

AMELIORATIVE POTENTIAL OF AQUEOUS CELL EXTRACT OF *SPIRULINA PLATENSIS* ON DIABETES ASSOCIATED METABOLIC ALTERATIONS

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ABSTRACT

Oral administration of aqueous extract of *Spirulina platensis* cells to diabetic rats protected the rats from the alterations induced in carbohydrate and lipid metabolism. The increase in the glycosylated hemoglobin is an indicator of progression in diabetes. In addition during diabetes there is an increase in the cholesterol and triglyceride contents. The supplementation of *Spirulina* extract (90mg/kg bw) brought the levels of lipids to almost normal by exhibiting antihypoglycemic and antilipidemic properties. The decrease in HDL cholesterol in diabetic rats can be used as a marker in the assessing the severity of diabetes.

INTRODUCTION

Diabetes mellitus is found in all parts of the world and rapidly increasing worldwide. This disease is quite alarming in most of the developing countries including India. India has more than 40 million diabetic individuals which represents nearly 20 % of total diabetes population worldwide. Many of the currently available antidiabetic agents have number of adverse effects on the body (Jung *et al.*, 2006). Therefore, managing diabetes without any side effects is still a challenging task for health care providers (Saxena and Kishore, 2004). Hence, the search for more effective and safer hypoglycemic agents with lesser side effects has continued to be an important area of investigation. Numerous diabetes associated metabolic alterations are reported (Kostner and Karadi, 1998; Szalczky *et al.*, 1999; Chandalia and Lamda, 2002). Even though antidiabetic activity of crude extracts and purified active constituents of many plants are identified, studies related to the restorative activity of medicinal plants with reference to the diabetes associated altered metabolic functions are very scanty. Therefore in this investigation edible organism and protein rich in nature *Spirulina platensis* has been chosen to study the crude extract effect in the restoration of enzyme activities related to the carbohydrate metabolism in STZ-induced metabolic alterations in diabetic albino rats.

MATERIALS AND METHODS

Animals: Male albino rats (Wistar strain, weighing 150-200g)

were purchased from Tamil Nadu Veterinary Animal Science University, Madhavaram, Chennai and housed under standard husbandry conditions (30°C ± 2°C, 60-70 % relative humidity and 12hr day night cycle) and allowed standard pelleted rat feed and water *ad libitum*. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (Sri Venkateswara University, Tirupati).

Plant material and extract preparation

The cells of *Spirulina platensis* were harvested and after washing with 5 mM phosphate buffer then were suspended in the same buffer. The cells were broken down using ultrasonic disintegrator. Cell homogenate was centrifuged and the extract was concentrated under vacuum to get a solid powder.

Induction of diabetes mellitus in rats

Diabetes was developed by injecting Streptozotocin (STZ) (Sigma, USA) at a dose of 35 mg/kg body weight (b/w) in 0.1 M cold citrate buffer of pH 4.5, intraperitoneally. STZ injected animals exhibited severe glycosuria and hyperglycemia and rats were stabilized over a period of 7 days (Sarkar *et al.*, 1996). Diabetes was confirmed in the overnight fasted rats by measuring blood glucose concentration 96 hr after injection with STZ. The rats with blood glucose above 250 mg/dL were considered to be diabetic and used for the experiment. Control rats were given citrate buffer (pH 4.5).

Experimental design

Animals were divided into six groups of six animals each.

Group I served as a control: group II had normal + SP (50 mg/kg bw) rats; group III had normal + SP (90 mg/kg bw) and Group IV acts as diabetic control, V as diabetic + SP (50 mg/kg bw) and VI comprised the diabetic + SP (90 mg/kg bw) rats treated with *Spirulina platensis* aqueous extract 50 and 90 mg/kg bw/day respectively for 6 weeks, by oral incubation method. Rats were sacrificed at the end of 6 weeks and the blood samples were collected to analyze the effect of SP on biochemical parameters.

Collection and processing of blood for estimation of glucose and other biochemical parameters

Total hemoglobin was estimated by the cyanomethaemoglobin method (Drabkin and Austin, 1932) and glycosylated hemoglobin (HbA_{1c}) was estimated by the method (Nayak and Pattabiraman, 1981; Bannon, 1982). Serum total cholesterol, triglycerides and serum HDL-cholesterol were using commercial kits (Dialab, Austria).

Toxicity studies

The aqueous extract was administered orally to different groups of rats (n=6) in doses ranging from 100 mg-1g/kg of bw/day to 2-5g/kg of bw/day. The rats were observed for any lethal effects.

Table 1: Effect of SP - cell extract on hemoglobin (Hb), glycosylated hemoglobin (HbA_{1c}) and hepatic glycogen levels in control and STZ - diabetic rats

Groups	Body weight (g)	Glucose (mg/dL)	Hb(mg/dL)	HbA _{1c} (mg/g of Hb)
Normal	+ 21.0 ± 1.8	78 ± 6.9	13.1 ± 1.13	0.58 ± 0.03
Normal + SP (50 mg/kg bw)	+ 22 ± 1.8	75 ± 6.6	12.9 ± 0.97 ^b	0.51 ± 0.02 ^b
Normal + SP (90 mg/kg bw)	+ 20.1 ± 1.7	77 ± 7.1	13.0 ± 0.99 ^b	0.49 ± 0.03 ^b
Diabetic control	- 37.2 ± 3.4	235 ± 17.2	5.9 ± 0.39 ^a	1.01 ± 0.07 ^a
Diabetic + SP (50 mg/kg bw)	- 10.1 ± 0.8	109 ± 8.9	11.2 ± 0.98 ^b	0.42 ± 0.02 ^b
Diabetic + SP (90 mg/kg bw)	- 10.4 ± 0.8	105 ± 7.9	11.3 ± 0.99 ^b	0.45 ± 0.03 ^{ab}

Each value is mean ± SD for 6 rats in each group; a: p < 0.05 by comparison with normal rats; b: p < 0.05 by comparison with STZ diabetic rats. No significance.

Table 2: Effect of SP - cell extract on tissue total cholesterol and triglycerides levels in control and STZ - diabetic rats

Groups	Liver Total cholesterol (mg/g wet tissue)	Tri glycerides (mg/g wet tissue)
Normal	6.78 ± 0.52	6.12 ± 0.51
Normal + SP (50 mg/kg bw)	5.01 ± 0.49 ^b	6.08 ± 0.49 ^b
Normal + SP (90 mg/kg bw)	6.18 ± 0.61 ^b	5.62 ± 0.45 ^b
Diabetic control	13.92 ± 1.12 ^a	12.93 ± 1.12 ^a
Diabetic + SP (50 mg/kg bw)	7.45 ± 0.51 ^b	8.23 ± 0.64 ^b
Diabetic + SP (90 mg/kg bw)	7.88 ± 0.62 ^b	7.01 ± 0.62 ^b

Each value is mean ± SD for 6 rats in each group; a: p < 0.05 by comparison with normal rats; b: p < 0.05 by comparison with STZ diabetic rats. No significance.

Table 3: Effect of SP - cell extract on serum HDL, LDL and VLDL levels in control and STZ - diabetic rats

Groups	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	VLDL-cholesterol (mg/dL)
Normal	41.48 ± 3.12	21.88 ± 1.69	16.12 ± 1.58
Normal + SP (50 mg/kg bw)	43.42 ± 3.88 ^b	24.11 ± 1.77 ^b	18.12 ± 1.49 ^b
Normal + SP (90 mg/kg bw)	57.66 ± 4.12 ^b	25.12 ± 1.71 ^b	16.92 ± 1.11 ^b
Diabetic control	22.18 ± 1.72 ^a	74.66 ± 5.12 ^a	39.68 ± 3.01 ^a
Diabetic + SP (50 mg/kg bw)	40.29 ± 3.77 ^b	43.42 ± 3.66 ^b	29.68 ± 2.12 ^b
Diabetic + SP (90 mg/kg w)	34.76 ± 2.12 ^b	32.11 ± 2.21 ^b	25.66 ± 1.82 ^b

Each value is mean ± SD for 6 rats in each group; a: p < 0.05 by comparison with normal rats; b: p < 0.05 by comparison with STZ diabetic rats. : No significance.

Statistical analysis

Statistical analysis was performed using the SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMART). All the results were expressed as mean ± SD for six rats in each group and p < 0.05 was considered as significant.

RESULTS

The yield of aqueous extract of SP cells was found to be 5.2 % (w/v). The SP extract treated rats appeared as normal. No toxic effect was reported with the effective dose of aqueous extract and there were no death in all the groups. The application of aqueous extract of SP on the change of body weight, plasma glucose, hemoglobin and glycosylated hemoglobin is mentioned in Table 1. In diabetic rats there are significant decrease in the levels of glycogen and glycosylated hemoglobin was observed when compared to the untreated normal rats Oral administration of aqueous extract of SP significantly increased the levels of glycogen and restored the normal levels of glycosylated hemoglobin in diabetic treated rats. In Table 2 and 3 serum lipids of normal and diabetic rats

were mentioned. Total cholesterol, triglycerides and LDL cholesterol levels were significantly increased in diabetic rats with significant decrease of HDL cholesterol levels in comparison with untreated control rats. Oral administration of aqueous extract of SP showed significant effect in the restoration of the normal levels of above mentioned lipids. Thus SP aqueous extract is able to protect the system from diabetic induced damage by altering both carbohydrate and lipid metabolism.

DISCUSSION

The present investigation was to evaluate the efficiency of the aqueous extract of SP on STZ-induced metabolic changes in diabetic rats. Decreased Hb content observed in diabetic rats might be due to increased formation of glycosalated Hb.

Generally total hemoglobin levels is much below the normal levels in diabetic subject (Chandalia and Krishnaswamy, 2002) and HbA_{1c} levels has been reported to be increased in patients with diabetes mellitus (Paulsen, 1973). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA_{1c} (Koenig *et al.*, 1976). The levels of HbA_{1c} is always monitored as a reliable index of glycemic control in diabetes (Gabbay, 1976). Elevated levels of HbA_{1c} and reduced levels of Hb observed in our study reveals that diabetes animals had prior high blood glucose levels. Administration of aqueous extract of SP cell extract (50 mg/ Kgbw/day) had brought back the elevated HbA_{1c} levels to near normal levels. It has already been reported that decreased liver glycogen content was due to insulin deficiency and associated glycogenolysis process (Vats *et al.*, 2004). The possibility of restoration of glycogen content in STZ-induced diabetic rats by the administration of SP cell extract may be due to increased insulin secretion and reactivation of glycogen synthase enzyme system. Hypercholesterolemia and hypertriglyceridemia in STZ-induced diabetic rats are well documented Insulin deficiency leads to increased serum lipids because of increased lipolysis (Shirwaikar *et al.*, 2004). The elevated levels of serum total cholesterol, triglycerides and LDL cholesterol were significantly decreased after treatment with SP. Similar findings were also reported with the methanolic extract of the aqueous extract of SP. From this study it can be concluded that the administration of aqueous extract of SP cell is beneficial in normalizing the alterations in carbohydrate metabolism during diabetes.

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