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Effect of *Spirulina platensis* on Lipid Profile of Long Evans Rats

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Abstract

To study the Effect of *Spirulina platensis* on lipid profile of Long Evan rats, an experiment was conducted by feeding the rats with diets supplemented with *Spirulina platensis* dry powder. Five different types of diets were included in the feeding. Group A containing high fat and high sugar considered as control-01. Group B with high fat, high sugar and 0.5% *Spirulina.* Group C with high fat, high sugar and 2.5% *Spirulina.* These three groups were included in Diet-1. Group D with laboratory diets considered as control-02 and Group E i.e., laboratory diet with *Spirulina* 150mg/kg/body weight. These two groups were included in Diet-2. This study describes the useful effect of *Spirulina* powder after administration in high fat and high sugar diet and in normal laboratory diet. *Spirulina platensis* powder showed an effective result in the lipid profile of Long Evan rats. Total cholesterol (TC) serum lipid significantly reduced in Group B than that of 2.5% of *Spirulina* powder in Group C rats. 0.5% and 2.5% *Spirulina* powder had the same effect in dropping low density lipoprotein (LDL) in both B and C Groups of rats. Conversely 2.5% *Spirulina* powder was found more effective in increasing high density lipoprotein (HDL) in Group C (50.54%) rats than the group B (24.18%) rats. On the other hand, the dose 150mg/kg b.w *Spirulina* powder mixed in laboratory diet showed an effective result in decreasing total cholesterol (TC) and low density lipoprotein (LDL) 19.21% and 46.06%, respectively. The findings clearly indicated that 150mg/kg b.w *Spirulina* powder had positive effect to increase the value of high density lipoprotein (HDL) in Group E (24.98%) rats compare to control group D rats. There were no significant differences in diet-1 groups' TC, TG and LDL-C of the groups of A (control- 01), B and C ($p<0.05$), although they varies statistically for HDL among these groups ($p>0.05$). On the other hand in diet-2 groups' TG, LDL and HDL of E groups of rats were significantly varies than control-2 groups of rats D ($p>0.05$), although TC was not significant statistically ($p<0.05$).

Keywords: High fat diet; Body weight (b.w); Hypercholesterolemia; Hypolipidaemic; *Spirulina platensis.*

Introduction

Hyperlipidaemia is considered a risk factor involved in the development of cardiovascular disease (Frishman, 1998; Smith *et al.,* 2004). The search for new drugs capable of reducing and regulating serum cholesterol and triglyceride levels has gained momentum over the years, resulting in numerous reports on significant activities of natural agents (Jahromi *et al.,* 1993). People are now well aware of the need to lower cholesterol levels in order to lower the risk of heart attacks and strokes. Besides dietary improvement, the search is underway to identify natural foods having a cholesterol reducing effect such as fish oil or oat bran. *Spirulina* is one of these foods (Nayaka *et al.*, 1988). *Spirulina* (family Oscillatoriaceae), a cyanobacteria, grows naturally in lakes and has been used for over a thousand years as a source of food because of its high protein content and good amino acid composition (Hayashi *et al.,* 1994). These blue green microalgae used in daily diets in natives in Africa and America have found to be a good source of carotenoids, micronutrients, tocopherols, beta-carotetene, polyunsaturated fatty acid in the form of linoleic acid and gamma-linoleic acid (GLA) and dietary fibre (Miranda *et al.,* 1998). It was shown that the IgA antibody level was significantly enhanced by *Spirulina* treatment (Hayashi *et al.,* 1998). *Spirulina* is commercially produced in some tropical and subtropical climatic regions of the world (Venkataraman and Becker, 1985, Henrikson, 1989, Bonnin, 1982). Commercial production of *Spirulina* have also been producing in Bangladesh (Jahan *et al.,* 1999). A number of clinical studies has been done by using *Spirulina* (Trisha, *et al.,* 2001). Researchers in West Germany had previously demonstrated cholesterol reduction in mice using diet supplemented with

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Spirulina (Devi and Venkataraman, 1983). The Japanese research showed lower cholesterol reduction was related to weight loss. *Spirulina* was chosen because it previously lowered serum cholesterol in rats (Devi and Venkataraman, 1983, Kato and Takemoto, 1984, Begum *et al.*1993). Some of the scientists have investigated the hypocholesterolaemic effects of *Spirulina*. Further studies are hereby necessary to reproduce the validity and consistency of hypolipidemic effects of *Spirulina*. On the basis of this information an attempt has been made to study the effect of *Spirulina* on lipid profile in Long Evan rats.

Materials and Methods

Long Evans rats were given various feeds supplemented with *Spirulina platensis* powder for 28 days as shown in Table I.

Table I. Types of feed given to different groups of rats. (Each group contains six rats)

No. of diets groups	No. of the treatments	Types of feed supplied	Spirulina platensis powder supplied
Diet-1	Group A (Control-01)	High fat and high sugar	nil
	Group B (low dose)	High fat and high sugar	0.5% Spirulina of total diet
	Group C (high dose)	High fat and high sugar	2.5% Spirulina of total diet
Diet-2	Group D (Control -02)	Laboratory Diet	nil
	Group E	Laboratory Diet	150mg/kg body weight.

Plant material

Spirulina platensis was supplied from Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka as a powder form. The collected *Spirulina* powder were dried up by sunlight and kept at a room temperature of about 25 °C for future use.

Animals

Thirty Long Evans rats of sex, aged 3-4 months, and weighed 135 to 200g were supplied by Institute of Food Science and Technology (IFST), Animal Research House, BCSIR, Dhaka, used in this study. They were kept in cages and maintained at 25°C room temperature under conditions of natural light and dark schedule.

Preparation of diet

Two types of diet were supplied to rats. First type included high fat and high sugar diet contained animal fat (10%), vegetable fat (10%), carboxy methyl cellulose (CMC) (10%), starch (20%), sugar (50%) and multi vitamin (1%) (ACI Limited) available in the market. Second type was laboratory diet containing wheat mash (23%), rice polish (23%), oil cake (8.6%), dried fish or fish meal (8.6%), wheat (29%), oil (2.9%), salt (1.45%), molasses (2.9%) and multi vitamin (0.5%) (ACI Limited) available in the market. In both the cases constituents were mixed thoroughly.

Experimental design

The experiment was carried out in 5 groups of rats supplemented with diets for 28 days. Each group contained six rats. The body weights (b.w) of the rats were measured before starting each experiment and also at weekly interval. Selected diet with or without *Spirulina* was given to each group of rats according to the group distribution of Table A.

The diet was given daily with equal volumes of water for the same period of time. The diet intake of each rat was also recorded. Initial diet for all groups was 15g/rat /day.

After 28 days of treatment, the animals were kept for fasting overnight and then anesthetized by diethyl ether for slaughter. The rat's necks were cut by spatula and blood samples were collected through the funnel in centrifuge tube without any anticoagulant. Then blood samples were centrifuged at 4000 rpm for 10 min. Serum was separated from the blood samples by 500µl micropipette and stored in refrigerator at 2^{o}C temperature for biochemical analyses. The experiment was carried out under the guidelines of IFST (Institute of Food Science & Technology), Animal Research House, BCSIR, Dhaka.

Biochemical analyses

TC (Total Cholesterol), TG (Triglyceride), HDL-C (High density lipoprotein- cholesterol), and LDL-C (Low density

lipoprotein-cholesterol) in serum were assayed by Spectrophotometer (Thermo Spectronic, England) and with the corresponding commercial kits (CHRONOLAB AG Switzerland).

Statistical analyses

The collected data were analyzed statistically and the mean, standard deviation and other calculations are evaluated considering 5% level of significance. For comparing means of different lipid profiles for diet-1, Analysis of Variance (ANOVA) and for comparing two groups means for diet-2, Student's t-test were used since sample size is small. All statistical analysis were done by a computer package programme called SPASS(Statistical Package for Social Science) of version 12, now frequently being used to analyze all sort of data and this version was developed by SPSS Inc.

Results and Discussion

Findings of the present investigation have been depicted in Fig.1 and Tables II-III.

Effect of diets on body weight

During the period of investigation it was found that feed in take in high fat and high sugar intake rats of group A, B and C decreased in from 10th, 14th and 18th days, respectively. Rats of group A became sick from 14th day and group B and C became sick from the day of 22nd. Amount of feed intake of these three groups rat had decreased from 15g to 11g/rat. Though the amount of feed intake reduced but body weight of the above groups' rats increased up to 7th day. In case of high fat and high sugar diet group A (control-01) rats weight was increased up to 14th and then decreased at 28th day. Rats of group B with Spirulina 0.5% also loose their weight

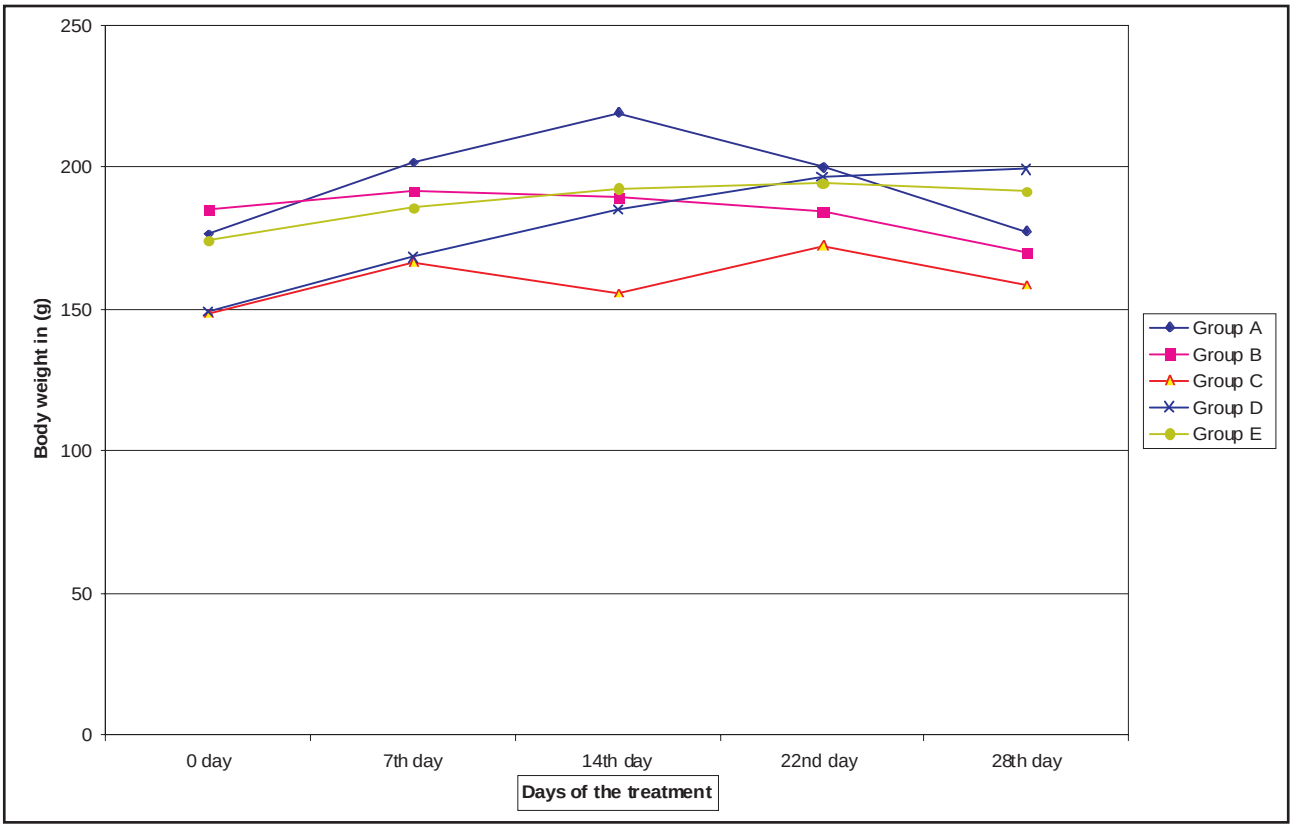

Fig. 1. Effect of diet on body weight after taking feed from 0-28 days. Values are expressed as mean of six rats per group

Table II. Total Lipid profile in Long Evan rats after feeding high fat and high sugar with different doses *Spirulina platensis*

Parameter	Group A (Control-01) (n= 6)	Group B (0.5% Spirulina of total diet) (n=6)	Group C (2.5% Spirulina of total diet) (n=6)	Significance Value (p value)
TC(mg/dl)	68.62±12.22	55.60±7.08 (18.97% ↓)	58.85±2.36(14.23% ↓)	0.040
TG (mg/dl)	43.84±7.10	68.18±22.45(55.52%↑)	54.36±9.85 (23.99 %↑)	0.038
LDL- C (mg/dl)	36.97±17.64	13.55±8.83 (63.34% ↓)	13.55±7.54 (63.34% ↓)	0.006
HDL-C (mg/dl)	22.87±12.03	28.40±7.94 (24.18% ↑)	34.43±8.3 (50.54% ↑)	0.149

but not like that of control group A. It was interestingly observed that rats of group C (with *Spirulina* 2.5%) loose their weight at 14th day and increased at 22th day, then again reduced at 28th day (Fig. 1). It was also observed that along with the reducing weight in group A rats, body hairs also dropped out after 14th day. Body hair of group B and C rats did not drop out though their weight reduced to some extents. On the other hand, 2nd type laboratory diet apparently increased the body weight of the group D (Control -02) and E. Rats of group E (with Spirulina 150mg/kg b.w) reduced minimum level at 28th day (Fig. 1). Feed intake of these groups' rat increased from 15g to 19g/rat. After increasing diet, the belonging groups did not become sick.

Table III. Total lipid profile in Long Evan rats after feeding Laboratory diet an Laboratory diet with *Spirulina platensis*

Parameter	Group D (control-02) (n=6)	Group E (Spirulina150mg/kg/body weight) . (n=6)	Significance Value (p value)
TC (mg/dl)	96.21 ±26.32	77.72± 6.44 (19.21%↓)	0.012
TG (mg/dl)	40.96± 5.51	42.57± 6.64 (3.93%↑)	0.235
LDL-C (mg/dl)	57.49 ±30.80	31.01± 18.89 (46.06%↓)	0.303
HDL-C (mg/dl)	30.54 ±12.00	38.17± 18.22 (24.98%↑)	0.323

Effects of diets on serum lipid profile

The effects of diets on serum lipids of rats of groups A, B and C have been summarized in Table I. The mean of TC in serum was reduced maximum in group B (55.60mg/dl) than in Group C (58.85 mg/dl) compared to control-01 group A (68.62mg/dl). TG of serum increased 68.18 mg/dl and 54.36 mg/dl in group B and group C rats respectively than control group A (43.84 mg/dl). LDL-C decreased 13.55mg/dl in group B and C uniformly in respect to control group A (36.97mg/dl). HDL-C of serum was increased accordingly in group C (34.43 mg/dl) and in group B (28.40 mg/dl) than control group A (22.87mg/dl). It was found that after administration of 0.5% and 2.5% Spirulina powder of total diet no statistical difference were found in rats of groups B, C than A in case of TC (p= 0.040), TG (p=0.038) and LDL (p=0.006), but was varied in HDL (p=0.149). From Table II it was evident that the mean of Group E having laboratory diet with *Spirulina* showed better result than Group D (control -02) with laboratory diet. TC reduced in group E (77.72 mg/dl) than in group D (96.21mg/dl). Consequently TG increased in group E (42.57 mg/dl) compare to group D (40.96 mg/dl). LDL-C decreased in group E (31.01mg/dl) compare to group D (57.49mg/dl) and HDL-C increased in group E (38.17mg/dl) than group D (30.54 mg/dl) rats.

The effect of 150mg/kg b.w *Spirulina* powder were found to be significant in TG (p= 0.235), LDL (p= 0.303) and HDL (p= 0.323) and was insignificant in TC (p= 0.012) rats of E. The Department of Internal Medicine of Tokai University, Japan conducted an experiment on thirty male employees with high cholesterol, mild hypertension and hyperlipidemia

(43.84 mg/dl) showed lower serum cholesterol, triglyceride and LDL (undesirable fat) after eating Spirulina for 8 weeks. The group consumed 4.2g (about 8 tablets) daily for a period of four weeks (from 244 to 233).

The other group stopped consumption of *Spirulina* after four weeks, and recorded a return of serum cholesterol to original level after initial decrease with the use. It was found that triglyceride (TG) decreased slightly and LDL cholesterol level reduced by a significant level of 6.1% within four weeks. (Nayaka, e*t al*, 1988). In the present study, the hyperlipidaemic activity of Spirulina was evaluated among the hypercholesterolaemic rats. Above findings are corresponding to the present study. Rats fed with 0.5% Spirulina were more effective in decreasing TC level and 2.5% positively effective in increasing HDL-C level. But effect of 0.5% and 2.5% *Spirulina* were same in dropping LDL-C level (Table - 01). In case of group D (control-02) with laboratory diet and group E having laboratory diet with *Spirulina* have also shown promising results on reducing cholesterol of serum (Table-02). TC, LDL-C and HDL-C decreased 19.21%, 46.06% and 24.98% respectively in Spirulina enriched laboratory feed than group D (control- 02). It was notified that TG of serum increased in group B (18.97%), group C (14.23%) and in group E (3.93%) than both control groups. It may be the presence of high fat and high sugar in diets that was responsible for the increase of TG in these groups. Further study on this aspect is solicited.

Conclusion

The present study indicated that the high concentration LDL-C in hypercholesterolaemic rats was significantly reduced by the administration of *Spirulina*. Therefore, *Spirulina* might constitute a good candidate for the treatment of atherosclerosis by lowering serum LDL-C level. In conclusion, the present study clearly specify the anti-hypercholesterolaemic and anti-hyperlipidaemic effects of *Spirulina* in animal models with undetected side effects. *Spirulina* have significant role on reducing the LDL/HDL ratio mainly through decreasing the serum level of LDL. This may lead to isolation and structure 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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