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In vivo study on the efficacy of hypoglycemic activity of *Spirulina plantesis* in long evan rats

Nurunnahar Fayzunnessa[1], M Alam Morshed[1,2,3*], Azim Uddin[3], Anzana Parvin[1], Rahman Saifur[1]

[1]*Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia 7003, Bangladesh.*

[2]*Department of Biochemistry and Cell Biology, Bangladesh Institute of Health Sciences (BIHS), Darus Salam, Dhaka 1216, Bangladesh*

[3]*Department of Pharmacy, North South University, Bashundhara, Dhaka 1229, Bangladesh*

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Abstract

The ethanol extract of *Spirulina platensis* was investigated for antihyperglycemic effects in Long Evans rats. Three tests were carried out to assess these activities. The extract caused a dose dependent inhibition of glucose absorption and showed hypoglycemic effects at rats weighing from 110 – 150 gram. The anti-diabetic effects were estimated by measuring the amount of glucose in the samples collected after the experiment. The extract at a dose level of 250mg/kg showed significant result ($p<0.05$) at 15 minutes and the dose level of 500mg/kg showed significant efficacy ($p<0.05$) at 10 and 15 minutes and the glucose absorption rates were 38.94±0.21, 34.99±1.91 and 40.86±0.07 respectively. The present study explored the extra pancreatic action of the plant in Long Evans rats. This study suggests that ethanol extract of *Spirulina platensis* has anti-diabetic effects in a dose dependant manner and these may be effective in the treatment of diabetes.

*Corresponding author: M Alam Morshed ✉ morshedbt@gmail.com

Introduction

Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and it is mainly linked with low blood insulin level or insensitivity of target organs to insulin. It is the most prevalent chronic disease in the world affecting nearly 25% of the population (Ghosh *et al*., 2001).

Hyperglycemia is an important character of diabetes mellitus, an endocrine disorder based disease. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus (Berger, 1985). Though pharmaceutic drugs like sulfonylureas and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications (Berger, 1985; Rang, 1991). In recent years, there has been renewed interest in plant medicine (Dubey, 1994; Prince, 1998; Ladeji, 2003) for the treatment against different diseases as herbal drugs are generally out of toxic effect (Geetha, 1994; Rao, 2003) reported from research work conducted on experimental model animal. Although in human, whether there is any toxic effect are not investigated, isolated studies screened various plants having "folk medicine reputation" by biochemical test for this for antidiabetogenic effect (Vats, 2002)

Spirulina is a microscopic blue-green aquatic plant and it is the nature's richest and most complete source of organic nutrition. The concentrated nutritional profile of *Spirulina* occurs naturally, so it is ideal for those preferring a whole food supplement to artificial nutrient sources. *Spirulina*, the blue-green alga, has a unique blend of nutrients that no single source can provide. It contains a wide spectrum of nutrients that include B-complex vitamins, minerals, good quality proteins, gamma-linolenic acid and the super antioxidants, beta-carotene, vitamin E and trace elements. *Spirulina* is fast emerging as a whole answer to the varied demands due to its impressive nutrient composition which can be used for therapeutic uses (Venkataraman, 1998).

Spirulina, a blue-green alga, is now becoming a health food worldwide. It is a multicellular, filamentous cyanobacterium belonging to algae of the class *Cyanophyta* (Anitha *et al*., 2006). The United Nations world food conference declared spirulina as "the best for tomorrow", and it is gaining popularity in recent years as a food supplement (Kapoor *et al*., 1993). The spirulina ability as a potent anti-viral (Hayashi *et al*., 1996; Hayashi *et al,* 1993; Patterson, 1993; Gustafson *et al*. 1989), anti–cancer (Babu *et al*. 1995; Lisheng *et al*. 1991; Qishen *et al*. 1988; Schwartz *et al*. 1986; Schwartz, 1988), hypocholesterolemic (Nakaya *et al*., 1988; Kato *et al*., 1984) and health improvement (Annapurna *et al*., 1991) agent is gaining attention as a nutraceutical and a source of potential pharmaceutical.

Diabetes mellitus, a metabolic disorder, is becoming a major health problem. Many groups have reported on the antidiabetic activity of *Spirulina platensis* (Anuradha *et al*., 2001; Anitha *et al*., 2006). Despite that, its hypoglycemic potential regarding to its mode of action remains largely untapped owing to the inadequate documentation and validation of its activity against known oral hypoglycemic drugs. In this regard the present study was undertaken to evaluate the hypoglycaemic activity of *Spirulina platensis* in the animal model. In the present study we explored the extra pancreatic action of the cyanobacterium in Long Evans rats.

Materials and methods

Plant materials and preparation of test samples

The powder of *Spirulina platensis* was collected from Science Laboratory, Dhaka, Bangladesh. The powder was then soaked in 80% ethanol. These suspensions were filtered with thin and clean cloth and then filtered by filter paper. The suspensions were evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 68^{o}C. In this case, 175mbar (to remove ethanol), 72mbar (to remove water) pressure

and 160rpm rotation speed were maintained constantly. Finally, freeze-drier (HETOSICC, Heto Lab Equipment, Denmark) was used to get complete extract from the gummy extract and preserved at +4°c.

Experimental animals

The study was conducted with adult (both sex) Long-Evans rats (weighing 110±150g). They were bred at the BIRDEM animal house and maintained at a constant room temperature of 22±5°C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure when animals were used after 12hrs fasting. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

Effect on sucrose absorption from gastrointestinal tract

Experiments were carried out on normal rats. Extracts of *Spirulina platensis* were fed to the rats by using a syringe (3ml) with a metallic tube that was smooth and curved at the end, which led the feed directly to the stomach. Rats were fasted for 12 h before receiving a 50% sucrose solution by gavage (2·5 g/kg/5ml body weight) with (for experimental) or without (for control) ethanolic extract of *Spirulina platensis* (0·5 g/kg body weight). Blood samples were collected by amputation of the tail tip under mild diethyl ether anesthesia (Mamun et al, 2001). Blood samples were collected at 30 min before sucrose load and at 30, 60, 180 and 360 min after sucrose administration to determine the glucose level. Finally rats were sacrificed to collect the gastrointestinal tract. The gastrointestinal tract was excised and divided into 6 segments: the stomach, the upper 20 cm, middle, and lower 20 cm of the small intestine, the cecum, and the large intestine. Each segment was washed out with ice-cold saline, acidified with H_2SO_4 and centrifuged at 3000 rpm (1000 g) for 10 min. The supernatant thus obtained was boiled for 2 h to hydrolyze the sucrose and then neutralized with NaOH. The blood glucose level and the amount of glucose liberated from residual sucrose in the gastrointestinal tract were measured. Then the gastrointestinal sucrose content was calculated from the amount of liberated glucose (Goto et al 1995). Glucose was measured by glucose-oxidase (GOD-PAP) method.

Effects on intestinal glucose absorption

An intestinal perfusion technique (Swintosky and Pogonowska-Wala, 1982) was used to study the effects of *Spirulina platensis* extracts on intestinal absorption of glucose in rats fasted for 36 hours and anesthetized with sodium pentobarbital (50 mg/kg). The plant extracts were added to a kreb's solution (g/L 1.02 $CaCl_2$, 7.37 NaCl, 0.20 KCl, 0.065 $NaH_2PO_4.6H_2O$, 0.6 $NaHCO_3$, pH 7.4), supplemented with glucose (54.0 g/L) and perfused at a perfusion rate of 0.5 mL/min for 30 min through the duodenum. The perfusate was collected from a catheter set at 40 cm. The system was set at a constant temperature of 37 °C. *Spirulina platensis* extracts were added to Kreb's solution to a final conc. of 5mg/ml and 10mg/ml, so that the amount of extract in the perfused intestine is equivalent to the dose of 250 g/kg and 500g/kg body weight respectively. The control group was perfused only with Kreb's buffer supplemented with glucose. The results were expressed as percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

Biochemical procedure

Serum glucose levels were estimated by glucose oxidase (GOD/POD) method (Sera Pak, USA). The absorbance was measured by microplate ELISA Reader (Bio-Tek EL-340, USA).

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science(SPSS) software

for windows version 17 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results were performed by using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for comparisons. The limit of significance was set at $p<0.05$.

Results

Effect on sucrose absorption from gastrointestinal tract

The six-segment study was performed to assess the amount of sucrose remaining in the GIT at six different positions. The various data for sucrose absorption in the gastrointestinal tract are presented in the Fig. 1. The amount sucrose unabsorbed in different GIT segments showed that in control rats vs. rats fed with 500mg/kg extract at 30, 60, 180, and 360 minutes in mmol/l were 0.120 vs. 0.149, 0.038 vs. 0.057, 0.003 vs. 0.003 and 0.004 vs. 0.085 respectively.

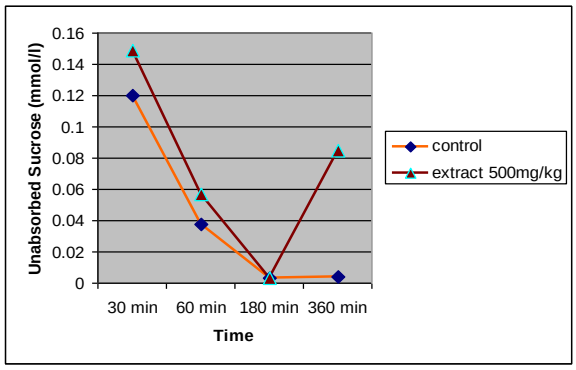

Fig. 1. Graph comparing the total sucrose content in the whole gastrointestinal tract at 30 minutes, 60 minutes, 180minutes, and 360minutes in a group of control rats vs. rats given a gavage with *Spirulina platensis* extract.

Effect on intestinal glucose absorption

As shown in Fig. 2, intestinal glucose absorption in non-diabetic control rats was gradually increased during 30 min of perfusion. Addition of *Spirulina platensis* to the glucose perfusate resulted an increase in intestinal glucose absorption at 10 min which were gradually decreased from 20 min to 30 min. The extract at a dose level of 250mg/kg showed significant result at 15 minutes ($p<0.05$) and the dose level of 500mg/kg showed significant efficacy at 10 and 15 minutes ($p<0.05$).

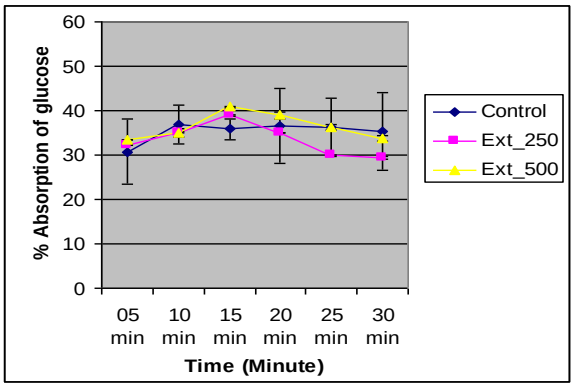

Fig. 2. The effect of *Spirulina platensis* on intestinal glucose absorption with respect to time compared to control rats.

Discussion

Hypoglycemic activity that is found when given with a simultaneous glucose load in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the gut by various mechanisms (Nahar *et al*., 2000; Vinik and Wing, 1990; Lempcke, 1987). It may be postulated that the plant extract might stimulate glycogenesis in the liver, which is enhanced by feeding (Creutzfeld et al., 1979).

One of the objectives of the present study was to investigate whether the antihyperglycemic effect is related to the inhibition of carbohydrate absorption in the gut. In order to confirm this hypothesis, we examined sucrose content in six segments of the rat gastrointestinal tract after simultaneous administration of sucrose. The extract of *Spirulina platensis* suppressed postprandial hyperglycemia after sucrose ingestion till 30 minutes in the stomach and upper 20cm of the small intestine and all throughout the small intestine till 60 minutes. The extract increased the residual sucrose content at these times after sucrose administration. After 180 min,

the concentration of sucrose detected throughout the gut indicated that most of the sucrose was absorbed. This result suggests that the extract can delay sucrose absorption without completely inhibiting it, which might be due to inhibition on rapid digestion and absorption of carbohydrate at the upper part of intestine and undigested carbohydrate is digested and absorbed after 180 min. These findings suggest that the reduction of hyperglycemia by the extract is, at least partly, related to the retardation of carbohydrate absorption in the gut. This was also confirmed in gut perfusion experiment, where the extract reduced intestinal glucose absorption, especially around 5 and 15 minutes, when the dose was administered at 250mg/kg body weight and around 5, 15, and 20 minutes, when the dose was administered at 500mg/kg body weight.

In conclusion, the present study demonstrated that the extract can delay sucrose absorption without completely inhibiting it in the intestine. The present study has evaluated potential anti-diabetic activity of *Spirulina platensis*

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