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Lycopene and Phycocyanin - biological properties in experimental diabetes: 1. Effects on blood parameters and liver carbohydrates

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SUMMARY. Type 2 diabetes is one of the fastest rising metabolic diseases of our time, mainly due to an unhealthy lifestyle, diet and lack of exercise. While medical treatment does indeed exist and the quality of life is improved under medication, one must take into account that the predominant geographical areas in which diabetes is on the rise are underdeveloped and as such, access to modern medicine may be hindered. The purpose of our study was to confirm the hypoglycemic and antioxidant effects of two biomolecules in the pathology of diabetes. For this study we used 40 albino male Wistar rats, divided into four groups. The control group (C) received a normal diet and tap water. The untreated diabetic group (D) was intravenously injected with a dose of 50mg/kg streptozotocin after a 12 hour fasting period and given a normal diet and water. Diabetes was induced in the same way for the two treated groups, with their diets being supplemented with 10 mg/kg lycopene (DL group) and an equivalent of 200 mg/kg phycocyanin in the form of *Arthrospira* powder (DS group). Blood was drawn every 7 days do determine glycemic status and after 14 days the animals were killed under anesthesia, with blood and liver being collected for morphological and biochemical analysis. Blood glucose significantly dropped in the DL group on the 7th day of treatment, with both treatments reducing fasting blood sugar to normal levels on the 14th day. Hepatic glucose was normalized in both DL and DS groups without any significant change in glycogen concentrations. RBC counts revealed a tendency of the erythrocytes to increase in numbers in the treated groups and lycopene appears to restore WBC numbers to normal levels. Taking into account these results, it can be said that both biomolecules have a potent hypoglycemic effect in diabetic hyperglycemia, while also improving carbohydrate metabolism in the liver. Lycopene seems to be the more effective antioxidant of the two, preventing oxidative-induced hemolysis of the red blood cells and restoring normal PCV and hemoglobin levels.

Keywords: *Arthrospira* powder, diabetes, lycopene, oxidative stress

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Introduction

Diabetes is probably the most common metabolic disease of our time, with the number of patients diagnosed having skyrocketed to 422 million in 2014 from 108 million in 1980 (World Health Organization, 2016). Statistics like these, and the fact that the most significant rise in the number of diagnosed patients occurs in underdeveloped areas of the world, motivate the search for cheap and effective diet supplements that can act as adjuvants in diabetes therapy.

Lycopene is a carotenoid that naturally occurs in red fruit and vegetables, where its main role is that of photoprotection. Recently, lycopene has been investigated for its antioxidant effect in cardiovascular disease (Arab and Steck, 2000) and especially cancer (Basu and Imrhan, 2007; Luo and Wu, 2011). As a therapeutic agent against diabetes, lycopene has proven to lower blood sugar levels (Bayramoglu *et al.*, 2013; Guo *et al.*, 2015) as well as stimulate and complement the antioxidant systems (Ali and Agha, 2009).

Phycocyanin is the main proteic compound in cyanobacteria such as *Arthrospira platensis* contributing to approximately 15-40% of their dried biomass. Structurally, it resembles bilirubin thus hinting towards its antioxidant capacity, especially considering protein and lipid oxidation. As an adjuvant therapeutic agent in diabetes, it was tested amongst others by Zhou *et al.* in 2005, who noticed a decrease in blood sugar concentrations and improved oxidative status.

Testing both compounds and observing which parameters they do or do not alter, one can draw conclusions as to how and at what level they function, considering their complex metabolism is not yet fully understood. Another possible conclusion arising from such studies could lead to complex supplement formulations that contain different biomolecules capable of complementing each other.

This study is part of a systemic study that also took into account the therapeutic effects of lycopene and phycocyanin on the liver, brain, kidney and pancreas of diabetic rats.

Materials and methods

All reagents used in this study were of analytical grade and were purchased from Sigma-Aldrich Chemie GmbH, Germany, Nordic Invest S.R.L., Romania and S.C. BioZyme S.R.L, Romania. Lycopene was from König Laboratorium, Canada, and *Arthrospira* poweder was from Adams Vision, Romania.

The experimental model consisted of 40 adult male Wistar rats distributed into four groups as follows: a control group (C, n=10), an untreated diabetic group (D, n=10), a diabetic group treated with lycopene 10 mg/kg (DL, n=10) and a diabetic group treated with *Arthrospira* powder 200 mg/kg (DS, n=10). Three days before

the treatment, diabetes was induced by a single dose of intravenously administered streptozotocin (50 mg/kg dissolved in ice cold 10 mM citrate buffer) in 3 of the groups (D, DL and DS). All the animals had *ad libitum* access to tap water and were fed a standard diet (S.C. Siamond Prod. S.R.L., Cluj Napoca, Romania) according to their weight, with the DL and DS groups having their diets supplemented with the aforementioned doses of lycopene and *Arthrospira* powder (AP) respectively.

The animals were sacrificed by exsanguination under anesthesia 14 days after the confirming their diabetic status. For the purpose of this experiment, blood samples were harvested by retro orbital bleeding at the start of the treatment and seven days into the treatment as well as blood and liver samples on the 14^{th} day for the following biochemical and morphological analysis: RBC, WBC, PCV, hemoglobin, blood glucose and liver glucose and glycogen.

Blood and liver glucose concentrations were measured using the Somogy-Nelson method (Nelson, 1944). Glycogen concentration was determined by the Montgomery method (1957) modified by Lo *et al.* (1970). Hemoglobin concentration was measured using the Drabkin assay (1935). RBC and WBC were counted in a Thoma counting chamber and a Burker-Turk counting chamber respectively. PCV was measured using centrifuged glass capillaries filled with blood. Eosin – hematoxylin staining was used on fresh blood smears to determine the differential WBC count.

Results were analyzed using the two tailed *t* test and considered statistically significant at $p \leq 0.05$.

Results and discussion

This study was conducted in an effort to establish the therapeutic effect that natural supplements have in the case of experimental diabetes in adult male rats. Both compounds were investigated for their capacity to reduce blood sugar and to prevent oxidative stress.

Blood glucose was measured three times during the experiment to establish the way in which the two supplements affect diabetes-induced hyperglycemia. The results (Table 1, Fig. 1) confirm literature data concerning the hypoglycemic effect lycopene has (Bayramoglu *et al.*, 2013; Ip *et al.*, 2013; Guo *et al.*, 2015), shedding light on how fast it acts to reduce blood glucose. Compared to AP, lycopene acts faster, reducing the blood sugar levels below the diabetes diagnostic threshold. However, even though AP acts slower, it proves to be a more potent hypoglycemic agent with similar results being obtained by Layam and Reddy, 2006. These results correlate with measurements obtained from the tissue, where glucose concentration in the DL and DS groups dropped to normal levels (Table 1, Fig. 2a).

Considering these two supplements have complex metabolic pathways that are in no way similar to each other, a plausible explanation to their action is that they can, at least partially, restore normal function in the pancreas and thus insulin signaling.

Table 1.

Effects of lycopene and *Arthrospira* powder on blood sugar, hepatic tissue glucose and glycogen concentrations in diabetic Wistar rats

Parameter	Control	D	DL	DS
Blood glucose concentration day 0 (mg/dL)	123.72 ± 10.4	484.19 ± 37.2 ***	432.57 ± 40.42 ***	395.23 ± 23.93 ***
Blood glucose concentration day 7 (mg/dL)	123.72 ± 10.4	346.63 ± 17.87 ***	135.97 ± 29.48 ***	280.92 ± 20.42 *
Blood glucose concentration day 14 (mg/dL)	117.27 ± 9.01	210.39 ± 41.81	70.54 ± 8.42 ** *	59.07 ± 8.17 *** **
Hepatic tissue glucose concentration (mg/g tissue)	1.14 ± 0.28	9.79 ± 2.73 **	2.84 ± 0.44 * *	2.15 ± 0.73 *
Hepatic glycogen concentration (mg/g tissue)	0.49 ± 0.05	0.59 ± 0.08	0.51 ± 0.10	0.56 ± 0.13

D – untreated diabetic; DL – diabetic treated with lycopene; DS – diabetic treated with *Arthrospira* (Spirulina) powder containing phycocyanin; Results are expressed as mean ± SE. Comparisons made: black – *vs* Control group; red – vs D group; ***** - p<0.05; ****** - p<0.01; ******* - p<0.001

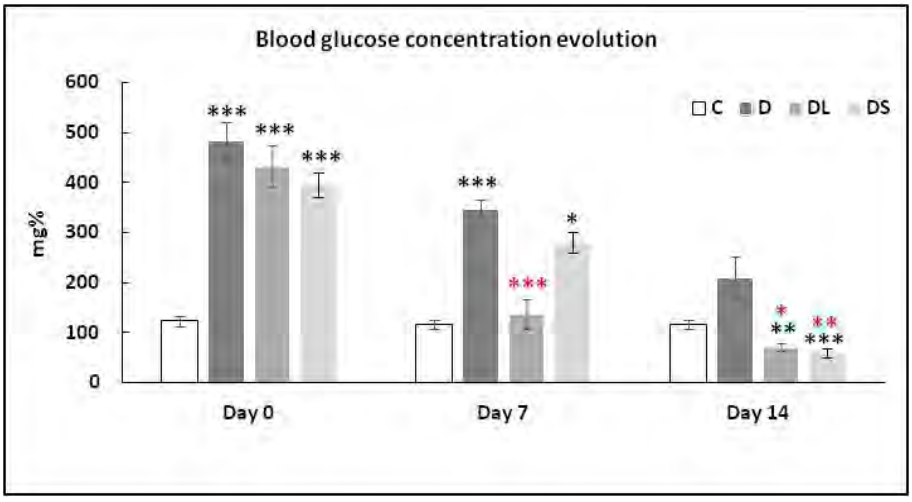

Figure 1. Effects of lycopene and *Arthrospira* powder on the concentration of blood glucose. The results are expressed as mean ± SE. Comparisons made: black - vs Control group; red - vs D group; * - p<0.05; ** - p<0.01; *** - p<0.001

A recent study has tried to explain the hypoglycemic effects of AP by its capacity to intervene in the pentose phosphate pathway and increase the activity of hexokinase by modulating the NADPH/$NADP^+$ ratio (Farouk *et al.*, 2013). Evidence of lycopene playing a role in glucose metabolism has emerged in 2016 when Eze *et al.* discovered that it can activate and stimulate glucokinase in the absence of insulin signaling. Interestingly, glycogen concentrations appear to not have been affected (Table 1, Fig. 2b).

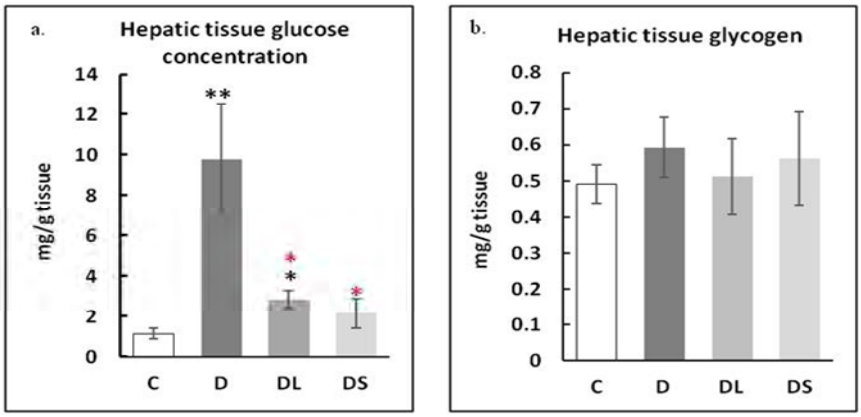

Figure 2. Effects of lycopene and *Arthrospira* powder on the concentration of hepatic glucose (a) and hepatic glycogen (b). The results are expressed as mean ± SE.

Morphological aspects of the blood (RBC, WBC, PCV) seem to have a tendency to normalize in the treated groups, especially in DL.

Lycopene has the intrinsic capacity to neutralize reactive oxygen species (ROS) in the plasma as described by Holzapfel *et al.* in 2013 as well as to prevent ROS-induced hemolysis *in vitro* (Chiste *et al.*, 2014) thus explaining the increase in RBC and consequently in PCV and the concentration of hemoglobin (Table 2, Figs. 3 a,c,d). The effect of AP on these parameters is not as potent, with only a slight statistically significant increase in RBC (Fig. 3a) and a tendency of the hemoglobin concentration to increase (Fig. 3c) probably due to the high concentration of bioavailable iron ion (Fe^{2+}) in AP (Khan *et al.*, 2005). Similar results were obtained by Romay and Gonzalez in 2010 on cultured human erythrocytes.

WBC counting data reveals that of the two supplements only lycopene acted as an immunostimulant by restoring leukocyte numbers to normal levels (Table 2, Fig. 3b). Lycopene has been proven to stimulate the secretion of cytokines (Luo and Wu, 2011) and consequently stimulate the proliferation of leukocytes. AP appears to have no effect on WBC numbers in the blood of diabetic rats, despite sources claiming it should (Milaðiu *et al.*, 2004).

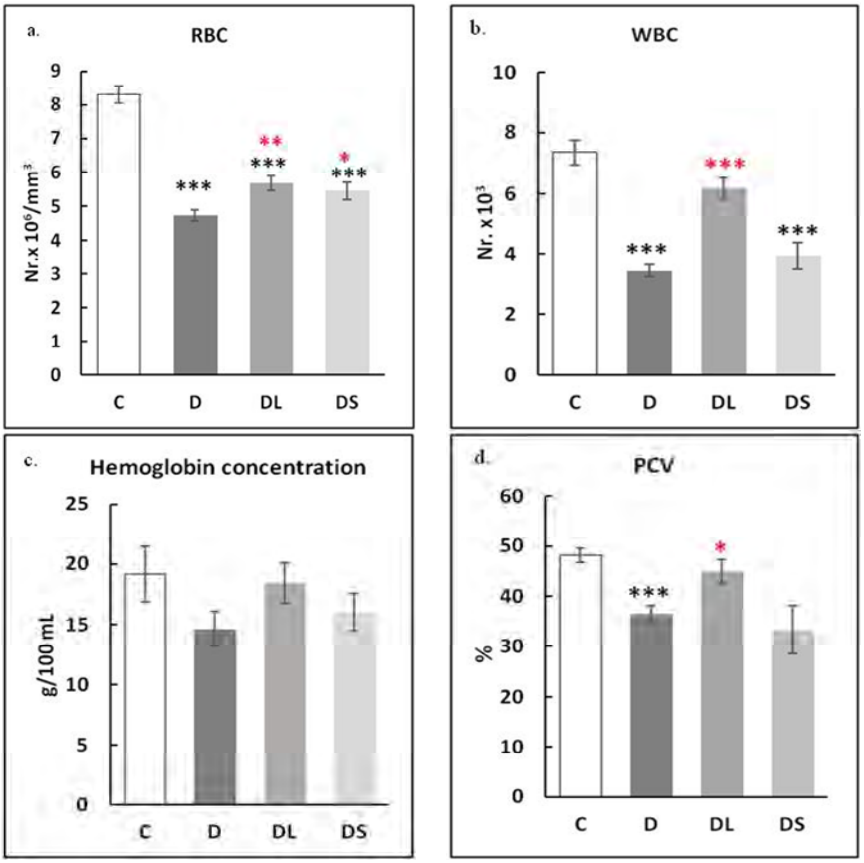

Figure 3. Effects of lycopene and *Arthrospira* powder on the number of red blood cells (a), white blood cells (b), the concentration of hemoglobin (c) and the packed cell volume (d). The results are expressed as mean ± SE.

The differential WBC count (Table 2, Figure 4) reveals a drop in the percentage number of lymphocytes in the untreated diabetic group as well as a normalization in the DL group and what appears to be an overstimulation of the lymphoid progenitor in the DS group. Even though the exact mechanism of phycocyanin activation of lymphocytes is unknown, data concerning is capacity to activate and modulate lymphocytes towards NHEJ DNA repair has been published recently (Stankova *et al.*, 2011). Phycocyanin does seem to be a more potent inhibitor of the hyperglycemia-induced hyperactivation of neutrophils (Xiu *et al.*, 2014) thus preventing neutrophil degranulation and oxidative burn. Lycopene lowered the number of neutrophils but in a lesser degree. Neither compound appears to have any effect on monocyte or eosinophil numbers in the DL and DS groups when comparing them to the untreated group.

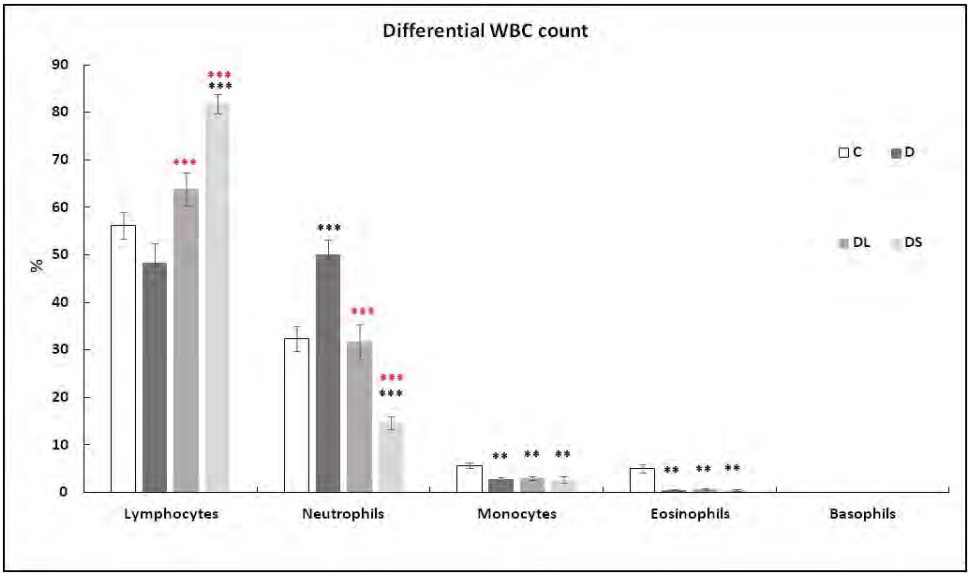

Figure 4. Effects of lycopene and *Arthrospira* powder on the differential WBC count. The results are expressed as mean ± SE.

Table 2.

Effects of lycopene and *Arthrospira* powder on morphological and biochemical parameters in the blood of diabetic Wistar rats

Parameter	Control	D	DL	DS
RBC (x 10^6/mm^3)	8.3 ± 0.26	4.72 ± 0.15 ***	5.68 ± 0.21 *** **	5.44 ± 0.26 *** *
Hemoglobin concentration (mg/dL)	19.14 ± 2.29	14.62 ± 1.44	18.41 ± 1.62	15.99 ± 1.55
PCV (%)	48.12 ± 1.43	36.51 ± 1.66 ***	44.91 ± 2.41 *	33.33 ± 4 .77
WBC (x 10^3/mm^3)	7.34 ± 0.41	3.44 ± 0.2 ***	6.19 ± 0.34 ***	3.93± 0.43 ***
% Lymphocytes	56.26 ± 2.81	48.29 ± 4.17	63.83 ± 3.44 ***	81.83 ± 2.03 *** ***
% Neutrophils	32.3 ± 2.64	50.08 ± 3.01 ***	31.69 ± 3.63 ***	14.62 ± 1.38 ***
% Monocytes	5.65 ± 0.59	2.83 ± 0.37 **	3 ± 0.34 **	2.63 ± 0.65 **
% Eosinophils	5.09 ± 0.85	0.46 ± 0.15 **	0.62 ± 0.25 **	0.47 ± 0.22 **
% Basophils	0	0	0	0

RBC – red blood cells; WBC – white blood cells; PCV – packed cell volume; D – untreated diabetic; DL – diabetic treated with lycopene; DS – diabetic treated with *Arthrospira* powder; The results are expressed as mean ± SE.

Conclusions

Both supplements had an impressive capacity to lower blood sugar concentrations in rats affected by experimental diabetes. While lycopene is a faster acting hypoglycemic agent, *Arthrospira* powder proved to be more potent in the end. The supplements seem to facilitate the internalization of glucose into the liver and stimulate glycolysis even in the absence of insulin signaling. In regards to oxidative stress, lycopene was superior to AP in almost all categories, managing to normalize to an extend all the parameters we have tested. Besides being a powerful antioxidant, lycopene also seems to be a potent modulator of the immune response, whereas AP did show promise in the well established process of hyperglycemia induced hyperactivation of neutrophils.

These results, we hope, validate the status of both supplements as hypoglycemic agents, and antioxidant in the case of lycopene.

REFERENCES

Ali, M. M., Agha, F. G. (2009) Amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene, *Scandinavian Journal of Clinical and Laboratory Investigation* **69**(3), 371–379

Arab, L., Steck, S. (2000) Lycopene and cardiovascular disease, *The American Journal of Clinical Nutrition* **71**(Supplement), 1691–1695

Basu, A., Imrhan, V. (2007) Tomatoes versus lycopene in oxidative stress and carcinogenesis: conclusions from clinical trials, *European Journal of Clinical Nutrition* **61**(3), 295–303

Bayramoglu, A., Bayramoglu, G., Senturk, H. (2013) Lycopene partially reverses symptoms of diabetes in rats with streptozotocin-induced diabetes, *Journal of Medicinal Food* **16**(2), 128–132

Chisté, R. C., Freitas, M., Mercadante, A. Z., Fernandes, E. (2014) Carotenoids are Effective Inhibitors of in vitro Hemolysis of Human Erythrocytes, as Determined by a Practical and Optimized Cellular Antioxidant Assay, *Journal of Food Science* **79**(9), 1841–1847

Drabkin, D. L., Austin, J. H. (1935) Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide, hemoglobin and sulfhemoglobin, *Journal of Biological Chemistry* **112**, 51

Eze, E. D., Tanko, Y., Tende, J. A., Ehinomhen, U. A. (2016) Effects of Lycopene on Liver Markers and Glucokinase Activity in Experimentally-induced Diabetes Mellitus Rat Model, *Journal of Basic and Applied Research* **2**(3), 353–362

Farouk, K. E., Hanan, F. A., El-Sayed, A. B., Amal, A. M. (2013) Role of Spirulina Platensis in the control of glycemia in DM2 rats, *International Journal of Scientific & Engineering Research* **4**(12), 1731–1740

Guo, Y., Liu, Y., Wang, Y. (2015) Beneficial effect of lycopene on anti-diabetic nephropathy through diminishing inflammatory response and oxidative stress, *Food and Function* **6**(4), 1150–1156

Holzapfel, N. P., Holzapfel, B. M., Champ, S., Feldthusen, J., Clements, J., Hutmacher, D. W. (2013) The potential role of lycopene for the prevention and therapy of prostate cancer: From molecular mechanisms to clinical evidence, *International Journal of Molecular Sciences* **14**(7), 14620–14646

Ip, B. C., Hu, K. Q., Liu, C., Smith, D. E., Obin, M. S., Ausman, L. M., Wang, X. D. (2013) Lycopene metabolite, apo-10′-lycopenoic acid, inhibits diethylnitrosamine-initiated, high fat diet-promoted hepatic inflammation and tumorigenesis in mice, *Cancer Prevention Research* **6**(12), 1304–1316

Khan, Z., Bhadouria, P., Bisen, P. S. (2005) Nutritional and Therapeutic Potential of Spirulina, *Current Pharmaceutical Biotechnology* **6,** 373-379

Layam, A., Reddy, C. (2006) Antidiabetic Property of Spirulina, *Diabetologia Croatica*, **62**(1), 29-33

Lo, S., Russel, J. C., Taylor, A. W. (1970) Determination of glycogen in small tissue samples, *Journal of Applied Physiology* **28**, 234-236

Luo, C., Wu, X. G. (2011) Lycopene enhances antioxidant enzyme activities and immunity function in N-methyl-N-nitro-N-nitrosoguanidine-induced gastric cancer rats, *International Journal of Molecular Sciences* **12**(5), 3340–3351

Milaðius, K., Peèiukonienë, M., Dadelienë, R. (2004) Effect of Spirulina Food Supplement on Blood Morphological and Biochemical Composition in Sportsmen, *Acta Medica Lituanica* **11**(1) 47-51

Montgomery, R. (1957) Determination of glycogen, *Archives of Biochemical Biophysics* **67** (2), 378-386

Nelson, N. (1944) A photometric adaptation of the Somogy method for the determination of glucose, *Journal of Biological Chemistry* **153**, 375-380

Romay, C., González, R. (2000) Phycocyanin is an Antioxidant Protector of Human Erythroytes Against Lysis by Peroxyl Radicals, *Journal of Pharmacy and Pharmacology* **52**(4), 367-368

Stankova, K., Ivanova, K., Nikolov, V., Minkova, K., Gigova, L., Georgieva, R., Boteva, R. (2011) The Biliprotein C-Phycocyanin Modulates the DNA Damage Response in Lymphocytes from Nuclear Power Plant Workers, *Nuclear Power - Operation, Safety and Environment*, Tsvetkov, P. (ed.), InTech, 327-340

Xiu, F., Stanojcic, M., Diao, L., Jeschke, M. G. (2014) Stress hyperglycemia, insulin treatment, and innate immune cells, *International Journal of Endocrinology*, **2014**: 486403, http://dx.doi.org/10.1155/2014/486403

Zhou, P., Liu, L., Chen, X. (2005) Factors that affect antioxidant activity of C-Phycocyanins from Spirulina platensis, *Journal of Food Biochemistry* **76**(2), 313-322

*** (2016) World Health Organization, Global Report on Diabetes, 88