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Study on Hypoglycaemic Effect of *Spirulina platensis* on Long -Evans Rats

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Abstract

Spirulina containing high concentration of functional nutrients is emerging as an important therapeutic food. So the present study was designed to evaluate the hypoglycaemic properties of *Spirulina platensis* on long-evans rats. Two sets of experiment were conducted, the 1st set was compared with the effect of *Spirulina platensis*, glibenclamide, glucose and water after feeding 60 minutes. Glibenclamide was used as a standard reference drug. The findings clearly indicated that the oral administration of *Spirulina platensis* (150mg/kg b.w) significantly tended to reduce blood glucose level of rat than that of glibenclamide. In the 2nd experiment long-term hypoglycaemic effect of *Spirulina platensis* 0.5% and 2.5% fed with high fat high sugar containing diet and 150mg/kg b.w with normal laboratory diet was also observed. The 2nd findings also indicated that the blood glucose level was significantly decreased in both the diet when supplemented with *Spirulina platensis*.

Key words : Diabetic mellitus, High fat diet, Hypoglycaemic, *Spirulina platensis*, Body weight

Introduction

A number of plant species worldwide are known to have hypoglycaemic (Kar *et al.* 2006, Kumer *et al.* 2006) hypolipidaemic (Lemhadri *et al.* 2006, Kumari *et al.* 2006) or both activities (Sharma *et al.* 2003). Despite the presence of known anti diabetic medicines in the pharmaceutical market, screening for new anti diabetic sources from natural plants is still attractive because they contain substances that have an alternative and safe effect on diabetes mellitus (Jung Bong Ju *et al.* 2008). Plant products are frequently considered to be less toxic and more free from side effects than synthetic agents. These properties have led to the discovery of new therapeutic agents including antioxidants, hypoglycaemics and hypolipidemics (Bahramikia and Yazdanparast 2008). *Spirulina* a microscopic blue-green alga is now becoming a health food worldwide. Spirulina is rich in protein, vitamin, minerals and also has a galaxy of nutrients like linolinic acid, gamma linolinic acid, antioxidants balanced amino acids, fatty acid profile and trace elements etc. This concentrated nutritional profile of *Spirulina* occurs naturally, so it is ideal for those preferring a whole food supplement of artificial nutrient sources. Due to its impressive nutrient component it can be used for therapeutic uses (Venkataraman 1998). The United Nations World Food conference declared *Spirulina* as "the best food for tomorrow" and it is gaining popularity day by day because of their valuable food content. In recent years *Spirulina* became popular because of their meditative value used as not only food supplement also it has ability to potent anti-viral (Hayashi and Hayashi 1996, Patterson 1993), anti-cancer (Babu 1995, Schwartz and Shklar 1988), reduced arsenic accumulation (Sikder *et al.* 2000, Fariduddin and Misbahuddin, 2004), hypocholesterolemic (Nakaya *et al.* 1988, Kato *et al.* 1984, Devi and Venkataraman 1983) and health improvement agent leading attention as a source of potential therapeutic and pharmaceutic use world wide. *Spirulina* in the dose of 200 mg/kg body weight for 28 days decreased serum lipid levels in normal rats but not significantly (Begum *et al.* 1993). Administration of *Spirulina* in normal rats in a dose of 100 mg/kg body weight orally once daily for 21 consecutive days did not produce any significant change in serum cholesterol, triglyceride and HDL-chol levels except LDL-chol level which was reduced significantly (Jahan 1991).

Diabetes mellitus is a non-communicable disease, which is considered one of the five leading cause of death in the world. About 100 million people around the world have been diagnosed with diabetes and by the year 2010, it is projected that 215 million people will have the disease (Zimmet 1999). There are number of artificial drugs available in the market which are more safe and effective but long time use may

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cause many side effects. Recently, the search for appropriate hypoglycaemic agents has been focused on plants used in tradional medicine, partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs (Rates 2001). So people are trying to find out natural sources that are effective for reducing blood glucose level. The present study was undertaken to evaluate hypoglycaemic effect of Spirulina induced on long Evan-rats.

Materials and Methods

Plant material

Spirulina platensis was suppliedin a powdered form Bangladesh Council of Scientific and Industrial Research. The collected *Spirulina platensis* were dried by sunlight. The dried sample was kept at room temperature for future use, in BCSIR.

Animals

Thirty long-Evans rats of either sex, aged 3-4 months, weight 135 to 200 gm, supplied by Animal research house, IFST (BCSIR), Dhaka, were used in this experiment. They were kept in cages and maintained at room temperature under conditions of natural light and dark schedule.

Experimental design

Two sets of experiment were performed with S*pirulina platensis* powder : a) oral glucose test tolerance (OGTT) and b) long term hypoglycaemic effect of *Spirulina platensis* with high fat high sugar and normal diet containing food.

a) Oral glucose test tolerance

Dose

Four gm of powdered *Spirulina platensis* was weighed and suspended with distilled water to make up to 10 ml and shaken it thoroughly. Ten gm glucose was dissolved in distilled water to make net volume 5 ml.

One tablet of Glibenclamide (Daonil, glibenclamide tablet BP 5 mg, Hoeshst Marion Roussel Limited, Aventis), was dissolved in distilled water to make net volume 1 ml.

Experiment

The initial glucose level in blood of experimental rats was measured. Then administered water or glibenclamide or *Spirulina platensis* administered, using an intragastric tube (Oral administration) and waited 60 min. Glucose solution was administered by the same procedure. Finally the blood from rats was collected and glucose in blood measured by glucose measurement machine.

Measurement of body weight and diet intake

Before day of the experiment measurement of the body weight (b.w.) of rats was recorded and the animals were fasted overnight. Body weight and blood glucose levels also were measured at the beginning of the experiment.

Treatment of rats

The rats were divided into four groups, each group containing five rats: the first group of rats was control (group-01), the second group was standard (group-02), and the third group was low dose of *Spirulina platensis* (150mg/kg b.w.) (group-03) and the fourth was high dose of *Spirulina platensis* (350 mg/kg b.w.) (group-04).

Biostatistical analyses

Glucose in blood was assayed using the glucose measurement machine. Data were analyzed using student's t-test and $p<0.05$ was considered significant. Values are expressed as mean ± standard deviation (S.D) for five rats per group.

b) Long term hypoglycaemic effect of *Spirulina platensis*

Diet

Two types of diet were supplied to rats, firstly, high fat and high sugar diet and secondly, laboratory diet. High fat high sugar diet consisted to animal fat (10%), vegetable fat (10%), CMC (10%), starch (20%), sugar (50%) and vitamin were mixed (Table I). In laboratory diet, wheat mash (23%), rice polish (23%), oil cake (8.6%), dried fish (8.6%) flour (29%), oil (2.9%), salt (1.45%), molasses (2.9%) and vitamin (Table II) were to be comprised. In both the cases constituents were mixed thoroughly.

Measurement of body weight and diet intake

The body weights of rats were measured before starting each experiment and also after each week of the treatment. The diet intake of each rate was also recorded. Equal volumes of water fed everyday for the same period of time. Before setting the experiment the rats were fasted overnight.

Table I. High fat High sugar content diet

No.	Name of chemical	Control-01 (gm) gr-1	%	Diet-01(gm) gr-2	%	Diet-02(gm) gr-3	%
01	Casein	267	10	267	10	267	10
02	Starch	534	20	534	20	534	20
03	Sucrose	1334	50	1334	50	1334	50
04	Dalda	267	10	267	10	267	10
05	CMC	267	10	253	9.5	197	7.5
06	Vitamin mixture	27 ml	1	27 ml	1	27 ml	1
07	*Spirulina platensis*	nil	0	14	0.5	68	2.5

Table II. Laboratory diet

No.	Name of chemical	Control-02 gr-4	%	Diet-03 gr-5	%
01	Wheat mash	8 kg	23	8 kg	23
02	Rice polish	8 kg	23	8 kg	23
03	Oil cake	3 kg	8.6	3 kg	8.6
04	Dried fish	3 kg	8.6	3 kg	8.6
05	Flour	10 kg	29	10 kg	29
06	Oil	1L	2.8	1L	2.8
07	Salt	0.5 kg	1.4	0.5 kg	1.4
08	Molasses	1 kg	2.8	1 kg	2.8
09	Vitamin	200 ml	0.5	200 ml	0.5
10	*Spirulina platensis*	nil	0	150 mg/kg/b.w.	0.015

Treatment of rats

In the experiment, a total of 30 rats were used. These rats were divided into five groups, each consisting of six rats. The first group of rats was fed with high fat and high sucrose diet as the first control group (group-01). The second group of rats was fed with high fat and high sucrose diet with *Spirulina platensis* (0.5% of total diet) as the diet-01 (low dose) (group-02). The third group of rats was fed with high fat and high sucrose diet with *Spirulina platensis* (2.5% of total diet) as the diet-02 (high dose) (group-03). The forth group of rats was fed with laboratory diet as the control-02 (group-4). The fifth group of rats was fed with laboratory diet with *Spirulina platensis* (150mg/kg b.w.) (group-05). But equal volumes of water were given every day for the same period of time. After 28 days of treatment, the animals were deprived of food overnight and then sacrificed under diethyl ether anesthesia. All experiments were carried out according to the guidelines for the care and use of experimental animals and approved by BCSIR Laboratory, Dhaka. Blood samples were taken from the animals of all groups and then centrifuged at 4000 rpm for 10 min. Serums were separated from the blood samples and were stored at 2^0 C temperature for biochemical analyses.

Biochemical analyses

Glucose levels in serum were assayed using the commercial kits (CHRONOLAB AG Switzerland).

Statistical analyses

Data were analyzed using student's t-test and $p<0.05$ was considered significant. Values are expressed as mean ± standard deviation (S.D) for six rats per group.

Results and Discussion

a. Oral glucose test tolerance (OGTT)

Effects of *Spirulina platensis* on blood glucose level

Numerous studies demonstrated that *Spirulina platensis* effectively lowered the glucose levels of rats. The single dose response to normal long-Evans rats at 2 h after admin-

istration glibenclamide or *Spirulina platensis* and 1 h after administration glucose (2g/kg b.w.) were initially determined. In Table III, the *Spirulina platensis* effectively reduced rat's blood glucose level at 33% (150mg/kg b.w.) and at 22.11% (350 mg/kg b.w.) where glibenclamide reduced rat's blood glucose level at 21.34% (2mg/kg b.w.). Final glucose levels of all were compared with control.

Spirulina platensis of group-01 diet reduced 13.04% and 2.5% *Spirulina platensis* of group-02 diet reduced 15.97% of total glucose level (Table IV). Group-02 and group-03 were compared with group-01 (control-01). Another laboratory diet with *Spirulina platensis* reduced 21.47% of total blood glucose. Here group-05 was compared with group-04 (control-02) (Fig. 1).

Table III. Effects of *Spirulina platensis* (OGTT) on blood glucose level

	Initial (mg/dl)	Final (mg/dl)
Control (water)	78.17±8.02	166.9±44.95
Standard (glibeclamide, 2mg/kg b. w.)	82.80±18.07	131.27±36.75, (21.34%) ↓
Dose-01 (*Spirulina*, 150mg/kg b.w.)	84.72±8.68	111.81±15.46, (33%) ↓
Dose-02 (*Spirulina*, 3 50mg/kg b.w)	95.26±15.51	129.99±17.59, (22.11%) ↓

b. Long term hypoglycaemic effect *Spirulina platensis*

Effects of diets on body weight

The daily diet intakes of the rats were found to be decreased in group-01, group-02 and group-03 respectively from the 10th days, 14th days and 18th days of the experiment. High fat diet apparently caused an increased in the body weight of rats despite their lower food intake. The rats of group-01 became sick from the 20th day and group-02, group-03 became sick from 26th day and they had taken average 65gm diet per day per group. But the laboratory diet apparently increased the body weight of rats the group-04 and group-05 and also increased food uptake. They didn't become sick and they had taken average 110 gm diet per day per group.

Effect of *Spirulina platensis* (long term use) on blood glucose level

The long term use of *Spirulina platensis* to long-Evans rats were initially determined and found that the *Spirulina platensis* effectively reduced blood glucose level. 0.5%, The significant hypoglycaemic effect of *Spirulina platensis* was found to be more effective than glibenclamide in lowering the blood glucose level of rats. A clinical use of glibenclamide is known to lower the blood glucose level by stimulating β-cells to release more insulin in streptozotocin-induced diabetic rats (Frishman 1998). In the present study, the glibenclamide revealed a mild hypoglycaemic effect, while the *Spirulina* showed a significant effect in the long Evans rats. Therefore the mechanism of *Spirulina* is probably different from that of glibenclamide, which is an insulin independent mechanism. Hyperglycemia, the primary clinical manifestation in diabetes, is associated with the development of certain complication of diabetes (Brownlee *et al.* 1981). Streptozotocin causes a significant elevation in the level of blood glucose in rats. But the administration of *Spirulina platensis* at doses of 150 mg, 350 mg/kg b.w. to long-Evans rats caused a significant decrease in the blood glucose. Thus, *Spirulina platensis* has an antihyperglycaemic activity. Changes in body weight show that *Spirulina platensis* has a significant effect in controlling the loss of body weight, which is caused during diabetes. The activities

Table IV. Result of long term hypoglycaemic effect of *Spirulina platensis*. (Student's t-test analysis)

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	Group-01 (Control-01)	Group-2 (0.5% *Spirulina* with group-01)	Group-03 (2.5% *Spirulina* with group-01)	Group-04 (Control-02)	Group-05 (*Spirulina*, 150m g/kg bw)
Mean ±SD	91.90±13.15	79.91±15.93	77.22±5.26	98.13±8.86	76.66±14.52
SE		11.30	7.08		9.48
Tcal		1.06	2.07		2.26
Significance level		Significance	P<0.05		P<0.05

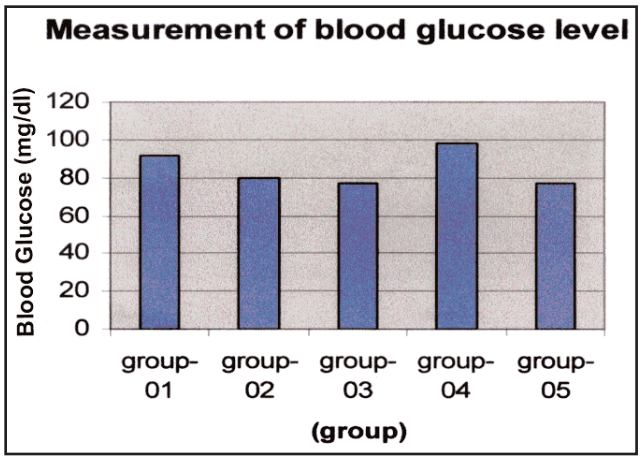

Fig. 1. Measurement of blood glucose level (long term use of *Spirulina platensis*)

were found to be dose dependent. The result suggested the presence of oral hypoglycaemic agents in this plant which may act by various mechanisms. The possible mechanism by which *Spirulina* brings about its anti hyperglycemic / hypoglycaemic action may be through potentiation of the pancreatic secretion of insulin from islet β-cell or due to enhanced transport of blood glucose to the peripheral tissue (Layam and Reddy 2007). They also mentioned that the antihyperglycaemic effect of *Spirulina* may be due to the down-regulation of NADPH and NADH, a cofactor in the fat metabolism. *Spirulina* also may be capable of oxidizing NADPH. One of the possible antihyperglycaemic actions of *Spirulina* may be due to its inhibition of endogenous synthesis of lipids. In this context, a number of other plants have also been reported to have an anti hyperglycaemic and insulin release stimulatory effect (Pari and Umamal swari 1999, Prince *et al.* 1998). Mani *et al*. (2000) and Parikh *et al.* (2001) suggested that the mechanisms of action include hypoglycemia caused by fiber content of possible insulin-stimulating action of peptides and poly peptides of *Spirulina proteins*.

Conclusion

The data of the present study clearly concluded that, Spirulina has anti-hyperglycemic effects in animal models with indictable side effects. So this hypoglycaemic effects of *Spirulina* may also solve the problem of human diabetes. However, further elaborate investigations are essential to establish on human trial to see the effect of *Spirulina* on diabetes patients. This may lead to help the isolation and structural elucidation of some of the bioactive constituents followed by establishing the most probable mechanism of action for each of the characterized compounds.

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