

Research Article



A Study on the Type II Antidiabetic Activity of Methanolic Extract of Marine Algae, *Gracilaria edulis* and *Sargassum polycystum*

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ABSTRACT

To evaluate the Antidiabetic activity of methanolic extract of marine algae *Sargassum polycystum* (SP) and *Gracilaria edulis* (GE) in fructose induced type II diabetic mellitus. The marine algae *Sargassum polycystum* and *Gracilaria edulis* were dried under shade and then powdered, and extracted with methanol. Preliminary phytochemical studies and acute toxicity studies were also carried out on methanolic extract of SP, and GE. For type II Diabetes was induced in Male rats (200-250g). Animals were fed with a high-fat, high-sugar diet (normal diet mixed with 66% (w/v) fructose, 10% coconut oil, and 20% protein). The high fructose diet consisting 10% (w/v) of fructose concentration was delivered ad libitum through drinking water for 10 weeks. Standard received Metformin (300mg/kg). During the study, body weight and fasting blood glucose level were taken at 0 and 30th day. At the end of study, animals in all groups were sacrificed, blood sample, pancreas were collected. Biochemical parameters such as total cholesterol, HDL cholesterol, TG, LDL cholesterol, serum insulin, histopathological studies of pancreas were performed. Oral administration of methanolic extract of SP, and GE (250 mg/kg & 500mg/kg) showed antidiabetic activity in fructose induced diabetic animals. This was evidenced by recovery of body weight, reduction in fasting serum glucose, hypolipidaemic effect. It can be promising in the management of complications and severity of the metabolic abnormality of type II diabetes.

Keywords: Methanolic extract, *Sargassum polycystum*, *Gracilaria edulis*, fructose, Antidiabetic.

INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by defects in carbohydrate, lipid, and protein metabolism. It is a leading cause of morbidity and mortality worldwide, due to diabetic complications such as coronary heart disease, stroke, retinopathy, nephropathy, liver disease, and peripheral neuropathy.¹ The majority (about 90%) of diabetes is of Type II (T2DM) or non-insulin-dependent diabetes mellitus (NIDDM), which is the result of deviations in pancreatic β -cells functions, insulin secretions, and insulin insensitivity.² Hyperglycemia increases the production of free radicals,³ and induces oxidative stress leading to liver injuries related to carbohydrate metabolism disorder.^{4,5} These injuries are represented by cellular degenerations, pyknotic nuclei, and cellular necrosis due to increased lipid accumulation and oxidation in the hepatocytes. However, the liver is able to regenerate even after initial injuries.⁶ Various diabetic complications are caused by defects in the body antioxidant defense systems,⁷ oxidative stresses, and damages to cellular membranes, sub cellular organelles,⁸⁻¹¹ DNA damage, and cell death.¹²

Type II diabetes mellitus is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction). Insulin resistance is a characteristic metabolic defect that precedes overt beta cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. The beta cells normally

compensate insulin resistance by secreting more amounts of insulin to maintain the glucose homeostasis. In the course of time, however, this beta cell function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes.^{13, 14} There occurs only a relative insulin deficiency as the day-long circulating insulin concentrations in patients with type II diabetes are almost comparable or slightly elevated in absolute terms to the values in normal individuals. Despite the role of genetic predisposition, aging, obesity and dietetic/sedentary life style are major risk factors involved in the development of type II diabetes. Most of the individuals diagnosed with type 2 diabetes are found to be obese.^{15,16} Although there exists a surplus of animal models (spontaneous as well as induced) available for the study of type II diabetes, the pattern of disease initiation and development in most of them do not appear to be closely analogous to the clinical situation in humans.

Natural antioxidants from plants retard these damages, and may be an effective, safe, and economical alternative therapy for diabetes management and organs protection. *In vivo* studies and histopathological examinations are necessary to prove their efficacy and safety on the liver, kidney, pancreas, and the other important organs, since biochemical measurements alone are not conclusive. The common edible brown seaweed *Sargassum polycystum* and *Gracilaria edulis* reportedly alleviated hyperglycemia and dyslipidemia in diabetic rats,¹⁷ possibly due to its good antioxidant and free radical scavenging properties.¹⁸ *Sargassum polycystum* is reportedly used for eczema,



scabies, and psoriasis, ulcer and lung diseases, renal dysfunction, viral hepatitis and heart ailments and to promote bile secretion,⁷ besides having antilipidemic, antioxidant and membrane stabilizing properties.^{8, 19} *Sargassum polycystum* also has drug metabolizing enzymes protective effects, prevents TNF- α elevation,⁹ inhibits lipid peroxidation, and preserves hepatic antioxidant defense system *in vivo*.^{10,12} It was reported to be hepatoprotective under high-fat/high cholesterol diet.¹⁶ The administration of *Sargassum polycystum* ethanolic or water extracts dose dependently reduced blood glucose, glycosylated hemoglobin (HbA1C) levels, and dyslipidemia in type II diabetic animals.¹⁷ *Sargassum polycystum* and *Gracilaria edulis* appeared to be an insulin sensitizer, beneficial in the management of T2 DM that can also help reduce atherogenic risk. Currently, there is no report the organ protective effect of *Sargassum polycystum* in type II diabetes animal model. This study reports on the protective or tissue restorative effects *Sargassum polycystum* and *Gracilaria edulis* methanolic extracts on the pancreas tissues in type II-induced diabetic rat model.

MATERIALS AND METHOD

Plant Material

The marine algae *Sargassum polycystum* and *Gracilaria edulis* was collected during August, from the Mandapam coast (latitude 90 17' Longitude 790 22, E), Gulf of manner. The sample was identified by Scientist in charge, at the Centre for Marine and Fisheries Research Institution (CMFRI), Mandapam Tamilnadu.

Extraction of the plant materials and sample preparation

The algae of *Sargassum polycystum* and *Gracilaria edulis* were, chopped into small pieces and dried under shade at room temperature for seven days. The dried algae were powdered and passed through the sieve (coarse10/40). The powder was used for the preparation of methanolic extract. Dried and powdered algae of *Sargassum polycystum* and *Gracilaria edulis* (each 1.0 kg) were extracted with boiling 70% MeOH in a reflux condition. After filtration, the extract obtained was concentrated in a rotary shaker and evaporated to dryness to get constant weight. The Qualitative phytochemicals screening were carried out on the Methanolic extract of *Sargassum polycystum* and *Gracilaria edulis*. To detect various phytoconstituents present in them.^{20, 21}

Experimental Animals

Male Wister rats weighing between 200-250g were procured from NIMHANS Bangalore, Karnataka. The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C \pm 2°C, Relative humidity 65 \pm 7.5% under 12 hours light /dark cycle. The animals were fed with rodent pellet diet (Gold Mohur Lipton India Ltd.) and water *ad libitum*. Ethical clearance for performing the experiments on animals was obtained from the

Institutional Animal Ethics Committee (IAEC). Studies were performed in accordance with the CPCSEA guidelines.

EXPERIMENTAL DESIGN

Acute Oral Toxicity Study

The acute oral toxicity study was performed according to the OECD guidelines no. 425.

Fructose induced type II diabetes mellitus

Diabetes was induced in Male rats (200-250g). Animals were fed with a high-fat, high-sugar diet (normal diet mixed with 66% (w/v) fructose, 10% coconut oil, and 20% protein). The high fructose diet consisting 10% (w/v) of fructose concentration was delivered *ad libitum* through drinking water for 10 weeks. The rats were divided into following groups consisting of six rats each.

Group 1	Administered vehicle serves as Normal control
Group 2	Administered fructose serves as diabetic control
Group 3	Administered Reference standard, Metformin (300mg, kg, p.o.)
Group 4	Diabetic rats treated with methanolic extract of <i>Sargassum polycystum</i> , dose obtained from acute toxicity
Group 5	Diabetic rats treated with methanolic extract of <i>Gracilaria edulis</i> , dose obtained from acute toxicity

Body weights of rats were taken at end of the treatment using electronic balance. Fasting blood glucose level of rats were taken on before and after the treatment i.e., 0, and 30th day of treatment by using one touch glucometer by vein puncture. Later withdrawal the blood for analyzed various biochemical parameters. At the end of experimental period sacrifice the animals with high dose of pentobarbital for tissue histology.

Biochemical parameters The biochemical parameters like Glucose, Triglyceride, Total Cholesterol, HDL, LDL, were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit. (DELTA LABS kit, Bangalore, India) using Semi Auto analyzer.

Histopathological Studies

Preparation of isolated pancreas

The animals were euthanized using high dose of pentobarbital and sacrificed. And the pancreas of each animal was isolated and was cut into small pieces, preserved and fixed with 10% formaldehyde. The samples were then dehydrated and embedded in paraffin. After sectioning (5 μ m thick) with a rotary slicer (LEICA RM2135, Wetzlar, Germany), hematoxylin and eosin stain (H&E) and luxol fast blue staining was performed to evaluate the status of pancreas.



Statistical analysis

The results are expressed as mean ± S.D from n=6 rats in each group. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey’s test.

RESULTS

Sargassum polycystum and *Gracilaria edulis* was analyzed qualitatively. It was observed that the mixture contains carbohydrates, phytosterols, flavonoids, and glycosides but does not contain alkaloids, tannins, saponin fixed oil, amino acids and proteins.

Table 1: Preliminary Qualitative analysis of *Sargassum polycystum* and *Gracilaria edulis*.

S.N.	Phytochemical Constituent	Observation	
		<i>G. edulis</i>	<i>S. polycystum</i>
1	Alkaloids	-	-
2	Carbohydrates	+	+
3	Glycosides	+	+
4	Proteins & amino acids	+	-
5	Saponin	-	-
6	Tannins	-	-
7	Phytosterol	+	+
8	Flavonoids	-	+
9	Fixed oils & fats	-	--

Acute Toxicity study

The LD50 of the extract of *Gracilaria edulis* (GE) and, *Sargassum polycystum* (SP) was found to be 5000mg/kg, after conducting the acute oral toxicity studies. So 1/10th and 1/20th of doses (250 and 500 mg/kg) were selected and the experiment was carried out.

For Fructose Induced Type II DM Model

Effect of methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg/kg.po/day/30days) on fructose induced treated rats on body weight, blood glucose level, serum insulin, cholesterol, triglyceride, HDL, LDL, after 30 days of treatment.

On Body Weight

Values are expressed as Mean + S.E.M (n=6) *Sargassum polycystum* low dose (SLD), *Sargassum polycystum* high dose (SHD), *Gracilaria edulis* low dose (ELD), *Gracilaria edulis* high dose (EHD), Standard (STD)

Administration of methanolic extracts of *Sargassum polycystum* and *Gracilaria edulis* (both 250 & 500mg/kg.po/day/30days) to the treated Wister rats showed significant increase (P<0.001) body weight on 30th day of treatment when compared with Diabetic

control and Standard control Metformin (300mg/kg.po/day//30 days).

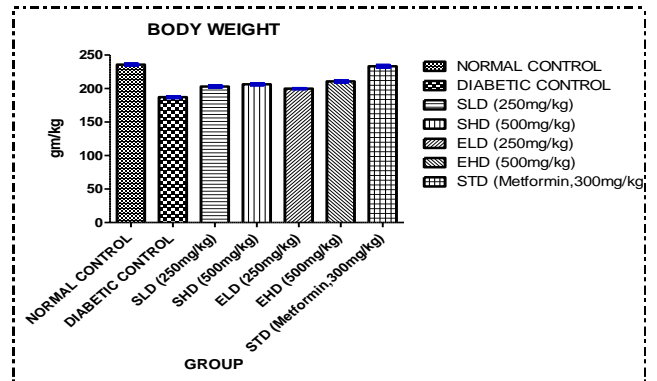


Figure 1: Effect of methanolic extracts of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg/kg.po/day/30days) on fructose induced treated rats on body weight after 30 days of treatment.

On Blood Glucose Level

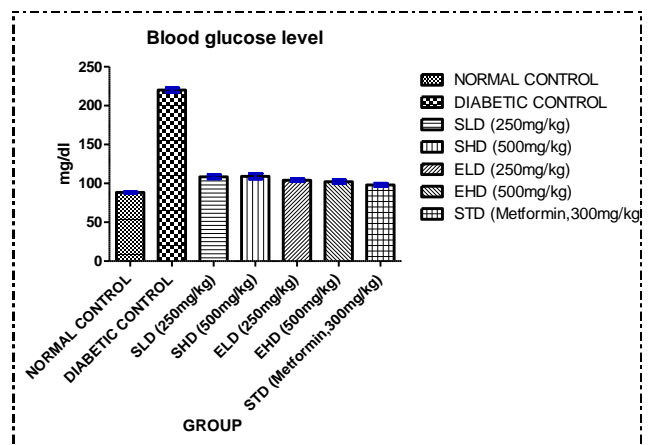
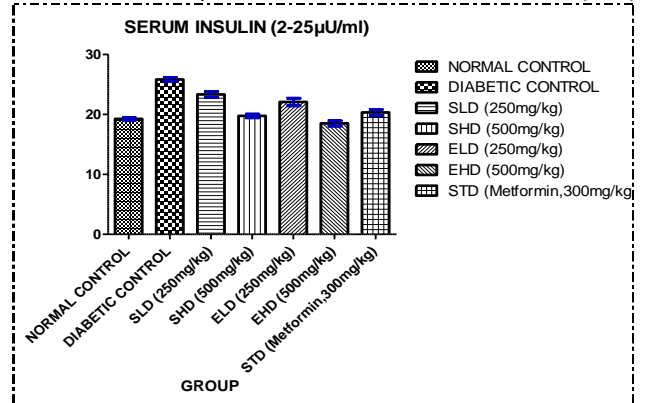


Figure 2: Effect of methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg/kg.po/day/30days) on fructose induced treated rats on blood glucose level (mg/dl) after 30 days of treatment

Values are expressed as Mean + S.E.M (n=6).



Values are expressed as Mean + S.E.M (n=6)

Figure 3: Effect of methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg/kg.po/day/30days) on fructose induced treated rats on serum insulin after 30 days of treatment



Administration of methanolic extracts of *Sargassum polycystum* and *Gracilaria edulis* (both 250 & 500mg/kg.po/day/30days) to the treated Wister rats Showed significant decrease ($p<0.01$) in the blood glucose on 30th day of treatment when compared with diabetic control.

On Serum Insulin

In diabetic rats, the insulin remained within the diabetic range, but gradual decrease in insulin was observed, which reached almost normal levels after 1 month's treatment. Treated diabetic rats showed significantly decreased serum insulin levels.

Table 2: Effect of Methanolic extracts of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg /kg.po/day/30days) on fructose induced treated rat on LDL, Triglyceride, Total Cholesterol, and HDL after 30 days of treatment

Normal Control	Diabetic Control	SHD 500mg/kg	SLD 250mg/kg	EHD 500mg/kg	ELD 250mg/kg	STD (Insulin,4U/kg)
LDL >130mg/dl						
99±0.33	136±0.56 ^{####}	110±0.30 ^{***}	99±0.43 ^{***}	98±0.40 ^{***}	99±0.97 ^{***}	101±0.76
Triglycerides 150-199mg/dl						
161±1.23	213±16.91 ^{####}	182±1.03 ^{***}	176±0.67 ^{***}	185±1.15 ^{***}	169± ^{***}	165±1.054
Total Cholesterol 200-239 mg/dl						
197±0.76	230±1.033 ^{####}	199±0.67 ^{***}	183±0.83 ^{***}	201±0.58 ^{***}	196±0.77 ^{***}	203±0.67
HDL 50-60mg/dl						
61±0.58	46±0.67 ^{####}	54±0.76 ^{***}	64±1.30 ^{***}	58±0.73 ^{***}	64±0.95 ^{***}	64±1.38

Values are expressed as Mean + S.E.M (n=6). *** $p<0.001$; when compared to diabetic control ^{####} $p<0.001$; when compared to normal controls.

Effect on serum total cholesterol (serum TC) level

Administration of fructose induced to Wister rats showed significant increase ($p<0.001$) in the serum TC on 30th day of treatment when compared with normal control. Administration of methanolic extract of GE, SP, and standard Metformin to the fructose induced treated Wister rats showed significant decrease ($p<0.05$) in the serum TC on 30th day of treatment when compared with diabetic control.

Effect on serum triglyceride (serum TG) level

Administration of fructose induced to Wister rats showed significant increase ($p<0.001$) in the serum TGs on 30th day of treatment when compared with normal control. Administration of methanolic extract of GE, SP, and standard Metformin to the fructose induced treated Wister rats showed significant decrease ($p<0.05$) in the serum TG on 30th day of treatment when compared with diabetic control.

Effect on serum LDL-C level

Administration of fructose induced to Wister rats showed significant increase ($p<0.001$) in the serum LDL-C on 30th day of treatment when compared with normal control. Administration of methanolic extract of GE, SP, and standard Metformin to the fructose induced treated Wister rats showed significant decrease ($p<0.05$) in the serum LDL-C on 30th day of treatment when compared with diabetic control.

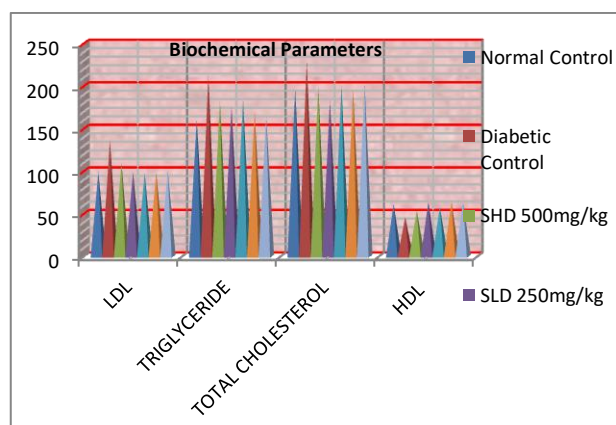


Figure 4: Effect of methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg/kg.po/day/30days) on fructose induced treated rats on LDL, Triglyceride, Total Cholesterol, and HDL, after 30 days of treatment.

Values are expressed as Mean + S.E.M (n=6)

Effect on serum HDL cholesterol (serum HDL-C) level

Administration of fructose induced to Wister rats showed significant decrease ($p<0.01$) in the serum HDL level on 30th day of treatment when compared with normal control. Administration of methanolic extract of GE, SP, and standard Metformin to the treated Wister rats showed significant increase ($p<0.001$) in the serum HDL level on 30th day of treatment when compared with diabetic control.

Pathological Changes On Pancreatic Islets: Histological examination of hematoxylin and eosin (H&E) on pancreas.

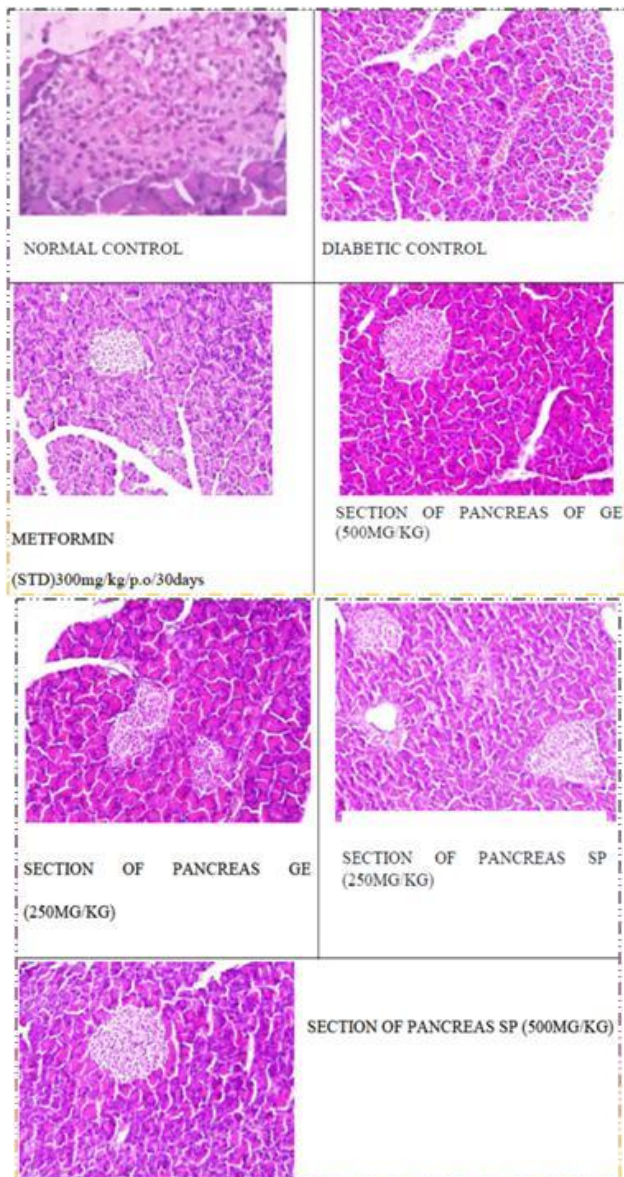


Figure 5: Effect of Methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* (250mg and 500mg/kg.po/day/30days) and Metformin (300mg/kg/p.o./30days) on fructose induced treated rats on pancreatic histology after 30 days of treatment (H&E=100)

Normal control Pancreatic lobules separated by connective tissue septa refer pancreatic lobules which consist largely of the exocrine acini. Most of the lobules show small, round, light - staining islets of langerhans. The center of islet cells consists of aggregates of small β -cells (75%) having basophilic granules, while the periphery comprised of large α -cells (25%) were having eosinophilic granules. Thin walled capillaries are seen in between these cells. **Diabetic control** Atrophied and deranged islets are visible. Space between acini represents disorganization of pancreatic tissue. Ducts appear normal but acini appear distended. **Standard (Metformin; 300mg/kg)** Overall pancreatic structure appears regular and the islet of langerhans seem completely recovered from toxic insult. Acini appear

normal. Presence of mild inflammatory infiltration cannot be ruled out. **Methanolic extract of GE 500mg/kg treated** Acini and islets appear completely normal. There is evidence of mild infiltration. Structure of cells is well maintained and there is no sign of toxic insult. **Methanolic extract of GE 250mg/kg treated** the acini appear normal but damage to islets is clearly evident. Islet-cell atrophy can also be seen. Blood streaks suggest internal hemorrhages. Incidence of infiltration is present in the acinar cells. **Methanolic extract of SP 500mg/kg treated** Extract treated diabetic rats shows significantly reduced necrosis, reduced degeneration, reduced irregular spaces, and normal acinar epithelial lining. **Methanolic extract of SP 250mg/kg treated** the islets appear of normal shape and morphology but mild infiltrations are present. Blood vessels show minor internal hemorrhages. The acinar structure appears marred around the edges. No signs of atrophy.

DISCUSSION

Hypoglycaemic herbs increase insulin secretion, and inhibit glucose absorption from intestine and glucose production from liver. Insulin lowers the concentration of glucose in blood by stimulating the uptake and metabolism of glucose by muscle. Enhance glucose uptake by adipose or muscle tissues. The Ethanolic extract of *Strobilanthes asperimus* (Pradeep Kumar Samal, 2013), MeOH-H₂O extract of *Grateloupia elliptica* (KY Kim *et al.*), aqueous extract of *Ulva fasciata* (Abirami *et al.*), ethanolic extract of *Sargassum duplicatum* and *Turbinaria decurens* (Hardoko *et al.*), reported that these algae having anti-diabetic activity. In present study we have observed that these algae *Sargassum polycystum* and *Gracilaria edulis* having anti-diabetic activity. Fructose is widely used as sweetener in food processing (Dai and McNeill, 1995). It is demonstrated that normal rats fed with fructose- enriched diet develop hypertension accompanied with metabolic abnormalities including hyperglycemia, insulin resistance, hyperinsulinemia and hyper triglyceridemia (Dai and McNeill, 1995; Vrana and Kazdova, 1986; Suzuki *et al.*, 1997; Katakam *et al.*, 1998; Lee *et al.*, 2002; Bunnag *et al.*, 1997). Feeding of a High Fructose Diet (HFD) can provide a type 2 diabetic dietary model associated with insulin resistance (Thorburn *et al.*, 1989; Tobey *et al.*, 1982) and hyper triglyceridemia (Tobey *et al.*, 1982; Zavaroni *et al.*, 1982). The glucose metabolism and glucose uptake pathways are disturbed through an overload of fructose. Methanolic extract of SP and GE significantly decreased blood glucose level in STZ treated rats and as well as fructose fed rats. Diabetes rats characteristic loss of body weight, this may be due to increased muscle wasting; due to loss of tissue proteins and fats. The MSP and MGE treated diabetic animals showed marginal increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in glucose uptake, insulin secretion and glycemic control. Uncontrolled diabetes

mellitus is associated with increase in total cholesterol, triglycerides and LDL cholesterol associated with decrease in HDL cholesterol. These individuals are at high risk for the development of cardiovascular disease, and have higher total serum cholesterol levels. In present study, in diabetic control group, there was marked increase in total cholesterol, LDL cholesterol and TG, while significant decrease in HDL cholesterol level, was found. Hyperlipidemia is a known complication of diabetes mellitus and coexists with hyperglycemia and is characterized by increased level of cholesterol, TG and LDL cholesterol, and all the lipid abnormalities associated with diabetes was significantly normalized by treatment with methanolic extract of algae of SP and GE. In the present study histological picture of fructose fed pancreas of rats showed degeneration and shrinkage vacuolar change and disruption of normal endocrine architecture and significant decrease in beta cell density, whereas methanolic extract of marine algae *Sargassum polycystum* and *Gracilaria edulis* shows significant increase in beta cell density and reduced necrosis reduced degeneration reduced irregular spaces lining. This effect by SP and GE is of important significance because cell necrosis is an irreversible process, whereas cell degeneration is reversible with the help of a good glycemic control agent to enable it to function normally again.

CONCLUSION

In conclusion, our data suggest methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* possess potential antidiabetic activity as it lowers blood glucose level significantly. Methanolic extract of SP and GE also possess significant antihyperlipidemic activity as it lowers serum cholesterol and triglycerides levels, LDL cholesterol and increase HDL cholesterol level. Methanolic extract of SP and GE also showed decrease serum insulin. The results suggest that the marine algae possess significant antidiabetic activity and may prove to be good therapeutic agent for managing and treating diabetic mellitus.

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