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# *Ulva reticulata*, a marine alga normalize streptozotocin induced lipid peroxidation in experimental diabetic rats

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## ABSTRACT

Increased oxidative stress is directly involved in the development and progression of diabetes which leads to impairment of antioxidant status and increased free radical production. The present study was aimed to evaluate the effects of ethanolic extract of marine algae *Ulva reticulata* on plasma glucose, insulin, TBARS, lipid hydroperoxides and non enzymatic antioxidants including vitamin E, vitamin C and reduced glutathione (GSH) in streptozotocin (STZ) induced diabetic rats. Diabetes was induced by single intraperitoneal injection of STZ at a dose of 45 mg/kg body weight. Male wistar rats were randomly divided in to four groups. Diabetic rats treated with oral administration of ethanolic extract of *U. reticulata* for 45 days resulted in a significant reduction in fasting plasma glucose, TBARS, lipid hydroperoxides and elevated the activities of plasma insulin, vitamin E, vitamin C and reduced glutathione (GSH) when comparison with diabetic control group. Seaweeds are a valuable food resource which contains low calories, and are rich in vitamins, minerals, proteins, polysaccharides, steroids and dietary fibers. These findings suggest that *U. reticulata* extract have potent protective effect on STZ induced lipid peroxidative changes in diabetic rats.

**Keywords:** Ulva reticulata, Diabetes, STZ, Oxidative stress, Antioxidant

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## INTRODUCTION

Seaweeds are major marine resources which are important to dietary source and pharmaceutical industries. Such seaweeds are widely consumed as food and it maintain the eco system of marine organisms. The marine seaweeds contain varieties of nutrients and its supplementations are beneficial to our health. The seaweeds are mainly used for agar, alginate and some therapeutic agents apart from they produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [1,2].

Diabetes mellitus is a group of metabolic disease characterized by combination of hyperglycemia and glucose intolerance and dyslipidemia due to defects in insulin secretion, insulin action or both [3]. Diabetic incidence is considered to be high all over the world, it was estimated that in 2017 there are 451 million people with diabetes worldwide. These will be expected to increase to 693 million in 2045 [4]. Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body is closely associated with number of diseases including cancer, cardiovascular diseases, diabetes and diabetic complications [5].

Free radicals are produced in the body, as byproducts of metabolism and as a result of exposure of environmental pollutants and sedentary life style. Because these free radicals are highly reactive, they can damage cellular components and are concerned in a variety of diseases under normal conditions, free radicals are neutralized by well organized systems in the body that include the nutrient derived antioxidants [6]. Lipid peroxidation (LPO) is a key marker of oxidative stress. It is a free radical-induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction. The significant extent of LPO products that was measured as thiobarbituric acid reactive substances (TBARS) has been reported in diabetes [7].

The modern drugs, including insulin and other oral hypoglycemic agents such as Thiazolidinediones, biguanides, sulphonylureas control the blood glucose level as long as they are regularly administered, but they also produced lots of undesirable effects and ultimately, all of them fail to restore the glycemic control [8]. Thus, it is essential to look for more effective antidiabetic agents

preferably from dietary sources, which should be economical and non-toxic or less toxic. So, we considering some alternative approaches, including by identifying natural marine sources, especially algae for treatment of diabetes. *Ulva reticulata* are green algae in the division chlorophyta are the type species of the genus Ulva also known by the common name sea lettuce, which are widely distributed in both inter tidal and deep water regions of the seas. These sea weeds are of immense pharmaceutical, agricultural value and used in several traditional medicinal systems for many medical purposes including nutritional support, cancer therapy, immune stimulation, body detoxification, weight control, treatment of viral diseases etc [9,10,11]. Many types of algae have been traditionally used and are established healthy food materials that are rich in minerals and dietary fibers and are typically used as a health food. Moreover radical scavenging activities in some common seaweed have been reported [12]. Ulva is one of the most important algae of the intertidal system and very common seaweeds distributed worldwide [13]. Several marine species have been traditionally used for human consumption.

To our best knowledge, the anti lipidperoxidative effect of this marine algae species has not been investigated scientifically. Therefore, the aim of the present study was to determine the anti lipidperoxidative activity of ethanolic extract of *Ulva reticulata* in streptozotocin induced diabetic rat.

## MATERIALS AND METHODS

### Chemicals and reagents

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals were of analytical grade and obtained from E. Merck and Himedia, Mumbai, India.

### Preparation of ethanolic extract of *Ulva reticulata*

*Ulva reticulata* marine algae were collected in and around areas of Mandabam region, Ramnadu district, Tamil nadu India. *Ulva reticulata* were dried and powdered. 250g of powder was extracted with 750mL of ethanol for 24h. After 24h the suspension was filtered through a fine muslin cloth and concentrated at 30ºC-45ºC. The extract was stored at -20ºC prior to experimentation. Whenever needed, the residual extract was suspended in distilled water and used for treatment. 2% solution of *Ulva reticulata* was prepared daily as oral administration for exposed groups (II, and IV) per each day.

### Experimantal Animals

Male Albino Wistar rats weighing (180-200g) were maintained in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalai nagar. The rats were kept in clean and dry cages with a bedding of paddy husk, exposed to 12h dark and light cycle, fed with rat pellets feed (Hindustan lever Ltd, Bangalore, India) and water *ad libitum.* The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Animal Ethical Committee of Annamalai University (Reg no: 681/160/1999/CPCSEA).

### Experimental induction of diabetes

The rats were fasted overnight and diabetes was induced by a single intraperitoenal injection of a freshly prepared solution of streptozotocin (45mg/kg) in 0.01 M citrate buffer (pH 4.5) [14]. STZ treated rats were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 72 hours of STZ administration, the blood glucose range above 200-300 mg/dL were considered as diabetic rats and used for this experimental study.

### Experimental Design

In the experiment, a total of 24 rats were used. Rats were randomized and divided into four groups of six rats each

Group I Normal untreated rats.

Group II Normal rats treated with 2% solution of 1 ml of *Ulva reticulata* by using an intragastric tube daily for a period of 45 days.

Group III Diabetic control rats

Group IV Diabetic rats treated with 2% solution of 1 ml of *Ulva reticulata* by using an intragastric tube daily for a period of 45 days.

At the end of experimental period the rats were anesthetized using ketamine hydrochloride (24 mg/kg body weight, intramuscular injection) and sacrificed by cervical dislocation after an overnight fast. Blood samples were collected and plasma was obtained after centrifugation. It had been used for the various biochemical estimations. Tissue like liver were excised immediately and used for histological examinations.

### Biochemical analysis

### Determination of plasma glucose and insulin

Fasting blood glucose was estimated by the oxidase/peroxidase method [15]. Plasma insulin was assayed by the solid phase amplified sensitivity immuno assay using reagent kits obtained from Medgenix- INS- ELISA, Bioscience, Europe, Belgium [16].

### Determination of thiobarbituric acid reactive substances, lipid hydroperoxides and assay of non-enzymic antioxidants in plasma.

The concentration of TBARS was estimated by the method of Niehaus and Samuelson [17]. Lipid hydroperoxide in plasma was estimated by the method of Jiang *et al*[18]. The levels of vitamins C, Vitamin E and reduced glutathione were estimated by the methods of Roe and Kuether [19], Baker et al [20] and Ellman [21] respectively.

### Statistical analysis

Results are presented as mean ± S.D. for six rats in each group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at $p<0.05$.

## RESULTS

### Effect of *Ulva reticulata* on plasma glucose and insulin level

The plasma glucose and insulin levels were measured in normal and diabetic rats. The results were illustrated in Table 1. There was a significant elevation in blood glucose and the level of insulin was decreased during diabetes when compared with corresponding control group. Oral supplementation of ethanolic extract of *U.reticulata* tended to bring the values to near normal compare with diabetic control groups.

### Effect *Ulva reticulata* on lipid peroxidation markers

Table 2 shows the concentration of TBARS and hydroperoxides in plasma of normal and experimental rats. During diabetes, there was a significant elevation of plasma TBARS and hydroperoxides when compared to corresponding control group. Oral administration of U. reticulata significantly decreased the level of TBARS and hydroperoxide in plasma of diabetic rats.

**Effect of *Ulva reticulata* on non-enzymatic antioxidant**

Table 3 shows the effect of ethanolic extract of *Ulva reticulata* on vitamin C, vitamin E and GSH in plasma of normal and experimental rats. The level of vitamin C, vitamin E and GSH significantly decreased in diabetic rats. Oral administration of ethanolic extract of *Ulva reticulata* showed a significant increase in the levels of vitamin C, vitamin E and GSH in diabetic rats.

**Table 1: Effect of *Ulva reticulata* on plasma glucose and insulin in normal and experimental rats**

| Groups | Plasma glucose (mg/dL) | Plasma Insulin (µU/mL) |
|---|---|---|
| Normal | 88.16 ± 6.92[a] | 15.19 ± 1.46[a] |
| Normal + URE | 92.28 ± 8.02[b] | 15.09 ± 1.45[b] |
| Diabetic Control | 291.11 ± 26.55[c] | 5.20 ± 0.50[c] |
| Diabetic Control + URE | 140.23 ± 11.15[d] | 10.75 ± 0.9[d] |

URE - *Ulva reticulata* ; Values are given as mean ±S.D. from six rats in each group. Values not sharing a common superscript letter (a-d) differ significantly at $p < 0.05$ (DMRT).

**Table 2: Changes in the levels of TBARS and lipid hydroperoxides markers in normal and experimental rats**

| Groups | TBARS (nmol/mL) | Lipid hydroperoxides ($X10^{-5}$mM / dL) |
|---|---|---|
| Normal | 1.25 ± 0.12[a] | 7.29 ± 0.70[a] |
| Normal + URE | 1.23 ± 0.11[a] | 7.51 ± 0.72[a] |
| Diabetic Control | 3.96 ± 0.38[b] | 13.54 ± 1.3[c] |
| Diabetic Control + URE | 2.84 ± 0.27[c] | 11.04 ± 1.06[d] |

URE - *Ulva reticulata*; Values are given as mean ±S.D. from six rats in each group. Values not sharing a common superscript letter (a-d) differ significantly at $p < 0.05$ (DMRT).

**Table 3: Changes in the levels of plasma vitamin C, vitamin E and GSH in normal and experimental rats**

| Groups | Vitamin C (mg/dL) | Vitamin E (mg/dL) | GSH (mg/dL) |
|---|---|---|---|
| Normal | 1.50 ± 0.18[a] | 1.98 ± 0.18[a] | 29.64 ± 2.1[a] |
| Normal + URE | 2.06 ± 0.13[b] | 1.81 ± 0.13[a] | 33.43 ± 3.0[a] |
| Diabetic Control | 0.72 ± 0.04[c] | 0.64 ± 0.04[b] | 17.18 ± 2.1[b] |
| Diabetic Control + URE | 1.48 ± 0.13[a] | 1.41 ± 0.11[c] | 23.87 ± 1.8[c] |

URE - *Ulva reticulata*; Values are given as mean ±S.D. from six rats in each group. Values not sharing a common superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

## DISCUSSION

Diabetes related complications are most dangerous for diabetic populations. It impairs the antioxidant levels and increasing free radicals which can damage body organs [22]. In this study, there was a marked increase in blood glucose in STZ induced diabetic rats. STZ enters the pancreatic β-cell via a glucose transporters2 and causes alkylation of deoxyriboneuclic acid. Furthermore, STZ induces activation of polyadenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β- cells are destroyed by necrosis [23]. The importance of the progressive loss of pancreatic β cell reduction in the course of diabetes has been focus the therapeutic targets in the development of novel and potential drugs acting by enhancing pancreatic β- cell growth and/or survival. STZ was found to be selectively toxic to the β cells of the pancreatic islets, the cells that normally regulate blood glucose levels by producing the hormone insulin. STZ treated rats receiving the ethanolic extracts of *U. reticulate* showed that normalization of blood glucose levels could be due to the possibilities of insulin releasing effect of *Ulva reticulata*.

Lipid peroxidation is one of the characteristic features of chronic diabetes. The elevated levels of free radicals may react with polyunsaturated fatty acids in cell membranes leading to tissue damage [24]. Insulin secretion is also closely associated with lipoxygenase derived peroxides Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type 1 diabetes. The most commonly used indicator of lipid peroxidation is TBARS and lipid hydroperoxides [22]. In the present study, the diabetic rats had shown increased concentration of TBARS and hydroperoxide compared with those in normal rats. Treatment with *Ulva reticulata* extract showed a significant decrease in the TBARS and lipid hydroperoxides in the diabetic rats. This result suggests that *Ulva reticulata* would be helpful to the prevention of diabetic complication through improving glucose metabolism and altering lipid peroxidation marker level.

Earlier research has shown that diabetes have low levels of vitamin C and vitamin E and that vitamin E supplementation can help to prevent the development of

glucose intolerance and diabetes [25,26]. Vitamin E is a well known physiological antioxidant and membrane stabilizer. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus protecting the cell structure against damage [27]. Vitamin C is a hydrophilic antioxidant because it disappears faster than other antioxidants. The observed decreased in the level of vitamin C could be caused by increased utilization of vitamin C as an antioxidant defense against reactive oxygen species or by a decrease in GSH which is required for the recycling of vitamin C. GSH, the glutathione antioxidant system has a fundamental role in cellular defense against reactive free radicals and other oxidant species [28]. The GSH concentration was less in the diabetic rats. In previous report shows that *Ulva reticulata* having number of beneficial role including antimicrobial and anti-inflammatory activities [29,30,31]. In our study, ethanolic extract of *Ulva reticulata* having varieties of phytochemicals that influence to elevated the activity of these non enzymatic antioxidants (GSH, vitamin C and vitamin E) in STZ induced diabetic rats.

## Conclusion

In conclusion, the present study results suggest that STZ mediated oxidative stress generated number of diabetic complications are potentially subject to modulated by marine algae *Ulva reticulata*. Promising protective effect of *Ulva reticulata* due to its insulin secretory response, and scavenges the free radicals. So, we conclude that *Ulva reticulata* could be a potential therapeutic agent for diabetes related complication.

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