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# Lycopene and Phycocyanin - biological properties in experimental diabetes: 2. Effects on biochemical, enzymatic and histological parameters

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**SUMMARY.** The economic impact of diagnosing, treating and monitoring diabetic patients is huge, for both patients and medical systems involved. Adequate diets are hard to follow especially in poor countries, and stress life significantly contributes to diabetes development. In addition, access to relevant treatment may be limited for many patients and some medical policies do not help.

Dietary supplements are a key to help patients raising their quality of life, to alleviate symptoms and in some cases, to reduce insulin doses.

40 adult male Wistar rats were used, divided in 4 groups of 10 animals each: Control (C), Diabetic (D), Diabetic+Lycopene (DL) and Diabetic+Spirulina (DS). Diabetes was induced with a single dose of streptozotocin (50 mg STZ/kg body weight) in the tail vein. The DL group was given 10 mg lycopene/kg/day, while the DS group received 200 mg phycocyanin/kg/day in the form of *Arthrospira* (Spirulina) powder containing 15% phycocyanin.

Treatment with *Arthrospira* powder reduced the activity of seric alanine aminotransferase (ALT) and seric aspartate aminotransferase (AST) in diabetic animals (DS). Serum catalase (CAT) activity in the DS group was significantly reduced, compared to both C and D groups. Hepatic CAT activity in both treated groups increased as compared to the control and diabetic group. Furthermore, phycocyanin and lycopene stimulated serum and hepatic lactate dehydrogenase (LDH) activity in DL and DS rats.

Some other effects of lycopene and Spirulina powder, such as lowering blood and hepatic cholesterol concentrations and normalizing the histological structure of brain, liver, kidney and pancreatic tissues, support the assumption that both compounds have a potential therapeutic role as adjuvants.

**Keywords:** diabetes, lycopene, phycocyanin, streptozotocin

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## Introduction

Diabetes is a complex of metabolic disorders mainly characterized by high blood sugar concentration, either because cells cannot respond to insulin, or because insulin production is insufficient (NIH, 2014).

Using adjuvant treatments in early stages of diabetes can improve patients' life and prevent, or slow down diabetes evolution. According to Pribac *et al.* (2011) vegetal compounds found in *Ganoderma lucidum* (reishi mushroom) or *Trigonella foenum-graecum* (fenugreek) could improve biological parameters and restore β-cells function in diabetic rats.

*Arthrospira platensis* (commercial name: Spirulina) is an aquatic cyanobacteria, rich in nutrients, vitamin B complex, minerals (Fe, Se, Cr etc.), proteins, gamma linoleic acid and antioxidants (vitamin E and β-carotene) (Layam and Reddy, 2006).

Spirulina contains large amounts of phycocyanin, a phycobiliprotein (PBP) with a chemical structure similar to bilirubin. PBPs are proteic aggregates soluble in water and responsible for light absorption (Devendra *et al*., 2014). PBPs are used as fluorescent markers, nutritive supplements, antioxidant and anti-inflammatory agents, and natural dyes. Cyanobacteria are grown at industrial scale as a source of many useful compounds, including phycocyanin (Hirata *et al*., 2000).

According to Nishanth *et al.* (2010), phycocyanin inhibits cyclooxygenase-2 (COX-2), stimulates cytokine expression and enhances expression of superoxide dismutase (SOD) and CAT, two enzymes that are essential for mammalian natural antioxidant system.

Selenium binds to phycobiliproteins, increasing their antioxidant properties. Chromium stimulates carbohydrate metabolism and increases insulin activity (Belokobylsky *et al*., 2004).

Lycopene is a carotenoid without provitaminc function found in tomatoes and other (mostly red) vegetables and fruits. Lycopene increases transcription nuclear factor E2 (Nrf2), which regulates oxidative response (Kensler and Wakabayashi, 2009). It was shown by Bayramoglu *et al*. (2013) that lycopene has hypoglycemic effect in diabetic rats, also lowering total cholesterol and triglyceride plasma concentrations. Ali and Agha (2009) reported normalization of antioxidant enzymes activity in diabetic rat erythrocytes. Lycopene also improved carbohydrate metabolism, serum lipid profiling, and enhanced antioxidant enzyme activities in the kidney of STZ-induced diabetes mice (Guo *et al*., 2015).

While certain beneficial effects of lycopene and phycocyanin have been documented in various pathologies, little attention has been given to their antidiabetic promise. This study was undertaken to investigate the biological properties of lycopene and Spirulina powder (containing 15% phycocyanin) in experimental diabetes, in order to document their high potential as adjuvants in diabetes treatment.

## 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. Arthrospira (commercial name: Spirulina) powder was from Adams Vision, Romania and Lycopene was from König Laboratorium, Canada.

The study was conducted on 40 male Wistar rats, weighing 150±50 g, divided into four groups: Control (C), Diabetic (D), Diabetic+Lycopene (DL) and Diabetic+Spirulina (DS). Each group included 10 individuals.

All animals had *ad libitum* access to tap water and received a standard diet. Those in groups DL and DS received the standard diet supplemented with lycopene, or Spirulina powder containing 15% phycocyanin. Lycopene and Spirulina were both administered orally: 10 mg/kg body weight lycopene, and the amount of Spirulina corresponding to 200 mg/kg body weight phycocyanin.

Diabetes was induced with a single dose of streptozotocin (50 mg/kg b.wt.) dissolved in 10 mM sodium citrate, pH 4.5, injected in the tail vene. The control group received only the vehicle.

On the $14^{th}$ day of the experiment, the animals were sacrificed under anesthesia. Blood and liver samples were harvested, to quantify the following parameters: whole cholesterol, protein concentration, and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and catalase (CAT). Tissue pieces of liver, kidney, brain and pancreas were taken for histological examination.

Total protein concentration was determined by Bradford (1976) colorimetric assay using the Bradford "ready-to-use" reagent. The colorimetric assay based on the reaction with iron chloride in presence of sulphuric and acetic acids was used for cholesterol measurement (Zlakis *et al*., 1953).

For CAT activity, the decay of $H_2O_2$ was monitored at 240 nm (Vives-Bauza *et al*., 2007). The activity of LDH was measured as the oxidation rate of NADH at 365 nm (Bergmeyer and Bernt, 1974). Reitman and Frankel (1957) photocolorimetric assay was used for the determination of AST and ALT activities.

The results were expressed as mean values±SE and multiple comparisons between experimental groups were made using the *t* test: all groups *vs* control group; DL and DS *vs* D group. Differences were considered statistically significant at $p \leq 0.05$.

## Results and discussion

The diabetic condition became evident 3 days after STZ administration, with glycaemia values ranging for 400 to 600 mg/dL. Water consumption and diuresis of diabetic animals increased dramatically, while body weight decreased (data not shown here).

STZ intoxication, raised **cholesterolemia** of rats in D group with 32.5%, as compared to the control group (Table 1). Vornoli *et al.* (2014) also reported a 4.5-fold increase in plasma total cholesterol, in STZ-injected rats put on a high fat diet for 8 weeks. In groups receiving either lycopene (DL) or Spirulina (DS), blood cholesterol concentration returned to control values (Table 1). Earlier works (Heber and Lu, 2002) reported that lycopene and carotenoids moderately lowered cholesterolemia, by inhibiting 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Sheu *et al.* (2013) showed an improvement of lipid profile and cholesterol reduction by C-phycocyanin extracted from *Arthrospira sp.*

**Table 1**

Effect of lycopene and phycocyanin on total cholesterol and total protein concentration, in serum and liver tissue

| Parameter | Control | D | DL | DS |
|---|---|---|---|---|
| **Cholesterolemia** (mg/dL serum) | 88.83±5.44 | 117.74±19.55 | 89.02±5.86 | 89.58±12.13 |
| **Hepatic cholesterol concentration** (mg/g tissue) | 4.70±0.50 | 8.31±1.05 ** | 4.21±0.25 ** | 5.22±0.50 * |
| **Proteinemia** (mg/dL serum) | 1.77±0.04 | 1.69±0.02 | 1.69±0.03 | 1.62±0.04 |
| **Hepatic protein concentration** (mg/g tissue) | 144.48±4.72 | 133.47±5.51 | 144.68±4.70 | 147.47±47 |

D–Diabetic group, DL–Diabetic+lycopene, DS–Diabetic+Spirulina. Multiple comparisons were made: black - *vs* Control group; red - *vs* D group; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

**Liver total cholesterol** increased with 76.8% in the liver of STZ-diabetic animals, while both Spirulina and lycopene reversed cholesterol values to the control (Table 1). We believe this parameter is relevant because excessive intracellular accumulation of cholesterol is the result of imbalance between plasma intake and cellular synthesis. Lowering of cholesterol synthesis by inhibiting HMG-CoA reductase, and suppression of the expression of LDL receptors in cell plasma membranes may prevent abnormal cholesterol accumulation (Palozza *et al.*, 2012). Our data are consistent with those reported by Xia *et al.* (2016), showing that C-phycocyanin prevented cholesterol accumulation in the liver of mice with subacute alcohol-induced injury. Ou *et al.* (2012) found a significant decrease of plasma and liver cholesterol in alloxan-induced diabetic mice pre- and post-treated with 200 mg/kg phycocyanin. Young rats fed for 5 weeks with tomato powder (10% of the diet) or lycopene (0.62g/kg diet) showed significant reduction of liver total cholesterol and LDL-cholesterol, in both presence and absence of 1% $H_2O_2$ in the drinking water (Alshatwi *et al.,* 2010). Although most authors emphasize the role of lycopene in reducing blood cholesterol in various chronic diseases, fewer data are available concerning liver total and LDL-cholesterol. However,

the distribution of absorbed lycopene in organs indicated liver as a major retention site (Kong *et al.,* 2010). Moreover, Kim *et al.* (2012) reported that lycopene-enriched tomato wine failed to decrease liver, plasma and faeces cholesterol in rats fed a high-fat diet. This inconsistency between studies may result from different factors such as the type and duration of dietary intervention, animal species, gender and age, lycopene source and processing.

**Serum and liver total protein** content were not influenced in a significant manner by STZ diabetes, spirulina or lycopene treatments (Table 1). However, in diabetic animals, Spirulina and lycopene administration totally reversed the 7.62% reduction of liver protein, as compared to the control group. Although there is no evidence of a direct stimulation of liver protein synthesis by lycopene or Spirulina, it is well-known that oxidative stress accompanying various diseases, including diabetes, deeply alter nucleic acids and protein structure and synthesis. Proteins that are rich in tyrosine, tryptophan, histidine and cysteine are particularly affected and subjected to proteasome degradation (Cichoz-Lach and Michalak, 2014). Consequently, compounds possessing antioxidant properties may protect both nucleic acids and proteins from oxidative damage.

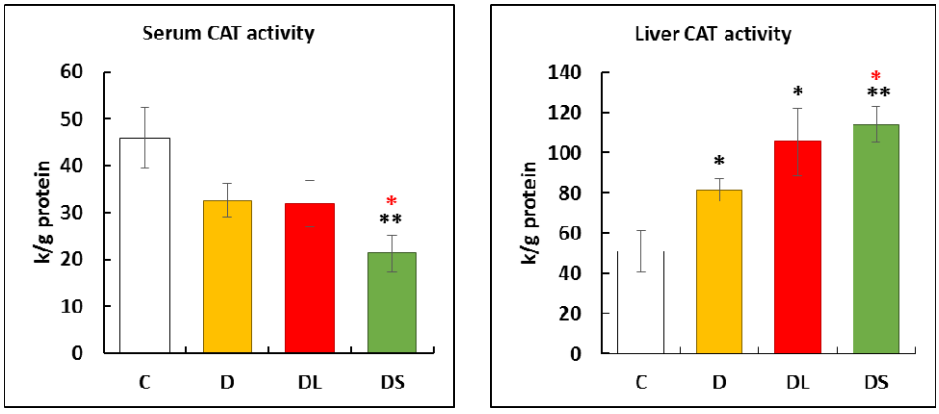


**Figure 1.** Effect of lycopene and phycocyanin on seric and hepatic CAT activity. Multiple comparisons were made: black - vs C group; red - vs D group; *p<0.05; **p<0.01. Results are expressed as k/g of protein in the sample. k=the rate of a first-order reaction.

**Catalase (CAT) activities** in sera and liver homogenates are shown in Fig. 1. The enzyme is ubiquitous in most tissues and catalyzes the decomposition of $H_2O_2$ to $H_2O$ and $O_2$, consecutive to the dismutation of superoxide anion to molecular oxygen and $H_2O_2$ in the superoxide dismutase reaction. Therefore, CAT is a crucial enzyme in protecting the cell from oxidative damage. In humans, it is believed that a decreased blood CAT activity may be associated with type 2 diabetes (Góth, 2008). Serum CAT

activity decreased with 21% in all three diabetic groups as compared to control; lycopene did not restore the control value, and Spirulina decreased enzyme's activity even more than diabetes. At a first glance, these results seem hard to explain; we have to take into consideration several factors that may influence CAT activity. First, most of the blood enzyme is located into erythrocytes; even a slight hemolysis of the serum can alter the measurements. Second, CAT is not the only enzyme that scavenges $H_2O_2$; it shares this function with glutathione peroxidase, and the contribution of each enzyme may change according to $H_2O_2$ concentration (Mueller *et al.*, 1997; Selvan *et al.*, 2011).

However, most of CAT is located into the liver. There was a significant increase of its activity in the liver of diabetic animals, as compared to C group, probably as a reaction to the increased reactive oxygen species (ROS) formation that accompanies diabetes development. Lycopene administered to diabetic animals slightly increased CAT activity above the one in the D group, while Spirulina was even more effective. Previous research provide good evidence for the antioxidant properties of lycopene and Spirulina. Kong *et al.* (2010) and Heber and Lu (2013) stated that lycopene is the most efficient antioxidant among carotenoids, in trapping singlet oxygen and reducing thiobarbituric acid reactive substances (TBARS). However, Alshatwi *et al.* (2010) believe that tomato powder is more efficient than lycopene supplement against lipid peroxidation in rats. A strong correlation between oxidative stress and diabetes has been demonstrated by Ou *et al.*(2012) in alloxan-injured mice. They also found that phycocyanin from Spirulina had a preventive effect on ROS generation, reducing malondialdehyde formation in liver, kidney and pancreas. In golden Syrian hamsters fed a hypercholesterolenic diet, phycocyanin enhanced SOD, CAT and glutathione peroxidase activities in the liver (Sheu *et al.*, 2013).

**LDH activity** in the liver was tremendously stimulated by both lycopene and Spirulina (Fig. 2), in diabetic rats. These data are in accordance with the considerable decrease in liver glucose found in DL and DS groups (see Moldovan *et al.*, 2016). Ou *et al.* (2012) reported that phycocyanin from Spirulina enhanced liver glucokinase (GK) activity in diabetic mice. Several years later, Eze *et al.* (2016) found a similar action of lycopene in diabetic rats. Liver cells contain the highest amount of GK, the enzyme accounting for 95% of the hexokinase activity in these cells. Phosphorylation of glucose by GK provides substrate for both glycogen synthesis and glycolysis. As in our experiment liver glycogen content was not affected, we believe that most of the glucose was directed *via* glycolysis. The rise in serum LDH closely parallels enzyme's activity in the liver.

**Serum transaminases** are specific (ALT) and non-specific (AST) markers of hepatocytes plasma membrane integrity. High activities of these enzymes usually account for altered membrane integrity and/or permeability. In our experiment, serum transaminases significantly increased in diabetic animals (Table 2) and lycopene administered for 2 weeks in a daily dose of 10 mg/kg could not reverse this effect.

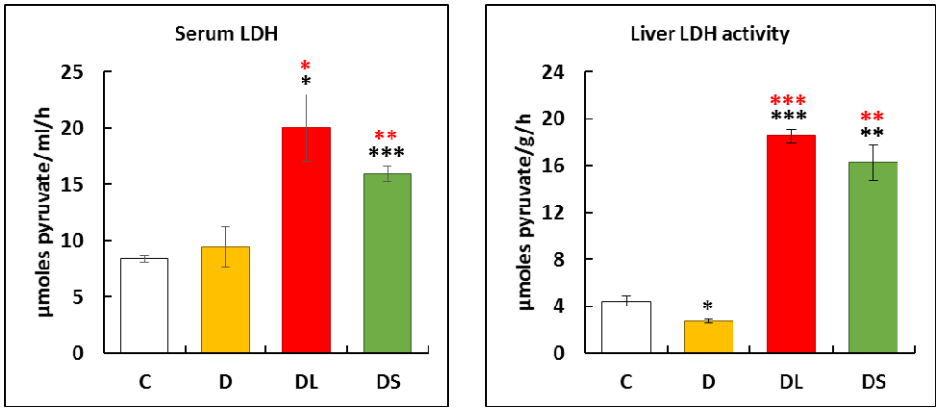


**Figure 2.** Effect of lycopene and phycocyanin on and hepatic LDH activity. Comparisons: black - vs C group; red - vs D group; *p<0.05; **p<0.01; ***p<0.001.

**Table 2.**

Effect of lycopene and Spirulina on membrane integrity biomarkers

| Parameter | Control | D | DL | DS |
|---|---|---|---|---|
| **Seric ALT activity** (µg pyruvate/mL/ hour) | 45.46±10.57 | 183.33±21.86 *** | 218.89±26.27 *** | 134.83±21.32 ** |
| **Seric AST activity** (µg pyruvate/mL/ hour) | 204.75±13.25 | 242.84±9.57 * | 247.31±12.42 * | 192.52±13.51 ** |
| **Hepatic ALT activity** (µg pyruvate/g tissue/ hour) | 127.83±3.52 | 138.20±3.48 * | 143.55±4.95 * | 143.10±6.15 |
| **Hepatic AST activity** (µg pyruvate/g tissue/ hour) | 48.70±1.30 | 44.72±1.46 | 44.51±3.38 | 49.97±4.70 |

D – Diabetic group, DL – Diabetic + lycopene, DS – Diabetic + Spirulina. Comparisons: black - *vs* Control group; red - *vs* D group; * p<0.05; ** p<0.01; *** p<0.001.

However, there is strong evidence (Eze *et al.,* 2016) that lycopene in higher doses (up to 40 mg/kg) and given for a longer time (4 weeks) is capable to reduce serum ALT and AST. Interestingly, Baymaroglu *et al.* (2013) reported similar results with 2.5 mg/kg lycopene, in a 7-day experiment. Only Spirulina (in a daily dose corresponding to 200 mg phycocyanin/kg) significantly reduced seric transaminases in DS group, as compared to D group. Our data are consistent with the ones reported by Gargouri *et al.* (2016), El-Sheekh *et al.* (2014), and Anwer *et al.* (2013).

**Liver ALT** activity (Table 2) increased with 8.1% in diabetic animals, and with 12.3% in DL and DS groups, showing the implication of this enzyme in the reversible conversion of glucose-derived pyruvate to alanine.

**Liver AST** is usually involved in the malate-aspartate shuttle, providing $NAD^+$ for glycolysis in the cytosol, and NADH in the mitochondria, for the electron transport chain. It seems that nor lycopene, neither Spirulina have not enhanced the shuttle's activity, as the elevated LDH (Fig. 2) could maintain an appropriate $NAD^+$ level in the cytosol.

### Histology of pancreas, liver, kidney and brain tissues

These tissues were chosen for histological examination due to their involvement in the pathology of diabetes.

In experimental diabetes, STZ enters β-pancreatic cells *via* the low-selectivity GLUT-2 glucose transporters and alkylates DNA molecules and proteins. DNA fragmentation and depletion of $NAD^+$ and energy stores ultimately result in β-cell necrosis (Lenzen, 2008; Wu and Yan, 2015).

Diabetes induced severe alterations in the ***pancreas*** (Fig. 3B): smaller and fewer Langerhans islets, with low cellularity and an obscure demarcation between islets and the acinar tissue. Similar modifications were reported by Ou *et al.* (2012) in the pancreas of alloxan-induced diabetic mice. Although the mechanisms by which alloxan and streptozotocin cause experimental diabetes are different, it seems that structural alterations are alike. Lycopene was not effective in reducing the dystrophy of Langerhans islets (Fig. 3C), while Spirulina (Fig. 3D) improved the appearance of pancreatic tissue, showing fewer, but larger islets, with better cellularity.

The ***liver*** of control rats had normal structure, with lobules separated by fibrous septa (Fig. 4A), while in the diabetic liver areas of micro- and macrovesicular steatosis, accompanied by biliary stasis, could be noticed (Fig. 4B). Similar alterations were reported by Salih *et al.* (2009) in STZ-induced diabetic mice. Left ventricle failure, specific for diabetes associated diseases, can be recognized by greatly dilated centrilobular veins (Fig. 4B). Both lycopene and Spirulina improved the histological appearance of hepatic tissue; however, small inflammation foci were still present in the DL group (Fig. 4C), while enlarged spaces between the cell strands could be noticed in the DS group (Fig. 4D).

***Kidney*** tissue in control rats had normal Bowman spaces, glomeruli and parenchima (Fig. 5A). The diabetic animals, (Fig. 5B) showed enlarged, or even disrupted Bowman capsule; tubular necrosis could also be seen. Lycopene and Spirulina visibly improved the renal tissue appearance: fewer altered renal corpuscles were present in DL group (Fig. 5C) without disrupted capsules; in the DS group (Fig. 5D) the renal corpuscles were quite normal, while small areas with tubular necrosis were still present. Our observations are consistent with those of Ahmed *et al.* (2015), who examined the alterations of liver and kidney tissue in STZ-induced diabetic rats and noticed a significant attenuation of these modifications by antioxidants Coenzyme Q10 and vitamin E. We believe that the beneficial effects of lycopene and Spirulina on tissue structure is also due to the free radical scavenging properties of these natural products.

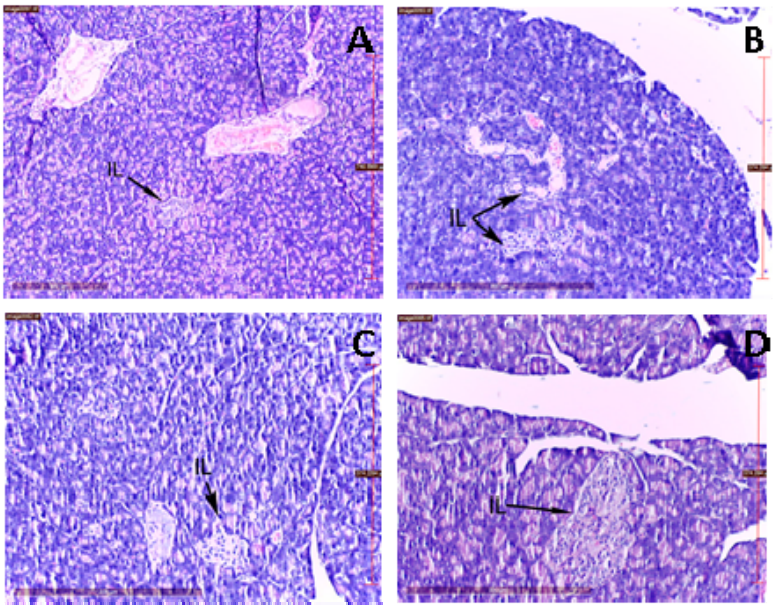


**Figure 3.** Histological aspects of pancreatic tissue in control (A), diabetic (B), DL (C) and DS (D) rats. IL – Langerhans islets. Bar – 300 µm.

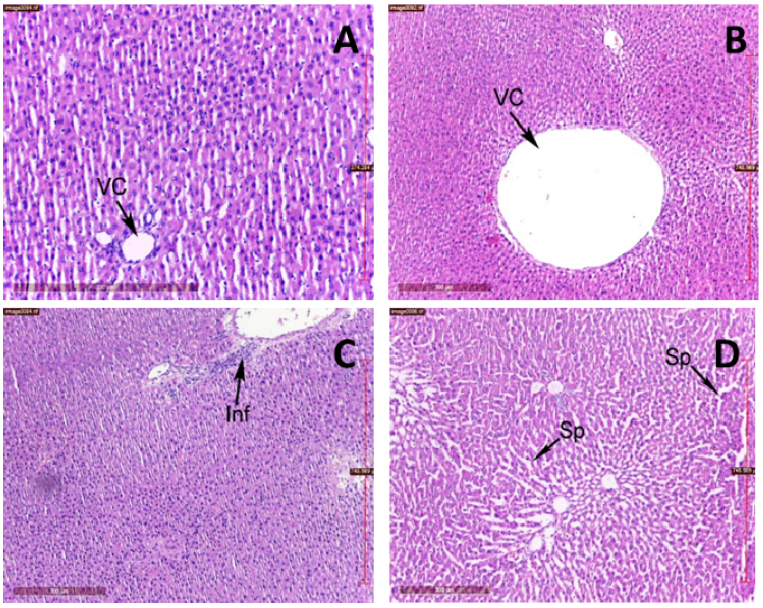


**Figure 4.** Histological aspects of hepatic tissue in control (A), diabetic (B), DL (C) and DS (D) rats. VC – centrolobular vein; Inf – inflammation focus; Sp – spaces between cell strands. Bar – 300 µm.

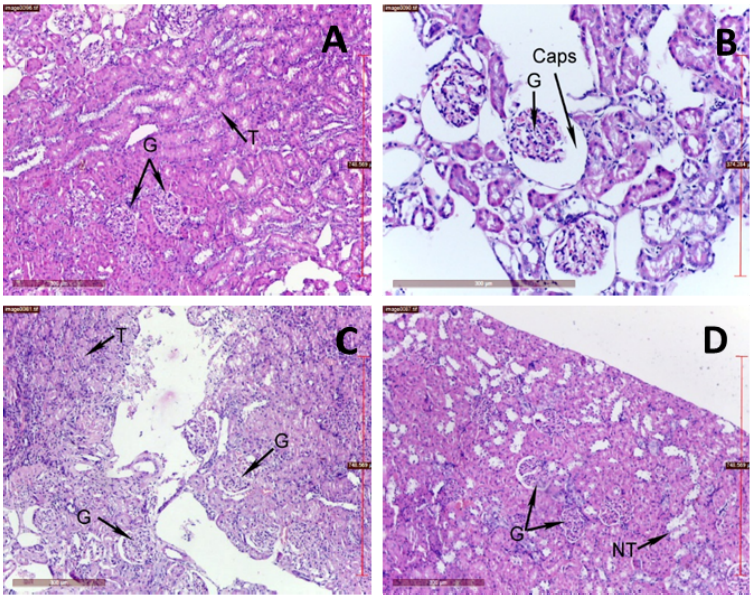


**Figure 5.** Histological aspects of kidney tissue in control (A), diabetic (B), DL (C) and DS (D) rats. G – glomeruli; T – tubule; Caps – Bowman capsule; NT – tubular necrosis. Bar – 300 µm.

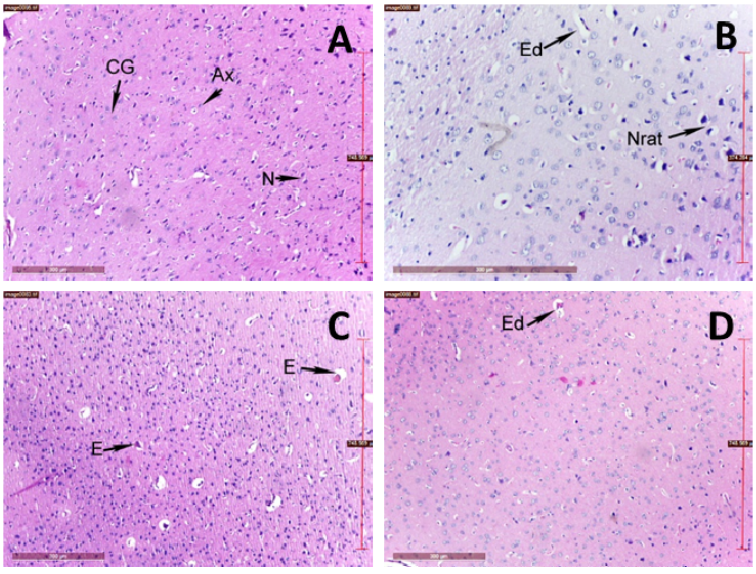


**Figure 6.** Histological aspects of brain in control (A), diabetic (B), DL (C) and DS (D) rats. CG-glia cell; Ax-axon; N-neuron; Ed, E-edema; Nrat-shrinked neuron. Bar-300 µm.

Diabetes brings about structural changes in the ***brain***, leading to cognitive dysfunctions, in both humans (Biessels *et al.*, 2014; Wrighten *et al.,* 2009) and rodents (Huang *et al.,* 2012). In our experiment, STZ-induced diabetes caused edema and neuronal damage (shrinking) (Fig. 6B), similar to the modifications reported by Huang *et al.* (2012): demyelination and axonal degradation, dystrophic neurons and abnormal oligodendrocytes. In the DL group (Fig. 6C) edema were also evident, while Spirulina (DS group, Fig. 6D) visibly reduced the extent of these alterations.

## Conclusions

According to our results we can say that lycopene and spirulina treatments improved biochemical and enzymatic parameters. The oxidative stress was reduced and membrane integrity partly restored. Glycolysis was stimulated by both adjuvants.

Histological examination of pancreas, liver, kidney and brain tissues confirm the biochemical and enzymatic results, by showing certain improvements of diabetes-induced alterations after lycopene and spirulina administration.

As lycopene and Spirulina have specific and different mechanisms of action, even though their effects are roughly similar, further directions of research would be to test them together, as a cocktail, on diabetic animals, for various periods of time and also as pre-treatments.

## REFERENCES

Ahmed, H. H., Aly, H. F., Ali, S. A. (2015) Potential impact of CoQ10 and vitamin E against (STZ) induced metabolic deterioration in the albino rats, *Int. J. Pharm. Pharm. Sci.*, **7**(11), 176-184

Ali, M. M., Agha, F. G. (2009) Amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene, *Scand. J. Clin. Lab. Invest.*, **69**(3), 371-379

Alshatwi, A. A., Al Obaaid, M. A., Al Sedairy, S.A., Al-Assaf, A. H., Zhang, J. J., Lei, K. Y. (2010) Tomato powder is more protective than lycopene supplement against lipid peroxidation in rats, *Nutr. Res.*, **30**, 66-73

Anwer, R., Alam, A., Khursheed, S., Kashif, S. M., Kabir, H., Fatma, T. (2013) Spirulina: possible pharmacological evaluation for insulin-like protein, *J. Appl. Phycol.*, **25**(3), 883-889

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

Belokobylsky, A., Ginturi, E., Kuchava, N., Kirkesali, E., Mosulishvili, L., Frontasyeva, M., Pavlov S.S., Aksenova N.G. (2004) Accumulation of selenium and chromium in the growth dynamics of *Spirulina platensis*, *J. Radioanal. Nucl. Chem.,* **259**(1), 65-68

Bergmeyer, H. U., Bernt, E. (1974) Lactate dehydrogenase, In: *Methods of Enzymatic Analysis*, Bermeyer, H. U. (ed), Academic Press, New York, **2**, pp. 574-579

Biessels, G. J., Reijmer, Y. D. (2014) Brain changes underlying cognitive dysfunction in diabetes: what can we learn from MRI?, *Diabetes*, **63**, 2244-2252

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analit. Biochem.,* **72**, 248-254

Cichoz-Lach, H., Michalak, A. (2014) Oxidative stress as a crucial factor in liver diseases, *World J. Gastroenterol.*, **20**(25), 8082-8091

Devendra, K., Dolly, W., Sunil, P., Neeraj, K., Suresh, W. (2014) Extraction and purification of C-phycocyanin from Spirulina platensis (CCC540), *Indian J. Plant Physiol.,* **19**(2), 184-188

El-Sheekh, M. M., Hamad, S. M., Gomaa, M. (2014) Protective effects of Spirulina on the liver function and hyperlipidemia of rats and human, *Braz. Arch. Biol. Technol.*, **57**(1), 77-86

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, *J. Basic appl. Res.*, **2**(3), 353-362

Gargouri, M., Magné, C., El Feki, A. (2016) Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation, *Nutrition Res.,* http://dx.doi.org/10.1016/j.nutres. 2016.09.011

Góth, L. (2008) Catalase deficiency and type 2 diabetes, *Diabetes Care*, **31**(12), c93

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

Heber, D., Lu, K. -Y. (2002) Overview of mechanisms of action of lycopene, *Exp. Biol. Med.,* **227**, 920-923

Hirata, T., Tanaka, M., Ookie, M., Tsunomura, T., Sakaguchi, M. (2000) Antioxidant activities of phycocyanobilin prepared from Spirulina platensis, *J. Appl. Phycol.,* **12**(3), 435-439

Huang, M., Gao, L., Yang, L., Lin, F., Lei, H. (2012) Abnormalities in the brain of streptozotocin-induced type 1 diabetic rats revealed by diffusion tensor imaging, *NeuroImage: Clinical*, **1**, 57-65

Kensler, T. W., Wakabayashi, N. (2009) Nrf2: Friend or foe for chemoprevention*? Carcinogenesis*, **31**(1), 90-99

Kim, A. -Y., Jeong, Y. -J., Park, Y. B., Lee, M. -K., Jeon, S. -M., McGregor, R. A., Choi, M. -S. (2012) Dose dependent effects of lycopene enriched tomato-wine on liver and adipose tissue in high-fat diet fed rats, *Food Chem.*, **130**, 42-48

Kong, K. -W., Khoo, H. -E., Prasad, K. N., Ismail, A., Tan, C. -P., Rajab, N. F. (2010) Revealing the power of the natural red pigment lycopene, *Molecules*, **15**, 959-987

Layam, A., Reddy, C. (2006) Antidiabetic Property of Spirulina, *Diabetologia Croatica,* **35**(2), 29-33

Lenzen, S. (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes, *Diabetologia*, **51**, 216-226

Mueller, S., Riedel, H. -D., Stremmel, W. (1997) Direct evidence for catalase as the predominant $H_2O_2$-removing enzyme in human erythrocytes, *Blood*, **90**(12), 4973-4978

National Institute of Diabetes and Digestive and Kidney Diseases - NIDDK (2014) Health information – Diabetes, *http://www.niddk.nih.gov*.

Nishanth, R. P., Ramakrishna, B. S., Jyotsna, R. G., Roy, K. R., Reddy, G. V., Reddy, P. K., Reddanna, P. (2010) C-phycocyanin inhibits MDR1 through reactive oxygen species and cyclooxygenase-2 mediated pathways in human hepatocellular carcinoma cell line, *Eur. J. Pharmacol.*, **649**, 74-83

Ou, Y., Lin, L., Pan, Q., Yang, X., Cheng, X. (2012) Preventive effect of phycocyanin from Spirulina platensis on alloxan-injured mice, *Environ. Toxicol. Pharmacol.*, **34**, 721-726

Palozza, P., Catalano, A., Simone, R. E., Mele, M. C., Cittadini, A. (2012) Effect of lycopene and tomato products on cholesterol metabolism, *Ann. Nutr. Metab.,* **61**, 126-134

Pribac, G., Craciun, C., Szoke-Nagy, T., Simeoni, I., Lang, C., Roşioru, C., Czapar, M., Mathe, E., Mos, L., Ardelean, A., (2011) Comparative ultrastructural study of pancreatic beta cells from diabetic rats treated with fenugreek seed flour or ganoderma flour, *Annals of RSCB,* **16(**1), 62-80

Reitman, S., Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, *Am. J. Clin. Pathol.,* **28**(1), 56-63

Salih, N. D., Muslih, R. K., Hamoodi, S. R. (2009) Histological liver changes in streptozotocin induced diabetic mice, *Int. Med. J. Malaysia,* **8**(1), 1-4

Selvan, V. K., Vijayakumar, A., Kumar, K. S.,Singh, G. N. (2011), Lycopene's effects on health and diseases. A comprehensive review of the literature, *Nat. Med. J.,* **3**(3), ISSN 2157-6769

Sheu, M. -J., Hsieh, Y. -Y., Lai, C. -H., Chang, C. -C., Wu, C. -H. (2013) Antihyperlipidemic and antioxidant effects of C-phycocyanin in golden Syrian hamsters fed with a hypercholesterolemic diet, *J. Trad. Comp. Med.*, **3**, 41-47

Vives-Bauza, C., Starkov, A., Garcia-Arumi, E. **(**2007) Measurements of the Antioxidant Enzyme Activities of Superoxide Dismutase, Catalase, and Glutathione Peroxidase, in: *Methods in Cell Biology*, Pon, L. A., Schon, E. A. (eds), Elsevier, **80,** pp 379-393

Zlatkis, A., Zak, B., Boyle, A. J. (1953) A new method for the direct determination of serum cholesterol, *J. Lab. Clin. Med.,* **41**, 486-92

Vornoli, A., Pozzo, L., Della Croce, C. M., Gervasi, P. G., Longo, V. (2014) Drug metabolism enzymes in a steatotic model of rat treated with a high fat diet and low dose of streptozotocin, *Food Chem. Toxicol.,* **70**, 54-60

Wrighten, S. A., Piroli, G. G., Grillo, C. A., Reagan, L. P. (2009) A look inside the diabetic brain: contributors to diabetes-induced brain aging, *Biochim. Biophys. Acta*, **1792**, 444-453

Wu, J., Yan, L. -J. (2015) Streptozotocin-induced type I diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity, *Diabetes, Metab. Sindr. and Obesity: Targets Therap.,* **5**(8), 181-188

Xia, D., Liu, B., Xin, W., Liu, T., Sun, J., Liu, N., Qin, S., Du, Z. (2016) Protective effects of C-phycocyanil on alcohol-induced subacute liver injury in mice, *J. Appl. Phycol.*, **28**, 765-772