INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Antidiabetic Activity of *Schizochytrium* sp. Against Streptozotocin-Nicotinamide-Induced Diabetes in Rats

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OPEN ACCESS

Received: 23-11-2023
Accepted: 22-12-2023
Published: 28-12-2023

Citation: Harshitha S, Sarkar M, Rachana G, Harshitha HA (2023) Antidiabetic Activity of *Schizochytrium* sp. Against Streptozotocin-Nicotinamide-Induced Diabetes in Rats. Indian Journal of Science and Technology 16(48): 4743-4751. https://doi.org/10.17485/IJST/v16i48.2997

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Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objective: To evaluate the antidiabetic effect of agal oil in Streptozotocin-nicotinamide-induced type 2 diabetes in rats. **Methods:** Diabetes was induced by a single IP injection of Streptozotocin (50 mg/kg bw) and Nicotinamide (120 mg/kg bw). Daily the algal oil (*Schizochytrium* sp.) was administered with a single dose of 400 mg/kg bw and 800 mg/kg bw for the period of 21 days. The algal oil effect on body weight, fasting blood sugar, as well as serum lipid profile were evaluated. Histopathological examination of the pancreas was carried out to characterize the changes due to diabetes. **Findings:** A significant reduction in blood sugar levels was noted with algae oil. After 21 days of treatment of diabetic rats with algal oil in dosages of 400 mg/kg bw as well as 800 mg/kg bw, we noted a marked decrease in the levels of fasting blood glucose, HbA1c, triglycerides, LDL cholesterol as well as total cholesterol. Also, we observed a significant increase in the levels of haemoglobin, HDL cholesterol and body weight when compared to untreated diabetic rats ($p < 0.05$). These findings provide evidence for antidiabetic as well as anti-obesity properties of algal oil by regulating the parameters of lipid profile. Furthermore, the oral administration of algal oil demonstrated the regeneration of pancreatic beta cells which was evident in the histopathological examinations of pancreatic tissue in diabetic rats. **Novelty:** Algal oil functions effectively as an antidiabetic agent with less chance of side effects. This study showed the potential benefits of algal oil as a suitable bioactive compound that can be utilized in functional foods as a natural diabetes treatment.

Keywords: Algal oil; Diabetes; Streptozotocin; Nicotinamide; Blood glucose level

1 Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia symptoms that necessitate continuous medical attention. It generally occurs due to the partial or

complete lack of insulin [1]. It is primarily categorized into two types. Type I diabetes mellitus (T_1DM) results from the complete absence of insulin production whereas Type II diabetes mellitus (T_2DM) is caused by the partial absence of insulin production and the tissue resistance to the insulin action. It is projected that around 400 million people who were aged from 18 to 99 had diabetes in 2015, and by 2045, that figure will likely be increased to around 700 million. There have been reports of utilizing seaweed in some Asian traditional medicines to treat serious illnesses [2]. Of the fatty acids that are produced, around 40 per cent (w/w) of them are Docosahexaenoic acid (DHA) in heterotrophic microalgae. Additionally, according to some research, intake of seaweed regularly reduces the risk of developing cancer, hyperlipidemia, cardiovascular disease, and other conditions [3]. Among the marine seaweeds, there are approximately 1500–2000 classes of marine species, most of which are under extensive investigation as potential medications, nutraceuticals, etc.

Algal oil is derived from different types of microalgae and is characterized by high concentrations of essential fatty acids, specifically omega-3 fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The oil produced by *Schizochytrium* strains is a mixture of n–6 and n–3 polyunsaturated fatty acids, comprising approximately 40% of total fatty acids as total n–6 polyunsaturated fatty acids and approximately 19% of total fatty acids as ARA. Despite producing less oil, these strains grow quickly and have high biomass densities. One essential component of the body's defense mechanisms is the presence of DHA as well as EPA in the bloodstream, which actively reduce inflammation and neutralize (Reactive oxygen species) ROS that are created at these cell levels. Another example of an extended-chain omega-3 fatty acid is EPA (C20:5 n-3). Its primary advantage to wellness lies in its role as a phospholipid, which plays a vital role in regulating the immunological as well as inflammatory responses in the human body. Algal oil is a great dietary choice for vegetarians because it is safe and effective and contains a significant amount of omega-3 fatty acids [4]. Taking into the amount of impact caused because of diabetes as well as knowing the use of the algal extract, this study was designed and conducted with the purpose of investigating the antidiabetic activity of *Schizochytrium* sp. on the diabetes-induced rat model.

2 Methodology

2.1 Experimental animals

Albino Wistar rats weighing the range between 150–200 grams were purchased from the Central Animal Shelter at Krupanidhi College of Pharmacy for conducting an *in vivo* experiment. A total of 8 rats were housed in each of the polypropylene cages, in under temperature-controlled area with a 12-hour light/dark cycle at approximately 24°C and 50% humidity. The rats were provided regular diet pellet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) with No. KCP/IAEC/PCOL/102/2022, and the protocols were followed as per the standards established by the Committee for the Control and Supervision of Animals used in experiments (CPCSEA).

2.2 Collection of the Marketed product of algal oil

The algal oil (*Schizochytrium* sp.) was procured from Amazon Mart (Catalogue No. 0809RX).

2.3 Primary phytochemical analysis of algal oil [5]

Primary phytochemical screening was conducted to identify the present phytoconstituents. These tests included:

2.3.1 Test for alkaloids

- Dragendorff's Test: The presence of alkaloids is indicated by the development of a brownish-red precipitate.
- Wagner's Test: The formation of a brown or reddish precipitate suggests the presence of alkaloids.
- Mayer's, Bertrand's, and Valser's Tests: Observation of a creamy white or yellow precipitate signifies the presence of alkaloids.

2.3.2 Test for phenols

- Ferric Chloride Test: The presence of phenols is suggested by the development of a bluish-black color.
- Iodine Test: Observation of a transient red color indicates the presence of phenols.
- Lead Acetate Test: Production of a white precipitate is indicative of the presence of phenols.

2.3.3 Test for Flavonoids

- Alkaline Reagent Test: An intense yellow color with 2% NaOH, turning colorless with dilute HCl, indicates the presence of flavonoids.
- Ferric Chloride Test: The formation of a green precipitate suggests the presence of flavonoids.
- Ammonia Test: A yellow color with dilute ammonia and conc. H_2SO_4 indicates the presence of flavonoids.
- Conc. H_2SO_4 Test: An orange color with concentrated H_2SO_4 suggests the presence of flavonoids.

2.3.4 Test for Tannins

- $FeCl_3$ Test: A brownish-green color indicates the presence of condensed tannins.
- Matchstick Test: Matchstick wood turns pink or red near the flame when dipped in the aqueous plant extract.

2.3.5 Test for glycosides

- Legal's Test: A color change to pink or red with the test solution, pyridine, and sodium nitroprusside indicates the presence of cardiac glycosides.
- Keller-Killani Test: Formation of a blue-colored solution in an acetic acid layer with filtrate, glacial acetic acid, 5% ferric chloride, and conc. H_2SO_4.

2.3.6 Test for cardenolides

Bromine Water Test: Formation of a yellow precipitate with bromine water, turning red with 20% NaOH, and fading to brownish yellow.

2.3.7 Test for saponins

Foam Test: The development of persistent, stable foam for at least 15 minutes shows the presence of saponins.

2.3.8 Test for terpenoids

Chloroform Test: Formation of a gray-colored solution with chloroform, plant extract (evaporated on a water bath), and conc. H_2SO_4 (boiled in a water bath).

2.3.9 Test for triterpenoids

- Salkowski's Test: A golden yellow layer forms at the bottom with the filtrate and concentrated H_2SO_4.
- Libermann-Barchord Test: Deep red color at the junction after boiling the extract with acetic anhydride and cooling, followed by the addition of conc. H_2SO_4.
- Sulfur Powder Test: Sulfur sinks to the bottom when the test solution is mixed with a small amount of sulfur powder.

2.3.10 Test for steroids

- Libermann-Burchard's Test: Development of green color in the test tube following the described procedure
- Salkowski Reaction: Formation of red color by shaking 2 mg of the dry extract with chloroform and slowly adding sulfuric acid to the chloroform layer.

2.4 Acute Toxicity Studies[6]

Rats were randomly grouped and marked for individual identification; the animals were housed for at least five days in their cages for acclimatization to laboratory conditions. The animals were kept without food overnight before being dosed but had access to drinking water. Acute toxicity assessment was carried out using a single dose of 100, 300, 750, 1000, 2000 mg/kg bw. The dosage was administered to a single Wistar rat based on body weight. The rats were given a standard rodent diet 3 hours after oral gavage of treatments. They were then observed for signs of acute toxicity, changes in skin, fur, eyes and mucous membranes, somatomotor activity, behavior patterns, tremors, convulsions, salivation, diarrhoea, lethargy, sleep, changes in gait, and mortality. Observations were made continuously for the first 4 h after treatment, then once daily for 14 days. All animals were weighed on the day of arrival, day 1 of treatment, day 7 and day 14. Feed residual was weighed daily, and the average feed consumption per animal was calculated. On day 15, the animals were anaesthetized with diethyl-ether and a macroscopic examination of the animals was carried out. This study was performed according to the OECD Guideline for the Testing of Chemicals No. 423, Acute Oral Toxicity.

2.5 Streptozotocin-Nicotinamide induced model

Albino Wistar rats, weighing 150 to 200 grams, were provided with a standard pellet diet as well as tap water *ad libitum*. The rats were fasted overnight and were then administered with a freshly prepared solution of streptozotocin (STZ) dissolved in citrate buffer pH 4.5 at a dose of 65 mg/kg intraperitoneal (i.p.), 15 min after i.p. administration of 110 mg/kg body weight nicotinamide. Normal rats received 1 ml of citrate buffer as vehicle. As administration of STZ can induce fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were provided with a 10% glucose solution after 6 hours of STZ administration for the next 24 hours to prevent hypoglycemia. After 72 hours of streptozotocin injection, the blood glucose level of each rat was assayed, and further on day 7 of the injection. The rats with a fasting blood glucose level above 200 mg/dl were considered to be type 2 diabetic and used in this investigation.

2.6 Experimental design

Group1: Normal group (Nondiabetic control was administered 1% tween 80 for 21 days).

Group2: Diabetic control group treated with single i.p. doses of Streptozotocin (50mg/kg bw) & nicotinamide (120 mg/kg bw) to induce diabetes.

Group 3: Diabetic rats were treated with a low dose of algal oil (400 mg/kg bwp.o.) once daily for 21 days.

Group 4: Diabetic rats were treated with a high dose of algal oil (800 mg/kg bw p.o.) once daily for 21 days.

Group 5: Diabetic rats were treated with the standard drug glibenclamide (2.5 mg/kg bw p.o.) Once daily for 21 days.

2.7 Parameters to be Evaluated

2.7.1 Physical parameters

Change in body weight was measured every week for 21 days.

2.7.2 Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted (18 h) normal animals. Rats divided into four groups were administered algal oil (400 mg/kg), algal oil (800 mg/kg) and glibenclamide (2.5 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation (to minimize the distress) at 0, 30, 60, 90 min of algal oil administration. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips (Accu-chek active).

2.7.3 Biochemical Parameter

"The management of diabetes necessitates the regular measurement of glucose levels in the blood, using test strips for glucose and an Accu-Check current blood sugar level monitoring device on days 0, 7, 14, and 21." For glucose determination, the tail vein was used to take the blood sample. Additionally, serum biochemical parameters, including cholesterol, triglyceride, HDL, and LDL levels, were assessed using Erba diagnostic kits.2 ml of blood samples for the serum parameters were collected through retro-orbital puncture under anaesthesia (ether) using capillary tubes. The biochemical parameters were determined with the assistance of a semi-auto analyzer.

The entire blood sample was utilized for haemoglobin and glycosylated haemoglobin estimation. Erythrocytes were incubated at 37°C for 15 minutes after being cleansed with saline and lysed with 5 ml of distilled water. After centrifugation, 0.5 ml of saline was added after discarding the supernatant. An aliquot of 2 ml was mixed with 4 ml of oxalate hydrochloride solution were heated for four hours at 100°C, then cooled and 2 mL of 40% trichloroacetic acid (TCA) was used to precipitate the solution. Following centrifugation, 0.5 mL of the supernatant was blended with 3 mL of concentrated sulfuric acid and 0.5 mL of 80% phenol. After 30 minutes, the resultant color was measured at 480 nm. Standard solutions ranging from 10 to 50 μg were created by mixing them with a 1% fructose solution. Subsequently, 3 mL of pure sulfuric acid and 0.5 mL of 80% phenol were introduced to these standard solutions, and the reading was taken at 480 nm after 30 minutes.

2.8 Histopathological examination

At the end of the study, all the rats were humanely euthanized under anaesthesia, and the pancreas was collected from each group. The collected tissues were rinsed with ice-cold saline and immediately dipped in a 10% buffered neutral ketamine solution for 1 hour for further histopathological examination.

2.9 Statistical Analysis

One-way analysis of variance (ANOVA) was used followed by Dunnett's test for comparison. The software GraphPad Prism 10.0.2 was utilized, and the outcome was provided as mean ± standard error of the mean (SEM). Significance was indicated with p-value< 0.05.

3 Results and Discussion

3.1 Preliminary phytochemical screening of Algal oil

A quantitative phytochemical screening on Algal oil indicated the presence of steroids, saponin, tannins, glycosides, phenols, alkaloids, terpenoids, and flavonoids. The results of preliminary qualitative screening studied by Munir et al., in their study indicated the presence of flavonoids, phenols, quinones, steroids, resins, anthraquinones, glycosides, lignin, proteins, saponins, tannins, reducing sugars, alkaloids, terpenoids, fats, and oils, whereas the quantification results revealed the high quantities of total ash, crude proteins, crude fiber, alkaloids, carotenoids, flavonoids, and chlorophyll [7].

3.2 Physical parameters

3.2.1 Oral glucose tolerance test

Both low and high doses of algal oil exhibited a decrease in glucose levels compared to the normal group, The high-dose algal oil group showed a glucose-lowering effect comparable to glibenclamide, a standard drug known for its antidiabetic properties. These findings suggest that algal oil may have a potential glucose-regulating effect, with higher doses demonstrating efficacy similar to the established antidiabetic medication, glibenclamide [Table 1].

Table 1. Effect of algal oil on body weight in normal and streptozotocin-nicotinamide induced diabetic rats

Sl.no	Days	Vehicle Control	Diabetic Control	Diabetic + Low dose (400 mg/kg bw)	Diabetic + High dose (800 mg/kg bw)	Diabetic + Glibenclamide (2.5 mg/kg bw)
1	O^{th} day	170.27±0.369	163.39±0.307*	147.29±0.649*	142.26±0.186*	149.413±0.301*
2	7^{th} day	173.94±0.526	152.70±0.784*	149.07±0.582*	142.27±0.640*	152.34±0.579*
3	14^{th} day	178.70±0.353	152.70±0.784*	148.27±0.369*	158.137±0.464*	162.29±0.353*
4	21^{th} day	180.66±0.33	143.97±0.064*	157.43±0.297*	166.27±0.369*	170.33±0.665*

*Significant

3.3 Effect on Body Weight

In comparison to the normal control group, the diabetic control group observed a significant decrease in bw ($p < 0.05$). However, the administration of algal oil at dosages of 400 and 800 mg/kg bw resulted in an improvement in bw when compared to the diabetic control group ($p<0.05$) [Table 2]. Additionally, in the diabetic group, a notable increase in water intake was observed in comparison to the normal group. However, after 21 days of administering algal oil and the standard drug, groups 3, 4, and 5 exhibited a significant reduction in water intake compared to the diabetic control group, indicating a positive response to the treatments. Certainly, fucoxanthin a major carotenoid present in the chloroplasts of brown seaweeds and diatoms has the ability to regulate energy expenditure, which in turn affects leptin levels and body weight [8].

Table 2. Effect of algal oil on oral glucose tolerance in normal rats

Days	Vehicle Control	Diabetic Control	Diabetic +Algal Oil (400mg/kg bw)	Diabetic+ Algal oil (800mg/kg bw)	Diabetic+Glibenclamide (2.5 mg/kg bw)
0	80.556±0.56	299.743±0.941*	313.326±7.866*	327.403±1.517*	332.667±3.282*
7	83.5036±2.073	318.3336±1.201*	269.396±0.872*	249.063±3.175*	257.723±0.925*
14	80.596±0.798	325.773±1.230*	190.66±3.179*	181.73±0.822*	159.2236±1.926*
21	80.3266±1.770	337.3266±0.0336*	113.556±3.816*	103.180±1.779*	98.056±1.204*

*Significant

3.4 Fasting blood glucose level

The suppression of blood glucose involves a multifaceted approach, including reduced gut absorption and potential extra-pancreatic actions stimulating peripheral glucose utilization, alongside increased enzymatic activities in peripheral tissues. This orchestrated interplay, coupled with the diminished secretion of counterregulatory hormones, intricately regulates blood glucose levels, underscoring the complexity of glucose homeostasis.

In our study, the blood glucose of all groups was observed on the 1st, 7th, 14th and 21st day. The diabetic rats treated with algal oil and glibenclamide showed a significant decrease in blood glucose levels. Compared to the vehicle control group, the diabetic control group had elevated blood glucose levels. In contrast to the diabetic control group, the treatment groups with Glibenclamide at 2.5 mg/kg bw and algal oil at both doses (400 and 800 mg/kg bw) for 21 days led to a statistical improvement in blood glucose levels, as shown in Table 3.

Table 3. Effect of algal oil on fasting blood glucose levels in diabetic rats

Groups	Dose mg/kg bw	0 minutes	30 minutes	60 minutes	90 minutes
Normal group	-	74±1.31	127±1.76	131±2.06	120±1.72
Low dose (Algal oil)	400	72±1.11	113±1.43	117±1.85*	119±1.81*
High dose (Algal oil)	800	72±1.769	99±1.47*	100±3.31*	98±1.66*
Standard dose (Glibenclamide)	2.5	73±1.25	96±1.25*	94±1.75*	92±2.15*

*Significant

Similar results were observed in the Oliyaei et al. study, wherein all seaweed-treated groups had a statistically significant decrease in FBS in comparison to the group with diabetes[8]. Seaweed's potential mechanism of action against diabetes could be attributed to inhibition or lowered activity of the enzyme α-glucosidase, which is responsible for converting carbohydrates into glucose. Therefore, elevated level of blood glucose is prevented by slowing down the carbohydrate absorption in the small intestine[2]. According to Zaharudin et al., fucoxanthin exhibits a high α-glucosidase inhibitory activity, with an IC50 value that is lower than that of the specific inhibitor acarbose. They also reported that administration of acetone extract from brown seaweeds such as *Undaria pinnatifida* and *Laminaria digitate* effectively inhibited α-glucosidase[2]. However, *U. pinnatifida* extract had significant inhibitory activity (>70%). Moreover, they suggested that the brown algae extract had better inhibitory effects compared with red algae[2].

3.5 Effect of serum lipids profile

Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and phospholipids, and changes in lipoprotein composition. Table 4 illustrates the changes that were noted in the HDL levels, LDL levels, total cholesterol, and triglycerides levels. A decrease in the serum triglycerides, total cholesterol, LDL (low-density lipoprotein) and an increase in the HDL (high-density lipoprotein) cholesterol levels were observed. This is similar to the study of Oliyaei et al., the total cholesterol, triglyceride, and low-density lipoprotein were lower in the seaweed-treated groups[8].

Table 4. Effect of algal oil on serum lipid profile in diabetic rats

Groups (n=8)	Treatment	Total Cholesterol	Triglyceride level	HDL	LDL
1	Vehicle control	71.14±4.25	74.85±1.00	54.50±3.12	22.84±0.74
2	Diabetic control	167.02±5.28	179.42±5.21	26.83±1.77*	99.32±2.38
3	Diabetic + 400mg/kg bw	99.08±4.96*	105.13±4.33*	35.67±2.29	52.22±1.95*
4	Diabetic+800mg/kg bw	92.02±4.50*	95.11±4.46*	47.33±1.89*	44.42±1.24*
5	Diabetic+Glibenclamide 2.5mg/kg bw	83.33±3.24*	88.22±3.35*	49.67±2.51*	36.36±0.663*

*Significant

3.6 Hematological parameter

3.6.1 Effect on HbA1c and hemoglobin

The impact of algal oil and glibenclamide on HbA1c and haemoglobin levels in STZ-NA-induced diabetic rats was presented in Table 5.

Table 5. Effect of algal oil on HbA1c and hemoglobin level in diabetic rats

Group	Treatment	HbA1c	Hemoglobin
1	Vehicle control	5.20±0.72	12.20±0.93
2	Diabetic control	11.58±1.80	9.04±0.54
3	Diabetic +Low dose (400 mg/kg bw)	8.46±0.93	11.70±0.82*
4	Diabetic + High dose (800mg/kg bw)	6.58±0.51*	12.10±0.85*
5	Diabetic+ Glibenclamide (2.5mg/kg bw)	6.50±0.84*	13.50±0.40*

***Significant**

Glycosylated haemoglobin (HbA1c) was significantly increased in diabetic animals, and this increase was found directly proportional to the fasting blood glucose level. During diabetes, the excess glucose present in the blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in diabetic rats [9]. In our study comparison to the normal group, the diabetic control and low-dose algal oil groups showed significantly higher HbA1c levels, indicating poor glycemic control. Conversely, the high-dose algal oil and standard drug (Glibenclamide) groups exhibited significantly lower HbA1c levels, suggesting improved glycemic regulation compared to the diabetic control group. Additionally, compared to the normal haemoglobin level, the diabetic control group exhibited a significantly lower level, indicative of potential diabetes-related anaemia. However, the groups receiving low-dose algal oil, high-dose algal oil, and the standard drug (Glibenclamide) demonstrated haemoglobin levels closer to normal, suggesting a potential beneficial effect on haemoglobin with these interventions.

3.7 Histopathology Results

3.7.1 Effect of Algal oil in histopathology of pancreas

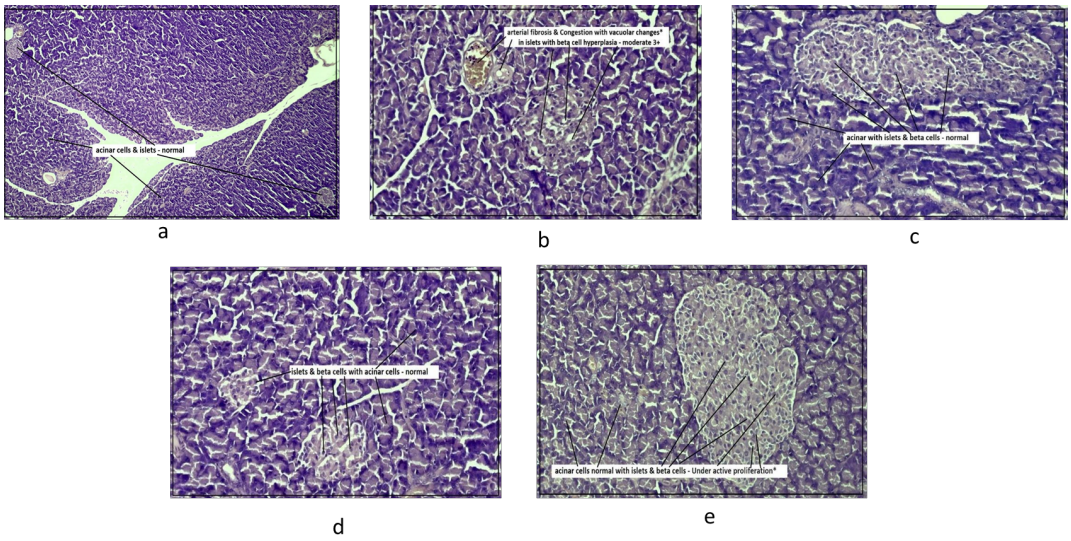

Fig 1. a. Normal Control Group Pancreas: showing islets & beta cells with acinar cells are normal -NAD+ (100),b. Disease Control group – Rat Pancreas: arterial fibrosis & Congestion with vacuolar changes* in islets with beta cell hyperplasia - it's a degenerative response*- moderate 3+ (X100),c. Standard - Glibenclamide treated Rat Pancreas: showing islets & beta cells normal – NAD + (X100),d. Low dose group Rat Pancreas: showing islets & beta cells with acinar cells normal – NAD + (X100),e. High dose group Rat Pancreas: showing acinar cells normal with islets & beta cells -Under activeproliferation* – could be due to drug response* (X100)

The study assesses the antidiabetic effects of drugs treated on STZ-NA-induced diabetic rat models by examining pancreatic tissue. Disease control rats exhibit degenerative responses, including arterial fibrosis, inflammation, and congestion. Glibenclamide-treated rats show normal pancreatic architecture, while low-dose group rats display mild fibrosis. High-dose group rats exhibit structural restoration and beta cell proliferation, suggesting a potential cytoprotective effect [Figure 1**:** beta cells are having some proliferation]. Notably, the underactive proliferation of beta cells in the high-dose group may be attributed to the drug response, indicating a promising outcome for the drug's efficacy in treating diabetes-induced pancreatic

damage. This finding was in agreement with the findings of Oliyaei et al., where the section of pancreatic tissue of nondiabetic group showed the normal appearance of islets of Langerhans, whereas in the diabetic group, islet cells underwent significant degenerative changes due to STZ, including a decrease in size as well as number, while pancreatic beta cells in diabetic groups were recovered and proliferated following metformin or fucoxanthin treatment[8]. In the present study, histopathological examination of the pancreas in STZ-induced diabetic animals revealed a significant reduction in the number of Langerhans islets and β cells, accompanied by fibrosis and inflammatory cell infiltration within the islets. The histopathological observation for the anti-diabetic activity of algal oil in STZ-NA-induced diabetes in rats was carried out in the rat pancreas. In diabetic control group showed arterial fibrosis and congestion with vacuolar changes in islets with beta cell hyperplasia. In treated groups showing islets & beta cells with acinar cells normal.

Our research aimed to understand its precise effects on albino Wistar rats with induced diabetes through the administration of streptozotocin at 50 mg/kg bw and nicotinamide at 120 mg/kg bw. This investigation seeks to give an in-depth awareness of the potential benefits of algal oil in managing diabetes and its implications for treatment strategies. Studies have indicated that the induction of experimental diabetes using streptozotocin in combination with nicotinamide exhibits beneficial characteristics such as sustained hyperglycemia, glucose intolerance, and insulin secretion stimulated by glucose in experimental models[10]. Therefore, in the current study, diabetes-induced in experimental rats using STZ-NA was selected as the rat model to assess the anti-diabetic potential of Algal oil.

In today's age, herbal preparations are more crucial than ever, owing to their effectiveness and convenient availability as well as fewer side effects as compared to synthetic drugs[11]. Algal oil, due to its omega-3 fatty acid content, has been explored for its potential to enhance insulin sensitivity. Insulin resistance is a key feature in the development of type 2 diabetes. The omega-3 fatty acids in algal oil may improve cellular responses to insulin, thereby helping to regulate blood glucose levels more effectively[12]. This has been a focal point in the search for alternatives to conventional medicine to treat this illness. An initial phytochemical examination was conducted on the algal oil showed the prominent presence of compounds such as phenols, flavonoids, alkaloids, steroids, tannins, terpenoids and glycosides.

In the present study, acute oral toxicity OECD 425 studies on Algal oil indicated that was found to be safe even at the highest tested dose of 2000 mg/kg bw, which corresponds to the maximum recommended limit for this test. Based on these results, two distinct doses, specifically 400 mg/kg bw and 800 mg/kg bw, were chosen for further preclinical investigations for algal oil.

A comprehensive assessment was conducted involving both physical and biochemical analyses, first after the induction of diabetes and subsequently following a 21-day treatment with algal oil. Glibenclamide is a standard antidiabetic drug known to produce its effect via selective blockage of adenosine triphosphate (ATP) sensitive K+ (KATP) channels in the plasma membrane. This leads to membrane depolarization, activates voltage-gated Ca^{2+} channels, a rise in cytosolic (Ca^{2+}) and release of endogenous insulin in â-cells of the pancreas[13].

Further research may be required to investigate the insight of understanding the mechanism of algal oil for antidiabetic activity and new therapeutic interventions for diabetes. The antidiabetic effects of the algal oil in diabetic rats are likely due to its insulin-mimicking properties, along with possible facilitation of glucose uptake in peripheral tissues, inhibition of natural glucose production, or promotion of gluconeogenesis within the liver and muscles.

4 Conclusion

The results obtained from the current study suggested that the agal oil could be used as a potential antidiabetic agent that demonstrated significant anti-hyperglycemic activity. Further elucidation of compounds present in *Schizochytrium* sp. could be used as an antidiabetic agent that could be targeted for the treatment of diabetes.

References

1) Soffe R, Nock V, Chase JG. Towards Point-of-Care Insulin Detection. *ACS Sensors*. 2019;4(1):3–19. Available from: https://doi.org/10.1021/acssensors.8b01253.
2) Zainuddin EN, Anshary H, Huyyirnah H, Hiola R, Baxa DV. Antibacterial activity of Caulerpa racemosa against pathogenic bacteria promoting "ice-ice" disease in the red alga Gracilaria verrucosa. *Journal of Applied Phycology*. 2019;31(5):3201–3212. Available from: https://doi.org/10.1007/s10811-019-01805-w.
3) Ganesan AR, Tiwari U, Rajauria G. Seaweed nutraceuticals and their therapeutic role in disease prevention. *Food Science and Human Wellness*. 2019;8(3):252–263. Available from: https://doi.org/10.1016/j.fshw.2019.08.001.
4) Rao A, Briskey D, Nalley JO, Ganuza E. Omega-3 Eicosapentaenoic Acid (EPA) Rich Extract from the Microalga Nannochloropsis Decreases Cholesterol in Healthy Individuals: A Double-Blind, Randomized, Placebo-Controlled, Three-Month Supplementation Study. *Nutrients*. 2020;12(6):1–14. Available from: https://doi.org/10.3390/nu12061869.
5) Sathiya RA, Murali A, Jayaraman A. Phytochemical screening and toxicological evaluation of Sargassum wightiiGreville in Wistar rats. *Turkish Journal of Pharmaceutical Sciences*. 2019;16(4):466–475. Available from: https://cms.galenos.com.tr/Uploads/Article_21108/TJPS-16-466-En.pdf.

6) Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. 2001. Available from: https://read.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en#page1.
7) Munir M, Khan AM, Qureshi R, Murtaza S, Munazir M. Preliminary Phytochemical Screening, Proximate Analysis, Antioxidant and Antibacterial Activities of an Algal Species of Hydrodictyon Reticulatum. *Journal of Bioresource Management*. 2020;7(4):120–148. Available from: https://corescholar.libraries.wright.edu/cgi/viewcontent.cgi?article=1258&context=jbm.
8) Oliyaei N, Moosavi-nasab M, Tamaddon AM, Tanideh N. Antidiabetic effect of fucoxanthin extracted from Sargassum angustifolium on streptozotocin-nicotinamide-induced type 2 diabetic mice. *Food Science & Nutrition*. 2021;9(7):3521–3529. Available from: https://doi.org/10.1002/fsn3.2301.
9) Wang Y, Yang P, Yan Z, Liu Z, Ma Q, Zhang Z, et al. The Relationship between Erythrocytes and Diabetes Mellitus. *Journal of Diabetes Research*. 2021;2021:1–9. Available from: https://doi.org/10.1155/2021/6656062.
10) Cruz PL, Moraes-Silva IC, Ribeiro AA, Machi JF, de Melo MDT, Santos FD, et al. Nicotinamide attenuates streptozotocin-induced diabetes complications and increases survival rate in rats: role of autonomic nervous system. *BMC Endocrine Disorders*. 2021;21(1):1–10. Available from: https://doi.org/10.1186/s12902-021-00795-6.
11) Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. *Frontiers in Pharmacology*. 2023;14:1–16. Available from: https://doi.org/10.3389/fphar.2023.1265178.
12) Sinha S, Haque M, Lugova H, Kumar S. The Effect of Omega-3 Fatty Acids on Insulin Resistance. *Life*. 2023;13(6):1–17. Available from: https://doi.org/10.3390/life13061322.
13) Glibenclamide. . Available from: https://en.wikipedia.org/wiki/Glibenclamide.