Antidiabetic Effect of *Sargassum wightii* and *Ulva fasciata* in High fat diet and Multi Low Dose Streptozotocin Induced Type 2 Diabetic Mice

Lucy Mohapatra¹*, Subrat Kumar Bhattamishra², Ramachandra Panigrahy¹, Sambit Parida³, Premalata Pati¹

¹Department of Marine Sciences, Berhampur University, Berhampur-760007, Odisha, India  
²Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Odisha, India  
³College of Pharmaceutical Sciences, Berhampur-760002, Odisha, India  
⁴Present address: Dept. of Life Sciences, School of Pharmacy, International Medical University, Buki Jall 57000, Kuala Lumpur, Malaysia

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**Abstract**  
This study was undertaken to evaluate the antidiabetic effect of edible seaweeds *Sargassum wightii* and *Ulva fasciata* with an objective to establish these seaweeds as add-on therapy for controlling diabetes. The ethyl acetate extract of *Sargassum wightii* (EAS) and *Ulva fasciata* (EAU) at 100 and 200 mg/kg dose was examined for antidiabetic activity in the high fat diet and multi-low-dose streptozotocin induced type 2 diabetic mice. Plasma glucose, oral glucose tolerance test [OGTT], lipid profile, liver and skeletal muscle glycogen content, triglycerides, superoxide dismutase [SOD], body and organ weight was estimated at the end of the study. Consequently, histopathology of liver was also studied. Both EAS and EAU showed significant reduction in plasma glucose level and the area under curve [AUC] of OGTT was significantly (p<0.001) attenuated. The extracts appreciably normalized lipid profile, body and organ weight. At higher doses of both EAS and EAU showed significant (p<0.05) improvement in SOD level of skeletal muscle and reduction in liver as well as skeletal muscle triglycerides. Both the extracts considerably enhanced hepatic and muscle glycogen content. Remarkable improvements were found in the histopathological observations of liver of all the treated groups as compared against the diabetes induced group. The findings suggest that these seaweeds offer a great opportunity to satisfy the food and nutrition requirement for rectifying and controlling diabetes.

1 Introduction

Type 2 diabetes mellitus is a metabolic disorder of hyperglycemia, caused either by insufficient insulin secretion or insulin resistance¹. Prevalence of this disease is rapidly rising in most parts of the world. The major cause of morbidity and premature death associated with diabetes are actually due to severe complications of this disorder. The important complications of diabetes include cardiovascular diseases, diabetic nephropathy, diabetic neuropathy, diabetic ketoacidosis, and diabetic myopathy etc.²,³ Specific therapeutic goals for these patients include achieving and maintaining normal blood glucose, optimal serum lipid levels, reasonable body weight and improving overall health by maintaining balanced intake of macro and micro nutrients⁴. Several types of oral hypoglycemic agents are available commercially to normalize blood glucose level, especially in type 2 diabetes. Very often two or more hypoglycemic agents of different types are used concomitantly to get better results and limited side effects. But undesirable side effects and limited access of such drugs motivate patients to use alternative therapies to counteract the complications associated with this disease⁵. These therapies usually include dietary modification, proper exercise regimen and use of effective and safe herbal medicines.

It has been found that there is a significant relationship between disordered food habits and diabetes. The increased prevalence of diabetes in the present scenario is due to an increase in desire to have the modern diet having the high amount of refined and processed products, which doesn’t contain sufficient dietary fibers,
minerals and vitamins necessary to keep our body healthy. Previous report reveals that consuming diets rich in soluble and insoluble fiber induces satiety, improves glycemic control and reduces total energy intake, adiposity and blood lipids. People of Asia frequently consume edible seaweeds as the good source of dietary fiber. These seaweed fibers have been found to produce hypcholesterolemic and hypolipidemic responses as it tend to reduce cholesterol absorption in the gut. In addition, fat contents of most of the seaweeds are very low (<2%) and these are rich source of poly unsaturated fatty acids [PUFA] including the essential omega (n)-3s linolenic acid [LNA], eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA] and omega-(n)-6 linoleic acid [LA]. Maeda et al., 2005 suggested that, fucoxanthin and its metabolite fucoxanthinol obtained from a brown marine alga Undaria pinnatifida, enhances the expression of uncoupling protein 1 [UCP1]. UCP1 is a protein that suppresses fat accumulation, particularly around internal organs in rats and mice. Hypolipidemic activities reported in ethanol extracts of Solieria robusta, Iyengaria stellata, Colpomenia sinuosa, Spatoglossum asperum and Caulerpa racemosa in rats. Some diabetologists suggest that alpha-glucosidase inhibitors are a cost-effective means to prevent the progression of diabetes as this enzyme reduces the absorption of glucose from the gut itself. Seaweeds stand as a unique source of natural alpha-glucosidase inhibitors from nature as per some of the previous studies. Recently some researchers revealed that fucoxanthin, a type of carotenoid found abundantly in edible brown seaweed are having good hypoglycemic effect.

Marine macro algae offer a great opportunity to satisfy the food and nutritional requirement for rectifying and controlling diabetes. The strong evidence in support of this contention is that, Japanese diet contains nearly 10-15% marine macro algae and the prevalence of diabetes and metabolic syndrome in this country is less compared to western countries. Incorporation of seaweeds into a balanced diet could definitely help in minimizing and reducing instances of diabetes to a greater extent.

Sargassum wightii is dark brown seaweed which is used as raw material for the production of sodium alginate. Traditionally this seaweed is used to treat goiter in Chinese medicine. In addition to that, this seaweed has been proven to have promising anti-Alzheimer, antibacterial, anticancer and anti-asthmatic potential. The green seaweed Ulva fasciata is commonly called sea lettuce. This seaweed has been proven to have potential algicidal activity due to the presence of some compounds like hexadeca-4,7,10,13-tetraenoic acid (HDTA), octadeca-6,9,12,15-tetraenoic acid (ODTA) and alpha-linolenic acid within it. In some recent studies, this seaweed has shown extensive anti-coagulant, anticancer and anti-hyperglycemic properties. In addition to that, this green alga has been also studied for its antioxidant, hypocholesteremic and antimicrobial properties.

Mohapatra et al. Antidiabetic effect of Sargassum wightii and Ulva fasciata Studies pertaining to pharmacological importance of seaweeds in India are at low key. Therefore, this study was undertaken to evaluate the antidiabetic effect of edible seaweeds S. wightii and U. fasciata with an objective to prove that these seaweeds are suitable to be used as add on therapy during diabetes treatment.

2 Materials and Methods

2.1 Materials

All chemicals used in this investigation were of analytical grade. Streptozotocin (STZ) was procured from Himedia Lab, India. Ethylene diamine tetra acetic acid (EDTA) and haematoxylin–eosin (H&E) dye were obtained from Loba Chemie Pvt. Ltd., India while other chemicals and solvents were obtained from Merck Specialities Pvt. Ltd., India. The biochemical parameters were tested using commercially available glucose kit (GOD/POD method), triglycerides kit (GPO/PAP method) and cholesterol kit (CHOD/PAP method) which were supplied by Crest Biosystems, a division of Coral Clinical Systems, India.

2.2 Seaweed extracts preparation

Sargassum wightii Greville ex J. Agardh (Family: Sargassaceae) was obtained from the Palk Bay and Gulf of Mannar, Indian east coast and Ulva fasciata Delle (Family: Ulvaceae) was collected from the Chilika Lake, Odisha, India, during October - November, 2012. They were properly identified and authenticated by Prof. R.C. Panigrahy, marine Biologist, Berhampur University, Berhampur, Odisha, India.

After collection, these seaweeds were washed thoroughly with sea water and then with tap water to make the samples free from debris and impurities. Properly washed and air dried seaweeds were cut into small pieces and powdered using electronic grinder and passed through the sieve no.16 mesh. Exactly, 400 g of each seaweed powder was first defatted with petroleum ether and then extracted with ethyl acetate using soxhlet apparatus. These ethyl acetate extracts were encoded as: Ethyl acetate extract of S. wightii (EAS) and Ethyl acetate extract of U. fasciata (EAU).

2.3 Toxicity study

The experimental protocol was approved by the institutional animal ethics committee (IAEC no. 66, dated 7/06/2012/ CPCSEA), Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India. Toxicity study of EAU and EAS on female Swiss albino mice was conducted as per OECD guidelines 423. The highest dose (2000 mg/kg) of each extracts was administered orally to the overnight fasted mice. The animals were then observed continuously for 24 h and up to 14 days under the following profiles:

• The behavioral profile includes observing the animals for their alertness, restlessness, irritability and fearfulness.
Neurological profile includes observing the animals for their spontaneous activities, reactivity, touch response, pain response and gait.

Whereas the autonomic profile includes observing for the defecation and urination of the animals.

### 2.4 Experimental animals and induction of type 2 diabetes mellitus

Swiss albino male mice weighing 22-27 g were maintained in our animal house for this specific study. All animals were housed in standard cages (48x35x22 cm) at room temperature (20±2°C) with artificial light from 7.00 am to 7.00 pm, and provided with normal chow pellet food [Rayan’s Ltd, Hyderabad, India] and water ad libitum; ambient relative humidity was maintained at 55-60%. After acclimation in our laboratory for one week, selected 35 numbers of male albino mice were divided into 7 experimental groups, each group containing five animals in it to receive the following treatments: 

Group 1 were normal non diabetic mice which received normal saline only throughout the treatment period. Group 2 were diabetic mice which were administered with 1% sodium carboxy methyl cellulose (CMC) orally. Group 3 and 4 consist of diabetic mice and were orally administered 100 and 200 mg/kg EAS respectively. Groups 5 and 6 consist of diabetic mice and were orally administered 100 and 200 mg/kg of EAU respectively. Group 7 were diabetic mice which received 10 mg/kg gliclazide orally.

This study was performed as per the established procedure. The extracts were solubilized in 1% sodium CMC and administered by oral gavages between 11.00–12.00 h from 7th to 28th day. All animals except animals in Group 1 were fed with high fat diet [HFD] during this period. This HFD was prepared by using major constituents like casein, DL-Methionine, sucrose, corn starch, hydrogenated vegetable shortening, cellulose, corn oil, vitamins, minerals and calcium carbonate. From 16th day onwards, all the animals except group 1 were injected with a multi low dose of STZ (MLDS) (40 mg/kg) dissolved in citrate buffer (pH 4.5) i.p. for five consecutive days. Prior to each study, the animals were subjected to fasting for 12 h with free access to R.O. water.

### 2.5 Collection of blood and determination of plasma parameters

Animals were fasted overnight for a period of 12 h. Blood (0.5 ml) was withdrawn via the retro-orbital sinus under mild ether anaesthesia and was collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 20 min to obtain clear plasma. The plasma was analyzed for plasma glucose, triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL) in auto-analyzer using respective diagnostic kits. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated as per Friedwald’s equation:

\[
\text{VLDL} = \frac{\text{TG}}{5}; \quad \text{LDL} = \text{TC} - \text{VLDL} - \text{HDL}
\]

### 2.6 Oral glucose tolerance test (OGTT)

OGTT was performed in overnight fasted mice. The animals were however allowed to drink water and the respective drugs were administered at the regular time to different treatment groups. Glucose (2 g/kg) was fed to each animal 10 min after collecting the blood samples which was taken as reference (0 min). Exactly 0.1 ml of blood was withdrawn from the retro orbital route of each mouse under mild ether anaesthesia at 30, 60 and 120 min after the glucose load. The plasma obtained was analyzed for glucose.

### 2.7 Organ and Skeletal muscle weight

Mice were sacrificed by over anesthesia (diethyl ether) at the end of the study period and then liver, pancreas and skeletal muscle (Gastrocnemius muscle from left and right legs) were isolated. The wet weight was taken immediately after drying on a blotting paper.

### 2.8 Estimation of liver and skeletal muscle TG

Frozen liver (5 mg) and skeletal muscle (15 mg) were used for TG extraction. Each frozen tissue was added to 0.3 ml heptane-isopropanol-tween mixture (3:2:0.01 by volume) and homogenized. This homogenate was centrifuged at 1500xg at 4°C for 15 min. Supernatants (upper phase contained extracted TG) were collected and evaporated with vacuum centrifuge. The TG content was estimated by spectrophotometry with commercial kit.

### 2.9 Estimation of liver and skeletal muscle Superoxide Dismutase (SOD)

Mice were sacrificed using anesthesia (diethyl ether) at the end of the study period. The gastrocnemious muscle and liver were quickly removed and washed with cold saline solution from which 10% (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4) using a glass Teflon homogenizer after cutting them into small pieces with scissors (for 5 min at 5000 rpm). The homogenate was then centrifuged at 5000×g i.e. (8600 rpm) for 60 min to remove debris. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume [v/v]). After centrifugation at 5000 ×g i.e., (8600 rpm) for 30 min, the clear upper layer (the ethanol phase) was taken and used for SOD activity estimation. All preparation procedures were performed at 4 °C.

### 2.10 Estimation of liver and skeletal muscle glycogen content

Muscle or liver (25 mg.) were ground with 5 ml of the deproteinizing solution in a centrifuge tube; a stainless-steel pestle with longitudinal grooves which fits closely into a cylindrical centrifuge tube has proven useful for this purpose. The fluid level was marked on the centrifuge tube, covered with a glass cap and was placed in a boiling-water bath for 15 min. Then the tube was cooled in running water, filled up to the mark with deproteinizing solution to

compensate for evaporation and centrifuged at 3000 rev/min for 5 min. One ml. of the clear supernatant fluid is added to 3 ml \( \text{H}_2\text{SO}_4 \) in a wide test tube and mixed by vigorous shaking. The mixture was heated in a boiling-water bath for exactly 5 min. and subsequently cooled in running tap water. The intensity of the pink colour produced is measured spectrophotometrically at 520 nm and the glycogen concentration was read from a standard curve in terms of glucose equivalents. 

2.11 Liver histopathology studies

Part of the liver from the left lobe of each sacrificed mice was harvested and fixed in 10% neutral buffered formalin, while the remaining tissue were stored at \(-80^\circ\text{C}\) for further analysis. For histological analysis, tissue sections were embedded in paraffin. Further these tissue sections were de-paraffinized, stained with haematoxylin–eosin (H&E) and examined under light microscopy at 100X magnifications.

2.12 Statistical analysis

Results were expressed as mean ± SEM. Data were analyzed with One-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison tests. The level of significance was set at \( p<0.05 \).

3 Results

3.1 Toxicity study of the seaweed extracts as per OECD guidelines

The toxicity of those extracts was recorded after single oral administration to the animals. During the observation period, no abnormal behavior and mortality were reported in the animals under study by using any of the seaweed extract and as such the extracts were found to be safe at the dose level of 2000 mg/kg body weight.

3.2 Plasma glucose

The effects of EAS and EAU treatment on plasma glucose of diabetic mice are presented in figure 1. Both EAS 200 and 100 mg/kg treatment showed significant (\( p<0.001 \) and \( p<0.05 \) respectively) reduction in plasma glucose whereas EAU showed significant (\( p<0.01 \)) reduction in plasma glucose only at the higher dose (200 mg/kg).

3.3 Plasma lipids

The effect of EAS and EAU treatment on plasma lipid profile of diabetic mice is illustrated in table 1. Both EAS and EAU at 200 mg/kg showed significant (\( p<0.001 \)) reduction in plasma TG. EAS showed significant (\( p<0.05 \)) reduction in plasma TG at lower dose whereas EAU didn’t show any significant result at this dose. Both EAS and EAU at 200 mg/kg showed significant (\( p<0.001 \) and \( p<0.01 \) respectively) reduction in plasma TC. EAS showed significant (\( p<0.05 \)) reduction in plasma TC at the lower doses whereas EAU didn’t show any significant result at this dose. EAS and EAU at 200 mg/kg brought significant (\( p<0.05 \)) rise in plasma HDL level. EAS 200 and 100 mg/kg treatment showed significant (\( p<0.001 \) and \( p<0.01 \) respectively) reduction in plasma LDL. EAU also showed significant reduction in plasma LDL at both doses [200 (\( p<0.001 \)) and 100 (\( p<0.05 \)) mg/kg]. EAS showed significant (\( p<0.05 \) and \( p<0.01 \) at 100mg/kg and 200mg/kg respectively) reduction in plasma VLDL at both doses. EAU reduced plasma VLDL significantly (\( p<0.001 \)) only at the higher doses.

3.4 OGTT

Impaired glucose tolerance is reflected in a larger incremental area under the curve (AUC) of the plasma glucose OGTT curve. The AUC of the plasma glucose was significantly (\( p<0.001 \)) higher in case of diabetic control. However, the AUC of OGTT curve was significantly (\( p<0.001 \)) attenuated in both EAS and EAU treated animals at both doses (100 and 200 mg/kg). The extent of reduction was also higher in Gli 10 mg/kg treated mice (\( p<0.001 \)). The AUC of the plasma glucose disappearance curve is shown in figure 2.

3.5 Body weight

At the end of the treatment, body weight gain was estimated for all groups. It was found that diabetic mice significantly (\( p<0.001 \)) lose their body weight as compared to normal mice at the end of the study period. However, EAS treated animals showed significant (\( p<0.001 \) at 200 mg/kg and \( p<0.05 \) at 100 mg/kg) gain in body weight compared to diabetic mice. EAU showed significant (\( p<0.01 \)) improvement in body weight only at the higher doses. The result is depicted in table 2.
Table 1: Effect of EAS and EAU treatment on lipid profile of HFD and MLDS induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Plasma TG</th>
<th>Plasma TC</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N)</td>
<td>67.25±2.136</td>
<td>89.25±3.544</td>
<td>42±3.894</td>
<td>33.8±4.303</td>
<td>13.45±0.427</td>
</tr>
<tr>
<td>Group 2 (D)</td>
<td>181.75±6.6</td>
<td>230.25±9.63</td>
<td>20±3.136</td>
<td>173.9±5.363</td>
<td>36.3±1.32</td>
</tr>
<tr>
<td>Group 3 (EAS 1)</td>
<td>153.75±5.344</td>
<td>186.75±4.385</td>
<td>39.75±5.218</td>
<td>116.25±8.743</td>
<td>30.75±1.069</td>
</tr>
<tr>
<td>Group 4 (EAS 2)</td>
<td>129.75±6.486</td>
<td>155.5±5.041</td>
<td>48.5±3.969</td>
<td>81.05±7.286</td>
<td>25.95±1.297</td>
</tr>
<tr>
<td>Group 5 (EAU1)</td>
<td>158.5±6.513</td>
<td>192.5±7.136</td>
<td>34.75±4.956</td>
<td>126.05±9.507</td>
<td>31.7±1.303</td>
</tr>
<tr>
<td>Group 6 (EAU 2)</td>
<td>142.25±6.382</td>
<td>168±16.6186</td>
<td>44.5±5.392</td>
<td>95.05±11.489</td>
<td>28.45±1.276</td>
</tr>
<tr>
<td>Group 7 (G)</td>
<td>92.75±3.25</td>
<td>121.5±8.799</td>
<td>54.25±7.793</td>
<td>48.7±14.092</td>
<td>18.55±0.65</td>
</tr>
</tbody>
</table>

N: Normal control, D: Diabetic control,  EAS 1: EAS 100 mg/kg, EAS 2: EAS 200 mg/kg, EAU 1: EAU 100 mg/kg, EAU 2: EAU 200 mg/kg, G: Gliclazide.

α: p<0.001 vs Gr.1; $: p<0.001 vs Gr.2; £: p<0.01 vs Gr.2; €: p<0.05 vs Gr.2

3.6 Liver, Pancreas and Skeletal muscle (Gastrocnemius muscle) weight

In comparison to normal mice, the diabetic group has shown significant (p<0.001) increase in the weight of the liver. However, in case of animals treated with EAS and EAU, there was a significant reduction (p<0.01) in the weight of the liver compared to diabetic animals at the higher doses (200 mg/kg). Whereas at the lower doses (100 mg/kg), EAS (p<0.01) showed better result than that of EAU (p<0.05). EAS and EAU also showed significant (p<0.01 and p<0.05 respectively) increase in the pancreas weight only at the higher doses (200 mg/kg). In case of total gastrocnemius muscle weight estimation of the animals it was found that use of EAS and EAU resulted in significant weight gain at both the dose treatment (at 200mg/kg; p<0.01 and 100 mg/kg; p<0.05). The detail results are depicted in table 2.

3.7 Liver and Skeletal muscle (Gastrocnemius muscle) TG

The TG of liver and skeletal muscle recorded after the treatment provided to the mice is depicted in table 3. Both EAS and EAU at 200 mg/kg showed significant (p<0.001) reduction in liver TG. EAS showed significant (p<0.01) reduction in liver TG at the lower doses whereas EAU didn’t show any significant result at this dose. In case of skeletal muscle TG, EAS and EAU showed significant (p<0.01 and p<0.05 respectively) reduction at the higher doses but didn’t show any significant result at lower doses.

3.8 Liver and Skeletal muscle (Gastrocnemius muscle) SOD

SOD is significantly (p<0.01) lower in the diabetic control group than that of the normal group in case of skeletal muscle SOD estimation. But EAS and EAU at higher dose treatment could bring significant (p<0.05) improvement in this antioxidant level compared to standard drug in case of skeletal muscle SOD estimation. However, no significant result was found in case of liver SOD antioxidant activity. Result is presented in table 3.

3.9 Liver and skeletal muscle (Gastrocnemius muscle) glycogen content

EAS treated groups at both the doses (100 mg/kg p< 0.05 and 200 mg/kg p<0.01) showed a significant enhancement in liver glycogen content at the end of treatment week. EAU could show improvement in results of glycogen content of liver at 200 mg/kg dose (p<0.05). Both EAS and EAU showed significant (p<0.05) increase in glycogen content of skeletal muscle at the higher dose treated groups. The detail result is depicted in table 3.

3.10 Liver histopathology

Due to HFD, the liver shows extensive microvesicular and macrovesicular fatty changes with fatty cyst formation. In diabetic...
control group, HFD causes destruction of hepatocytes/ parenchyma cells and results in liver steatosis. EAS and EAU treated groups show less number of fatty changes with the least number of fatty cysts in their liver and showed hepatoprotective effect on liver cells. The detailed results of liver histopathology study are depicted in figure 3 (A, B, C, D, E, F, G).

Table 2: Effect of EAS and EAU treatment on body and organ weight of HFD and MLDS induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Bodyweight (g)</th>
<th>Skeletal muscle weight/100g body weight</th>
<th>Liver weight/100g body weight</th>
<th>Pancreas weight/100g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1(N)</td>
<td>34.775±1.949</td>
<td>2.002±0.979</td>
<td>5.057±0.337</td>
<td>0.694±0.081</td>
</tr>
<tr>
<td>Group2 (D)</td>
<td>20.375±0.551a</td>
<td>0.241±2.631a</td>
<td>12.706±0.886a</td>
<td>0.3067±0.0999β</td>
</tr>
<tr>
<td>Group3 (EAS 1)</td>
<td>27.125±1.548c</td>
<td>1.276±1.360c</td>
<td>8.614±0.339c</td>
<td>0.567±0.1</td>
</tr>
<tr>
<td>Group4 (EAS 2)</td>
<td>30.275±1.002d</td>
<td>1.815±2.201d</td>
<td>6.994±0.625d</td>
<td>0.611±0.483dβ</td>
</tr>
<tr>
<td>Group5 (EAU 1)</td>
<td>25.782±1.251a</td>
<td>0.238±1.656a</td>
<td>9.477±0.441f</td>
<td>0.562±0.055β</td>
</tr>
<tr>
<td>Group6 (EAU 2)</td>
<td>29.1±0.55f</td>
<td>1.658±1.939f</td>
<td>7.548±0.873f</td>
<td>0.597±0.423fβ</td>
</tr>
<tr>
<td>Group7(G)</td>
<td>31.125±1.328f</td>
<td>1.864±0.954f</td>
<td>6.658±0.549f</td>
<td>0.651±0.269fβ</td>
</tr>
</tbody>
</table>

N: Normal control, D: Diabetic control, EAS 1: EAS 100 mg/kg, EAS 2: EAS 200 mg/kg, EAU 1: EAU 100 mg/kg, EAU 2: EAU 200 mg/kg, G: Gliclazide. α: p<0.001 vs Gr.1; β: p<0.01 vs Gr.1; $: p<0.001 vs Gr.2; £: p<0.01 vs Gr.2; €: p<0.05 vs Gr.2

Table 3: Effect of EAS and EAU treatment on TG, SOD and glycogen content in liver and skeletal muscle tissue of HFD and MLDS induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TG (mg/g) of tissue</th>
<th>Glycogen (mg/g) of tissue</th>
<th>SOD (u/g) of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Skeletal muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Group1(N)</td>
<td>116.5±3.5</td>
<td>59.5±0</td>
<td>32.375±2.5</td>
</tr>
<tr>
<td>Group2 (D)</td>
<td>271±7α</td>
<td>124.75±0α</td>
<td>10.5±1.875β</td>
</tr>
<tr>
<td>Group3 (EAS 1)</td>
<td>227±9α</td>
<td>103.5±0β</td>
<td>22.375±2.5€</td>
</tr>
<tr>
<td>Group4 (EAS 2)</td>
<td>189.5±2.54β</td>
<td>84.75±0€</td>
<td>28.625±2.54β</td>
</tr>
<tr>
<td>Group5 (EAU 1)</td>
<td>241.5±2.5β</td>
<td>109.75±0€</td>
<td>19.875±1.25</td>
</tr>
<tr>
<td>Group6 (EAU 2)</td>
<td>197±1.85€</td>
<td>91.75±1.0€</td>
<td>25.5±1.875€</td>
</tr>
<tr>
<td>Group7(G)</td>
<td>175±8ό</td>
<td>79.5±0ό</td>
<td>1.75±1.875€</td>
</tr>
</tbody>
</table>

N: Normal control, D: Diabetic control, EAS 1: EAS 100 mg/kg, EAS 2: EAS 200 mg/kg, EAU 1: EAU 100 mg/kg, EAU 2: EAU 200 mg/kg, G: Gliclazide. α: p<0.001 vs Gr.1; β: p<0.01 vs Gr.1; $: p<0.001 vs Gr.2; £: p<0.01 vs Gr.2; €: p<0.05 vs Gr.2

4 Discussions

There are several reasons behind the elevated plasma glucose levels in type 2 diabetic patients. The most important factors are insulin resistance and the failure of pancreatic β cells to synthesize and release sufficient amount of insulin. Hence, an experimental animal model which imitates the pathogenesis and clinical features of this disease should preferably have these two distinguishing characteristics. Currently, from many studies it has been proved that feeding HFD to rodents develops insulin resistance and release sufficient amount of insulin. Therefore, HFD followed by MLDS induced diabetes in Swiss albino male mice was chosen for our study that would closely mimic the natural history of the disease. MLDS injection (40mg/kg body weight) after feeding HFD has shown significant abnormalities in blood glucose, lipid profile, OGTT, body weight and organ weight in diabetic animals. Antioxidant status, TG, glycogen level in liver and skeletal muscle was very low in diabetic animals as compared to other treatment groups.

In this study, both seaweeds showed the significant decrease in plasma glucose at the end of the treatment which was similar to these kinds of previously done studies.

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Fig 3: Effect of EAS & EAU treatment on liver histopathology of HFD and MLDS induced diabetic mice. A: Group 1 (Normal control) shows normal lobular architecture, B: Group 2 (Diabetic control) with marked steatosis and destroyed architecture, C: Group 3 (EAS 100 mg/kg) shows some fatty infiltration, D: Group 4 (EAS 200 mg/kg) representing mild steatosis and almost normal architecture, E: Group 5 (EAU 100 mg/kg) shows marked fatty infiltration, F: Group 6 (EAU 200 mg/kg) with mild steatosis, G: Group 7: (Gliclazide) shows normal lobular architecture

Diabetic patients generally have specific type of blood lipid profile characterized by an elevated serum TG, TC and low HDL cholesterol. This is because insulin has a role in inhibiting the hormone sensitive lipase and activating lipoprotein lipase. Therefore, in diabetic individuals, the transportation of free fatty acids from adipose tissues is increased and as a result of which total TG levels of serum are elevated. Seaweed extracts treatment resulted in lower levels of TG and TC. EAS and EAU at 200 mg/kg could bring about significant (p<0.05) change in plasma HDL and LDL level. Only small but not statistically significant changes were seen in VLDL levels. These results were somewhat similar to earlier studies, which reported decreased lipid levels with diets containing gel-forming fiber and seaweeds supplement.

OGTT is an easy diagnostic test used extensively for the diagnosis of type 2 diabetes. This test displays the competence of the diabetic
individual to effectively utilize glucose after a meal or oral glucose load. The OGTT or meal tolerance test resembles the glucose and insulin dynamics of physiological conditions closely. Impaired glucose tolerance is reflected in a larger incremental AUC of the plasma glucose disappearance curve. Results of OGTT revealed that AUC of the plasma glucose was significantly (p<0.001) higher in case of diabetic control. However, the AUC of OGTT curve was significantly (p<0.001) attenuated in both EAS and EAU treated animals at both low and high doses.

Uncontrolled diabetes is evidenced by constant high blood glucose levels and can cause of weight loss in our body. This may be because of insufficient amounts of insulin made by the pancreas. When there isn’t enough insulin around, glucose stays in blood and can’t get into the cells to be used for energy. The body needs fuel from somewhere, so in the absence of glucose, it starts to burn fat and muscle proteins. This mechanism of muscle wasting can be attributed to the main reason of weight loss in diabetics. It was found that diabetic mice significantly (p<0.001) lose their body weight as compared to normal mice at the end of the study period. However, both EAS and EAU treated animals showed significant (p<0.001) gain in body weight at both doses as compared to diabetic mice. These findings agreed with the results of Abirami and Kowsalya (2013).

Liver weight was significantly (p<0.001) increased in diabetic control groups as compared to the normal group. It may be because of accumulation of fat or formation of fatty patches on liver cells as a result of HFD. However, treatment groups showed significant reduction in liver weight as compared to diabetic control groups. A study suggested that, patients with diabetes usually show a reduction in muscle strength. The loss of muscle weight in diabetic control may be due to the muscle wasting or muscle protein degradation, which may be due to several causes one of the most important being the infraction of muscle tissues. In this study also there was the significant loss of muscle weight in diabetic animals. However, EAS and EAU showed the significant gain in the muscle weight at both the dose treatment [at 200mg/kg; p<0.01 and 100 mg/kg; p<0.05].

It is known that the quantity of TG in the skeletal muscle and liver is significantly increased in uncontrolled diabetes mellitus, which results from increased free fatty acids levels in the plasma. During a study, fucoxanthin rich brown seaweed extract was evaluated to find out its usefulness as nutraceutical, hypolipidemic and as an anti-obesity agent in C57BL/6J mice. The results of this study indicated that the extract reduced hepatic TG significantly. Our findings also agree with this result.

During diabetic state, increased generation of ROS occur and cause an imbalance between the oxidant and antioxidant status. Sustained hyperglycemia is the main cause of increased ROS generation in diabetes. This might be the result of suppression of beta-cell proliferation and inhibition of insulin gene transcription that causes impairment of insulin release. Literature has revealed that diabetic patients and diabetic rats usually show an elevated level of lipid peroxide. Studies have proven that, antioxidants can provide beneficial effects on pancreatic β-cells function in diabetes by rendering protection against glucose toxicity. Seaweeds are rich source of antioxidants like astaxanthin, fucoxanthin, phenolic acid, tannins, flavonoids etc which act by scavenging reactive oxygen species and inhibiting lipid peroxidation. In this study we found that, the diabetic mice show decreased SOD levels in their liver and skeletal muscle tissues, which follow the same connotation.

Glycogen is the primary form of glucose that can be intracellular. Its levels in various tissues, especially in liver and skeletal muscle are direct reflection of insulin activity. Insulin stimulates the activity of glycogen synthase and inhibites glycogen phosphorylase. This is the way how it regulates intracellular glycogen deposition. Our results showed that both seaweed extracts treatment to diabetic rats significantly elevated both muscle and hepatic glycogen contents. The decrease in hepatic glycogen content in diabetes is probably due to lack of insulin in the diabetic state that result in the inactivation of glycogen synthase enzyme. The significant increase in the liver glycogen content of the seaweed treated groups may be because of reactivation of this enzyme. Hence, another important way to control diabetic conditions is the improvement of glicogenesis of the individual.

The important findings of liver histopathological study displayed severe swelling and disarrangement in hepatocytes in diabetic livers. Necrotic tissue damage and microvesicular vacuolization were also found. These characteristic histopathological findings during this study were also similar to the results of a previous study. These observations may be due to insulin deficiency and altered mitochondrial β-oxidation of fatty acids. As a result of which fatty acids get esterified to TG in the cytoplasm and this is the reason why several triglyceride droplets were found within the hepatocytes. However, in our study seaweed extracts treatment improved the hepatic morphology to a greater extent, which has already been proved in previous study.

5 Conclusions

In this work, it was demonstrated that ethyl acetate extracts of S. wightii and U. faciata influence glycemic control and are effective in lowering blood lipids. Further these extracts are also helpful in improving antioxidant enzyme activities and glycogen content in liver and skeletal muscle. Consequently, the findings of this study suggest that the use of these seaweeds may minimize the chances of developing serious complications in type 2 diabetic individuals. But further studies are necessary to corroborate these results and to make dietary recommendations of these seaweeds for patients with diabetes.
type 2 diabetes. The exact mechanism involved in lowering plasma glucose and improving lipid profile are also needed to be established in further studies.

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7 Conflict of interests

The authors have no current conflict of interests in this work.

8 Author’s contributions

LM, PP and SP carried out literature review and draft the manuscript. SKB and RCP designed and monitored the experimental protocol. LM and PP collected the material and performed whole experimental procedures. All authors read and approved the final manuscript.

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