

Evaluation of Hypoglycemic Effect of Spirulina in Alloxan Induced Diabetic Mice

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Spirulina (SP) is a blue-green microalga used to treat and prevent cancer, arterial hypertension, obesity and dyslipidemia. The aim of this study consists in evaluating the antidiabetic effect of SP in alloxan - induced diabetic mice during a 28 - day study. SP has proved a significant antidiabetic effect, especially due to its strong antioxidant effects. Moreover, SP has also ameliorated the diabetic symptoms including polyphagia and polydipsia.

Keywords: Spirulina, antidiabetic effect, fasting blood sugar, alloxan

Diabetes mellitus (DM) is one of the most common chronic disease [1] being considered the third serious chronic illness after cardiovascular disease and cancer [2].

DM is characterized by an increased prevalence of death, being responsible for 4.6 million deaths each year [3]. In this regard, DM is considered one of the major social and economic burdens worldwide [4]. According to statistics more than 336 million people had diabetes in 2011, and their number is increasing. Furthermore, there is expected a number of 552 million of diabetic patients in 2030 [5].

Moreover, DM, due to its overproduction of reactive oxygen species, is also responsible for endothelial dysfunction being involved in atherosclerosis [6] and other severe complications including nephropathy, retinopathy, neuropathy, vasculopathy, cardiomyopathy, dermatopathy, psychological distress, encephalopathy, memory deficits and changes in intestinal flora [7].

It is known that more than 70,000 plant species are commonly used in folk medicine for their beneficial effects on health all over the world [7a] The active compounds from plants known for their hypoglycemic effect are: flavonoids, carotenoids, glycosides, alkaloids and terpenoids [8].

SP is a blue-green microalga [9], used in traditional medicine to treat and prevent various chronic diseases including cancer, arterial hypertension, obesity, dyslipidemia [10] due to its antioxidant, anticancer, anti-inflammatory, antiviral and antibacterial properties [11].

The aim of this study consisted in evaluating the antidiabetic effect of SP in alloxan-induced diabetic mice. There were also assessed body weight changes, food and water intake during the 28 - day study.

Experimental part

Materials and methods

SP was purchased as a green powder (Analysis Bulletin: 1543/2014) from FAVISAN Laboratories, Lugoj, Romania and alloxan powder was purchased from Sigma Aldrich, Germany. The fasting blood glucose (FBG) was measured using an Accu-Check Active Glucometer.

The study protocol was according to the Universal Declaration of Animal Rights from Paris (1978) and to the Declaration of Helsinki, the European Convention (ETS No. 123) amended in 1998 (ETS No. 170) and Council Directive 86/609/EEC on the protection of vertebrate animals used for experimental and other scientific purposes. The study protocol was approved by the Ethics Committee of the Faculty of Pharmacy, Victor Babes University of Medicine and Pharmacy, Timisoara.

This animal study was developed on healthy male SKH1 mice, 8 - 12 weeks old. The mice were housed at room temperature of 22°C (+3°C), humidity around 55% and artificial lighting (12 h light/12 h dark). During the experiment the mice were fed with special pellets and water *ad libitum*.

The mice were acclimated to the laboratory conditions for 5 days before the experiment and they have been marked for individual identification.

After an overnight fasting, the mice were intraperitoneally injected with alloxan (150mg/kg body weight) dissolved in saline solution (0.2 mL/mice). The mice with a blood glycemia of more than 200 mg/dL were included in the experimental study.

The mice were divided in three groups (n=10): a control group of healthy animals (group 1), a control group of diabetic animals (group 2) and one group of diabetic

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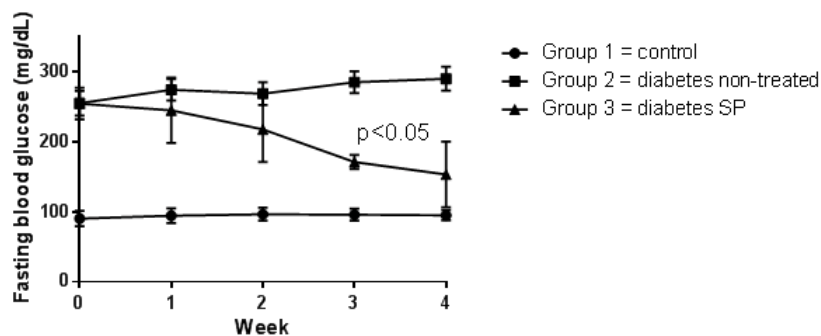


Fig 1 The fasting blood glucose assessment during the 28-days experimental study

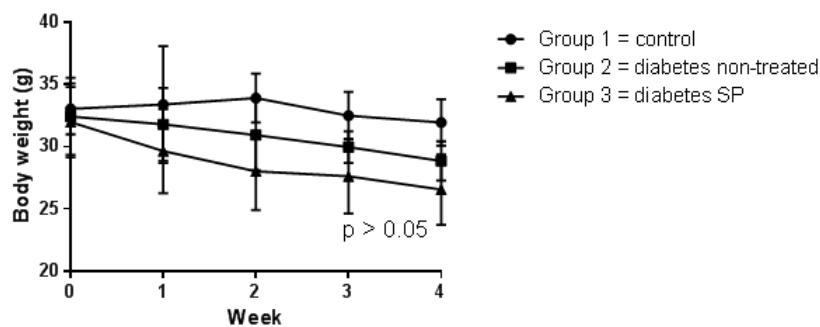


Fig 2 Mice body weight assessment during the 28-days experimental study

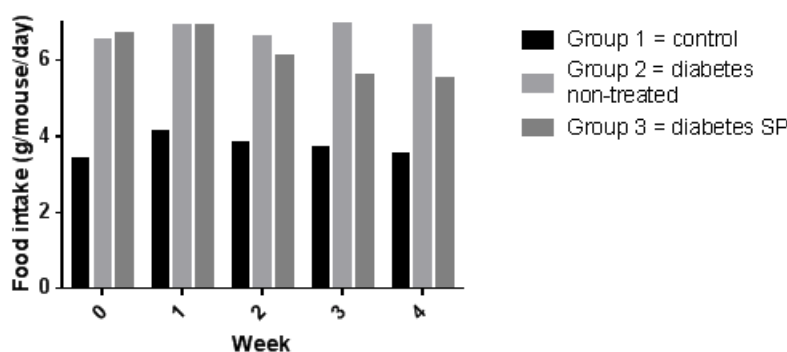


Fig 3 Food intake assessment during the 28-days experimental study

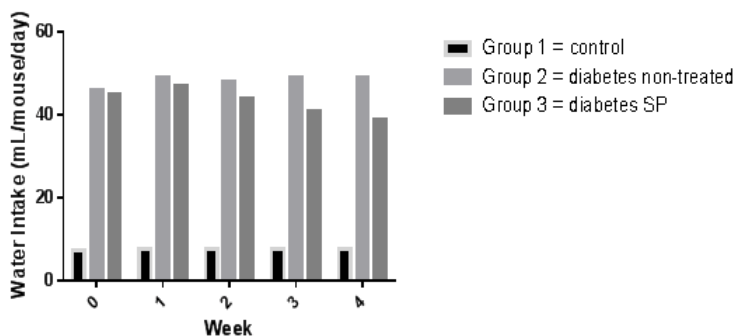


Fig. 4 Water intake assessment during the 28-days experimental study

animals treated with SP 100 mg/kg body weight/day (group 3). During the study period of 28 days, the body weight and FBG levels of the animals were checked out weekly. After one month, the animals were sacrificed (under anesthesia).

We calculated the average daily water and food intake for each mouse during the experiment.

Results and discussions

As it can be seen in the figure 1, the FBG levels of mice from groups 2 and 3 were significantly higher than in healthy control mice. These results have revealed that the alloxan-induced diabetes is a truthful experimental model of diabetes which carefully simulates the main characteristics of diabetes including hyperglycemia. The FBG levels of mice treated with SP were significant lower than in diabetic control group ($p=0.000$, $p<0.05$), but significant higher than in healthy control group ($p=0.000$, $p<0.05$). Moreover, the hypoglycemic effect of SP has proved to be time dependent.

According to the figure 2, a decrease in body weight was observed only in the two groups (groups 2 and 3) which received alloxan. The greatest weight loss was observed in group 3, a fact that suggests the role of SP in body weight control. The body weight loss observed in diabetic mice treated with SP was not statistically significant as compared to diabetic non-treated mice ($p = 0.118$, $p > 0.05$).

The water and food intake (fig. 3,4) have been significantly higher in diabetic mice (group 2) than in healthy ones (group 1), polydipsia and polyphagia being one of the major warning signs in DM. The water intake has been significantly lower in diabetic mice treated with SP, than in untreated diabetic mice ($p = 0.01$, $p < 0.05$), although the food intake did not show significant differences between the diabetic mice untreated and those treated with SP ($p = 0.07$, $p > 0.05$).

According to Kashif *et al.* the possible mechanism by which SP decreased the blood glucose concentration in diabetic rats, might be by stimulating the pancreatic β -cells secretion of insulin or by enhancing the transport of

blood glucose to the peripheral tissues [13]. It was previously demonstrated that SP may improve insulin resistance and the uptake of glucose [14]

Experimental studies on diabetic rats have also revealed that SP had improved the hexokinase activity, had decreased the glucose-6-phosphatase activity and it had a beneficial effect on plasma insulin and C-peptide [11b].

The crude, aqueous and ethanolic extracts of SP decreased glycosylated hemoglobin levels in diabetic animals probably by its rich iron content that contributed to the elevated levels of hemoglobin [13].

Another hypothesis to explain the antidiabetic effect of SP is related to its fiber composition that can reduce glucose absorption and also to the peptides and polypeptides that may stimulate insulin secretion [15].

Moreover, according to an experimental study in rats, SP had a beneficial effect on lowering serum glucose levels because of its rich content in PC which has been able to reduce the circulating glucose levels [16]

Moreover, the 5 - 10 % aqueous extracts of SP *maxima* revealed a multi-target effect through decreasing blood glucose, lipid profile levels (triglycerides, cholesterol and LDL, VLDL) and liver function markers (SGPT and SGOT) in diabetic rats after 30 days therapy [17]

Clinical evidence revealed that PC has decreased fasting blood glucose and glycosylated serum protein, being useful for diabetic patients [18].

Conclusions

In our study, SP has proved a significant antidiabetic effect in alloxan - induced diabetic mice. We emphasize that this effect was revealed to its high content of PC, beta-carotene and polyphenols, that are well recognized for their strong antioxidant effects. SP has also ameliorated the diabetic symptoms including polyphagia and polydipsia.

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Abbreviations

DM = diabetes mellitus
FBG = fasting blood glucose
PC = phycocyanin
SP = spirulina

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