

Antidiabetic, antihyperlipidemic and antioxidant activity of marine brown seaweed *Padina tetrastromatica*

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ABSTRACT

The abnormal increase of sugar and lipids in blood is the major health problem throughout the world population. *Padina tetrastromatica* is one of the most important marine brown seaweed having lot of pharmacological applications. In this present investigation the marine macroalgae *Padina tetrastromatica* is used for antidiabetic, antihyperlipidemic and antioxidant activities. The male albino rats are used for this study. The streptozotocin is used as an inducing agent for diabetes and other health implications. The biomarkers such as glucose, glycosylated haemoglobin, insulin, cholesterol (High density Lipoprotein and Low density lipo protein), triglycerides, Vitamin C, Vitamin E and reduced glutathione (GSH) levels are tested after and before induction and treatment. After the treatment with our brown seaweed aqueous extract the markers are near to the normal value or control and standard drug values. The results reveal that the eco-friendly algae are very good to diabetes and other disease and metabolic disorders.

KEY WORDS: Antidiabetic, Antihyper Lipidemic, Antioxidant, Marine algae, *Padina tetrastromatica*.

1. INTRODUCTION

Diabetes mellitus, one of the coarsest hormone metabolic disorders has induced substantial morbidity and mortality due to micro vascular (neuropathy, retinopathy and nephropathy) and macro vascular (stroke, heart attack and peripheral vascular disease) complication (Patel, 2011; 2012). Presently in India many people with diabetes around 40.9 million and it is expected to rise to 69.9 million by 2025 (Ayesha Noor, 2013; Mohan, 2007). India has got issued the diabetic capital of the world (Joshi, 2007). Unless the immediate preventive steps are taken, it's get a major health trouble. The Indian Diabetes Federation (IDF) estimated 3.9 million deaths for the year 2010, which constituted 6.8% of the total world-wide mortality rate (International Diabetes Federation, 2009). A number of people on diabetes multiply worldwide; the disease accepts an ever enhancing proportion of national and international health care budgets. It is designed to get one of the world's main disablers and killers within the next 25 years. It is nearly coarse serious metabolic disease in the world. The number of people bearing from the disease worldwide is enhancing at an alarming degree with a probable 552 million people likely to be diabetic by the year 2030 as against 366 million estimated 2011 (Venkatachalam, 2013; King, 1998). Insulin is the nearly significant hormone assuring the intermediary metabolism of our body. It is overall consequences are to continue energy by helping to inform and storage of glucose, amino acids and fats after meals. Acutely, it reduces the sugar level in blood. Accordingly, a plunge in plasma insulin increases sugar level in the blood. The main affair checking the synthesis and secretion of insulin is the concentration of glucose in blood. There is a stationary basal release of insulin and also response to adjust in blood glucose. Insulin plays a crucial role in the metabolism of carbohydrate, fat and protein (Bhavani, 2014; Atkinson, 1994). *Padina tetrastromatica* is the family of Dictyotaceae one of nearly pertinent marine brown seaweed and it will contains the major element of fucopyranosyl residues comprising sulfated fucans called as fucoidan, Alginic acid and glucans were isolated from the seaweed and minor amount of glucuronic acid and uronic acid are also determined and they will play a crucial role in the field of Antibacterial and Antiviral activities (Paramita, 2009).

***Padina tetrastromatica* Characteristic Features:** Divison: Phaeophyta; Order: Dictyotales; Family: Dictyaceae; Genus: *Padina*; Species: *Tetrastromatica*.

Padina tetrastromatica (Fig.1) is yellowish brown fan shaped algae these are olive green become drying (Mica, 1966). It grows in shallow and sand covered rocky pools at the adjacent coastal areas (Shameel, 1990). It proliferates in tropical waters these algae are mainly used as seasoning in dried flake from and as table salt replacement for high blood pressure patients (Novaczek, 2001). These are studies which showed that it contains alginic acid, major polysaccharides which show high anticoagulant (Prasada Rao, 1984) and antiviral properties (Chatterji, 2004). The research carried out over last several decades gets established that plants based therapies are used all around the world to assure and treat diabetes and its complications. Therefore, there is a call for phytochemicals that have anti hyperglycemic potential, which are cost-effective and also safe without long-term side effects (Sohn, 2010).



Figure.1. *Padina Tetrastromatica* (Dried)

2. MATERIALS AND METHODS

Source of chemicals: *Streptozotocin* purchased from Sigma Chemical Company (St. Louis, MO, USA) and Ranbaxy Chemicals Ltd., (Mumbai, India). All other chemicals used were of analytical grade.

Animals and diets: Adult male Wistar rats (150–180 g) were maintained at the Biochemistry Department Animal house, Adhiparasakthi College of Arts and Science. Throughout the experimental period, the rats were housed in polypropylene cages in an air-conditioned room with controlled temperature (20–22°C) and automatic lighting (alternation 12-h periods of light and dark) and free access to standard pellet diet (Pranav Agro Industries, Pune, India) and drinking water. The experimental protocol was approved by the Animal Ethical Committee of Adhiparasakthi College of Arts and Science in accordance with the Indian National Law on Animal Care and use.

Algal extraction and Dosing: The brown algae *P. tetrastromatica* was collected from Tuticorin coastal area in Tamilnadu, South India. The algae were washed with water and dried under shade, then smashed by hand in a small pore sieve and stored in dark dry place at room temperature. The extract was orally administered by using special stomach tube with a smooth tip to protect the interior lining.

Induction of type 2 diabetes by STZ injection: Type 2 diabetes was induced by a single intraperitoneal (i.p) injection of STZ (45 mg/kg body weight (BW)) freshly dissolved in sodium citrate buffer (pH 4.5, 0.1M), 15 minutes. STZ injection cause partial destruction of pancreatic beta cells mimicking type 2 diabetes. STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. The development of diabetes was confirmed by measuring plasma glucose concentrations at 72 h after injection of STZ. Rats with plasma glucose level > 250 mg/dL were used for the study.

Experimental Design: Animal were divided in to 6 groups. Group I served as Control rats administered with 0.9% saline; Group II served as rats with 1g/Kg body weight algal extract of *Padina tetrastromatica*; Group III rats administered with Intra peritoneal administration of STZ 50 mg/kg body weight; Group IV rats administered with Intra peritoneal administration of STZ 50 mg/kg body weight and oral administration of 250 mg/Kg algal extract of *Padina tetrastromatica*; Group V rats administered with Intra peritoneal administration of STZ 50 mg/kg body weight and oral administration of 500 mg/Kg extract of *Padina tetrastromatica*; Group VI rats administered with Intra peritoneal administration of STZ 50 mg/kg body weight and oral administration of 1g/Kg extract of *Padina tetrastromatica*.

Statistical Analysis: The data are expressed as means \pm S.D. The data for biochemical and physiological parameters were analyzed statistically using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test by using statistical software package of social science (SPSS, version 15.01 for windows). The 0.05 level of probability was used as the criterion for statistical significance.

Biochemical Analysis: All animals were sacrificed at the end of the experimental period, blood was collected and serum was separated immediately, glucose was estimated by the method of (Dubowski, 1962), Glycosylated hemoglobin, Plasma insulin was estimated ELISA assay kit (for rats) supplied by Linco Research Inc. (Stat Diagnostics, Mumbai). Total cholesterol (Zlatkis, 1953) and Triglycerides (Esposti, 1996), Fatty acids (Froguel, 1992) Low density lipoprotein-cholesterol, High density lipoprotein-cholesterol, Ascorbic acid (O'Byrne, 1990), α -Tocopherol and Reduced glutathione were methods are described elsewhere.

3. RESULT AND DISCUSSION

A substantial decrease in the body weight and an increase in food and water intake in diabetic control rats were observed. Diabetic rats treated with *Padina tetrastromatica* (250, 500mg/kg and 1g/kg) showed a significant increase in body weight and a significant decrease in food and water intake when compared with diabetic control rats. However, rats treated with *Padina tetrastromatica* to normal rats did not show any significant changes in the body weight, food and water intake when compared with normal control rats.

Effect of *Padina tetrastromatica* on STZ Influenced Plasma Glucose Level: An important ($p < 0.05$) increase in the levels of plasma glucose and glycosylated hemoglobin and decreased levels of plasma insulin were observed in diabetic control rats as compared to normal rats. Supplementation of *Padina tetrastromatica* diabetic rats reversed the STZ induced changes in diabetic rats.

Padina tetrastromatica distributed to diabetic rats importantly increased plasma insulin, total hemoglobin and decreased plasma glucose and glycosylated hemoglobin when compared with diabetic control rats. *Padina tetrastromatica* has the ability to scavenge free radicals and to inhibit lipid peroxidation prevents STZ-induced oxidative stress and protects β -cells, resulting in increased insulin secretion and decreased plasma glucose levels to near normal glycemic concentration and is an essential trigger for the liver to revert its normal homeostasis during experimental diabetes. *Padina tetrastromatica* has the ability to trigger the proinsulin synthesis and also insulin release, which might be helpful to reduce the plasma glucose.

Mixed administration of *Padina tetrastromatica* to diabetic rats showed the levels of plasma glucose and insulin towards near normality when equated with their individual effects. *Padina tetrastromatica* by its ability to salvage free radicals and to inhibit lipid peroxidation prevents STZ-induced oxidative stress and protects β -cells, resulting in increased insulin secretion and decreased plasma glucose levels. The combined administration of *Padina tetrastromatica* to diabetic rats also improved the body weight and this could be due to a better control of hyperglycemic state in the diabetic rats

Table.1. Effect of *Padina tetrastromatica* and Streptozotocin the Level of Glucose, Glycosylated Hb and Insulin levels in Plasma of Control and Experimental Rats

| Parameters | Glucose (mg/dL) | Glycosylated Hb(g/dL) | Insulin(IU/ml) |
|------------------------------------------------|-------------------|-----------------------|------------------|
| Control | 119.21 \pm 3.09 | 5.29 \pm 0.11 | 21.96 \pm 2.28 |
| <i>Padina tetrastromatica</i> (1g/kg) | 114.79 \pm 3.03 | 5.89 \pm 0.29 | 24.21 \pm 1.46 |
| Streptozotocin (1.25 mg/kg) | 234.38 \pm 6.12 | 9.02 \pm 0.58 | 14.06 \pm 2.02 |
| STZ& <i>Padina tetrastromatica</i> (250 mg/kg) | 184.66 \pm 5.32 | 7.27 \pm 0.33 | 16.05 \pm 1.87 |
| STZ& <i>Padina tetrastromatica</i> (500 mg/kg) | 156.84 \pm 4.89 | 6.45 \pm 0.39 | 19.37 \pm 2.13 |
| STZ& <i>Padina tetrastromatica</i> (1g/kg) | 142.07 \pm 4.14 | 6.06 \pm 0.51 | 20.73 \pm 2.03 |

Values are expressed in mg/dl for Glucose, Glycosylated Hb (g/dL) Insulin (IU/ml), Values are means \pm S.D for six rats Values differ significantly at $p < 0.05$ (DMRT)

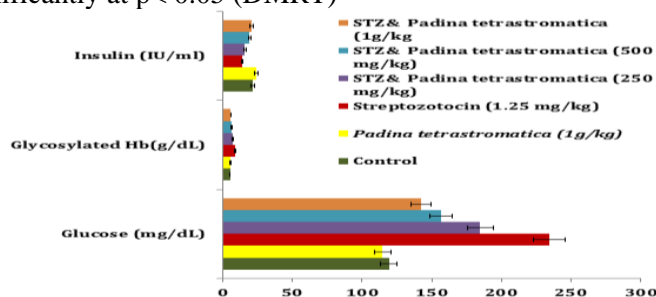


Figure.2. Changes in the Activity of Insulin, Glucose and Glycosylated Hb levels Experimental Animals
Lipid Profile: Low density and very low-density lipoproteins (LDL and VLDL), and a decrease in the concentration of high-density lipoproteins (HDL) were noticed in STZ alone-administered diabetic rats. Administration of *Padina tetrastromatica* more potentially ameliorated the STZ-induced changes in lipids, lipoproteins.

Increased insulin levels in the diabetic rats treated with *Padina tetrastromatica* decreased the activities of HMG-CoA reductase and increased the activities of LPL and LCAT. Treatment of *Padina tetrastromatica* when administered pre-orally to diabetic rats exerts more potential antihyper lipidemic effects as shown by the reversed levels of LDL, VLDL and HDL-C. The lipid lowering activities of *Padina tetrastromatica* could be due to its antioxidant property.

A *Padina tetrastromatica* when administered per orally to diabetic rats exerts more potential anti hyperlipidemic effects as shown by the reversed levels of LDL, VLDL and HDL-C. The lipid lowering activities of *Padina tetrastromatica* could also due to its antioxidant property.

Table.2. Effect of *Padina tetrastromatica* and Streptozotocin the Level of Tri glycerides, HDL Cholesterol, LDL Cholesterol in Control and Experimental Rats

| Parameters | Triglycerides (mg/dL) | HDL Cholesterol (mg/dL) | LDL Cholesterol (mg/dL) |
|------------------------------------------------|-----------------------|-------------------------------|--------------------------------|
| Control | 165 \pm 3.81 | 31.78 \pm 2.97 | 126.96 \pm 4.23 |
| <i>Padina tetrastromatica</i> (1g/kg) | 152.79 \pm 2.95 | 33.44 \pm 1.99 | 115.21 \pm 5.62 |
| Streptozotocin (1.25 mg/kg) | 238 \pm 0.12 | 24.02 \pm 2.63 | 151.06 \pm 4.12 |
| STZ& <i>Padina tetrastromatica</i> (250 mg/kg) | 217 \pm 5.22 | 25.32 \pm 2.37 ^a | 145.05 \pm 5.87 |
| STZ& <i>Padina tetrastromatica</i> (500 mg/kg) | 194 \pm 6.43 | 29.44 \pm 2.14 ^a | 139.33 \pm 4.89 ^b |
| STZ& <i>Padina tetrastromatica</i> (1g/kg) | 177 \pm 4.42 | 36 \pm 2.87 ^a | 130.27 \pm 4.76 |

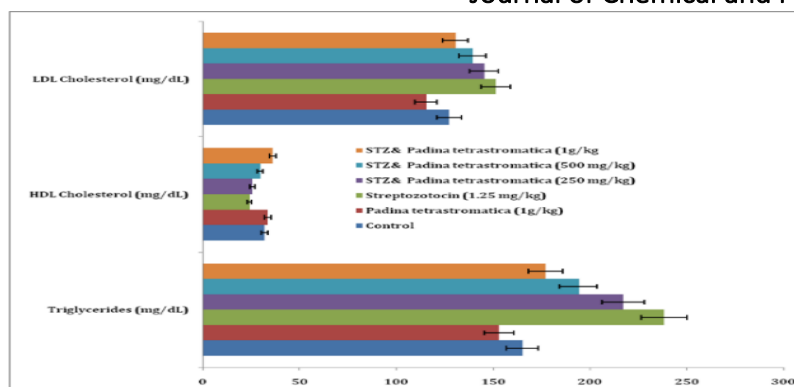


Figure.3. Changes in the activity of Triglycerides, HDL Cholesterol and LDL Cholesterol

Antioxidant: Untreated diabetic rats showed a significant decrease in the levels of plasma non-enzymic antioxidants (reduced glutathione (GSH), vitamin C, vitamin E) as compared to normal control rats. Administration of *Padina tetrastromatica* to diabetic rats leads to a significant increase in the levels of plasma GSH, vitamin C, vitamin E. Vitamin C is a primary preventive antioxidant in the cells and body fluids scavenges the free radicals and serves as a metabolic marker of toxicity (Pharikal, 1988). Reduction in reduced glutathione (GSH) levels indicates degenerative and necrotic alterations in the kidneys and liver. Samah (2012), identified an increase in the production of many reactive oxygen species (ROS) such as superoxide and H_2O_2 (Miller, 1990) causes lipid peroxidation and subsequently oxidative tissue damage (Huang, 1996; Linden, 2008) associated with superoxide and radical generation and glutathione reduction (Girardi and Elias, 1995; Miura, 1995).

At the end of the experimental period (28 days), fasting plasma glucose levels were significantly increased in diabetic control rats when compared with normal control rats. Administration of *Padina tetrastromatica* rat three different doses (250, 500 mg/kg and 1g/kg) significantly decreased the plasma glucose levels when compared to diabetic control rats. Oral administration of *Padina tetrastromatica* (1g/kg) to normal rats did not show any significant effect on fasting plasma glucose levels in this study. The effect of *Padina tetrastromatica* at a dose of 1g/kg was more effective when compared to other doses of *Padina tetrastromatica* (250, 500 mg/kg). Hence, *Padina tetrastromatica* at 1g/kg was used for further biochemical studies.

Table.3. Effect of *Padina tetrastromatica* and Streptozotocin the Level of Vitamin C, Vitamin E and GSH in Plasma of Control and Experimental Rats

| Parameters | Vitamin C(mg/dL) | Vitamin E(mg/dL) | GSH(U/mg protein) |
|-------------------------------------------------|------------------|------------------|-------------------|
| Control | 2.21 ± 0.09 | 1.78 ± 0.17 | 33.96 ± 3.28 |
| <i>Padina tetrastromatica</i> (1g/kg) | 2.79 ± 0.05 | 1.89 ± 0.09 | 35.21 ± 2.46 |
| Streptozotocin (1.25 mg/kg) | 1.38 ± 0.12 | 1.02 ± 0.18 | 21.06 ± 3.12 |
| STZ & <i>Padina tetrastromatica</i> (250 mg/kg) | 1.67 ± 0.12 | 1.32 ± 0.13 | 25.05 ± 2.87 |
| STZ & <i>Padina tetrastromatica</i> (500 mg/kg) | 1.94 ± 0.16 | 1.45 ± 0.09 | 29.33 ± 2.34 |
| STZ & <i>Padina tetrastromatica</i> (1g/kg) | 2.07 ± 0.14 | 1.66 ± 0.11 | 30.27 ± 2.19 |

Values are expressed in mg/dl for Vitamin C and E, U/mg protein for GSH Values are means ± S.D for six rats Values differ significantly at $p < 0.05$ (DMRT)

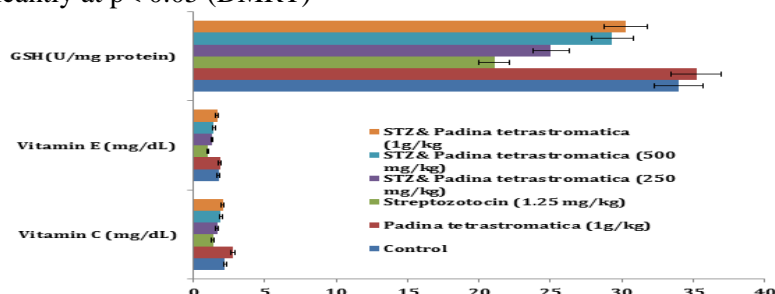


Figure.4. Changes in the activity of Vitamin C, Vitamin E and GSH

Histopathological examination of pancreas of diabetic rats revealed morphological changes. The effect of *Padina tetrastromatica* on the histopathological changes of diabetic pancreas is very promising. *Padina* treated diabetic liver showed only mild sinusoidal dilatation. *Padina tetrastromatica* treated diabetic kidney showed normal glomeruli with normal tubules. Diabetic pancreas showed marked micro vesicular and macro vesicular changes in islets. *Padina tetrastromatica* alone treated diabetic pancreas showed a normal cell of β -cell islets of Langerhans. In addition, combined administration of *Padina tetrastromatica* to diabetic rats preserved near normal cell morphology in the pancreas as compared to normal pancreas. This reflects that *Padina tetrastromatica* restore pancreas histology

by alleviating oxidative stress induced by STZ. Further *Padina tetrastromatica* increases the size of the islets by its ability to regenerate β -cells.

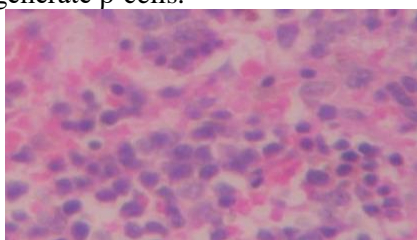


Figure.5. Control pancreatic cell

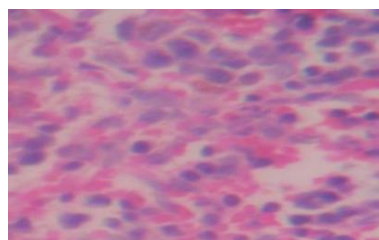


Figure.6. Positive control Pancreas (0.9 % saline + 1g / kg *Padina tetrastromatica*) – exhibits normal Pancreatic lobules.

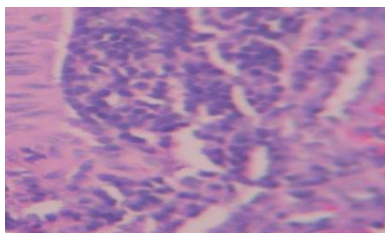


Figure.7. Negative control pancreas (0.9 % saline + 45mg / kg STZ) – exhibits micro vesicular and macro vesicular changes in islets in pancreas

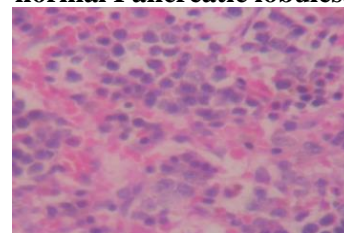


Figure.8. Treatment (STZ + 250 mg *Padina tetrastromatica* / kg body wt)- section shows congested Pancreatic lobules with less inflammation.

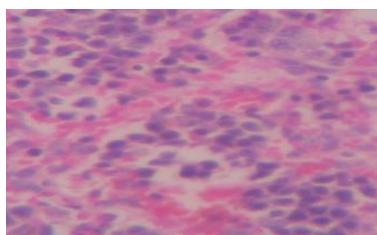


Figure.9. Treatment control Pancreas (STZ + 500 mg *Padina tetrastromatica* / kg body wt) - section shows minimum micro vesicular and macro vesicular changes in islets in pancreas

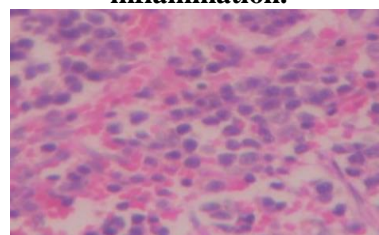


Figure.10. Treatment control Pancreas (STZ + 1000 mg *Padina tetrastromatica* / kg body wt) - section shows minimum micro vesicular and macro vesicular changes in islets in pancreas

4. CONCLUSION

The present study suggests that *Padina tetrastromatica* is having good antidiabetic character, antioxidant and antihyperlipidemic properties in STZ induced type 2 diabetic rats. It also exerts protective effect against pancreas injury associated with diabetes. *Padina tetrastromatica* enhances insulin stimulated glucose uptake by translocating glucose transporters in skeletal muscle membrane and modulates carbohydrate metabolism. *Padina tetrastromatica* also increases the insulin secretion and enhances insulin stimulated glucose uptake and modulates the carbohydrate metabolism. The biochemical studies were supported by histopathological studies. In many countries marine algae is very good food for human beings, now the very good pharmaceutical applications are proved will increase the largely available usage of marine algae for world wide applications.

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