flow-cytometry (FC500-BECKMAN Coulter). CBC was performed using haematology analyser Advia 2120 (Siemens Diagnostica).

Statistical analysis

All results are shown as percentage ± SEM. The mean value of each parameter in the control groups was taken as 100% and values in the experimental groups were calculated and compared.

The statistical package SPSS 19.0 was employed for the evaluation. The normal distribution was tested with one sample Kolmogorov-Smirnov test. In a case of normal distribution, one way ANOVA with Tuckey *post hoc* test was used; non-parametric Wilcoxon signed rank test and Mann Whitney test were conducted in the other case. Results were considered significant at $p < 0.05$.

Results and Discussion

According to Mikirova *et al.* [10] cells associated with hematopoietic activity express CD34 biomarker. Elevated CD34+ levels in peripheral blood may be related to elevated levels of HPSCs in blood stream.

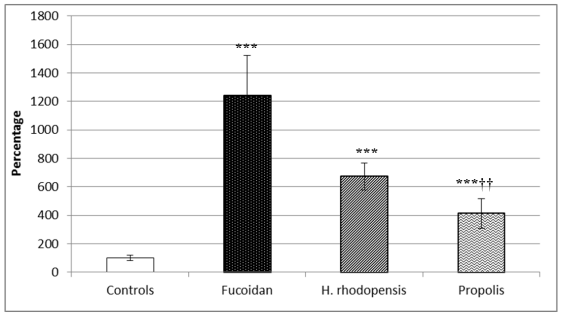

Figure 1.
Effect of fucoidan, *H. rhodopensis* and propolis on CD34+ level in peripheral blood of rats
(*** $p < 0.0005$ *versus* controls; †† $p < 0.005$ – compared to fucoidan)

Significant increase of circulating cells expressing CD34 biomarker was observed 2 hours after treatment with fucoidan (1243.75% ± 281.39), *H. rhodopensis* (673.61% ± 104.36) and propolis (413.19% ± 46.21) compared to controls, as shown in Figure 1.

Fucoidan has well defined effect on HPSC. Elevated levels of circulating HPSCs are registered after intravenous administration [15]. These authors reported an increase in the colony-forming cells and WBC. The cells levels reported by them was 10.9 fold on the 3rd hour after the treatment, compared to 12.43 fold registered by us on the second hour, in rats. In another study based on oral treatment with fucoidan no significant difference was found in the haematological parameters when compared to the

control group [8]. Irhimeh *et al.* reported a slight decrease in the lymphocytes and leucocytes after 12-day oral treatment with fucoidan (a clinical trial). Nevertheless, these authors found a small increase in the CD34+ cells [6]. Regarding the effect of propolis, it is possible that the increased levels of CD34+ cells are related to pinocembrin. Treatment of mice with pinocembrin (a flavonoid found in propolis) leads to elevated CD34+ cells levels in peripheral blood [18].

Circulating WBC count reached levels of 145.02% ± 17.60 after treatment with fucoidan as presented in Figure 2 left. We registered a significant decrease in WBCs levels in rats treated with *H. rhodopensis* (78.66% ± 7.80) when compared to the group treated with fucoidan (145.02% ± 17.60). No significant difference in RBC count was evaluated in the tested groups when compared to controls, as shown in Figure 2 right. A tendency of increase was observed in rats treated with fucoidan. *H. rhodopensis* (92.29% ± 7.78) and propolis (92.83% ± 8.18) groups revealed lower erythrocytes levels in comparison to rats treated with fucoidan (102.09% ± 17.60; $p < 0.05$).

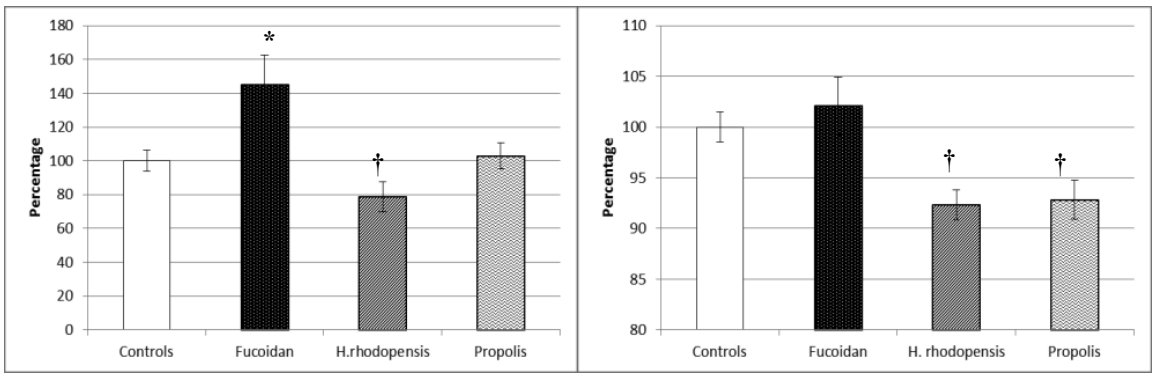

Figure 2.
Effect of fucoidan, *H. rhodopensis* and propolis on WBC (left) and RBC (right) levels in peripheral blood of rats (* $p < 0.05$ *versus* controls; † $p < 0.05$ *versus* fucoidan)

Our findings are consistent with Sweeney *et al.* [15] who reported an increase in the WBC levels after treatment with fucoidan. The elevated count may be due to the mobilization of leucocytes into the peripheral blood from the bone marrow. Another possibility is the differentiation of the mobilized CD34+ cells into leucocytes.

The elevated levels of WBCs in the peripheral blood may be related to the antibacterial and antiviral properties of fucoidan, as mentioned by Coneac *et al.* [2].

Based on the slight decrease of WBC and RBC and the increase of PLT after treatment with *H. rhodopensis*, we can conclude that the total plant extract leads the differentiation of CD34+ cells to platelets. Our results are consistent with Georgieva *et al.* [4] who also reported lack of positive effect on immune function in rabbits.

We found no significant difference in the number of circulating leucocytes in contrast to Orsolic *et al.* [12] who registered an increase after treating mice with a water soluble derivative of propolis.

The percent of circulating platelets showed no significant difference after treatment with the tested substances when compared to controls taken as 100% (data not shown). A tendency of increase was registered in the group treated with *H. rhodopensis*. Eventhough the platelets play a major role in the thrombosis, the process of thrombocyte aggregation is more complex and can be influenced by many factors (e.g. the presence of cations) [13]. Hence, further studies may enlighten the role of the herbal extract on the blood coagulation.

Conclusions

Fucoidan, *H. rhodopensis* and propolis elevated the CD34+ cells levels in the peripheral blood of rats. Fucoidan may increase the count of circulating WBC; *H. rhodopensis* decreases this value, while propolis does not influence leukocytes level in peripheral blood.

In addition, these results provided the first evidence that *H. rhodopensis* and Bulgarian propolis acts directly on CD34+ cells, augmenting their mobilization in the peripheral blood.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgement

The expert assistance of Tanya Deneva, Pavel Pavlov, Dimitar Penkov and Radoslav Radev in CBC and CD34+ cells analysis and *H. rhodopensis* extract preparation is gratefully acknowledged. This work was partially supported by a Project No 14/2013 (Medical University – Plovdiv).

References

1. Apostolova E., Deneva T., Peychev L., Pavlov P., Peychev Z., Kokova V., Fucoidan, AFA, *Haberlea rhodopensis*, propolis induced changes in imuno globulins levels in rats. *Science & Technologies*, 2015; 5(1): 18-22.
2. Daliborca C.V., Popescu R., Dumitrascu V., Cimporescu A., Vlad C.S., Vágvölgyi C., Krisch J., Dehelean C., Horhat F.G., Phytocomponents identification in mistletoe (*Viscum album*) young leaves and branches, by GC-MS and antiproliferative effect on HEPG2 and MCF7 cell lines. *Farmacia*, 2016; 64(1): 82-87.
3. Fitton J.H., Stringer D.N., Karpiniec S.S., Therapies from Fucoidan: An Update. *Marine Drugs*, 2015; 13(9): 5920-5946.
4. Georgieva S., Sotirov L., Popov B., Koynarski T., Impact of the *Haberlea rhodopensis* extract on the innate immune system and response in rabbits following KLH-hemocyanin immunization and cyclophosphamide treatment. *Turk. J. Vet. Anim. Sci.*, 2013; 37: 659-663.
5. Han Y.S., Lee J.H., Jung J.S., Noh H., Baek M.J., Ryu J.M., Yoon Y.M., Han H.J., Lee S.H., Fucoidan protects mesenchymal stem cells against oxidative stress and enhances vascular regeneration in a murine hindlimb ischemia model. *International Journal of Cardiology*, 2015; 198:187-195.
6. Irhimeh M.R., Fitton J.H., Lowenthal R.M., Fucoidan ingestion increases the expression of CXCR4 on human CD34+ cells. *Exp. Hem.*, 2007; 35(6): 989-994.
7. Katayama Y., Battista M., Kao W.M., Hidalgo A., Peired A.J., Thomas S.A., Frenette P.S., Signals from the Sympathetic Nervous System Regulate Hematopoietic Stem Cell Egress from Bone Marrow. *Cell*, 2006; 124(2): 407-421.
8. Li N., Zhang Q., Song J., Toxicological evaluation of fucoidan extracted from *Laminaria japonica* in Wistar rats. *Food Chem. Tox.*, 2005; 43(3): 421-426.
9. Martin L.F., Rocha E.M., Garcia S.B., Paula J.S., Topical Brazilian propolis improves corneal wound healing and inflammation in rats following alkali burns. *BMC Complementary and Alternative Medicine*, 2013, 13: 1-7.
10. Mikirova N.A., Jackson J.A., Hunninghake R., Kenyon J., Chan K.W., Swindlehurst C.A, Minev B., Patel A.N., Murphy M.P., Smith L., Ramos F., Ichim T.E., Riordan N.H., Nutraceutical augmentation of circulating endothelial progenitor cells and hematopoietic stem cells in human subjects. *J. Trans. Med.*, 2010; 8(34): 1-10.
11. Mahmoud Hashemzaei, Saeed Pudine Karami, Amin Delaramifar, Alireza Sheidary, Kaveh Tabrizian, Ramin Rezaee, Shabnam Shahsavand, Andreea Letiția Arsene, Aristidis Michael Tsatsakis, Seyed Mohammad Taghdisi, Anticancer effects of co-administration of daunorubicin and resveratrol in MOLT-4, U266 B1 and RAJI cell lines. *Farmacia*, 2016; 64(1): 36-42.
12. Ogawa M., Differentiation and proliferation of hematopoietic stem cells. *Blood*, 1993; 81(11): 2844-2853.
13. Oršolić N., Tadić Z., Benković V., Horvat A., Lisičić D., Bašić I., Stimulation of hematopoiesis by a water–soluble derivative of propolis in mice. *Pharmacologyonline*, 2006; 3: 698-705.
14. Solti Á., Mihailova G., Sárvári É., Georgieva K., Antioxidative defence mechanisms contributes to desiccation tolerance in *Haberlea rhodopensis* population naturally exposed to high irradiation. *Acta Biologica Szegediensis*, 2014; 58(1): 11-14.
15. Sweeney E.A., Priestley G.V., Nakamoto B., Collins R.G., Beaudet A.L., Papayannopoulou T., Mobilization of stem/progenitor cells by sulfated polysaccharides does not require selectin presence. *Proceed. Nat. Acad. Sci. USA*, 2000; 97(12): 6544-6549.
16. Todorova R., Atanasov A.T., *Haberlea rhodopensis*: pharmaceutical and medical potential as a food additive. Nat. Prod. Res., 2016; 30(5): 507-29.
17. Winkler I.G., Lévesque J.P., Mechanisms of hematopoietic stem cell mobilization: When innate immunity assails the cells that make blood and bone. *Exp. Hem.*, 2006; 34(8): 996-1009.
18. Yang N., Qin S., Wang M., Chen B., Yuan N., Fang Y., Yao S., Jiao P., Yu Y., Zhang Y., and Wang, J. (2013). Pinocembrin, a major flavonoid in propolis, improves the biological functions of EPCs derived from rat bone marrow through the PI3K-eNOS-NO signaling pathway. *Cytotechnology*, 2013; 65(4): 541-551.