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ANTIDIABETIC PROPERTY OF SPIRULINA

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SUMMARY

To evaluate the antidiabetic property of spirulina, experimentation was studied in an animal model before proceeding to clinical trials. The levels of blood glucose, plasma insulin and serum C-peptide, and activities of the glucose metabolizing enzymes hexokinase and glucose-6-phosphatase were estimated and studied in streptozotocin diabetic rats. The findings were compared between normal, diabetic and spirulina supplemented diabetic rats. The findings indicated that the administration of spirulina tended to bring the parameters significantly towards the normal. The effect of spirulina at a dose of 15 mg/kg body weight yielded a higher level of significance than the doses of 5 and 10 mg/kg body weight, therefore the former was used in further biochemical and clinical studies. The activity of hexokinase in the liver decreased markedly, while the activity of glucose-6-phosphatase increased significantly in diabetic control rats. Treatment with spirulina in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase activity.

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INTRODUCTION

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 anti-oxidants, 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 (1).

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*. 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 (2). The spirulina ability as a potent anti-viral (3-6), anti–cancer (7-11), hypo-cholesterolemic (12-17) and health improvement (18) agent is gaining attention as a nutraceutical and a source of potential pharmaceutical.

Diabetes mellitus, a metabolic disorder, is becoming a major health problem. Although there are a number of drugs available on the market, long time use may cause a number of side effects. Hence, a large number of studies are in progress to find natural sources, which are effective in reducing the intensity of diabetes. The present study was undertaken to evaluate the antidiabetic effect of spirulina on streptozotocin induced (45 mg/kg body weight) diabetes in male albino Wistar rats. Blood glucose levels were elevated in diabetic rats. The levels of blood glucose, plasma insulin and serum C-peptide, and activities of the glucose metabolizing enzymes hexokinase and glucose-6-phosphatase were estimated using standard protocols. Oral administration of spirulina was carried out for 45 days. Findings are presented in tables.

MATERIALS AND METHODS

To demonstrate the antidiabetic property of spirulina and its effect on blood glucose levels, male albino Wistar rats aged seven to eight weeks (180-200 g) were used. A freshly prepared solution of streptozotocin (45 mg/kg i.p.) in 0.1 M citrate buffer, pH 4.5, was injected intraperitoneally. After 48 hours of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with a blood glucose of 200-300 mg/dL) were taken for the experiment.

In the experiment, a total of 36 rats (30 diabetic surviving rats and six normal rats) were used. The rats were divided into six groups of six rats each: group 1, normal untreated rats; group 2, diabetic control rats; group 3, diabetic rats given spirulina (5 mg/kg body weight); group 4, diabetic rats given spirulina (10 mg/kg body weight); group 5, diabetic rats given spirulina (15 mg/kg body weight); and group 6, diabetic rats given glibenclamide (600 µg/kg body weight).

The experimentation was carried out for 45 days, with oral administration of spirulina. At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes, i.e. one with anticoagulant, potassium oxalate and sodium fluoride for plasma, and another without anticoagulant for serum separation. Serum was separated by centrifugation. Liver was immediately dissected, washed in ice cold saline, patted dry and weighed. Fasting blood glucose was estimated by the kit method. Plasma insulin level was assayed by the radio-immunoassay method. C-peptide level was assayed by the chemiluminescence immunoassay method. Hexokinase and glucose-6-phosphatase were assayed by standard protocols (19,20).

RESULTS AND DISCUSSION

Table 1 shows the levels of blood glucose, plasma insulin, C-peptide and total hemoglobin, and changes in body weight in normal and experimental rats. There was a significant increase in blood glucose levels, whereas plasma insulin, C-peptide and total

Table 1**. Blood glucose, plasma insulin, total hemoglobin, C-peptide and body weight in normal and experimental animals**

No.	Group	Body weight (g)		Fasting blood glucose (mg/dL)	Plasma insulin (µ/mL)	C-peptide (ng/mL)	Hemoglobin (g/dL)
		Initial	*Final*				
1	Normal	196.33±7.42	203.67±3.55	92.67^{a}±3.01	15.33^{a}±0.81	1.5^{a}±0.32	11.75^{a}±0.52
2	Diabetic control	197.67±10.48	158.5±4.63	232.33^{b}±4.84	4.98^{b}±0.50	0.50^{b}±0.12	5.88^{b}±0.38
3	Diabetic + spirulina 5 mg/kg	192±2.85	197.17±11.89	220.8^{b}±12.30	4.93^{b}±0.30	0.73^{b}±0.26	6.78^{c}±0.49
4	Diabetic + spirulina 10 mg/kg	196.5±13.12	209.67±6.65	159.5^{c}±10.87	6.99^{c}±0.43	1.10^{c}±0.20	9.41^{d}±0.86
5	Diabetic + spirulina 15 mg/kg	202.67±12.97	213.5±8.57	114.0^{a,d}±7.15	14.11^{d}±0.44	1.42^{d}±0.15	11.53^{e}±0.70
6	Diabetic + glibenclamide 600 µg/kg	194.83±7.65	205.33±8.43	123.5^{d}±7.42	12.78^{e}±0.30	1.36^{e}±0.33	10.29^{d}±0.85

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

hemoglobin levels decreased significantly in streptozotocin diabetic rats when compared with normal rats. The administration of spirulina and glibenclamide tended to bring the parameters significantly towards the normal. The effect of spirulina at a dose of 15 mg/kg body yielded a higher level of significance than the doses of 5 and 10 mg/kg body weight, therefore the former was used in further biochemical studies.

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (21). Intraperitoneal administration of streptozotocin (45 mg/kg) effectively induced diabetes in normal rats, as reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and body weight loss when compared with normal rats (22). In the present study, it was observed and demonstrated that oral administration of spirulina could reverse the above mentioned diabetic effects. The possible mechanism by which spirulina brings about its antihyperglycemic 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. This was clearly demonstrated by the increased levels of insulin and C-peptide in diabetic rats treated with spirulina. In this context, a number of other products have also been reported to have an antihyperglycemic and insulin-release stimulatory effect (23,24).

It is also evident from Table 1 that there was a decrease in total hemoglobin from normal to diabetic control albino rats, and this may be due to the formation of glycosylated hemoglobin. The increase in the level of hemoglobin in animals given spirulina may have been due to the decreased level of blood glucose, that would automatically lead to a decrease in glycosylated hemoglobin. Another reason might be that spirulina, which is a rich source of iron, contributed to the elevated levels of hemoglobin. The administration of spirulina to streptozotocin dosed animals reversed their weight loss. The ability of spirulina to recover body weight loss seems to be due to its antihyperglycemic effect.

The activities of carbohydrate enzymes are presented in Table 2. The activity of hexokinase in liver decreased markedly, whereas the activity of glucose-6-phosphatase increased significantly in diabetic control rats. Treatment with spirulina in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase activity.

Table 2. **Changes in hexokinase and glucose-6-phosphatase activity in the liver of normal and experimental animals**

No.	Group	Hexokinase (units[a]/g protein)	Glucose-6-phosphatase (units[b]/mg protein)
1	Normal	148.76 ± 6.09[a]	0.158 ± 0.012[a]
2	Diabetic control	109.68 ± 5.74[b]	0.232 ± 0.013[b]
3	Diabetic + spirulina 15 mg/kg	130.72 ± 7.44[c]	0.176 ± 0.010[a,c]
4	Diabetic + glibenclamide 600 µg/kg	125.30 ± 6.30[c]	0.190 ± 0.007[c]

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT); [a]µmoles of glucose phosphorylated/min; [b]µmoles of Pi liberated/min

The antihyperglycemic effect of spirulina may be due to the down-regulation of NADPH and NADH, a cofactor in the fat metabolism. The higher activity of glucose-6-phosphatase provides H^+, which binds with $NADP^+$ in the form of NADPH and is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, the pentose phosphate pathway still remains active in the liver to break down glucose that continuously provides NADPH, which converts acetyl radicals into long chain fatty acid chains. Spirulina may be capable of oxidizing NADPH. The enhanced hexokinase activity in spirulina treated rats suggests a greater uptake of glucose from blood by liver cells. The activities of enzymes suggest that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilization of glucose at peripheral sites. One of the possible actions of spirulina may be due to its inhibition of endogenous synthesis of lipids.

The decreased activity of glucose-6-phosphatase through pentose phosphate shunt results in a high reduced glutathione to oxidized glutathione ratio

(GSH/GSSG), which is coupled with conversion of NADPH to NADP (25). Spirulina may produce high $NADP^+$, which results in down regulation of lipogenesis and lower risk of the tissues for oxidation stress and high resistance for diabetes.

Accordingly, it can be concluded that spirulina has a beneficial effect on plasma insulin, C-peptide and hexokinase activity. Moreover, its antihyperglycemic effect in clinical trials (26-30) could represent a protective mechanism against the development of atherosclerosis and to maintain euglycemia.

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