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ASSESSMENT OF ANTI-DIABETIC ACTIVITY OF SOME SELECTED SEAWEEDS

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ABSTRACT
Three seaweeds one from each group was taken to test their antidiabetic activity against alloxan induced diabetic rats. The experiment was carried out for 28 days and their results are tabulated. In our study we found that the red seaweed *A.spicifera* had good antidiabetic activity. It can be a good natural remedy to maintain the body weight, glucose levels, glycogen and hepatic enzymes. A brief haematological study was also carried out to find out the WBC, RBC, Haemoglobin and platelet counts.

KEYWORDS: antidiabetic, seaweeds, alloxan induced diabetes, haematology.

INTRODUCTION
Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. The underlying process attributed to hyperglycemia ultimately result in oxidative stress, alteration in enzyme activity, protein glycosylation and several structural changes.[1] Enzyme activities of glucogenesis have been shown to increase in the glucogenolytics and lipolytic pathways. Diabetes also has been accompanied with the decrease in the enzyme activity of the glycolytics and pentose phosphate pathways.[2]

Despite the immense strides that have been made in the understanding and management of diabetes, disease related complications are increasingly unabated. In spite of the presence of series of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Many marine sources treatments for diabetes are used throughout the world and there is an increasing demand from patients to use the natural products with anti-diabetic activity.[3] Seaweeds offer a wide range of therapeutic possibilities both internally and externally. All essential minerals are provided by dietary seaweeds. No land plant even remotely approaches seaweeds as sources of metabolically-required minerals. Brown seaweeds are the only known non-animal sources of thyroid hormones. Most seaweeds are rich in vitamins, especially the B vitamins, including B12.Hence, the present study was carried out to find whether seaweeds had antidiabetic activities.

MATERIALS AND METHODS
Seaweed (*Acanthophora spicifera, Padina tetrastomatica* and *Caulerpa scalpelliformis*,) samples which were healthy and fully grown and submerged underwater from the tidepools were collected from Tuticorin coast (08° 46’ 2.15”N lat; 78°11’ 16.05” E long). Representative species from each group of macro algae such as Red (*Acanthophora spicifera*) Brown (*Padina tetrastomatica*) and Green (*Caulerpa scalpelliformis*), were collected. The samples were washed with seawater and freshwater to remove salt, epiphytic microorganisms and other suspended materials. The clean algae were frozen and lycophilized. The dry material was stored at -20°C.

Preparation of Extract: Extracts of the freeze dried and powdered biomass were prepared using ethanol, as solvent using a soxhlet apparatus. The resultant crude extracts were filtered and then concentrated in a rotary evaporator at a temperature less than 40°C. The crude extracts were weighed and deep frozen (-20°C) until further use.

Selection and acclimatization of animals
Wistar strains of albino rats weighing between 180-200g were used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature (22 ± 5°C) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

LD_{50} Determination: LD_{50} is the dose that is lethal to 50% of a population. LD_{50} of the extract was determined as per OECD guidelines (423).[4]

Experimental procedure: IAEC, K. M. College of Pharmacy, Madurai has approved this experimental procedure which has the approval from the appropriate Institutional Animal Ethics Committee in accordance with "Principles of Laboratory Animal Care".

In the experiment a total of 36 rats (30 diabetic surviving rats and 6 normal rats 6 in each group.) were used. Diabetes was induced in rats 3 days before starting the experiment. The rats were divided into 6 groups after the induction of alloxan diabetes.

Treatment protocol

Group-I: (Normal control) consist of normal rats treated with 10ml/Kg of normal saline, orally.

Group-II: (Toxic control) Diabetic control received 150mg/Kg of Alloxan monohydrate through Intra peritoneally.

Group-III: (Positive control) Diabetic rats received Glipizide (10mg/Kg i.p) (Kavalali *et al*.,2003) for 28 days, orally.

Group-IV: (Treatment group) Diabetic rats received extract of *A.spicifera* at a dose of (200mg/Kg) daily using intra-gastric tube for 28 days.

Group-V: (Treatment group) Diabetic rats received extract of *P. tetrastomatica* at a dose of (200mg/Kg) daily using intra-gastric tube for 28 days.

Group-VI: (Treatment group) Diabetic rats received extract of *C. scalpelliformis* at a dose of (200mg/Kg) daily using intra-gastric tube for 28 days.

Biochemical analysis

Estimation of blood glucose

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson and Johnson based on glucose oxidase method.[5]

Glucose-6-phosphatase activity

Glucose phosphorylation was assayed by means of glucose 6 phosphate dependent spectrophotometric method.[6]

Glycogen Content

Glycogen content was determined calorimetrically.[7]

Haematological and Biochemical parameters

Blood samples were assessed for RBC, WBC, HB and Platelets with an auto analyzer (MISPA-EXCEL, Japan).

Statistical analysis: Data was put across as the mean ± SEM. For statistical analysis of the data, group means were compared by one way analysis of variance (ANOVA) followed by Newman Keul's multiple range tests, which was used to identify difference between groups. P value <0.05 was considered significant.

RESULT

Table 1 shows the values of body weight of normal and experimental animals in each group. The mean body weight of diabetic rats (155.40 ± 2.45) was significantly decreased as compared to normal animals (215.45 ± 3.20).The body weight of diabetic rats treated with ethanol extracts of the three seaweeds at a dose of 200 mg/kg was significantly increased to 226.32 ± 3.40, 218.8±3.65 and 226.50 ± 3.80 respectively as compared to non-treated diabetic animals. In group III treated animals also showed an increase in body weight significantly as compared to diabetic rats.

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

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group I Normal control	207.18 ± 4.18	215.45 ± 3.20
Group II Diabetic control	200.80 ± 4.70^{*a}	155.40 ± 2.45^{*a}
Group III Positive control	210.20 ± 4.76^{*b}	220.35 ± 4.50^{*b}
Group IV	222.65 ± 5.50^{*b}	226.32 ± 3.40^{*b}
Group V	218.35 ± 4.52^{*b}	226.5 ± 3.80^{*b}
Group VI	212.45 ± 4.20^{*b}	218.8 ± 3.65^{*b}

Values are expressed as Mean ± SEM.
Values were calculated by using one way ANOVA followed by Newman Keul's multiple range tests.
**a values were significantly different from normal control (Group I) *b values were significantly different from diabetic control (Group II).*

Table 2: Effect of extracts of *A. spicifera, P. tetrastomatica*, and *C. scalpelliformis* on glucose levels (mg %) in alloxan diabetic rats.

Groups	0 Day	14th Day	28th Day
Group I Normal control	75.6 ± 2.80	77.85 ± 2.30	72.20 ± 2.40
Group II Diabetic control	156.55 ± 5.65^{*a}	178.20 ± 4.28^{*a}	220.8 ± 5.38^{*a}
Group III Positive control	190.8 ± 5.25^{*b}	147.40 ± 4.17^{*b}	130.65 ± 3.72^{*b}
Group IV	190.58 ± 4.10^{*b}	156.60 ± 4.44^{*b}	148.32 ± 3.18^{*b}
Group V	195.30 ± 4.15^{*b}	152.40 ± 4.08^{*b}	152.58 ± 3.54^{*b}
Group VI	194.65 ± 4.20^{*b}	156.60 ± 4.38^{*b}	146.20 ± 3.24^{*b}

Blood Glucose Levels: In all groups prior to alloxan administration the basal level of plasma glucose of the rats were not significantly higher in the rats selected for the study. In contrast non-diabetic control remained steadily euglycemic throughout the course of study.

In pilot study (mild diabetics) the table 2 values show the effect of treatment of extracts of *A.spicifera*, *P.tetrastomatica* and *Caulerpa scalpelliformis* at a dose of 200 mg/kg on plasma glucose levels. Blood glucose level was increased significantly to 178.20 ± 4.28 and 220.8 ± 5.38 at 14th and 28th day of treatment respectively, in the diabetic animals as compared to normal animals.

In the treated groups (groups treated with seaweed extracts), significant anti-hyperglycemic ($p<0.001$) effect was evident from the 2nd week onwards, the decrease in blood sugar was maximum on completion of the 4th week in the group receiving 200 mg/kg of the extracts, whereas in group III treated animals receiving Glipizide at a dose of 10mg/kg also restored the blood sugar level near to normal range.

Glycogen Content

Glycogen content of liver tissue was estimated on the 28th day in non-diabetic control, diabetic control drug, treated group and positive control group as shown in Table 3. In diabetic control rat liver glycogen content decreased significantly by 79.89 % as compared to non-diabetic control. Treatment with Glipizide and the 3 seaweed extracts of *A.spicifera*, *P.tetrastomatica* and *Caulerpa scalpelliformis* at a dose (200mg/kg) led to 74.47 %, 66.05 %, 68.79% and 70.56% increase in liver glycogen content in comparison to diabetic control.

Table 3: Effect of *A. spicifera, P. tetrastomatica* and *C. scalpelliformis* on glycogen content (mg/gm tissue)

Groups	Liver Tissue Glycogen Content (mg/g tissue)
Group I Normal control	41.28 ± 2.42
Group II Diabetic control	8.30 ± 0.60*a
Group III Positive control	32.52 ± 1.65*b
Group IV	26.60 ± 1.42*b
Group V	24.45 ± 1.15*b
Group VI	28.20 ± 1.55*b

Hepatic Enzymes

To establish diabetic, plasma glucose was determined 72hr after alloxan administration. Only those rats with over 180 mg% were included in the study. On the 28th day, hepatic enzymes Hexokinase, Glucokinase and substrate Glucose-6-phosphatase were estimated in saline control (group I), diabetic control (group II) and treatment controls (groups III, IV, V and VI).

The result has been compiled in Table 4. As compared to non-diabetic control values, mean level of enzymes Hexokinase, Glucokinase and substrate Glucose-6-phosphatase values decreased in diabetic control. The respective percentage decrease was 56.19%, 79.96% and 67.69% in diabetic control. Treatment with extracts of *A.spicifera*, *P.tetrastomatica* and *Caulerpa scalpelliformis* at a dose of (200mg/kg) for 28 days led to rise in percentage of these parameter by 22.03% and 56.03%, 45.21% and 34.28%,, 67.78% and 47.5% and 33.33%,67.88% and 45.76% respectively ($p<0.001$) as compared to diabetic control. Also, treatment with Glipizide 10mg/kg for 28 days led to rise in percentage of these parameters by 27.55%, 65.39% and 58.0% respectively ($p< 0.001$) as compared to diabetic control.

Table 4: Effect of *A. spicifera, P. tetrastomatica* and *C. scalpelliformis* on enzymes involved in carbohydrate metabolism in rats

Groups	Hexokinase (µg/mg)	Glucose-6-Phosphatase (µg/mg)	Glucokinase (µg/mg)
Group I Normal control	0.216 ± 0.013	0.392 ± 0.010	23.40 ± 1.38
Group II Diabetic control	0.090 ± 0.004*a	0.128 ± 0.007*a	4.90 ± 0.28*a
Group III Positive control	0.126 ± 0.007*b	0.301 ± 0.010*b	15.20 ± 0.92
Group IV	0.141 ± 0.006*b	0.242 ± 0.008*b	16.16 ± 0.90*b
Group V	0.120 ± 0.005*b	0.231 ± 0.007*b	12.12 ± 0.50*b
Group VI	0.135 ± 0.005*b	0.236 ± 0.007*b	15.26 ± 0.94*b

Table 5: Effect of *A. spicifera, P.tetrastomatica* and *C.scalpelliformis* on haematological parameters

Groups	WBC × 10^3/µL	RBC × 10^6/µL	HB % gm/dL	Platelet × 10^3/ml
Group I Normal control	8.45 ± 0.62	6.55 ± 0.32	12.35 ± 0.66	312.46 ± 14.05
Group II Diabetic control	8.16 ± 0.75*a	6.98 ± 0.25*a	13.25 ± 0.48*a	296.52± 13.30*a
Group III Positive control	6.50 ± 0.45*b	6.56 ± 0.48*b	14.33 ± 0.40*b	280.45 ± 31.60*b
Group IV	8.85 ± 0.85*b	6.80 ± 0.45*b	11.85 ±0.63*b	315.45 ± 12.35*b
Group V	7.15 ± 0.45*b	7.80 ± 0.26*b	12.86 ± 0.60*b	316.80 ± 12.88*b
Group VI	8.90 ± 0.92*b	6.75 ± 0.52*b	11.40 ±0.56*b	306.65 ± 11.20*b

Haematological Parameters

Table 5 values show the haematological parameters of group I to VI treated animals. At the end of 28 days of the study period, no statistically significant differences were seen in the mean WBC and RBC counts, HB and Platelet values as compared to the non-diabetic animals.

DISCUSSION

In the present study all the three seaweeds taken for study showed promising antidiabetic activity. However, the activity was profound in the seaweed. *A.spicifera*.

Hardoko et al (2008) [8] has reported that seaweeds may reduce blood sugar levels in the blood in invivo. According Limantara and Heryanto (2011)[9], fucoxanthine found in the brown seaweed can inhibit the accumulation of fat thus preventing obesity and can be used as an antidiabetic drug. In extract treated diabetic rats, reduction in blood glucose levels may be associated with degranulation in pancreatic β-cells, peripheral utilization of glucose or decrease in glucose uptake in intestine, which is in line with earlier reports. The increased hepatic glucose output in diabetes may be derived from glycogenolysis and/or gluconeogenesis.[10] In general, increased hepatic glucose production plus decreased hepatic glycogen synthesis and glycolysis are the major symptoms in type 2 diabetes that results in hyperglycemia.[11] Our results revealed an immense depletion in hepatic glycogen contents. These results are in accordance with those of Lavoie and Van de Werve, (1991)[12] and Ahmed *et al.* 2010[13] who found that streptozotocin induced diabetes, reduced hepatic glycogen content and increased glucose - 6- phosphatase activity in diabetic rats. We can thus state that the seaweeds are a good source of bioactive substances and these can be further studied to bring out their therapeutic nature.

Ethics

This experiment has the approval from the appropriate Institutional Animal Ethics Committee in accordance with "Principles of Laboratory Animal Care"

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