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LETTERS

Evaluation of anti-hyperglycemic effect of *Gracilaria corticata* extract in Normal and Streptozotocin -induced Diabetic rats

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ABSTRACT

Diabetes is a growing health concern worldwide and now emerging as an epidemic world over. The management of diabetes is still a major challenge. Diabetes is characterized by excessive blood sugar due to body's failure to produce insulin or the consequence of insulin resistance. The present study was designed to investigate the anti-hyperglycemic effect of different extract (Ethyl Acetate, Methanol and Aqueous) of *Gracilaria corticata* seaweed on normal and streptozotocin (STZ) induced in diabetic rats. Diabetes was induced into male albino Wistar rats by intraperitonial administration of STZ. The *Gracilaria corticata* different extract was administered orally at three different doses to normal and STZ-diabetic rats for 0hrs-2hrs and 0-15 days. Effect of *G. corticata* (15 day) on blood glucose and body weight in normal and STZ-diabetic rats was evaluated. Significant increase in blood glucose level and decrease in body weight was observed in diabetic rats when compared to normal. Treatment with *G. corticata* extracts lowered plasma glucose and elevated body weight significantly as compared to STZ-diabetic rats. The results suggested that methanolic extract of *G. corticata* at a dose of 100 mg/kg body weight exhibited a considerable hypoglycemic effect and could be developed as a potential hypoglycemic agent for the management of diabetes.

Keywords: Body weight, Blood glucose, Diabetes mellitus, Gracilaria corticata, Streptozotocin

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] The majority (about 90%) of diabetes cases are of type 2 (T2DM) or non-insulin-dependent diabetes mellitus (NIDDM), which is the result of deviations in pancreatic β-cell functions, insulin secretions, and insulin insensitivity.[2] Diabetes mellitus is a common disease affecting the citizens of both developed and developing countries. It has been estimated that 2.1% of the world population may have diabetes, predicted to rise to 3% by the year 2010.[3] More recently, a survey estimates that by 2030 more than 439 million people will suffer from diabetes mellitus (DM), one of the most common chronic diseases in nearly all countries.[4] India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes would affect approximately 57 million people by the year 2025 in India.[5] The main reasons of morbidity and mortality in the diabetic patients are heart disease and stroke as patients are 2 to 4 times more susceptible to have heart disease and 5 times more likely to have stroke.[6,7]

Streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity and has been extensively used for induction of diabetes mellitus in different animal models.[8] Streptozotocin induced diabetes is a well-documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the dose of STZ employed, animal and species used.[9]

The control and treatment of diabetes, and its complications mainly depend on the chemical and biochemical agents. The distinctive traditional medical opinions and natural medicines, mainly originating from herbs show a bright future in the

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therapy of diabetes mellitus and alleviation of its complications.[10] In India and other Middle East countries, diabetes mellitus has been treated orally with herbal remedies based on folk medicine since ancient times.[11] Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research.[12] The medicinal plants used for the treatment of hypoglycemic and hyperglycemic conditions are of considerable interest to ethno botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and the major merits of herbal medicines seem to be their efficacy, low incidence of side-effects and low cost.[13]

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities.[14,15,16] Seaweed has plenty of essential nutrients, especially trace elements and several other bioactive substances. That explains why today seaweeds are considered as the food supplement for 21st century as a source for proteins, lipids, polysaccharides, mineral, vitamins and enzymes.[17] Seaweeds are presently a center of focus as a natural source of biological compounds which have beneficial effects for human health.[18]

Algae are a great source of natural compounds which are widely known and consumed in Asian countries.[19] Marine algae are known to generate an abundance of bioactive compounds with great potential in the pharmaceuticals, food, and biomedical industries. *Gracilaria corticata* is a predominant red seaweed species of west coast of Madagascan south island, belonging to the family *Gracilaria*cea and order *Gracilariales*. This species is also found along the east coast of Madagascar particularly at coast of Mahambo.[20] Red algae display a variety of biological activities. *Gracilaria corticata* has several biomedical properties such as anti-bacterial, anti-viral, anti-fungal, anti-protozoal, anti-inflammatory, anti-oxidant, cytotoxic, contraception, gastrointestinal, cardiovascular, hypoglycemia, spasmolytic and allelophatic effects.[21,22] In this study, we attempted to study the anti-hyperglycemic effect of *Gracilaria corticata* extract on STZ-induced diabetic rats.

MATERIALS AND METHODS

Collection of *Gracilaria corticata* seaweed

Seaweeds of *Gracilaria corticata* were collected in the month of June from sea coast of Mandapam, Rameshwaram, Tamil Nadu, India. The collected *Gracilaria corticata* seaweed was botanically identified by P. Anandaraman, Department of CAS Marine Biology, Annamalai University, Annamalainagar, Tamil Nadu.

Preparation of different solvent extract

Gracilaria corticata seaweed was cleaned and the necrotic parts were removed. The seaweeds washed with tap water to remove any associated debris and shade dried at room temperature (25±2 °C) for 5-8 days or until they are brittle. After complete drying, the seaweed materials (1.0 kg) were ground to a fine powder using electrical blender. Forty gram of powdered seaweeds were extracted successively with 200 mL of solvents (methanol, ethyl acetate and water) using a Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness under reduced pressure using a rotary vacuum evaporator and the resulting pasty extracts were stored in a refrigerator at 4 °C for future use.

The three type of different *Gracilaria corticata* seaweed extracts are namely

(i) Ethyl acetate extract of *Gracilaria corticata* (EAGC)

(ii) Methanolic extract of *Gracilaria corticata* (MGC)

(iii) Aqueous extract of *Gracilaria corticata* (AqGC), respectively.

Experimental animals

Healthy adult male albino Wistar rats, bred and reared in Animal House, School of Pharmacy, Vels University were used for the experiment. Weight matched animals (180-200g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 h dark). The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India). Animal ethical were prepared and submitted to department of pharmacy, School of Life Sciences, Vels University. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Vels University (CPCSEA Approval No. (XV/VELS/PCOL/11/2000/CPCSEA/IAEC/30.10.2013).

Chemicals

Streptozotocin (STZ.) was purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals used were of analytical grade obtained from E. Merck or HIMEDIA, Mumbai, India.

Experimental induction of Diabetes

The animals were made diabetic by an intraperitoneal injection of streptozotocin (STZ, 40 mg/kg body weight) in a freshly prepared citrate buffer (0.1M, pH 4.5) after an overnight fast. STZ injected animals were given 20 % glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. STZ injected animals exhibited massive glycosuria (determined by Benedict's qualitative test) and hyperglycaemia (by glucose oxidase method) within a few days. Diabetes in STZ rats was confirmed by measuring the fasting blood glucose concentration, 96 h after injection with STZ. The animals with blood glucose more than 240 mg/dL were considered diabetic and used for the experiments.

Dosage studies

Dosage fixation studies were carried out to determine the optimum dose of different extracts of EAGC, MGC and AqGC which exhibited maximal hypoglycaemic activity on diabetic rats.

Animals were randomly divided into ten groups of six animals each. Group I served as diabetic control and Group II, III and IV were diabetes induced rats treated with 50,100,200 mg/kg b.wt of EAGC. Group V, VI and VII were diabetes induced rats treated with 50,100,200 mg/kg b.wt of MGC. Group VIII, XI and X were diabetes induced rats treated with 50,100,200 mg/kg b.wt of AqGC. The extract was suspended in 2% gum acacia vehicle solution and fed by oral intubation.

Animals were fasted for 12 h, blood collected by retro orbital puncture followed by the extract administration. Subsequently blood was withdrawn for every 30 minutes for the next 2 h.

Plasma glucose levels were estimated using semi-auto analyzer (Qualigens AR 601)

For effective dosage fixation of *G. corticata* on glycaemic control in STZ-diabetic rats, an experiment was carried out for 15 days. Body weight and plasma glucose were assessed in STZ-induced diabetic rats.

Animals were randomly divided into five groups of six animals each. Group I served as normal control and Group II consisted diabetes induced rats treated with 100 mg/kg b.wt of EAGC. Group III consist of diabetes induced rats treated with 100 mg/kg b.wt of MGC. Group IV consist of diabetes induced rats treated with 100 mg/kg b.wt of AqGC.

Statistical analysis

All quantitative measurements were expressed as means ± SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS/PC* (statistical package for social sciences, personal computer) Ver. 10 and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the *p* value is less than 0.05.

Biochemical analysis

Plasma glucose was estimated by the method of Trinder using a reagent kit.[23]

RESULTS

Induction of diabetes by STZ provoked the severe alterations in blood glucose level as well as body weight in the experimented Wistar rats. Administration of EAGC, MGC and AqGC almost restored the blood glucose levels to near normal level in diabetic rats.

Effect of *G. corticata* extracts on Blood Glucose level

Table 1 shows the effect of *G. corticata* on blood glucose level on STZ-diabetic rats at 0h to 2 h. Diabetic rats showed a significant increase in blood glucose level by 4.24%. Oral administration of the three extracts at three different doses 50, 100 and 200 mg/kg of b.wt. decreased blood glucose level value ranging from 7.19% to 18.67%. Almost all extracts showed significant reductions in plasma glucose level when compared to the diabetic rats, thereby showing its beneficial effect in maintaining the blood glucose level. MGC showed maximal reduction of 18.67% at a dose of 100 mg/kg of b.wt. Based on this result, the optimum dosage for drug administration was determined.

Table 2 shows the effect of three extracts EAGC, MGC and AqGC on blood glucose level after administration for 15 days to STZ-diabetic rats. Group II diabetic rats indicated increase in blood glucose levels as compared to normal controls rats. Oral administration of EAGC, MGC and AqGC for 15 days showed significant reduction blood glucose levels in all three groups,

Table 1 Effect of different concentrations (2 h) of *G. corticata* extracts on blood glucose in STZ-induced diabetic rats

Extracts	Blood glucose (mg/dl)					2 h change (•/•)
	0 min	30 min	60 min	90 min	120 min	
Diabetic control	252.07±15.74	254.33±14.09	257.24±16.26	259.95±13.05	262.76±14.48[a]	(+) 4.24
EAGC extract 50 mg/kg BW	248.62±15.24	247.03±15.62	243.14±14.53	237.39±17.63	230.74±14.30[b]	(-) 7.19
EAGC extract 100 mg/kg BW	251.23±14.22	246.81±16.09	238.14±13.94	229.5±15.76	220.69±16.71[c]	(-) 12.15
EAGC extract 200 mg/kg BW	253.17±18.64	248.06±14.10	244.75±17.21	239.63±16.53	230.17±13.01[b]	(-) 9.08
MGC extract 50 mg/kg BW	247.84±14.45	245.73±15.28	239.35±16.30	232.62±12.65	222.01±12.42[c]	(-) 10.42
MGC extract 100 mg/kg BW	250.87±15.32	246.6±14.1	231.54±12.47	210.15±10.09	204.02±11.48[d]	(-) 18.67
MGC extract 200 mg/kg BW	256.15±14.27	246.69±13.33	242.97±16.25	236.12±12.56	222.82±14.64[c]	(-) 13.00
AGC extract 50 mg/kg BW	257.14±14.56	250.93±13.53	242.09±16.21	239.1±12.71	235.23±14.17[b]	(-) 8.62
AGC extract 100 mg/kg BW	249.21±13.26	246.78±14.34	240.63±17.51	232.18±12.61	221.42±12.42[c]	(-) 11.15
AGC extract 200 mg/kg BW	245.61±13.64	242.43±17.19	238.76±13.43	230.65±12.17	222.87±14.56[c]	(-) 9.25

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

maximal hypoglycemic activity shown by MGC was statistically near to normal values.

Table 2 Effect of *G. corticata* extracts on blood glucose in STZ-diabetic rats after 15 days

Groups	Blood glucose (mg/dl)				Changes (•/•)
	0 day	5th day	10th day	15th day	
Normal control	81.53±5.34	83.75±4.29	80.16±6.18	78.45±4.49[a]	(+) 3.77
Diabetic control	244.07±15.52	256.17±17.08	265.83±16.37	272.6±15.14[b]	(-) 11.71
Diabetic + EGC (100 mg/kg BW)	251.23±13.22	233.18±16.21	228.44±16.16	200.37±14.30[c]	(+) 20.24
Diabetic + MGC (100 mg/kg BW)	247.84±14.45	237.62±15.02	215.79±14.62	186.26±13.22[d]	(+)24.84
Diabetic + AGC (100 mg/kg BW)	249.21±14.35	235.15±16.54	223.35±14.80	199.48±16.17[c]	(+) 19.20

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT)

Table 3 Effect of *G. corticata* extracts on body weight (g) in STZ-diabetic rats after 15 days

Groups	Body weight (g)				Changes (•/•)
	0 day	5th day	10th day	15th day	
Normal control	180.24±15.13	183.57±14.84	186.52±14.60	192.65±13.97[a]	(+) 6.88
Diabetic control	182.02±16.86	178.68±17.09	171.19±16.45	164.4±16.13[c]	(-) 9.68
Diabetic + EGC (100 mg/kg BW)	180.95±15.62	183.53±15.30	187.7±16.10	190.82±17.43[b]	(+) 5.45
Diabetic + MGC (100 mg/kg BW)	181.14±17.16	184.36±16.90	188.47±8.03	193.34±17.81[a]	(+)6.73
Diabetic + AGC (100 mg/kg BW)	182.18±17.41	183.32±16.32	186.93±15.29	190.1±16.12[b]	(+) 4.34

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT)

Effect of *G. corticata* extracts on body weight

Table 3 show the effect of oral administration of *G. corticata* after 15 days on body weight on normal and STZ- diabetic rats. Administration significantly minimized the weight loss in STZ-diabetic rats as compared to the untreated diabetic group. Particularly, the methanolic extract of *G. corticata* (MGC) showed the maximal increase in body weight as compared to EAGC and AqGC thereby strongly confirming its beneficial effect in maintaining the body weight (or minimization of weight loss due to diabetes).

DISCUSSION

Algae are alternative nutrient sources that contain abundant secondary metabolites with a broad range of biological activities, such as antibiotics, anti-virals, anti-oxidants and anti-inflammatories.[24] The secondary metabolites are well known to have protective effect as anti-oxidants that neutralize or inactivate highly unstable and extremely reactive free radicals that attack the bodycells each day and even serve as scavengers of singlet oxygen and free radicals.[25,26,27] The previous studies have revealed that the secondary metabolites found in *G. corticata* are Alkaloids, Flavonoids, Catechin, Phenols, Quinones, Steroids, Tannins, Sugar, Glycosides, Amino acids and Xanthoproteins.[28,29] In a recent review, the antidiabetic potential of various phytochemicals from medicinal plant was figured out for drug development.[31]

While many studies have shown the beneficial effects of marine algae,[30] the present study is focused on the anti-hyperglycemic effect of *G. corticata* extract on STZ induced diabetic rats. The results clearly indicate that the extracts of red seaweed *G. corticata* have immune anti-hyperglycemic potential. In particular, the methanolic extracts (MGC) was found to be very effective in lowering blood glucose, as evidenced from the fact that they produce a significant fall in blood glucose after 2h and 15days study of treatment at a dose of 100 mg/kg body weight. Statistically significant weight loss was observed in the diabetic group and improvement in weight was observed in the treated groups. Particularly, methanolic extracts of *G. corticata* showed significant improvement in

body weight when compared to ethyl acetate (EAGC) and Aqueous extracts (AqGC) of *G. corticata*. These effects could be attributed due to the presence of secondary metabolites of *G. corticata*. Similarly, anti-hyperglycemic effects have been observed by restoration of blood glucose level to normal in diabetic rats and mice through different solvent extracts of brown algae *cystoseira moniliformis*[32] and *Padina arborescens*[33] and soft corals of *Sinularia firma* and *S. erecta*[34] in STZ-diabetic rats.

In general, the selective cytotoxic effect of STZ on pancreatic β-cells imbalance the insulin level and results in hyperglycemia. One of its cytotoxic intracellular effects is through the generation of free radicals, as has been demonstrated both in vivo and in vitro.[35,36] Studies have also shown that free radicals may cause disruption in insulin action and mitigate glucose tolerance states.[36] The mechanism of the anti-hyperglycaemic effect of *G. corticata* extracts is not clear at the moment. Reports are available to show that anti-diabetic plants may affect circulating insulin levels.[37]

The medicinal plant having hypoglycemic activity act through multiple mechanisms such as improving insulin sensitivity augmenting glucose-dependent insulin secretion and stimulating the regeneration of islets of langerhans in pancreas of STZ –induced diabetic rats. The fundamental mechanism underlying hypoglycemic action of the fraction of herbal plants in diabetic rats may be possible through the insulinomimetic action or by preventing the death of cell and /or it may permit recovery of partially destroyed cells or by the other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver.[38]

In conclusion, the efficacy of Methanolic extract of *Gracilaria corticata* (MGC) of *G. corticata* showed hypoglycemic effect in STZ induced diabetic wistar rats during 2h and 15 days study. This would conclude that *G .corticata* extract could be a good candidate in developing new pharmaceutics. However, comprehensive pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this alga as a therapeutic target in diabetes research.

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