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Research Article

Antidiabetic activity of *Ulva fasciata* and its impact on carbohydrate metabolism enzymes in alloxan induced diabetic rats

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ABSTRACT

A group of 30 Wistar albino rats were selected and divided into five groups of six rats each. The hypoglycemic effect of aqueous extract *Ulva fasciata* was evaluated in diabetic rats using two different dose (200 and 400 mg/kg) in terms of blood glucose, glycosylated hemoglobin and hepatic glycogen were examined in control and extract treated diabetic rats. The activities of carbohydrate metabolic enzymes like hexokinase, glucokinase and glucose 6 phosphatase in the liver were assessed. The extract was orally administered for 28days. The results showed that aqueous extract of *Ulva fasciata* significantly ($p<0.05$) reduced blood glucose level, and restored hepatic glycogen content and hexokinase, glucokinase and glucose 6 phosphatase activities. Though glycosylated hemoglobin levels were reduced, it was not statistically significant, the body weight of the treated group rats showed a gain in weight. These results prove the antidiabetic activity of *Ulva fasciata*.

Keywords: *Ulva fasciata;* hexokinase; glucokinase; glucose 6 phosphatase

INTRODUCTION

Diabetes mellitus (DM) is a group of heterogenous, hormonal and metabolic disorders characterized by hyperglycemia and glucosuria, with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, both (WHO, 1999). Insulin therapy affords effective glycemic control, yet its shortcomings such as ineffectiveness of constant refrigeration and in the limits its usage. Treatments with sulfonylureas and biguanides are also associated with side effects. The plant drugs are frequently considered to be less toxic when compared to synthetic drugs. Herbal medicines are used in primary health care by about 80% of the world population particularly in developing countries because of better cultural acceptability, safety, efficacy, inexpensive, and lesser side effects (Pari and Umamaheswari, 2000).

Edible seaweeds contain a range of components which have potential health benefits (MacArtain, 2007). They are good sources of dietary fibre, especially soluble fibre such as alginates, which can influence satiety and glucose uptake from foods (Brownlee et al., 2005). These soluble polysaccharides may also act as prebiotics, stimulating growth of "beneficial" bacteria in the colon (Wang et al., 2006), edible seaweeds can contain appreciable amounts of polyphenols (Rodriguez et al., 2010), which are effective antioxidants and may have particular biological activities. Polyphenols from edible seaweeds have also been suggested to influence responses relevant to diabetes through modulation of glucose-induced oxidative stress (Lee et al, 2010), as well as through inhibition of starch-digestive enzymes (Lee et al., 2008).

The use of natural products or their active components for the prevention and/or treatment of chronic diseases are based primarily on the traditional medicine of various ethnic societies and on epidemiological data (Ribeiro and Salvadori, 2003). In present time, searching for safe and efficacious medicinal plants, possessing antidiabetic, antigenotoxic and antioxidant activities, are very important for therapy of complications of chronic diabetes. Seaweeds have traditionally been used as food and folk medicine for curing helminthes infectious, gout and eczema, particularly by coastal peoples in several countries (Celikler, 2009). Therefore the primary objectives of this study were to assess the hypoglycemic efficacy of selected seaweed against different intensities of hyperglycemia in rat models utilizing different diabetogenic agents in different doses for 28 days.

MATERIALS AND METHODS

Selection of sample

The green algae, *Ulva fasciata,* samples were collected at the seashore of Gulf of Mannar coast. The identification and authentication was carried out at CSMCRI, Mandapam-Ramanathapuram district. Epiphytes, salt and sand were cleaned and necrotic parts were removed. The surface of microflora was removed by suc-

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cessively rinsing with sea water and then with distilled water. Then, they were dried in the shade and finely powdered.

Preparation of extracts

Powdered seaweed (100 g) was dipped into 20 volumes of distilled water and kept at room temperature for 2 h, then homogenized and refluxed at 100°C for two hours. After cooling, the resulting material was centrifuged 10,000 g for 15 min. The supernatant was collected and centrifuged again at 8000 rpm for 15 min to obtain a clarified mixture. The supernatant so obtained was lyophilized (Alpha 1-2 LD Plus, Martin Christ, Germany) and stored at 4 ºC in a refrigerator for the further use as crude aqueous extract (Boopathy et al., 2010).

Selection and acclimatization of animals

Wistar strains of male albino rats weighing between 180-200 g were selected and were housed in large spacious cages and they were fed with commercial pellets and had access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature (22°c ± 5°c) and humidity (55 ±5%) and 12 h light dark cycles throughout the experimental period and the study was approved by the (R.G.ABIRAMI/A47/05/2010/PhD)

Induction of Diabetes mellitus

Diabetes mellitus is induced in Wistar rats by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (150mg/kg BW) in physiological saline after overnight fasting for 12 h. The development of hyperglycemias in rats is confirmed by plasma glucose estimation 72 h post alloxan injection. The rats with a fasting plasma glucose level of 200-260 mg/dl were used for this experiment.

Experimental procedure

In this experiment a total of 30 rats (24 diabetic surviving rats and six normal rats) were used. Diabetes was induced in rats three days before starting the experiment. The rats were divided into five groups after the induction of alloxan diabetes. In the experiment six rats were used in each group. Group-I consisted of normal rats given 10ml/Kg of normal saline. Group-II consisted of diabetic control received 150mg/Kg of Alloxan monohydrate through i.p. Group-III consisted of positive control i.e diabetic rat which received glipizide (10mg/Kg i.p) for 28 days. Group-IV was treatment group which consisted of diabetic rats received low dose (200mg/Kg) of aqueous extract of *Ulva fasciata* daily for 28 days. Group-V consisted of diabetic rat received high dose (400mg/Kg) of aqueous extract of *Ulva fasciata* daily for 28 days.

Sample collection

After 28 days of treatment, the blood glucose level and body weight was measured. Then blood was collected retro-orbitally from the eye under light ether anesthesia using capillary tubes. Blood was collected in fresh vials containing EDTA as anticoagulant agent and plasma was separated in a T8 electric centrifuge at 2000 rpm for two minutes. Then the animal was sacrificed by decapitation. Liver and pancreas were immediately dissected out, washed in ice-cold saline to remove the blood. And the liver was used for estimation of enzyme activity.

Estimation of blood glucose

Fasting blood glucose levels was determined in all experimental rats initially to determine the diabetic status and thereafter every week during the 28 day study period. Blood was obtained by snipping tail of rat with the help of sharp razor and blood glucose levels were determined using glucometer (Ultra Touch Two, Johnson and Johnson). Each time the tail of the rat was sterilized with spirit.

Hepatic glucokinase and hexokinase activity

The liver was perfused with ice cold 0.15M KCl and 1mM EDTA solution and homogenized with twice its weight of ice cold buffer (0.01 cysteine and 1mM EDTA in 0.1ml Tris-HCL., pH 7.4) and centrifuged for 20minutes at 40°C. Glucose phosphorylation was assayed by means of the glucose 6 phosphate dependent spectrophotometric method at 340nm (Chou and Wilson, 1975).

Glucose-6-phosphatase activity

The liver glucose-6-phosphatase activity was measured according to standard protocol (Swanson, 1955). Tissue was homogenized in ice cold of 0.1 M phosphate buffer saline (pH=7.4) at the tissue concentration of 50 mg/ml. In a calibrated centrifuge tube, 0.1ml of 0.1 M glucose-6-phosphate solution and 0.3 ml of 0.5 M maleic acid buffer (pH=6.5) were taken and brought to 37 °C in water bath for 15 min. The reaction was stopped with 1 ml of 10% trichloroacetic acid (TCA) followed by chilling in ice and centrifuged at 3000 × g for 10 min. The optical density was noted at 340 nm.

Glycogen content

The tissue sample was digested by hot concentrated 30% KOH and treated with anthrone reagent. Glycogen content was determined colorimetrically as glucose. (Swanston et al., 1990)

Estimation of glycosylated hemoglobin

After 28 days experimental period, the 12 h fasted rats were sacrificed by cervical decapitation, blood was withdrawn by retro orbital puncture under light ether anesthesia and the glycosylated hemoglobin was estimated (Sadasivam and Manickam, 1996).

Statistical analysis

The data thus obtained was processed and the results were interpreted. The haematological and biochemical

parameters of the control and treated groups II, III, IV and V were subjected to statistical analysis by one way Analysis of Variance to determine the significant difference between the groups. ANOVA was done with Graphpad Instat 3; Software by Student- Newman Keul's multiple range tests. Data were accepted as statistically significant difference at p <0.05. Diabetic control was compared with the normal, experimental groups were compared with diabetic control and non diabetic control.

RESULTS AND DISCUSSION

Effect of body weight of normal and experimental animals in each group

Table 1 shows the mean body weight of the diabetic rats observed throughout the study period.

Table 1 shows the values of body weight of normal and experimental rats in each group. The mean body weight of diabetic rats 157.33g significantly decreases as compared to normal animals 206.33g.

Table 1: Mean body weight of normal and experimental animals in each group

GROUPS	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)
Group I	202 ± 4.09	206.33 ± 3.10
Group II	195.83 ± 4.72	157.33 ± 2.41[a]
Group III	202.5 ± 3.81	227.33 ± 5.54[b]
Group IV	206.66 ± 5.72	212.33 ± 3.86[b]
Group V	196.66 ± 4.41	221.0 ± 1.80[b]

Group I -Normal Control; Group II- Diabetic Control; Group III - Positive control (Glipizide);Group IV - Treatment group 200mg/kg; Group V - Treatment group 400mg/kg. Values are expressed as Mean ± SEM for six rats. a – Values are significantly different from control (Group I) at p <0.01. b – Values are significantly different from diabetic control (Group II) p < 0.001, [ns]– not significant

The body weight of diabetic rats treated with aqueous extract of *U.fasciata* at different doses 200 mg/kg & 400 mg/kg was significantly increased to 212.33 & 221.00 respectively as compared to non-treated diabetic animals. Group III treated rats also showed a significant increase (227.33g) in body weight as compared to diabetic rats.

In diabetes mellitus, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as an energy source. This leads to decrease in protein storage which in turn (Guyton and Hall 2000) reduces body weight. In the present study alloxan induced diabetic rats showed a decrease in body weight throughout the experimental period. Oral treatment with aqueous extract of *Ulva fasicata* significantly improved the body weight loss in diabetic rats as compared to diabetic control indicating the possible role of the extract in restoration of protein metabolism.

Effect of *Ulva fasciata* on Liver glycogen content and Glycosylated Heamoglobin of experimental rats.

In the present study, decrease in glycogen content in alloxan-diabetic rats i.e 8.05mg/g, the decrease in glycogen content may result due to disturbances in glycogen synthetase system. Improvement in liver glycogen was noticed in group IV and V i.e 22.23mg/g and 24.78mg/g of diabetic rats after chronic treatment with aqueous extract of *Ulva fasciata* which indicates that the possible way of antidiabetic activity of the extract may be by the improvement of glycogenesis and or suppression of glycogenolysis. On the other hand group III showed more liver glycogen (32.51mg/g) which was equivalent to the normal group I.

Table 2: Liver Glycogen and Glycosylated Heamoglobin

Groups	Liver Glycogen Content (mg/g tissue)	Glycosylated Heamoglobin %
GROUP I	39.16 ± 2.10	5.38±0.21
GROUP II	8.05 ± 0.38[b]	8.53±1.78[ns]
GROUP III	32.51 ± 1.46[a]	6.13±0.56[ns]
GROUP IV	22.23 ± 1.45[b]	6.91±1.01[ns]
GROUP V	24.78 ± 1.01[b]	6.86±0.98[ns]

Group I -Normal Control; Group II- Diabetic Control; Group III - Positive control (Glipizide); Group IV - Treatment group 200mg/kg; Group V - Treatment group 400mg/kg. Values are expressed as Mean ± SEM for six rats. a – Values are significantly different from control (Group I) at p <0.01. b – Values are significantly different from diabetic control (Group II) p < 0.001, [ns]– not significant

As reported earlier (Yoon, 2001) in the present study also, hepatic glycogen content decreased significantly in diabetic controls as compared to non diabetic control i.e 39.16mg/g. On the other hand, treatment with aqueous extract of *Ulva fasicata* at lower dose (200mg/kg) was 22.23mg/g and upper dose (400mg/kg) was 24.78mg/g to exert any statistically significant changes between two groups but compared to control group it was significant at P<0.001, whereas the drug treated group III rats showed high content of glycogen 32.5l mg/g almost nearer to the normal rats. Thus glycogen in the liver is possibly due to earlier stimulation of insulin release from β cells or due to insulinomimetic activity of bioactive components present in the seaweed resulting in direct peripheral glucose uptake or due to a combination of two. The significant increase of glycogen levels was observed in the both dosage levels of aqueous extract treated diabetic rats.

Table 3: Fasting Blood Glucose (mg%)

GROUPS	Day 0	7th DAY	14th DAY	21th day	28th DAY
GROUP I	78.16±4.96	71.5± 5.67	75.83± 5.38	79.08±2.92	76.33± 4.42
GROUP II	258.5±5.68[a]	270.6± 5.48[a]	76.16± 6.18[a]	280.34±2.01[a]	303.5± 7.43[a]
GROUP III	254.83±5.91[a]	210.5± 4.74[a]	198.33± 4.99[b]	193.98±2.78[a]	109.66± 3.20[b]
GROUP IV	252.8±3.00[a]	198.18±6.11[a]	195.65±6.96[a]	168.3± 4.47[b]	151.16 ± 3.41[b]
GROUP V	257.98±1.20[a]	218± 4.57[a]	187.16± 4.40[b]	161.16±8.6	133.96± 1.14[b]

Group I -Normal Control; Group II- Diabetic Control; Group III - Positive control (Glipizide); Group IV - Treatment group 200mg/kg; Group V - Treatment group 400mg/kg. Values are expressed as Mean ± SEM for six rats. a – Values are significantly different from control (Group I) at p <0.01. b – Values are significantly different from diabetic control (Group II) p < 0.001, [ns]– not significant

The glycosylated heamoglobin of diabetic rats treated with *Ulva fasciata* at lower and higher doses were decreased to 6.91% and 6.86% respectively. In group II the level was 8.53%, when compared to the normal group 5.38% it was higher but changes were not statistically significant, whereas the group III glipizide treated rats showed low level of glycosylated heamoglobin (6.13%) which was nearer to the normal levels. During the extract-induced hypoglycemia, blood glucose was reduced and there was an increase in liver glycogen content. This may be due to mobilization of blood glucose towards liver glycogen reserve.

Glycosylated hemogolobin is formed progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of test. Therefore, glycosylated hemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level (Kameswararao et al., 2003).

Animals treated with aqueous extract of *Ulva fasciata* indicated a significant decrease in the glycosylated hemoglobin level which may be due to an improvement in insulin secretion, whereas glycosylated hemoglobin level increased significantly in untreated diabetic control group, which confirm the antidiabetic action of the extract.

Effect of *Ulva fasciata* on glucose levels in alloxan induced diabetic rats

The effect of alloxan and aqueous extracts of *Ulva fasciata* on blood glucose level is shown in Table 3. On repeated administration of aqueous extract of *Ulva fasciata* for 28 days, a sustained and significant (p<0.01) decrease in blood glucose level of diabetic rats was observed in a dose dependent manner as compared to the diabetic control group. In diabetic rats blood glucose level was reduced by 40.21% and 48.07% at 200 and 400 mg/kg doses of the extract respectively. The standard oral hypoglycemic drug glipizide (10mg/kg) showed more potent antidiabetic activity by reducing blood glucose level by 56.97%. The aqueous extract of *Ulva fasicata* indicates that the overall blood glucose level was controlled, probably may be due to an improvement in insulin secretion. The extract-induced decrease in the concentration of blood glucose in alloxan-treated rats may be the result of increased glycolysis.

Effect of *Ulva fasciata* extract on glucose levels in alloxan induced diabetic rats

As compared to non-diabetic control values, mean level of enzymes hexokinase and glucokinase and glucose-6-phosphatase values decreased in diabetic control. The respective percentage decrease was 56.9%, 79.9% and 67.2% in diabetic control when compared to the normal control group. Treatment with *Ulvafasciata* at lower doses for 28 days led to rise in the percentage of these parameters by 52.2%, 5.74% & 40.85% and for higher dose (400mg/kg) 54.44%, 20.97% & 46.34% respectively (P<0.001) as compared to diabetic control. Also treatment with glipizide 10mg/kg for 28 days led to rise in the percentage of these parameters by 98.8%, 52.34% and 79.26% respectively (P< 0.001) as compared to diabetic control.

Table 4: Carbohydrates metabolism enzymes of treated groups

GROUPS	HEXOKINASE (µg/mg)	GLUCOKINASE (µg/mg)	G-6-P (µg/mg)
GROUP I	0.209 ±0.012	24.34 ± 1.40	0.39±0.01
GROUPII	0.090±0.00[a]	5.96 ± 0.25[a]	0.16±0.01[a]
GROUP III	0.18± 0.006[b]	9.08 ± 0.90[b]	0.29±0.01[b]
GROUP IV	0.14± 0.004[b]	6.30± 0.55[b]	0.23±0.01[b]
GROUPV	0.14±0.003[b]	7.21 ± 0.87[b]	0.24±0.01[b]

G-6-P: Glucose 6 phosphatase. Group I -Normal Control; Group II- Diabetic Control; Group III - Positive control (Glipizide); Group IV - Treatment group 200mg/kg; Group V - Treatment group 400mg/kg. Values are expressed as Mean ± SEM for six rats. a – Values are significantly different from control (Group I) at p <0.01. b – Values are significantly different from diabetic control (Group II) p < 0.001, [ns]– not significant

The reduced glycogen content observed in diabetic rats may be associated with an increase in glycogen phosphorylase activity. Disturbed activities of carbohydrate metabolizing enzymes in diabetic rats indicate that the carbohydrate metabolic pathways (glycolysis, glycoge-

nolysis, glycogenesis and gluconeogenesis) were severely affected, which was probably due to insulin deficiency.

Our results indicated that the activities of hexokinase, glucokinase and glucose 6 phosphatase were significantly depressed in untreated diabetic rats. These results correspond with the reports of other researchers. Decrease enzymatic activity of glucokinase, hexokinase has been reported in diabetic animals resulting in depletion of liver and muscle glucose. Treatment with an upper dose of *Ulva fasciata* extract increases the glucose-6-phosphatase content in the liver, indicating an overall increase in glucose influx. The lower dose of *Ulva fasicata* also alters the enzyme level but could not normalize it. The polyphenolic constituents of *A. nodosum,* red seaweed exhibit promising antidiabetic characteristics and are amenable for use as food supplement ingredients (Zhang et al., 2007).

Administration of *Ulva fasicata* extract to diabetic animals increased the activity of glucokinase in liver. The activity of gluconeogenic enzyme, glucose 6 phosphatase is enhanced during diabetes (Babu, 2008). Treatment of diabetic rats with the extract of *Ulva fasicata* showed an increase hexokinase, glucokinase activity. At the same time, the activity level of the enzyme is considerably lower than normal in diabetes. Under these conditions, the high blood glucose level might overcome a lower glucokinase activity as well as an elevated hepatic glucose 6- phosphatase activity to maintain a high liver glycogen level. Glucose-6-phosphatase plays an important role in the homeostasis of blood glucose (Berg et al., 2001).

Conclusion

This work demonstrates the efficacy of an aqueous extract of *Ulva fasciata* to reduce the glycemic response consecutive to carbohydrate metabolism enzymes *in vivo*. The mechanism of the reaction of *Ulva fasciata* is not yet known exactly but from its effect on these glycolytic enzymes it seems to increase the flux of glucose into the glycolytic pathway in an attempt to reduce high blood glucose concentrations and increase the metabolism rate. Further work has to done to know the bioactive component responsible for antidiabetic activity.

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