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Research paper

The effect of the association of *Bifidobacterium breve* - *Spirulina platensis* on Diabetes Induced by Alloxan in Female Rats

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Abstract: Nutraceuticals and a functional foods-based diet have been suggested as a novel dietary approach for management of type 2 diabetes and its complications. In this context, both *Spirulina* and *Bifidobacterium breve* could have beneficial effects. We aimed to investigate the effect of the association between the *Spirulina* and *Bifidobacterium breve* on glucose level in diabetic rats. *Spirulina* and/or *Bifidobacterium breve* were administered after the induction of diabetes with alloxan in rats. Both *Bifidobacterium breve* and *Spirulina* decreased glucose levels in diabetic rats. We observed a synergistic effect of *Bifidobacterium breve –Spirulina* on in glucose control in diabetic rats. Despite more studies are needed in order to investigate the mechanisms that account the synergistic effect, our results suggest that the association of *Bifidobacterium breve – Spirulina* could be a promising functional food for diabetic patients.

Keywords: Diabetes, *Spirulina platensis*, *Bifidobacterium breve*, Alloxan, antidiabetic activity, nutritional Quality, Functional food,

Introduction:

Diabetes is a chronic metabolic disorder characterized by a persistent elevation of fasting blood glucose above 200 mg/dL, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (Tahrani et al., 2010; Mark et al., 2021). Different commercial drugs are frequently used to protect diabetic from the high sugar complications, but some of them are

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associated with considerable side effects, such as hypoglycemia, drug-resistance, dropsy, and weight gain (Tahrani et al., 2010; Mark et al., 2021). For this reason, the World Health Organization (WHO) has introduced a program for the use of traditional herbal medicines in the treatment of diabetes complications (WHO, 1980). In this context, nutraceuticals are interesting topics for research and development, and a functional foods-based diet has been suggested as a novel dietary approach for management of type 2 diabetes and its complications (Mirmiran et al., 2014).

Spirulina, a filamentous of blue-green (cyanobacteria) alga, recently attracted the interest of researchers as a one of the most promising sources of biological activity compounds (Vyacheslav et al. 2020). *Spirulina* is well known as a rich source of protein (60-70 g/100 g), vitamins, mainly vitamin B12 and pro-vitamin A, minerals and essential fatty acids (Masten Rutar et al., 2022). Due to its high nutritional values it has been successfully used as food supplement for undernourished children (Masten Rutar et al., 2022) and HIV infected subjects (Yamani et al., 2009).

In particular, *Spirulina* is an iron rich food so it is can be used to correct anaemia and weight loss in HIV-infected children, and even more quickly in HIV-negative undernourished children (Masten Rutar et al., 2022).

On the other hand, the omega-3 fatty acids *Spirulina* would prevent the accumulation of cholesterol in the body, as suggested by the results of (Ramamoorthy and Premakumari, 1996; Samuels et al., 2002; Al-Dhabi and Arasu, 2016). In fact recent results in HIV-infected subjects found that there is a significant increase in HDL-cholesterol and a significant decrease in total cholesterol, LDL-cholesterol and triglycerides in the group of patients who consumed *Spirulina* (Ngo-Matip et al., 2014).

In terms of quality of nutritional improvement for HIV-infected patients on *Spirulina* gave better results than on soya beans (Azabji-Kenfack et al., 2011). Also the insulin sensitivity in HIV patients was improved more when using *Spirulina* rather than soya bean as a nutritional supplement (Marcel et al., 2011).

Furthermore, *Spirulina* contains many different antioxidants including selenium, B -carotene and vitamin C, and the total antioxidant capacity of the serum in HIV patients treated with *Spirulina* was significantly different comparing to the decrease observed in the placebo group (Winter et al., 2014).

In this context, it is known that diabetes is associated with oxidative stress (Arif et al., 2010) and that probiotics inhibit oxidative stress via reducing inflammation and increasing antioxidant enzymes such as SOD and glutathione peroxidase (D'Souza et al., 2010). Besides, probiotics increase the antioxidant capacity of plasma, liver and intestines of animals, and decrease the malondialdehyde content in plasma (Uskova and Kravchenko, 2009). Furthermore, it has been recently reported that *Bifidobacterium breve* decreases serum levels of lipid and glucose and improve insulin resistance in high-fat diet-induced obese rats (Yu et al., 2013).

Spirulina treated rats were protected against the diabetogenic effect of alloxan which can cause severe necrosis of pancreatic β cells (Joventino et al., 2012), induced by oxidative stress.

In this study we aimed to investigate the effect of the association between the *Spirulina* and *Bifidobacterium breve* on glucose level after the induction of diabetes with alloxan in rats.

Material and Methods:

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The study has been made on White Wistar rats female (n=54), with 180 – 190 gr of body weight, these rats were provided by the Institut Pasteur of Algiers (breeding center Kouba -Algiers). All animals were exposed to an environment of 12 hour light: 12 hour dark period, at a room temperature between 23°C and 25°C for 2 weeks prior to the initiation of the experiment. Rats were maintained on a stock diet and tap water that were allowed ad libitum.

Rats fasted overnight for at least 8 hr. Hyperglycaemia was induced in each fasted rat by administering alloxan monohydrate (120 mg/ Kg body weight; intraperitoneal) in normal saline. At 72 hr post-induction of hyperglycaemia, blood glucose was assayed by the glucose oxidase method, using a glucometer On-Call-Plus ACON. The control group was administered normal saline intraperitoneally. Only those rats with established hyperglycaemia were included for subsequent treatment (Faidallah and Khan, 2012; Osman et al., 2012).

The rats were randomly divided into 9 groups (n = 6):

GI-Control group: Normal control animals without any treatment;

GII- Diabetic group: animals intraperitoneally injected with a single dose of 120 mg/kg b.w. of alloxan;

GIII- Treatment of diabetic rats: animals intraperitoneally injected with a single dose of 120 mg/kg b.w. of alloxan, receiving a prepared treatment:

GIIIa: 1mL inoculum *Bifidobacterium breve* in 9mL MRSc both (Man, Rogosa & Sharpe both + cysteine at 0.05%) per os for 7days;

GIIIb: 1mL inoculum *Bifidobacterium breve* in 9 mL MRSc both + 1mg/ml of *Spirulina* per os for 7 days;

GIIIc: 1mL inoculum *Bifidobacterium breve* in 9 mL MRSc both + 5mg/ml of *Spirulina* per os for 7 days;

GIIId: 1mL inoculum *Bifidobacterium breve* in 9 mL MRSc both + 10mg/ml of *Spirulina* per os for 7 days;

GIIIe: 1mg/mL of *Spirulina* in 9mL sterile distilled water per os 7days;

GIIIf: 5mg/mL of *Spirulina* in 9mL sterile distilled water per os 7days;

GIIIg: 10mg/mL of *Spirulina* in 9mL sterile distilled water per os 7days;

Blood glucose was assayed by the glucose oxidase method for 7 days (Osman et al. 2012).

Statistical analysis

The results were analyzed using analysis of variance (STATISTICA, 12). A p value < 0.05 was regarded as statistically significant.

Results:

Antidiabetic activity

The blood glucose responses after different treatments in alloxan-induced diabetic rats are presented in figures 1 and 2. Alloxan treated rats (GII) had significantly higher levels of glucose compared to controls (GI) ($p<0.001$). *Spirulina* 1mg/ml (GIIIe) and 5mg/ml (GIIIf) decreased glucose levels compared to GII from day 2, while at the higher dose (10mg/ml, GIIIg) glucose levels compared to GII from day 1 (Fig. 1A). In particular, glucose levels down to the levels of the control animals (GI) after 6, 5 and 2 days for the doses 1mg/ml, 5mg/ml and 10mg/ml, respectively (Fig. 1A). Therefore, *Spirulina* gave different results based on the concentration and the best effect was recorded in the GIIIg group (10mg/mL of *Spirulina*).

On the other hand *Bifidobacterium breve* (GIIIa) significantly decreased glucose levels at all time points compared to GII (Fig. 1B), but only after 6 days glucose levels were no different compared to controls (GI).

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The effect of the association of *Bifidobacterium breve- Spirulina* is described in figure 2. Despite the group treated with *Bifidobacterium breve* and 10mg/ml of *Spirulina* (GIIId) gave the best result compared to other treatment groups (Fig. 2C), the synergistic effect of the two nutraceuticals appeared at the low doses of *Spirulina* (Fig. 2A and B). In particular glucose levels after the association of *Bifidobacterium breve* with *Spirulina* 1gm/ml (GIIIb) became non significant versus control a day before than *Spirulina* 1gm/ml alone (GIIIe) (Fig. 2A). The synergistic effect was more evident with the dose of 5mg/ml of *Spirulina* (Fig. 2B). Glucose levels down to the levels of the control animals (GI) after 5 and 2 days for GIIIf and GIIIc, respectively (Fig. 2B).

Discussion and Conclusion:

Spirulina represents an important source of natural compounds for human nutrition, it is characterized by a high nutritional quality (Azabji-Kenfack et al., 2011) and could have a protective effect against diabetes (Narmadha et al., 2012).Our *Spirulina* has characteristic comparable with those previous reported (Clément, 1975; Dillon et al., 1995; Johnson-Delaney, 1996; Fox, 1999 and Pierlovisi, 2007).

The major protein constituents with significant beneficial health effects are the phycobiliproteins phycocyanin C and allophycocyanin. Phycocyanins constitute about 15-25% of the dry weight of the microalgae (Bermejo et al., 1997; Romay et al., 2003). Phycocyanins can be considered as a safe natural food colorant in non-acidic foodstuffs such as chewing gum, confectionaries and dairy products (Downham and Collins, 2000). In mice phycocyanin obtained from *Spirulina* led to the protection of pancreatic islets from alloxan injury, decreased fasting blood glucose, maintain total antioxidative capability and decreased malondialdehyde, cholesterol and triglycerides (Ou et al., 2012). In a previous study the treatment with *Spirulina* (25, 50 or 100 mg/kg, p.o.), started 48 h after the alloxan injection and continuing for 5 or 10 days, decreased glycemia after a 5-day treatment (Joventino et al., 2012). In our study lower doses of *Spirulina* (1mg/ml, 5mg/ml and 10mg/ml) decreased glucose levels. In particular, glucose levels down to the levels of the control animals after 6, 5 and 2 days for the doses 1mg/ml, 5mg/ml and 10mg/ml, respectively.

It has been recently reported that *Bifidobacterium breve* decreases serum levels of glucose in high-fat diet-induced obese rats (Yu et al., 2013). In our study, *Bifidobacterium breve* significantly decreased glucose levels at all time points compared to diabetics animals, but only after 6 days glucose levels were no different compared to controls.

For the first time we have evaluated the effect of the association of *Bifidobacterium breve* and *Spirulina* at different doses used on glucose levels by using the alloxan-induced diabetes models in Female Rats.

We observed a synergistic effect of *Bifidobacterium breve* and *Spirulina*. The synergistic effect was more evident with the dose of 5mg/ml of *Spirulina*. Glucose levels, after treatment with 5mg/ml of *Spirulina*, down to the levels of the control animals after 5 and 2 days in the absence and presence of for *Bifidobacterium breve*, respectively. The interaction between nutraceuticals is well known (Peluso et al., 2014). In this context, it has been reported that *Spirulina* promotes the growth of probiotics in vitro (Parada et al., 1998; Varga et al., 2002) and alters colonic microbiota in mice (Rasmussen et al., 2009). The effect could be due to the fatty acid

content. Fatty acids were identified in our alga *Spirulina*: Lauric acid (3.10%), Myristic acid (3.60%) Palmitic acid (42.79%), palmitoleic acid (0.52%) Stearic acid (1.81%), oleic acid (0.33%), linoleic acid (9.43%), Gamma linolenic acid (18.41%) and behenic acid (20.01%). In vitro studies reported that linoleic and gamma-linolenic acids could affect the growth and adhesion of probiotics (Laparra and Sanz 2010).

In conclusion, despite more studies are needed in order to investigate the mechanisms that account the synergistic effect of *Bifidobacterium breve –Spirulina* on in glucose control in diabetic rats, our results suggest that the association of *Bifidobacterium breve –Spirulina* could be a promising functional food for diabetic patients.

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Conflict of Interest:
The authors declare that they have no conflict of interest.

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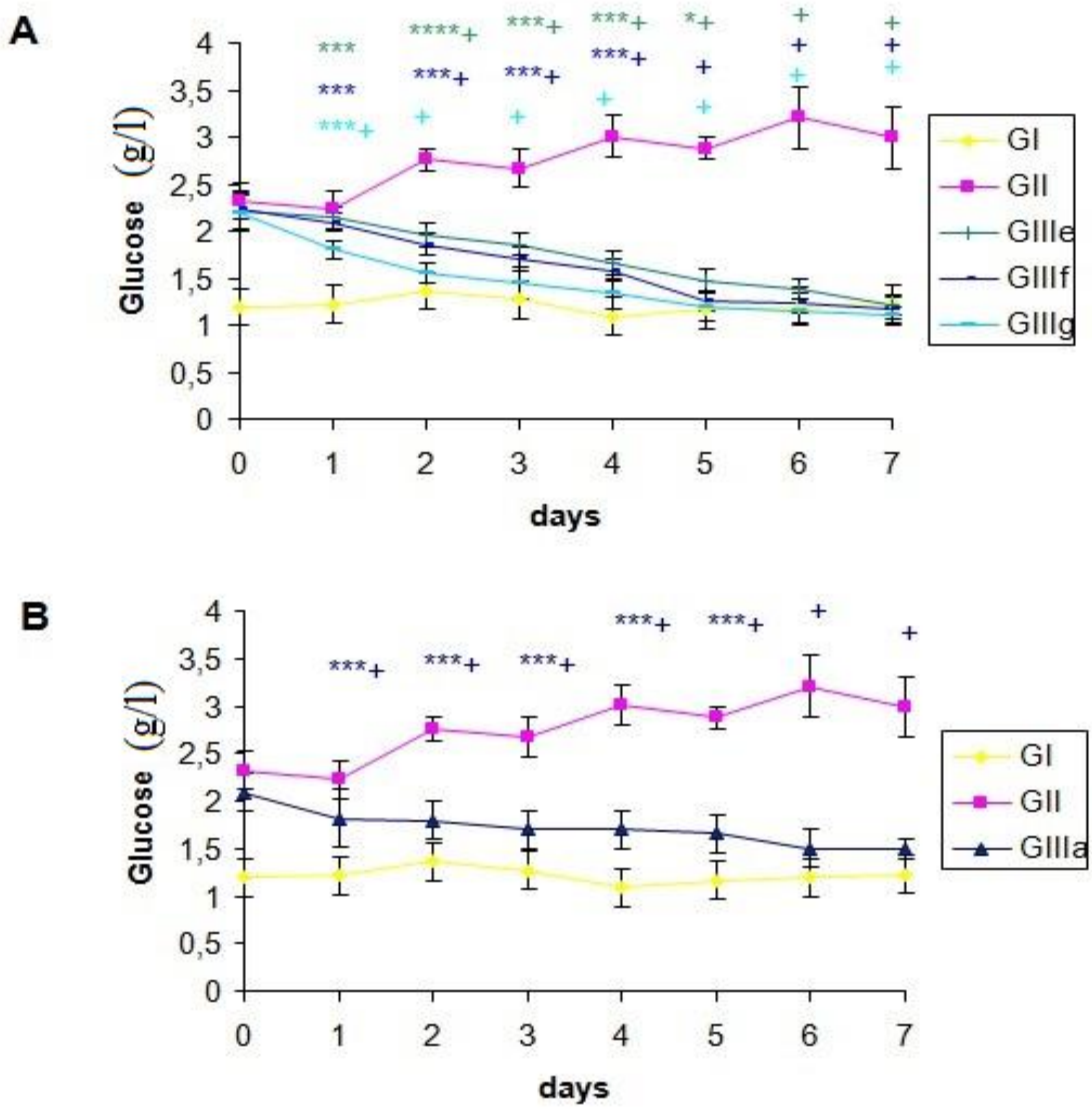

Fig 1. Effect of different doses of *Spirulina platensis* (**A)** and *Bifidobacterium breve* (**B)** on glucose levels in diabetic rats. GI-Control group, GII- Diabetic group, GIIIe: diabetic rats treated with 1mg/ml of *Spirulina platensis*; GIIIf: diabetic rats treated with 5mg/ml of *Spirulina platensis*; GIIIg: diabetic rats treated with 10mg/ml of *Spirulina platensis*; GIIIa: *Bifidobacterium breve*. *p<0.05 and ***p<0.001 versus GI; +p<0.001 versus GII.

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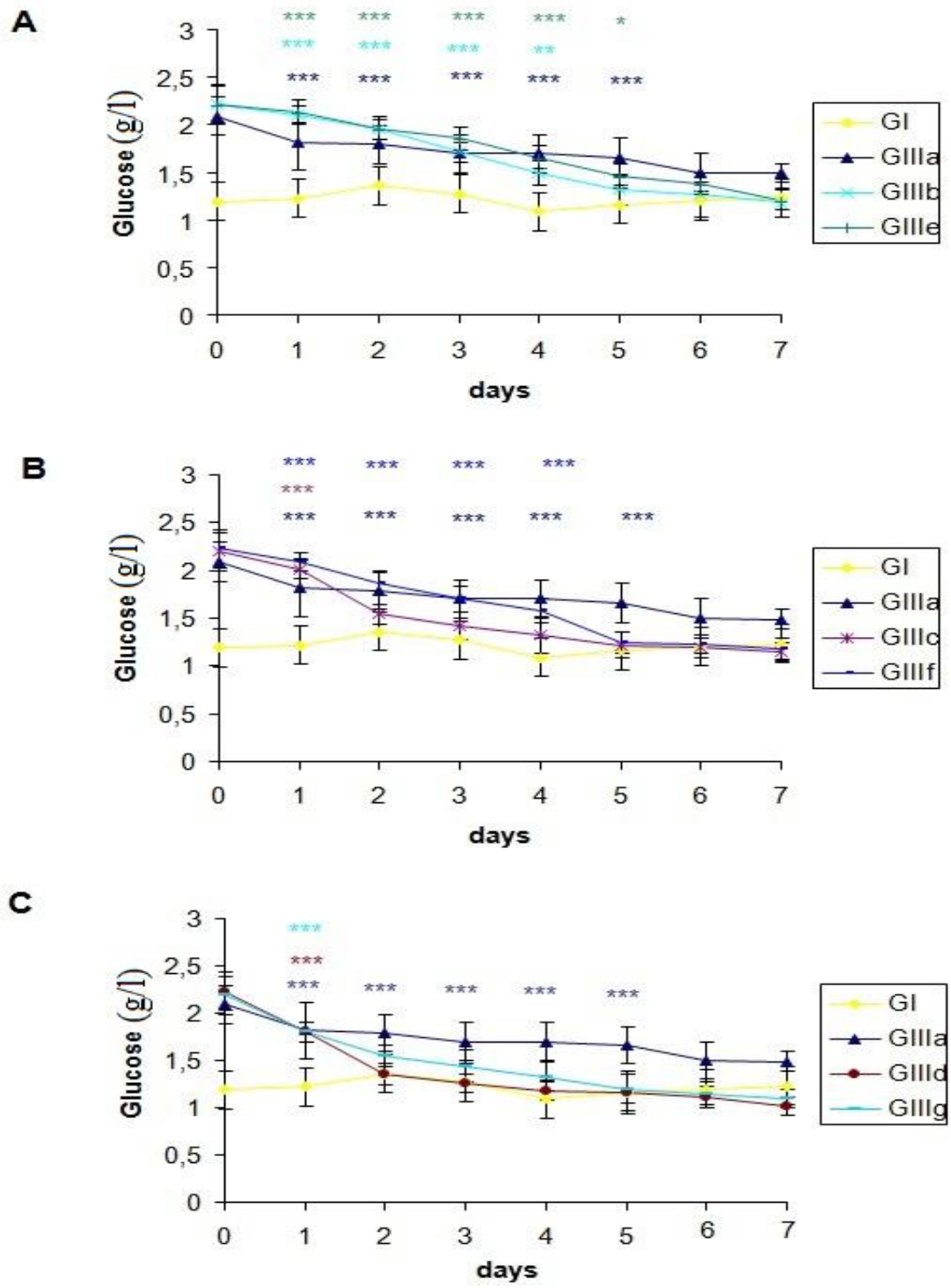

Fig 2. Effect of *Bifidobacterium breve* alone (**GIIIa)** or with 1mg/ml of *Spirulina platensis* (A, **GIIIb**); 5mg/ml of *Spirulina platensis* (B, **GIIIc**), or 10mg/ml of *Spirulina platensis* (C, **GIIId**) on glucose levels in diabetic rats. GI-Control group, GII- Diabetic group. *p<0.05 *p<0.01 *p<0.001