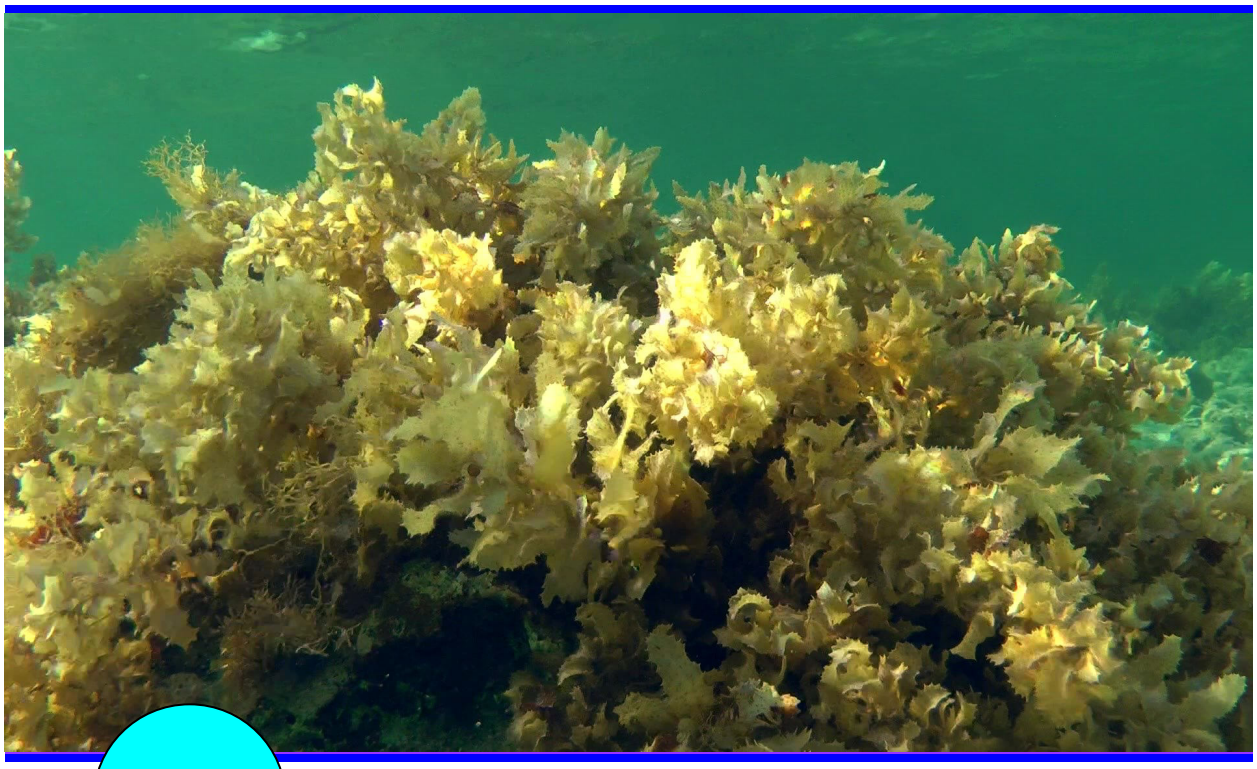

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Investigations on the anti-diabetic potential of novel marine seaweed *Sargassum longiotom* against alloxan-induced diabetes mellitus: A pilot study

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Abstract

The present study evaluated the hypoglycemic effect of brown algae *Sargassum longiotom* in alloxan-induced diabetic rats. After the treatment with *S. longiotom* extract, there is a significant reduction ($p<0.001$) in blood glucose when compared with the diabetic control group. Moreover the ethanolic extract of *S. longiotom* significantly reduces ($p<0.001$) the levels of triglycerides, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol dose-dependent manner. Alternatively, it increases the high-density lipoprotein cholesterol level in the treated groups. The values of SGOT, SGPT and ALP have been significantly reduced ($p<0.001$) in the treated rats when compared to diabetic control. Thus the present study indicates that the ethanolic extract of seaweed *S. longiotom* posses very effective hypoglycemic and hypolipidemic effect on the alloxan induced diabetic rats compared to the reference drug glibenclamide.

Introduction

Diabetes mellitus is one of the most dreadful endocrine disorders. It has a significant impact on human health, life expectancy of the patients suffered by diabetes mellitus (Alberti and Zimmet, 1998). According to WHO report, every year the incidence of diabetes mellitus is dramatically increased so WHO made an attempt to reduce the burden of diabetes mellitus by the use of traditional medicine systems. Several reports in the literature indicated that plant phytochemicals such as flavonoids and alkaloids possess excellent anti-diabetic effect by increasing insulin secretion and activating several metabolic pathways related to carbohydrate metabolism which is comparable with the commercial available allopathic drugs like metformin (Dembinska-Kiec et al., 2008). Because of the scarcity of medicinal plants availability and more need for new molecules for the treatment of diabetes mellitus, scientist paid more attention for the use of other natural source like the plants in the ocean.

Ocean posses several biological active compounds which are helpful for the discovery of novel molecules for the treatment of hormonal based disorders such as cancer, diabetes, arthritis etc., Ocean weeds has maximum nutritional value that can be used as dietary protein supplement for the treatment of protein mal-nutrition conditions (Aravindan et al., 2013; Guerra Dore et al., 2013). *Sargassum longiotom (S. longiotom)* is large brown seaweed of the division Phaeophyta. The hypolipidemic effect has been studied by several workers, however, there exists meager studies on the antidiabetic potential of *S. longiotom*. Hence, this study has been taken up to identify the antidiabetic potential of *S. longiotom*.

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Materials and Methods

Sample collection and preparation of the extract

The brown algae *S. longiotom* were collected from Thonithurai, Mandapam, South coast of India. The Samples were dried under shade at room temperature, pulverized by a mechanical grinder and sieved through 40 meshes. The powdered material (100 g) was extracted with 99.9%ethanol by a cold percolation method in a Soxhlet apparatus. The extract was then concentrated and dried under reduced pressure. The ethanol free semi solid mass obtained was used for the experiment.

Animals

Healthy Swiss albino rats of 6-8 weeks age and of either sex, weighing 150-180 g, were used. The animals were kept in clean and dry plastic cages, with 12/12 hours light/dark cycle at 25 ± 2°C temperatures and 45-55% relative humidity. The animals were fed with standard pellet diet (Gold Mohor rat feed; M/S Hindustan Leaver Ltd, Mumbai) and water was given *ad libitum*.

Induction of experimental diabetes

A single dose (150 mg/kg body weight, i.p.) of alloxan monohydrate (1%, SD Fine Chemicals Pvt. Ltd., Biosar) dissolved in sterile normal saline was used for induction of diabetes mellitus in the rats. Diabetes was confirmed 48 hours after alloxan injection by determining the plasma glucose concentration; only animals with plasma glucose of 150-200 mg/dL were used for the experiment. The diabetic animals were allowed free access to tap water and pellet diet and were maintained at room temperature in plastic cages (Dixit and Kar, 2010).

Table I

Effect of ethanolic extract of *S. longiotom* on blood glucose

Groups	Treatment days		
	1	10	21
Control (Normal saline)	91.6 (7.4)	91.8 (8.5)	92.0 (7.9)
Diabetic control (Alloxan 150 mg/kg)	218.2 (15.4)	220 (16.9)	215.4 (17.2)
S. longiotom extract (100 mg/kg)	216.3 (16.9)	196.7[a] (13.8)	119.5[a] (9.6)
S. longiotom extract (200 mg/kg)	215.2 (19.3)	162.9[a] (13.8)	109.3[a] (8.4)
Glibenclamide (5 mg/kg)	207.6 (14.8)	109.4[a] (7.9)	96.8[a] (6.5)

n = 6; Data are expressed as mean and SE in parenthesis; [a]p<0.001 vs control by Student 't' test

Drug administration

The quantities of the individual drug to be administered were calculated and suspended in vehicle (1%W/V suspension of carboxymethyl cellulose (CMC) in water 10 mL/ kg body weight).

Experimental group protocol

Animals were classified into five groups of six rats each. Group I served as control and received 1% w/v suspension of CMC in water at a dose of 10 mL/kg b.w. Group II treated with alloxan monohydrate 150 mg/kg served as diabetic control. Group III and IV received an ethanolic extract of *S. longiotom* in 1% CMC at a dose of (100 and 200 mg/kg. body weight) respectively. Group V treated with glibenclamide (5 mg/kg, b.w) and served as reference standard.

Collection of blood samples and estimation of biochemical parameters

At the end of the experimental periods, experimental rats were sacrificed. Plasma and serum were separated from the blood by centrifuging the samples at 5,000 rpm for 10 min and stored under refrigeration until analyzed. Plasma glucose was estimated before starting the treatment weekly and up to the end of treatment period (10 and 21 days). Lipid profile was estimated at the end of the study (21 day).

Glucose (Middleton, 1959; Salib et al., 2013), total cholesterol (Bowman and Wolf, 1962; Salib et al., 2013), plasma triglycerides (Nagaraju et al., 2013; Salib et al., 2013), high-density lipoprotein (HDL) cholesterol (Salib et al., 2013), serum glutamate pyruvate transaminase (Salib et al., 2013) (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (Salib, et al., 2013) (ALP) were determined by using commercially available kits. The total protein was also estimated (Salib et al., 2013). VLDL (very low-density lipoproteins) cholesterol was calculated as: TG/5; LDL cholesterol was calculated by the equation: LDL cholesterol = TC – (HDL + VLDL). All estimations were done using the auto analyzer.

Statistical analysis

Statistical analysis was performed with SPSS 12 statistical software package. All the values were expressed as mean ± standard error mean (SEM). Significance between the groups was estimated by students 't' test. P value <0.001 considered as significant and the minimum level of significance was fixed at p<0.05.

Results

The blood glucose levels in the untreated diabetic group were increased significantly 220.0 ± 16.9 (p<0.001) when tested against the control group (Table I). After the treatment with *S. longiotom* extract, there was significant reduction of total blood glucose when compared with the diabetic control group.

Lipid profile have been estimated and compared with

Table II

Effect of ethanolic extract of *S. longiotom* on lipid profile

Groups	TGL mg/dL	HDL mg/dL	VLDL mg/dL	LDL mg/dL
Control (Normal saline)	76.7 (5.7)	48.2 (2.5)	15.3 (1.1)	23.0 (2.9)
Diabetic control (Alloxan 150 mg/kg)	121.2 (10.3)	32.5 (2.7)	24.2 (2.1)	189.9 (12.6)
S. longiotom extract (100 mg/kg)	104.5 (8.3)	36.7[a] (2.5)	20.9 (1.7)	167.3[a] (12.5)
S. longiotom extract (200 mg/kg)	97.4[a] (7.3)	40.6[a] (3.4)	19.5 (1.5)	88.4[b] (5.7)
Glibenclamide (5 mg/kg)	113.6 (6.7)	39.8[a] (2.8)	22.7 (1.3)	95.8[b] (2.7)

Values are expressed as mean and S.E. Within parenthesis; n = 6; [a]p<0.01 vs control; [b]p<0.001 vs control by Students 't' test

Table III

Effect of ethanolic extract of *S. longiotom* on choles-terol and protein

Groups	Total cholesterol	Protein
Control (Normal saline)	86.5 (6.5)	3.6 (0.2)
Diabetic control (Alloxan 150 mg/kg)	246.6 (17.4)	3.7 (0.4)
S. longiotom extract (100 mg/kg)	167.3[a] (12.5)	3.5 (0.6)
S. longiotom extract (200 mg/kg)	148.4[a] (10.6)	2.9 (0.2)
Glibenclamide (5 mg/kg)	158.3[b] (6.8)	2.8 (0.5)

Values are expressed as mean and SE is within parenthesis; n = 6; [a]p<0.01 vs control; [b]p<0.001 vs control by Students 't' test

Table IV

Effect of ethanolic extract of *S. longiotom* on SGOT, SGPT and ALP

Groups	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)
Control (Normal saline)	96.5 (2.8)	36.3 (1.5)	15.9 (0.7)
Diabetic control (Alloxan 150 mg/kg)	117.3[a] (8.6)	53.2[a] (4.8)	33.5[b] (3.7)
S. longiotom extract (100 mg/kg)	103.5[a] (7.6)	41.9[a] (3.5)	28.4 (3.2)
S. longiotom extract (200 mg/kg)	98.3[a] (9.4)	39.8 (3.2)	21.5[b] (1.9)
Glibenclamide (5 mg/kg)	99.3 (6.4)	40.4 (3.2)	19.5 (1.4)

Values are expressed as mean and SE is within parenthesis; n = 6; [a]p<0.01 vs control; [b]p<0.001 vs control by Students 't' test

treated, untreated and control groups of rats (Table II). There was a significant increase in the values of TG, VLDL cholesterol, LDL cholesterol and decrease in HDL cholesterol in the untreated diabetic control rats compared to standard. After the treatment with 100 and 200 mg/kg of ethanolic extract of *S. longiotom*, there was significant reduction in these values, when the dose is increased there was a linear reduction in the amount of TG and VLDL cholesterol and LDL cholesterol. HDL cholesterol level has been raised in the treated groups. This reveals that the ethanolic extract of *S. longiotom* acts as a stimulator for lipid degradation and to increase the concentration of good cholesterol which is used to reduce the risk of hypercholesterolemia.

Total cholesterol levels in the untreated diabetic group were increased 246.6 ± 17.4 significantly ($p<0.01$) when tested against the control group (Table III). After the treatment with *S. longiotom* extract, the reduction of total cholesterol and the protein level 148.4 ± 10.6 was significant at ($p<0.001$) and the mean values are comparable with that of the control groups.

SGOT, SGPT and ALP are considered as very important metabolic enzymes. The levels of SGOT, SGPT and ALP have been compared with treated and untreated diabetic rats (Table IV). The enzyme levels are gradually increased in the untreated diabetic rats when compared to control. The values of SGOT, SGPT and ALP have been significantly reduced in the treated animals when compared to standard. There is no difference between standard and *S. longiotom* extracts (200 g/kg) against the level of these enzymes.

Discussion

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency. It produces an inadequate glucose control and leads to acute and chronic complications (Ikewuchi et al., 2011). Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk of cardiovascular disease (Hamden et al., 2009). The lowering of plasma glucose and lipid levels through dietary modification and drug therapy seems to be associated with a decrease in the risk of vascular disease (de Sousa et al., 2004).

Alloxan, selective beta cytotoxin, induces "chemical diabetes" in many animal species by damaging the insulin-secreting cells of the pancreas (Bilic, 1975) and the animals became permanently diabetic (Bilic, 1975). However, these animals have existing beta cells and regeneration is possible. It is well known that the glibenclamide act by directly stimulating the beta cells of the Islets of Langerhans to release more insulin and these compounds are active in mild alloxan induced diabetes (Kumar et al., 2013). Since the present findings show that glibenclamide decreased the glucose, TC, TG, LDL cholesterol, VLDL cholesterol, SGOT, SGPT, ALP levels and increase in HDL cholesterol level in the diabetic animals, the extent of diabetes is not severe.

In the present study, treatment with ethanolic extract of

S. longiotom in alloxan induced diabetic rats produced a more significant decrease in blood glucose level. The hypoglycemic effect may be due to increased secretion of insulin from the beta cells of pancreas, i.e., pancreatotrophic action (El-Alfy et al., 2005). The results were comparable to that of glibenclamide, which acts by stimulation of insulin release thus further confirming that the extract lowers blood glucose by a pancreatotrophic action.

Furthermore, *S. longiotom* produced significant beneficial effects on the lipid profile in alloxan induced diabetic rats by reducing TG, TC, LDL and VLDL and increasing HDL, significantly. The ethanolic extract of *S. longiotom* may increase the secretion of insulin from beta cells of the pancreas; this increased secretion of insulin stimulates fatty acid biosynthesis and also the incorporation of fatty acids into TG in the liver and adipose tissue. In the present study the decrease of TC, TG and LDL cholesterol levels achieved by administration of leaf extract, demonstrates a possible protection against hypercholesterolemia (Salib et al., 2013).

Moreover the ethanolic extract of *S. longiotom* caused a significant reduction in the levels of SGOT, SGPT and ALP. The activity of SGOT, SGPT and ALP are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. By the reduction of these enzymes in the diabetic rats suggesting that the extract of *S. longiotem* may prevent hepatic injury associated with diabetes.

From overall study, it is concluded that the ethanolic extract of *S. Longiotom* showed a significant hypoglycemic and hypolipidemic activity in the model of of alloxan-induced diabetic rats. There were no toxic effects during this period in regards to hepatotoxicity. Therefore, the ethanolic extract of the seaweed *S. longiotem* can be used as an alternative treatment for diabetes.

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