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EFFECT OF SUPPLEMENTATION OF SPIRULINA ON BLOOD GLUCOSE AND LIPID PROFILE OF THE NON-INSULIN DEPENDENT DIABETIC MALE SUBJECTS

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ABSTRACT

A sample of sixty male diabetic subjects (40-60 years) of MIG were selected from Punjab Agricultural University Hospital, Ludhiana and divided equally into three groups *viz*, E_1, E_2 and C. Spirulina supplementation was given daily in the form of SUNOVA capsules with two capsules (1g) and four capsules (2 g) to E_1 and E_2 group, respectively for a period of two months and C group was not given any supplementation. The impact of spirulina supplementation was studied individually on blood glucose and lipid profile of subjects before and after the study. It was observed that the mean energy intake decreased significantly (p=0.01) in group E_1 and E_2 after the supplementation. A non-significant decrease was observed in C group. The mean carbohydrate and protein intake also decreased significantly (p=0.01) in group E_2 while non-significantly in group E_1 and C. The present study indicated that the mean fasting and PP blood glucose level decreased significantly (p=0.01) i.e. from 178.4 and 242.7 g/dl to 149.3 and 212.4 g/dl, respectively in group E_1 and from 157.6 and 238.7 g/dl to 123.2 and 193.7 g/dl, respectively in group E_2, after the supplementation. However non-significant change was observed in blood glucose levels of the subjects of group C. A significant decrease was observed in TC, TG, LDL-C and VLDL-C levels in the E groups. Hence it is suggested that 2 g of spirulina supplementation can prove to be effective tool in management of diabetes with more favourable results.

INTRODUCTION

Diabetes is a major threat to global health that is rapidly getting worsened day by day. The biggest impact is on adults of working age in developing countries, worldwide. It is estimated that 3.2 million deaths are due to diabetes every year. Further, one in 20 deaths is attributed to diabetes; 8,700 deaths every day; 6 deaths every minute. At least one in 10 deaths among adults aged between 35 to 64 years old is due to diabetes. India had 23 million diabetic patients in the year 2000 and will rise to 57 million by the year 2025 (King, 2001). Diabetes mellitus, a complex metabolic disorder, has been associated with diet for centuries. Diabetic diet need not be a complete deviation from normal healthy diet. Normal Indian diet is composed of high carbohydrate and low fat, with carbohydrate providing 60-65 per cent and fat providing 15-25 per cent, and rest 15-20 per cent of the calories are supplied by protein. Daily intake of carbohydrate should be from 100 g to a maximum of 240-260g. The ill effects of inactivity, fast foods crept into our lives which are high in calories and fat. Psychosocial stress, changing life style and food habits, together lead to higher chances of developing obesity and diabetes. Further, obesity especially the central obesity has been strongly correlated with insulin resistance (Pradeepa *et al*, 2002). Spirulina, the green super food with its high concentration of functional nutrients is emerging as an important therapeutic food for diabetic patients in recent years. The pigment phycocyanin present in spirulina helps to strengthen the body's defence system and also reduces the blood sugar levels. Spirulina supplementation too had better control of glucose and lipid variables because it is the only vegetable source of vitamin B_{12}. It has been reported that for the conversion of linolenic acid to gamma linolenic acid (GLA), the enzyme delta 6-desaturase is required which is only present in spirulina. Further, the deficiency of GLA in the system resulted in the thickening of arteries, cholesterol accumulation. (Rao *et al* 2005).

Hence, the present study has been undertaken to find out effect of supplementation of spirulina on nutrient intake and blood parameters of the non-insulin dependent diabetic male subjects.

MATERIAL AND METHODS

Selection of the subjects : A sample of sixty male subjects aged 40-60 years belonging to middle income group, free from serious complications were selected from the OPD (out patient department) of the Hospital of Punjab Agricultural University, Ludhiana and equally divided into three groups, *viz.* E_1, E_2 and C. Collection of data pertaining to general and diabetic information was done through a questionnaire.

Dietary survey : Dietary intake of the subjects was recorded for three consecutive days by "24 hours recall-cum-weighment method", using standardized containers, of all the three groups before and after the study. The average daily nutrient intake of the diet was calculated by using MSU nutriguide computer programme (Song *et al* 1992). The average raw amounts in g of each and every item of food consumed for three consecutive days for each subject was fed in the hardware and nutritive value of diets were recorded.

Blood parameters : Blood analysis of the three groups were done for fasting blood sugar (FBS) and post-prandial blood sugar (PPBS), serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) before and after supplementation.

Supplementation of spirulina : Spirulina in the form of "SUNOVA spirulina" capsules were procured from market. As per composition each capsule contained 500 mg of spirulina, so 2 capsules were used to supplement 1 g of spirulina to E_1 and 4 capsules were used to supplement 2 g of spirulina to E_2, whereas subjects of group C were not given any supplementation. The supplementation was done for a period of 2 months. The subjects were advised to consume capsules before breakfast and lunch and were monitored regularly. All the subjects were also on oral hypoglycemic drugs as prescribed by the physician during the study period.

Statistical analysis : The data was analyzed with the help of various simple statistical tools such as mean, standard error (SE), percentages etc. Student 't' test was applied to test and their statistical significance was ascertained using a

Table 1. Diabetic information of the subjects (n = 20 each)

Particular	E_1	E_2	C
Age of onset (yrs) (Mean±SE)	46.3±1.7	45.95±1.6	47.6±1.5
Duration of diabetes (yrs) (Mean±SE)	8.37±1.5	7.89±1.3	6.32±1.1
Mode of onset			
Typical symptoms	14 (70)	19 (95)	14 (70)
Under physical stress	6 (30)	1 (5)	6 (30)
Medical problems diagnosed*			
Hypertension	6 (30)	8 (40)	8 (40)
Foot problems	–	–	2 (10)
Kidney	1 (5)	–	–
Vision	11 (55)	13 (65)	10 (50)
CAD	1 (5)	–	1 (5)
Skin problems	1 (5)	–	–

Figures in parenthesis are percentages

* Multiple responses

Microsoft Excel Computer Programme package on all the parameters.

RESULTS AND DISCUSSION

Diabetic information of the subjects : As per demographic information of the present study, the mean age of onset and duration of diabetes was 46.3±1.69, 45.95±1.58 and 47.6±1.49 yrs and 8.37±1.48, 7.89±1.33 and 6.32±1.06 yrs in E_1, E_2 and C groups, respectively. Majority of the subjects i.e. 55, 65 and 50 per cent had vision problems and 30, 40 and 40 per cent subjects had hypertension in the three groups, respectively. The most common symptoms observed in the three groups were tiredness, frequent urination, excessive thirst and frequent hunger. It was observed that the family history of the disease due to diabetic mother was more prevalent among the subjects as compared to diabetic father or both diabetic parents (Table 1).

Nutrient intake of the subjects : The average daily nutrient intake before and after the study period is given in Table 2.

Energy and protein : The initial mean energy intake of subjects decreased from 2001, 2231 and 2062 Kcal to 1867, 1922 and 2024 Kcal after study in E_1, E_2 and C group, respectively. It was observed that the mean energy intake decreased significantly (p=0.01) in E_1 and E_2 groups due to decreased intake of cereals, fat and sugar. Whereas, non-significant decrease in the energy intake was observed incase of C group after the study. The maximum decrease was observed in E_2 group. Kapoor (2001) reported a significant decrease in energy intake in diabetic subjects after intervention. The average daily intake of protein before and after the study was 68, 77 and 73 g and 65, 67 and 72 g in E_1, E_2 and C group, respectively. There was a non-significant decrease in the protein intake of the subjects of group E_1 and C and a significant decrease was observed (p=0.01) in the protein intake of the subjects of group E_2 after two months of the study. It was observed that initially protein intake in all the three groups was higher than the suggested intake of 65 g and after two

Table 2. Mean daily nutrient intake of the subjects (Mean±SE) (n = 20 each)

Food group	Initial	Final	Difference	t-value	Suggested intakes ++
Energy (k cal)					
E_1	2001	1867	-134	2.91**	1500
E_2	2231	1922	-309	8.11**	
C	2062	2024	-38	0.54 ^{NS}	
Carbohydrates (g)					
E_1	268±11.0	262±9.0	-6	0.77 ^{NS}	235
E_2	296±11.6	267±10.4	-29	2.83**	
C	283±8.5	284±6.5	1	-	
Protein (g)					
E_1	68±15.3	65±9.0	-3	1.34 ^{NS}	65
E_2	77±3.4	67±2.6	-10	3.82**	
C	73±3.0	72±1.8	-1	0.12 ^{NS}	
Total fat (g)					
E_1	73±6.0	62±3.0	-11	2.26*	34
E_2	82±4.8	65±2.5	-17	4.08**	
C	71±4.7	66±3.7	-5	0.96 ^{NS}	

Figures with different superscripts in a column differ significantly

++ Raghuram *et al* (1993) NS non significant NA data not available

** Significant at 1% * Significant at 5%

months protein intake was marginally adequate in E_1 and E_2 groups but higher in C group.

Carbohydrates : The mean daily intake of carbohydrates was 268, 296 and 283 and 262, 267 and 284 g/day before and after the study in E_1, E_2 and C group, respectively. There was a non-significant decrease in the intake of carbohydrates by the subjects of group E_1 and C and a significant decrease (p=0.01) in the carbohydrates intake in the subjects of group E_2 was observed after two months of the study. Further, it was observed that the carbohydrates intake in the three groups were higher than the suggested value of 235 g/day given by Raghuram *et al* (1993). Aggarwal (2003) reported a low carbohydrates intake in the elderly diabetic subjects. Diet plays an important role in both the development and control of diabetes. Wolever and Mehling (2002) reported that high-carbohydrate, low glycemic dietary advice, improved ß-cell function in subjects with impaired glucose tolerance (IGT).

Fats and oils : The daily intake of total fats and oils among the subjects of all the three groups was 73, 82 and 71 g/day before the study. The corresponding values after two months of the study were 62, 65 and 66 g/day in E_1, E_2 and C group, respectively. There was a significant decrease (p=0.05) in the intake of fats and oils by the subjects of group E_1 and a significant decrease (p=0.01) in the intake of fats and oils was observed in the subjects of group E_2. Whereas, incase of C group a non-significant decrease was observed after two months of the study. Wang *et al* (2003) reported that the incidence of diabetes was significantly and positively associated with proportion of total saturated fat in diet. The decreased fat intake in all the three groups could be attributed to knowledge gain via media, pamphlets and internet.

Per cent Contribution of Carbohydrates, Protein and Fat to the Total Energy Intake : The initial dietary carbohydrates intake contributed to 53.6, 53.1 and 54.9 per cent of total calories in E_1, E_2 and C group, respectively, while, the percentage increased to 56.1, 55.7 and 56.2 per cent in the three groups at the end of the study. Initially, the average contribution of dietary protein to total energy was 13.6, 13.8 and 14.2 per cent in E_1, E_2 and C group, respectively which increased significantly. Though, the total protein intake reduced after the study, but, the per cent contribution of protein to total energy significantly increased to 13.9 and 13.8 in E_1 and E_2 groups, respectively after the supplementation of spirulina. While, incase of C group corresponding values remained constant at end of study. The initial dietary total fat intake contributed to 32.8, 33.1 and 30.9 per cent of total calories in E_1, E_2 and C group, respectively, while, the percentage decreased to 29.9, 30.4 and 29.6 per cent in the three groups at the end of the study. The reduction in per cent contribution of carbohydrates, fat and protein to total energy intake in all the three groups could

Table 3. Mean fasting and post prandial blood glucose levels of the subjects before and after the study (Mean±SE) (n = 20 each)

Blood glucose (mg/dl)	Group	Initial	Final	Difference	t-value	Normal values++
Glucose (Fasting)	E_1	178.4±7.02	149.3±7.55	-29.1	9.59**	<120
	E_2	157.6±9.75	123.2±8.78	-34.4	6.81**	
	C	162.3±13.53	158.8±11.72	-3.5	0.68^{NS}	
Glucose (PP)	E_1	242.7±11.69	212.4±10.81	-30.3	7.68**	120-180
	E_2	238.7±15.29	193.7±13.47	-45	7.57**	
	C	272.5±22.06	260.6±19.04	-11.9	2.01^{NS}	

Figures with different superscripts in a column differ significantly

** Significant at 1% NS non significant ++ Raghuram *et al* (1993) be due to seasonal changes, information via media, internet, nutrition pamphlets and other such sources (Table 3).

Blood glucose levels : The mean fasting and post prandial blood glucose levels before and after supplementation are presented in Table 3. The mean initial fasting blood glucose levels of 178.4 and 157.6 mg/dl decreased significantly (p= 0.01) to 149.3 and 123.2 mg/dl in E_1 and E_2 group, respectively at the end of the supplementation. However, the mean fasting blood glucose levels of 162.3 mg/dl decreased non-significantly to 158.8 mg/dl in C group. The mean levels of fasting glucose were higher initially and after the supplementation in all the three groups, when compared to the normal range (80-115 mg/dl). A very noteworthy observation was seen among the subjects of group E_2, where 30 per cent of the subjects discontinued their medicine of diabetes while 10 per cent of the subjects decreased their dosage to half just after one month of spirulina supplementation. Similar findings were also reported by Mani *et al*, (2001) who reported reduction in fasting blood glucose levels after supplementation of 2 g of spirulina for a period of 4 months.

The mean post prandial blood glucose level (PPBS) was 242.7, 238.7 and 272.5 mg/dl before the study and it was observed that after the study the levels decreased to 212.4, 193.7 and 260.6 mg/dl in E_1, E_2 and C group, respectively. The PPBS glucose levels were non-significantly different among the three groups before the supplementation while, after two months of supplementation the values were significantly ($p \leq 0.01$) different among the experimental groups. Further, improvement in the PPBS values in E_1 and E_2 groups could be due to the effect of spirulina supplementation which was thought to make insulin work effectively. In another study conducted by Mani *et al*, (2000) reported that spirulina supplementation (2g) resulted in an appreciable lowering of fasting blood glucose and post-prandial (PP) blood glucose levels. Similar results were also observed by Anuradha and Vidya, (2000) who reported

Table 4. Lipid profile of the subjects before and after the study (n = 20 each)

Variable	Group	Initial	Final	Difference	t-value	Reference standard ++
TC (mg/dl)	E_1	218±7.08	196±5.88	-22	5.96**	< 200
	E_2	211.9±6.42	194.8±5.34	-17.1	4.58**	
	C	214.6±8.00	213.9±7.82	-0.7	0.16 NS	
TG (mg/dl)	E_1	192±7.24	171±5.66	-21	3.66**	< 150
	E_2	185.9±4.57	167.8±5.43	-18.1	3.97**	
	C	192.7±7.21	195.8±7.44	3.1	-	
LDL-C (mg/dl)	E_1	138.4±7.66	116.9±7.16	-21.5	5.51**	80-160
	E_2	134.1±5.71	117.7±4.52	-16.4	5.14**	
	C	135.6±8.05	133±7.64	-2.6	0.53 NS	
HDL-C (mg/dl)	E_1	41±1.43	44.6±2.31	3.6	-	40-70
	E_2	40.6±1.38	43.5±1.18	2.9	-	
	C	40.5±1.53	41.7±1.19	1.2	-	
VLDL (mg/dl)	E_1	38.3±1.45	34.2±1.13	-4.1	3.66**	< 40
	E_2	37.2±0.91	33.6±1.09	-3.6	3.97**	
	C	38.5±1.44	39.2±1.49	0.7	-	

Figures with different superscripts in a column differ significantly

++ Raghuram *et al* (1993) ** Significant at 1 % NS non significant hypoglycemic effect on NIDDM patients after supplementation of 4 g for 2 months.

Lipid Profile : Table 4 depicted various lipid levels of the subjects before and after the study. The mean initial levels of serum total cholesterol before the study were 218±7.08, 211.9±6.42 and 214.6±8.00 mg/dl in E_1, E_2 and C group, respectively. It was observed in the E groups that, the levels reduced statistically significant ($p \leq 0.01$) to 196±5.88, 194.8±5.34, respectively, while there was very marginal decrease in the C group. It was also observed that the mean values of serum total cholesterol were on higher side in three groups than the desirable values (<200 mg/dl) during the study period as suggested by Raghuram *et al.* (1993). It was reported in another study conducted earlier that spirulina had a direct availability of gamma linolenic acid (GLA), which was a unique feature and compensated for the metabolic inability of the individual cholesterol control by spirulina supplementation. Further, a study conducted by Nakaya *et al* reported decrease in serum cholesterol, triglycerides, and LDL levels after consuming 4.2g/day of spirulina for 8 weeks.

The present study indicated the mean triglycerides levels was 192±7.24, 185.9±4.57 and 192.7±7.21 mg/dl, in E_1, E_2 and C groups respectively in the beginning of study. However, after a period of two months of supplementation, the TG levels significantly (p£0.01) decreased to 171±5.66 & 167.8±5.43 in the subjects of E_1 and E_2 groups, respectively, on the contrary there was non-significant increase in the C group. It was also observed that the mean initial values of serum triglycerides were on higher side in three groups during the study period when compared to the desirable values (<150 mg/dl). It has been reported in the present study that reduction in triglycerides and total cholesterol could be due to the presence of Gamma linolenic acid (GLA) in the spirulina as it dissolved fat deposits and thus prevented heart problems.

Table 4 too depicted the LDL-cholesterol levels of the three groups as 138.4±7.66, 134.1±5.71 and 135.6±8.05 mg/dl, respectively. A significant ($p \leq 0.01$) decrease was observed in both experimental groups after supplementation. Further, the mean values of LDL-C were within the normal range (80 – 160 mg/dl) in three groups. The decrease in LDL-C in both experimental groups could be due to possible effects of spirulina supplementation. Similarly, Anuradha and Vidya (2000) reported a significant reduction in TC, TG, LDL-C and VLDL-C after supplementation of 4 g spirulina daily for 60 days. In another study, Ramamoorthy and Premakumari (1996) reported significant effect of spirulina supplementation of 4g/day for 3 months in reducing blood lipid profile.

The mean initial levels of VLDL-C before the study were 38.3±1.45, 37.2±0.91and 38.5±1.44 mg/dl in E_1, E_2 and C groups, respectively, whereas corresponding levels after the study were 34.2±1.13, 33.6±1.09and 39.2±1.49 mg/dl in all the three groups, respectively. A significant ($p \leq 0.01$) decrease was observed in VLDL-C levels in E_1 (38.3 to 34.2 mg/dl) and E_2 (37.2 to 33.6 mg/dl) after two months of supplementation, while the reduction in VLDL-C values in C group increased from 38.5 to 39.2 mg/dl though was non-significant. Further, it was observed that VLDL-C values of all the subjects were on the higher side of normal range i.e. 20 – 40 mg/dl before the study but reduced significantly at the end of study.

The data in Table 4 indicated the mean values of serum HDL-C were 41±1.43, 40.6±2.31 and 40.5±1.38 and 44.6±1.18, 43.5±1.53 and 41.7±1.19 mg/dl before and after the study in E_1, E_2 and C groups, respectively. It was observed that there was non-significant increase in all the three groups. Further, the levels of HDL-C were within the normal range of 40 – 70 mg/dl. A similar finding has also been reported the higher levels of HDL-C after taking spirulina supplementation.

The results of the present study inferred that there was a significant decrease in blood glucose levels and various lipid levels i.e. TC, TG LDL-C, and VLDL-C of both the experimental groups as compared to control group due to spirulina supplementation as it has hypoglycemic and hypolipidemic effects on non insulin dependent diabetics.

Coefficient of correlation (r) between different parameters :

Relationship of Nutrient Intake with Blood Glucose Levels : It was observed that energy intake was positively and significantly ($p \leq 0.05$) correlated to FBS (r=0.456) and PPBS (r=0.462). Protein intake was significantly ($p \leq 0.05$) correlated to FBS (r=0.445) and PPBS (r=0.472). Further, CHO intake was also positively and significantly correlated to FBS (r=0.472) and PPBS (r=0.443).

Relationship of Nutrient Intake with Lipid Profile : It was observed that energy intake was positively and significantly ($p \leq 0.05$) correlated to triglycerides (r=0.454), HDL-C (r=0.427), LDL-C (r=0.489) and VLDL-C (r=0.475). Whereas, it was significantly ($p \leq 0.01$) correlated to total cholesterol (r=0.570). A positive and significant ($p \leq 0.05$) correlation was observed between carbohydrates to triglycerides (r=0.456), HDL-C (r=0.459) and LDL-C (r=0.448). Further, carbohydrate intake was significantly ($p \leq 0.01$) correlated to VLDL-C (r=0.487) and total cholesterol (r=0.566). Total fat intake was positively and significantly ($p \leq 0.05$) correlated to LDL-C (r=0.482), VLDL-C (r=0.475) and total cholesterol (r=0.470). Whereas, it showed highly significant ($p \leq 0.01$) correlation with triglycerides (r=0.569).

CONCLUSION

In the light of above discussion, the scrutiny of data indicated that diabetic subjects definitely benefits from spirulina supplementation as it helps insulin to work efficiently, thereby improving glycemic control. Further, spirulina also increase insulin binding to cells, activates insulin receptor kinase leading to increased insulin sensitivity. So, the perusal of data clearly indicated that 2 g of spirulina supplementation is an effective measure to bring favourable and significant improvement in diabetic state as compared to 1 g dosage.

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