HYPOGLYCAEMIC ACTIVITY OF MARINE CYANOBACTERIA IN ALLOXAN INDUCED DIABETIC RATS

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Summary

The present study was designed to investigate the hypoglycemic effect of 80% ethanol extracts from six marine cyanobacteria such as *Chroococcus minor, Synchocystis pevalakii, Phormidium corium, Spirulina platensis, Oscillatoria chalybea, and Spirulina labrynthiformis.* Among the six marine cyanobacterial species, the *Spirulina platensis* showed a highly hypoglycemic effect against the alloxan induced diabetes mellitus rat. Orally administrated the ethanolic extract of *Spirulina platensis* (2.5mg/kg of body weight) to the normal and alloxan induced diabetic rats for 30 days. After 30th day, the experimental rats were sacrificed and analyzed some biochemical investigations. The blood glucose level, cholesterol, SGOT, SGPT, ACP, and ALP were significantly reduced whereas, increase in the level of plasma protein and liver glycogen. These results suggest that the *Spirulina platensis* ethanol extract has antihyperglycaemic activity and prevent diabetes mellitus in experimental animals.

Keywords: Marine Cyanobacteria, *Spirulina platensis*, Diabetes mellitus, Blood glucose, Hypoglycemic

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Introduction

Diabetes mellitus is becoming a serious threat to the health of mankind and third killer of the human beings after cancer, cardiovascular and cerebrovascular diseases (1). It alters the carbohydrate, lipid and protein metabolism (2). It increases the risk of several macro and micro vascular complications such as hypertension, nephropathy, retinopathy, cardiomyopathy, and coronary heart diseases (3). Patients, who have achieved good glycemic control with diet and exercise, usually show a significant improvement. When a patient does not show reasonable improvement with diet and exercise, pharmacotherapy should be added to treatment plan. In modern medicine, no satisfactory effective therapy is till available to cure the diabetes mellitus (4). Though many new oral hypoglycemic drugs are now available in commercial market, there is difficulty of choosing right medication for a longer period either because of their side effects or its effectiveness. Hence, the patients are looking for an alternate treatment through Folk medicine, Siddha, Ayurvedha and Unani traditional systems of medicines for antidiabetic.

In recent years, there has been renewed interest in plant medicine (5) for antidiabetic (6). Many indigenous Indian medicinal plants have been found to be successfully used to manage diabetes (7). Since most of the terrestrial plants are screened for antidiabetic, next best alternate source is marine cyanobacteria. The marine cyanobacteria has many potential pharmaceutical activity and they produce novel and biologically active natural products such as acetogenins, bromophenols, fattyacids, terpenes, sterols, alkaloids, etc.,. They have potentially useful biological activities such as antibiotic, antifungal, antitumour and anti-inflammatory activities (8). There are some cyanobacterial strains (including *Spirulina*) have been well-characterized or exploited commercially. Further research is needed to identify new cyanobacterial strains of high value products and enhancement of synthesis of medicinal products. And therefore, we investigate some clinical studies of marine cyanobacteria on diabetic rats.

Materials and methods

Plant material

Marine cyanobacterial samples were collected between Chennai and Kanniyakumari coastal area. The samples were identified by Light microscope and their techniques (9). The individual species were transferred to 500 ml Erlenmeyer flasks containing 300 ml of ASN III medium (10) for mass culture. The cultures were grown under fluorescent light (1500 lux)

14L/10Dhrs cycle at 27° C ± 2º temperature conditions. From the collection, the following species of marine cyanobacteria undertake for observation.

1. *Chroococcus minor*
2. *Synechocystis pevalakii*
3. *Phormidium corium*
4. *Spirulina platensis*
5. *Oscillatoria chalybea*
6. *Spirulina labrynthiformis*

Preparation of Ethanolic (80%) extract

The marine cyanobacteria was harvested after 30 days using a clean nylon cloth filter and washed thoroughly with tap water quickly to remove salts and other adhering substances and followed by distilled water. The biomass was placed in a filter paper to remove excess moisture and weighed. The wet biomass was grand with 80% ethanol and the slurry was kept at 4ºC for 12 hrs later the supernatant was collected after centrifugation at 10000 rpm for 10 minutes. The process was repeated till the biomass become gray in colour. The pooled supernatant was dried in vacuum. The dried extract was resuspended with 1 ml of phosphate buffer and administered through orally (2.5 mg / kg body weight) to the experimental rats.

Chemicals

Alloxan and Bovine Serum Albumin were purchased from Sigma chemical company, St. Louis, USA. All other chemicals used for biochemical analysis were purchased from Ranboxy Research Laboratories, Glaxo Laboratories, Nice Pharmaceuticals Company and Dr. Reddy's Laboratory-India.

Animals

Experimental animals were healthy male Swiss Albino Rats (6- 8 weeks old) having weight around (180 g – 230 g) were used for the investigation. They were maintained in an appropriate laboratory condition. All animals were fed standard pellet diet (Gold Mohor Rat Feed, Hindustan (p) Ltd., Mumbai) and water *ad libitum*.

Induction of the diabetes mellitus

The experimental rats were injected intraparetonially with alloxanmonohydrate (150mg/Kg bodyweight) dissolved in normal saline (11). After 5^{th} day, the rats were sacrificed and determine the blood glucose and the results are 240 – 280 mg/100 ml of blood. After induction of diabetes mellitus the rats were used for the experimental study.

Experimental protocol

In the present study, ten groups of rats were used. Each group consisting of five rats of same weight. The animals were treated for 30 days as follows.

Group I - Normal rats
Group II– Control rats (2.0 ml normal saline only)
Group III – Diabetic rats (Alloxan monohydrate150mg/Kg bodyweight)
Group IV – Diabetic treatment control rats (Phosphate buffer - 2.0 ml)
Group V – Diabetic rats treated with *Chroococcus minor*(2.5 mg/kg of b.w.)
Group VI - Diabetic rats treated with *Synechocystis pevalakii* ,,
Group VII - Diabetic rats treated with *Phormidium corium* ,,
Group VIII - Diabetic rats treated with *Spirulina platensis* ,,
Group IX - Diabetic rats treated with *Oscillatoria chalybea* ,,
Group X - Diabetic rats treated with *Spirulina labrynthiformis* ,,

Biochemical analysis

The blood was collected from the experimental rats and analyzed the following biochemical parameters.

1. Blood glucose (12).
2. Plasma protein (13).
3. Serum cholesterol (14).
4. Liver glycogen (15).
5. Serum alkaline phosphatase (16).
6. Serum acid phosphatase (16).
7. Serum Glutamate-Oxaloacetate Transaminase (17).
8. Serum Glutamate – Pyruvate Transaminase (17).

Statistical analysis

The values of the biochemical parameters were used to calculate mean, standard deviation and the data was subjected to Turkey – Kramer multiple comparison by one way ANOVA method.

Results

Experimental Swiss albino rats (180 – 230 g) were induced diabetic by a single dose intraparetonially injection of alloxan monohydrate (150 mg/ kg body weight). Five days later the blood samples were drawn and glucose level was estimated to confirm the diabetes. Treatment was carried out by the way of injecting test samples for thirty days. Thirty days after, the rats were sacrificed and collected the blood sample and liver for estimating various biochemical parameters (Table 1).

Effect on blood glucose and liver glycogen

As shown in Table 1 the treatment of diabetes rats with six marine cyanobacteria. From the results it was found that the ethanolic extract of

Spirulina platensis showed high hypoglycemic effect over any other cyanobacteria tested against diabetes. Hence, the *Spirulina platensis* species was selected for further biochemical analysis. The same experiment was repeated only to *Spirulina platensis* ethanolic extract to analyze the various biochemical parameters. After 30 days, the blood glucose level in the untreated diabetic rat was drastically increased to 284.3 ± 20.5. The *Spirulina platensis* extract treated, it was significantly reduced to 89.2 ± 14.8

(P<0.001) (Table 2). The liver glycogen level in the untreated diabetic rat was decreased significantly to 1.80 ± 0.12 (P< 0.001). After treatment with *Spirulina platensis*, it was increased significantly 4.0 ± 0.6 (P<0.001) and reached close to normal value.

Table 1. Screening of antidiabetic activity from marine cyanobacteria

Content	Blood glucose mg/100ml
Diabetes control	88.4 ± 10.6
Diabetes	284.3 ± 20.5**
Diabetes treatment control	334.6 ± 18.7
Diabetes treatment with *Cm*	286.2 ± 15.6*
Diabetes treatment with *Sp*	245.6 ± 16.0*
Diabetes treatment with *Pc*	186.4 ± 14.5
Diabetes treatment with *Spl*	89.2 ± 14.8$^{\#\#}$
Diabetes treatment with *Oc*	120.0 ± 20.3$^{\#}$
Diabetes treatment with *Sl*	226.8 ± 23.6

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's Kramer multiple comparison test using GraphPad Instat software, *P<0.05, **P<0.001, diabetic animals compared with control animals, $^{\#}$P<0.05, $^{\#\#}$P<0.001 diabetic treated animals compared with diabetic animals.

(Cm – *Chroococcus minor*, Sp – *Synechocystis pevalakii*, Pc – *Phormidium corium*, *Spl* – *Spirulina platensis*, Oc – *Oscillatoria chalybea*, Sl – *Spirulina labrynthiformis*)

Effect on Plasma Protein and Serum Cholesterol

In untreated diabetic rat, the plasma protein was decreased significantly to 4.28 ± 2.6 ($P<0.05$) and marked elevated level of serum cholesterol 142.9 ± 9.2 in the untreated diabetic rats. After administration of ethanolic extract of *Spirulina platensis* the plasma protein was increased to 5.8 ± 1.6 ($P< 0.05$) and considerably decreases the serum cholesterol to 87.2 ± 4.3 ($P<0.001$)(Table 2).

Effect on SGOT and SGPT

In the untreated diabetic rat the SGOT and SGPT was found elevated to 106.12 ± 9.6 and 87.30 ± 8.6 respectively. After administration with, the ethanolic extract of *Spirulina platensis* was significantly reduced 47.0 ± 6.8 and 62.22 ± 8.5 ($P<0.001$) respectively. (Table 3).

Table –2. Antidiabetic activity of *Spirulina platensis*

Content	Blood sugar mg/100ml	Liver glycogen mg/100ml	Plasma protein mg/100ml	Serum cholesterol mg/100ml
Diabetes control	98.2 ± 8.5	4.16 ± 0.48	6.16 ± 1.2	85.3 ± 7.5
Diabetes	284.3 ± 20.5	1.80 ± 0.12	4.28 ± 2.6$^{\#}$	142.9 ± 9.2
Diabetes treatment control	282.6 ± 12.4	1.75 ± 0.24	4.15 ± 1.2	139.0 ± 8.6*
Diabetes treatment with *Spirulina platensis*	102.0 ± 12.6*	4.0 ± 0.6*	5.8 ± 1.6$^{\#}$	87.2 ± 4.3*

Values are mean ± S.D. from 6 rats in each group; diabetic group is compared with diabetic control; experimental groups are compared with diabetic group; values are statistically significant at $^{*}P<0.001$ and $^{\#}P<0.05$.

Effect on ACP and ALP

In the untreated diabetic rat, the ACP and ALP was increased significantly to 102.14 ± 10.7 ($P<0.001$) and 240.0 ± 10.4 respectively. After administration of ethanolic extract of *Spirulina platensis* the ACP and ALP was decreased to 103.2 ± 10.2($P< 0.001$) and 160.0 ± 16.1($P<0.001$) (Table 3).

Discussion

Alloxan is a β – cytotoxin that induces chemical diabetes in a wide variety of animal species through damage of insulin secreting cell (18). It is a toxic agent for pancreas β cells; its proposed mechanism for diabetes induction includes: sulfhydryl groups attack, chelant action, enzyme and metabolic modifications; membrane transport changes on electrolytes (19) and increased lipoperoxidation (20). The toxic action of alloxan on pancreatic β cell gulcokinase, generation of free radicals and disturbances in intra cellular calcium homeostasis (21) and induce free radical formation that cause tissue injury. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over production and decreased utilization of glucose by the tissues (22).

Table –3. Toxicological study of *Spirulina platensis*

Content	SGOT IU/100ml	SGPT IU/100ml	ACP KA/100ml	ALP KA/100ml
Diabetes control	46.28 ± 7.5	59.26 ± 10.2	102.14 ± 10.7	154.0 ± 10.0
Diabetes	106.12 ± 9.6*	87.30 ± 8.6*	224.42 ± 9.8*	240.0 ± 10.4*
Diabetes treatment control	109.67 ± 8.6*	95.43 ± 6.2*	222.0 ± 8.8*	248.2 ± 14.5*
Diabetes treatment with *Spirulina platensis*	47.0 ± 6.8**	62.22 ± 8.5**	103.2 ± 10.2**	160.0 ± 16.1**

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's Kramer multiple comparison test using GraphPad Instat software, *P<0.05, diabetic animals compared with control animals, **P<0.001diabetic treated animals compared with diabetic animals.

From the results obtained, it is obvious that the *Spirulina platensis* statistically decreases the blood glucose concentration significantly in alloxan induced diabetic rats. The similar results was reported by Drapeau *et al.,* 2001 (23) from cyanobacteria. The *Spirulina maxima* treated alloxan induced male rats

are prevented hyperglycemia significantly, but not in female rats (24). *Ps. schmidlei* also reduced to the blood glucose level and would probably function like insulin or stimulate the β cells of islets of Langerhans to increase the output of insulin which could result in lowering of blood sugar level (25).

Insulin is the main regulator of glycogenesis in liver. The liver glycogen content was decreased in diabetic rats that have been observed earlier by others (26). The decrease in liver glycogen observed in this study may be due to lack of insulin in the diabetic state and it is due to the inactivation glycogen synthetase enzyme. After treatment with *Spirulina platensis* for 30 days in diabetic rats resulted significantly improve glycogen level. This may be due to improve the enzyme activity and induction of glycogenesis process in liver. The similar results were discussed in earlier reports (27).

The present observation of the plasma protein shows significant decreases in diabetic condition while after treatment with *Spirulina platensis,* it was improved. Excessive breakdown of plasma protein in inadequate supply or defective utilization during diabetes and it may accompany by hypoalbuminemia (28).

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (27). Administration of the extract of *Spirulina platensis,* significant decrease in serum cholesterol level but not in normal. Already reported, that the cyanobacteria, *Spirulina maxima* (29) and *Arthrospira maxima* (30) has prevent hypercholesterolemic activity.

Treatment with *Spirulina platensis,* the rat was not increases the SGOT, SGPT, ACP and ALP level. The earlier reports contrast, *Synecocystis elongates*, *Oscillatoria Formosa, P. angustissimum and Lyngbya sp.,* were increases the SGOT and SGPT Level, whereas, the *Phormidum tenue, Oscillatoria salina, P. valderianum* were decreases (25). From our observations, there was no elevated level of SGOT, SGPT, ACP and ALP in the *Spirulina platensis* treated rats and reduced all the four parameters level after *Spirulina platensis* treatment with diabetes. Considerably, from the above results, the *Spirulina platensis* has potent curative property of diabetes mellitus and hypoglycemic drug without cause any toxic effect.

Conclusion

The ethanolic extract of *Spirulina platensis* exhibits a significant hypoglycaemic activity which may be due to the presence of phytochemicals like flavanoids, phytopigments and sterols that is present in this blue green algae. Further studies are to be carried out and find out the active principle(s) of this species regarding hypoglycaemic activity.

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