INVESTIGATION OF POTENTIAL BIOACTIVE COMPOUNDS AND ASSESSMENT OF ANTI-DIABETIC PROPERTIES OF *HYDROCLATHRUS* SP. COLLECTED FROM THE BAY OF BENGAL, BANGLADESH

Bioresearch Communications
Volume 11, Issue 1, January 2025

DOI:
doi.org/10.3329/brc.v11i1.78884

Shomaya Akhter[1], Sheikh Shohag[1], Md Abdul Alim[1], Md. Tohidul Islam[2], and Mohammad Nazir Hossain[1]*

[1]Department of Genetic Engineering and Marine Biotechnology, Faculty of Earth and Ocean Science, Bangabandhu Sheikh Mujibur Rahman Maritime University, Dhaka-1216, Bangladesh.
[2]Department of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100, Bangladesh

ABSTRACT

Diabetes mellitus, a chronic metabolic disorder, has become a global health concern, prompting the exploration of alternative and sustainable sources for potential therapeutic agents. Seaweed extracts provide various benefits over synthetic medications, including lower toxicity and fewer side effects with diverse pharmacological properties, making them promising candidates for diabetes management. For this investigation, brown seaweeds, *Hydroclathrus sp.*, were obtained from Saint Martin Island, Bangladesh. Following phytochemical analysis, the anti-diabetic efficacy of *Hydroclathrus sp*. was evaluated both in vitro and in vivo. In vitro studies showed that alpha-amylase inhibitory activity of 50% ethanolic extract of *Hydroclathrus sp.* was closely related to positive control acarbose. Besides, in vivo studies were conducted on alloxan-induced diabetes mice models, exploring the effects of *Hydroclathrus sp.* on blood glucose levels, and their biochemical profile was evaluated and found the algal extract at 100mg/kg body weight was more potent than the reference medicine (Glibinclamide) after 14 days of treatment. Lipid profiles and liver and kidney function tests also revealed the potent antidiabetic effects of 50% ethanolic extract of *Hydroclathrus sp*. These findings offer valuable information for the development of novel marine-derived anti-diabetic medications. However, further studies are required to elucidate and validate its true potential in diabetes.

KEYWORDS: Anti-diabetic, *Hydroclathrus sp.,* Phytochemical Screening, In vivo, Alloxan, Alpha-Amylase.

RECEIVED: 22 August 2024, ACCEPTED: 26 November 2024

TYPE: Original Research

***CORRESPONDING AUTHOR: Dr. Mohammad Nazir Hossain, Department of Genetic Engineering and Marine Biotechnology, Faculty of Earth and Ocean Science, Bangabandhu Sheikh Mujibur Rahman Maritime University, Dhaka-1216, Bangladesh.**
Email: nazir.geb@bsmrmu.edu.bd

Introduction

Seaweed occurs naturally in coastal waters worldwide. Seaweeds, also known as macroalgae, are a diverse group of marine plants that play a crucial role in the world's oceans. There are currently 193 species of seaweed, 19 of which have commercial value (1). Seaweeds are marine algae that play a crucial role in the aquatic ecosystem (2). Seaweed has become a very versatile product widely used as a food or supplement in many countries (3). However, the seaweed industry in Bangladesh is at its initial stage, and people in Bangladesh are still unaware of the potential of seaweed (4). The Bay of Bengal, situated in the northeastern part of the Indian Ocean, is home to a diverse range of seaweeds. Seaweeds have been used since ancient times as food, fodder, fertilizer, and as a source of medicine. Seaweeds have garnered increasing attention recently for their rich biochemical composition and diverse bioactive compounds, contributing to their significant medicinal potential (5, 6). Diabetes is a global epidemic that affects millions of people worldwide. Diabetes is a metabolic illness characterized by hyperglycemia caused by abnormalities in insulin secretion, insulin action, or both. Complications of diabetes can lead to heart disease, kidney failure, blindness, and amputations (Deshpande, Harris-Hayes, and Schootman 2008; Hippisley-Cox and Coupland 2016). According to the International Diabetes Federation, there are currently over 537 million adults (20-79 years) living with diabetes (7), and this number is expected to rise to 700 million by 2045 (8). The current treatments for diabetes include insulin therapy, oral medications, and lifestyle changes such as diet and exercise (9, 10). While these treatments can effectively manage blood sugar levels, they have significant limitations and side effects (11, 12). Insulin therapy, for example, requires regular injections and can cause hypoglycemia, weight gain, and other complications. Oral medications can also cause side effects such as nausea, diarrhea, and liver damage. Lifestyle changes

can be challenging to maintain and may not be sufficient for some patients with advanced diabetes (13). These limitations and side effects highlight the urgent need for new treatments for diabetes. Seaweeds have been found to have antidiabetic properties and may offer a promising alternative to current therapies (14). *Hydroclathrus sp*. exhibits a distinct phytochemical composition compared to other seaweeds studied for their anti-diabetic effects. The presence of phenolic compounds and flavonoids distinguishes *Hydroclathrus sp.* as a unique source with the potential for novel therapeutic applications (15). One of the significant advantages of using natural products like these seaweed extracts is that they are often less toxic and have fewer side effects than synthetic drugs. Additionally, natural products tend to be more readily available and affordable, which could make them an attractive option for treating diabetes in low-income populations.

Seaweeds represent a promising source of antidiabetic compounds that could complement existing strategies and treatments for diabetes. Brown seaweed has shown promise as a potential antidiabetic agent due to its ability to modulate key pathways involved in glucose metabolism and insulin sensitivity (16). *Hydroclathrus sp.*, a unique seaweed variety found in the Bay of Bengal, has captured the attention of researchers for its promising medicinal properties (17). The present study aims to examine the phytochemical composition and evaluate the possible anti-diabetic properties of *Hydroclathrus sp*., obtained from the Bay of Bengal in Bangladesh. The research encompasses in vivo and in vitro evaluations to thoroughly investigate Hydroclathrus sp.'s effectiveness in managing diabetes. The findings of this research could have significant implications for developing novel therapeutic agents, especially in the context of diabetes management. The unique marine environment of the Bay of Bengal, coupled with the unexplored potential of Hydroclathrus sp., presents an exciting opportunity to discover new bioactive compounds with therapeutic applications.

Materials and Methods

Study Design

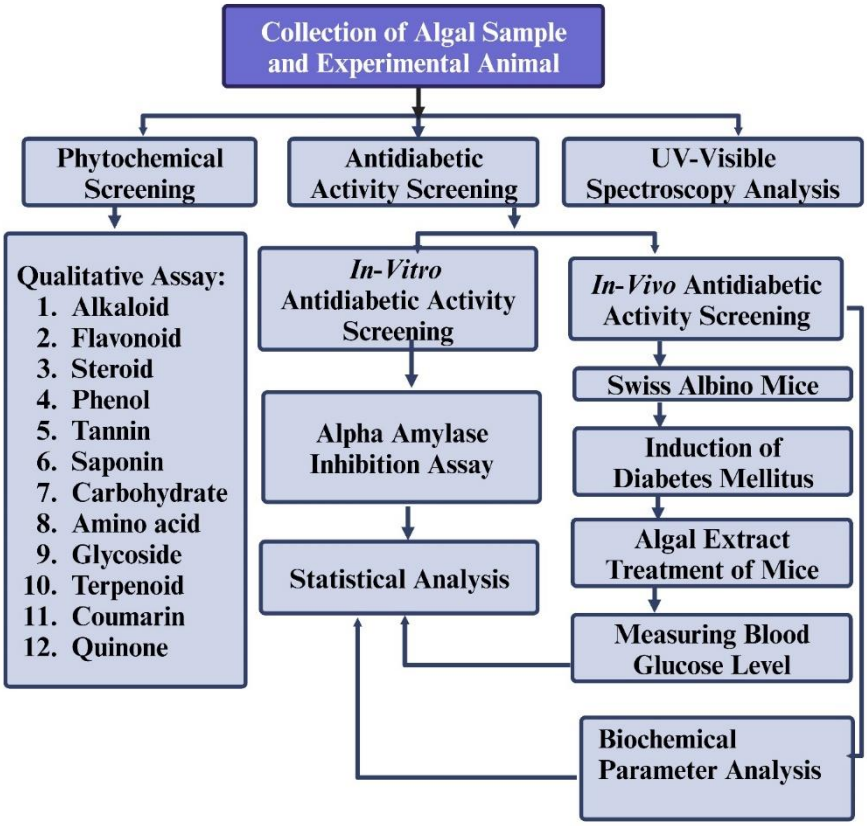

Figure 1. A brief depiction of the experimental design of the present study.

Sample Collection and Processing

Seaweed samples were collected from the shallow waters near Chhera Dwip on 5th March 2023. Collected samples were cleaned with seawater and then distilled water to remove sand particles, epiphytes, etc. The collected seaweed was subsequently identified as *Hydroclathrus sp.* based on its morphology (18). After washing, the samples were preserved in a 50% ethanol solution. Then, the samples were kept in a dry heat sterilizer (Model: JSON 030S, JSR, Korea) at 45°C until grinding. A conical flask contained a powdered sample weighing 20 grams, which was subsequently soaked in 200 ml of ethanol solution with a concentration of 50%. At room temperature (37° C), the conical flask was shaken at 150 rpm by a shaking incubator (Model: JSSI-070C, JSR, Korea) for five days. The extracts were filtered through Double Rings of 11.0 cm filter paper (Qualitative, 102).

Phytochemical Screening

The phytochemical test is a preliminary screening of algal extracts for the presence of medicinally active compounds, which are indicated by different colors and pigmentation by chemical reaction. These were identified by characteristic color changes using standard procedures (19–22)**.**

UV-Visible Spectroscopy Analysis

A Shimadzu 1900 UV-Visible double beam spectrophotometer with a slit width of 1.0 nm was used to perform spectrophotometric analysis on the *Hydroclathrus sp.* extract, which was kept at room temperature. In the proximal analysis, the extract was examined with visible and ultraviolet light ranging from 190 to 1100 nm in wavelength (23).

The extract was subjected to this procedure before further analysis with a UV-VIS spectrophotometer. The sample was diluted with a 50% ethanol solution at a ratio of 1:10. The initial value was calibrated using the same solvents, in this case, a 50% ethanol solution.

In vitro Antidiabetic (α-amylase inhibition) Assay

The procedure outlined by Mitra et al. was used to conduct the experiment (Tamil et al., 2010). 6mg of starch and 0.6 milliliters of a 0.01 M CaCl2 (pH 6.9) and 0.5 M Tris-HCl buffer (pH 6.9) solution made up the substrate solution. After being pipetted into test tubes, the substrate solution was heated to 37 degrees Celsius for five minutes, then it was preincubated. By dissolving them in DMSO, various quantities of acarbose and algal extract were created. The test tube holding the substrate solution was first filled with the algal extract or acarbose solution (0.6 mL) at various concentrations. Next, 0.3 mL of porcine pancreatic alpha-amylase (in TrisHCl buffer (2 units/mL)) was added (24). 1.5 mL of 50% acetic acid was added to each test tube to complete the procedure after it had been incubated for 10 minutes at 37° C. After centrifugation (for five minutes at 4° C at 3000 rpm), the supernatant optic density was measured at 595 nm. Alpha-amylase inhibitor acarbose was used as a positive control in this study. Three copies of each test were administered. The tests were performed in triplicates, and the inhibitory activity was calculated as percentage inhibition using the formula

% Inhibition = ((Abs control – Abs sample) /Abs control) × 100

In vivo Antidiabetic Assay

Animals

Swiss albino mice (female) 2 and 3 months of age, weighing 20 to 30 gm, were used for this study. They were obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). All experimental procedures were performed per the guide for the care and use of experimental animals. The ethical permission to conduct this research was taken from Bangabandhu Sheikh Mujibur Rahman Maritime University, Dhaka. They were housed in polypropylene cages at an ambient temperature of 25°C–27°C and maintained with a 12 h light and dark cycle. There was free access to standard mice feed and tap water. Before starting the experiment, the animals were acclimatized to the laboratory environment for one week.

Induction of Experimental Diabetes

The animals were fasted overnight, and their blood glucose levels were measured using a glucometer before being injected with alloxan. According to their specific weights, each animal's alloxan was calculated separately. Thus, just before injection, it was dissolved in 0.9% (w/v) normal saline (25, 26). After fastening overnight, the mice were given an intraperitoneal (IP) injection of 180 mg/kg/body weight of alloxan solution. Alloxan, which was freshly generated, was injected intraperitoneally to develop experimental diabetes. A solution of 10% glucose in tap water was given through a water bottle over 24 hours to reduce the risk of hypoglycemia shock and death.

Treatment Protocol

The animals were divided into six groups after the induction of alloxan diabetes.

Group I (Normal control) consists of mice treated with a normal diet.

Group-II: (Diabetic untreated) Diabetic untreated mice received distilled water 10ml/kg body weight.

Group-III: (Treatment group) Diabetic mice received an extract of Hydroclathrus sp. at a dose of (100mg/Kg) daily using the intra-gastric tube for 14 days.

Group-IV: (Treatment group) Diabetic mice received an extract of Hydroclathrus sp. at a dose of (50mg/Kg) daily using the intra-gastric tube for 14 days.

Group-V: (Positive control) Diabetic mice received (standard) glibenclamide at 0.66mg/kg body weight.

Group-VI: (Negative control) Diabetic mice received 50% ethanol as a negative control.

After four days of alloxan injection, fasting blood glucose (FBG) values above 200 mg dL−1 were considered diabetic mice. The treatment started on the fourth day, and diabetic animals were considered for further study and continued for 14 days. Each mouse's blood glucose levels (BGL) were measured by taking blood from its tail. Blood samples were taken at 0, 2, and 4 hours post-administration for the acute trials. Blood glucose levels (BGL) were measured weekly for two weeks during the chronic studies. The blood glucose levels were assessed using a One Touch Select Simple TM glucometer, while the weights of the individuals were routinely monitored.

Biochemical Analysis

Biochemical analysis techniques refer to a set of methods, assays, and procedures that enable scientists to analyse the substances found in living organisms and the chemical reactions underlying life processes (27). At the end of the experiment, the animals were made to fast overnight, and the blood was collected. The collected blood was incubated for 15–30 min at room temperature; the serum was separated by centrifugation (3000 rpm). The serum was collected to perform a lipid profile study, comprise the quantification of triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels. The tests have been performed with the help of experts from the Lions Eye Institute & General Hospital.

Statistical Analysis of the Result

Groups of data were compared with ANOVA, followed by Tukey's multiple comparison tests used to compare results.

Values were expressed as mean ± SEM, and the level of statistical significance was taken at $p < 0.05$. Microsoft Excel 2013 was used for data analysis in the study.

Result

Phytochemical Screening

In qualitative phytochemical screening, a specific class of chemicals can be detected by observing color changes or the production of precipitates when specific reagents are applied to extracts (**Table 1**).

Table 1. Phytochemical Screening of *Hydroclathrus sp.* By Chemical Treated Method.

SI No	Name of the Compound	Test Name	Extract			
			Ethanol	Methanol	Chloroform	Isopropanol
1	Alkaloid	Mayer's Test	-	-	-	-
		Wagner's Test	-	-	-	-
2	Flavonoid	Alkaline Reagent Test	+	+	+++	+
		Lead Acetate Test	+++	+++	+	+++
		Sulfuric Acid (H_2SO_4) Test	-	++	+++	++
3	Steroid	Salkowski Reaction	+++	+	+	+
4	Phenol	Ferric Chloride Test	+++	++	+	++
		Lead Acetate Test	+++	+++	-	++
5	Tannin	5 % Ferric Chloride Test	+	-	-	+
6	Saponin	Foam Test	-	-	-	-
7	Carbohydrate	Molisch Test	+	++	+	++
8	Amino Acid	Ninhydrin Test	++	+++	+++	+++
9	Glycosides	Salkowski Reaction	+	+	+	+
10	Terpenoid	Alkaline Reagent Test	+++	++	++	+

11	Coumarin	Alkaline Reagent Test	-	+	+	++
12	Quinone	Acid Test(H_2SO_4)	++	++	+	++
		Acid Test(HCl)	++	+	+	+

(-): not detectable, (+): low quantities, (++): moderate quantities, (+++): high quantities

UV-Visible Spectroscopy Analysis

A UV-vis spectrophotometer is an analytical instrument that measures the amount of ultraviolet (UV) and visible light absorbed by a sample. A UV-VIS spectrum profile of an ethanolic extract of *Hydroclathrus* species was selected from 190 to 1100 nm due to the sharpness of the peaks in the spectrum. The profile showed peaks at 664.00, 401.50, 270.50, and 262.50 nm with absorption values accordingly of 0.002, 0.007, 0.017, and 0.017 (UV-Visible spectrum of ethanol extract of *Hydroclathrus sp.* Is displayed in **Table 2).** Based on the present study's findings, it has been observed that the ethanolic extract derived from *Hydroclathrus sp.*, a type of brown seaweed, has a substantial quantity of primary and secondary phytochemical compounds. These compounds are believed to potentially play a role in the biological activities exhibited by the extract.

Table 2. UV-visible spectrum of ethanol extract of *Hydroclathrus sp.*

Compound detection of *Hydroclathrus sp.* extract on UV-Vis Spectrophotometer.				
Wavelength range	Wavelength range Scanning Speed	Sample Interval	Detection (nm)	Absorption values
190-1100nm	slow	0.5	664.00	0.002
			401.50	0.007
			270.50	0.017
			262.50	0.017

In vitro Antidiabetic (α-amylase inhibition) Assay Analysis

Alpha-amylase is widely recognized as an essential enzyme involved in digestion, predominantly present in both saliva and pancreatic juice. This enzyme plays a significant role in the breakdown of polysaccharides. One potential strategy to avoid elevated postprandial blood glucose levels is to target and inhibit this enzyme. (24). The inhibitory effects of acarbose and *Hydroclathrus sp.* on alpha-amylase activity are shown in Table. The inhibitory potential of acarbose was 11.064±2.59%, and the inhibitory potential of *Hydroclathrus sp.* was 8.511±3.52, which is close to the positive control acarbose (**Table 3**).

Table 3. Comparison of Alpha Amylase % inhibition between Acarbose (positive control) and *Hydroclathrus sp.*

Groups	**Average (% Inhibition)**
Acarbose	11.064±2.59
Hydroclathrus sp.	8.511±3.52

In vivo Antidiabetic Activity Analysis

Effect of Hydroclathrus sp. on Diabetic Mice after Acute Treatment

The current study evaluated the antihyperglycemic activity of ethanolic extract of *Hydroclathrus sp*. in alloxan-induced diabetes mice. Different doses and time intervals (0h, two h, and four h) were used to evaluate the effects. A time-dependent and dose-dependent reduction in BGL was shown in the data displayed in **Table 4**. Statistically significant differences ($p<0.05$) were discovered when comparing the results to those of Group I, which functioned as the Normal Control group. After four hours of extract administration, subjects treated with 100 mg/kg of ethanolic *Hydroclathrus sp.* indicated a 4.37% reduction in plasma glucose levels and subjects treated with 50 mg/kg showed a -13.82% reduction. Glibenclamide (0.66 mg/kg) also produced no significant decrease of -1.19 % in the BGL after four hours of drug administration. There was no stable considerable reduction in plasma glucose level after acute treatment.

A statistical significance test for comparison was done by ANOVA, followed by Turkey's test using Microsoft Excel 2013. Data are expressed as SEM; n=5.

Table 4. Effect of 50% Ethanolic Extract of *Hydroclathrus sp.* on Diabetic Mice after Acute Treatment.

Group	Treatment	BGL (mg/dl) (Mean±SEM)		
		0h	2h	4h
i.	NC: Normal control	133.92±9.12	137.52±6.38	115.92±3.76
ii.	DCTH (10 ml/kg)	558.36±16.26[a]	548.64±15.61[a]	513.36±32.92[a]
iii.	DTE (100 mg/kg)	583.92±10.08 [a]	577.8±14.89 [a]	558.36±21.93[a]
iv.	DTE (50 mg/kg)	455.76±36.86 [a]	528.12±29.8[a]	518.76±46.22[a]
v.	DTG (0.66 mg/kg): positive control	421.56±56.11[a]	441.72±64.68[a]	426.6±69.93[a]
vi.	DTEt 50% (1ml/kg): Negative control	456.48±60.20[a]	522.72±30.14[a]	441.72±57.44[a]

Notes:

- NC: Normal Control (Untreated)
- DCTH (10 ml/kg): diabetic control treated with H_2O (receiving distilled water 10 ml/kg);
- DTE (100 mg/kg): diabetic mice treated with extract (receiving 100 mg/kg ethanolic extract);
- DTE (50 mg/kg): diabetic mice treated with extract (receiving 50 mg/kg ethanolic extract);
- DTG (0.66 mg/kg): diabetic mice treated with glibenclamide (receiving 0.66 mg/kg glibenclamide with dH2O);
- DTEt 50% (1ml/kg): diabetic mice treated with 50% ethanol (receiving 1 ml/kg 50% ethanol).

Significant values at $p<0.05$ compared to the group- I

Table 5. Reduction % on Acute Test.

Group	Treatment (mg/kg)	Reduction Percentage (%)		
		0h to 2h	2h to 4h	0h to 4h
i.	NC: Normal control	-2.68	15.71	13.44
ii.	DCTH (10 ml/kg)	1.74	6.43	8.05
iii.	DTE (100 mg/kg)	1.05	3.364	4.38
iv.	DTE (50 mg/kg)	-15.88	1.77	-13.82
v.	DTG (0.66 mg/kg): positive control	-4.78	3.42	-1.19
vi.	DTEt 50% (1ml/kg): Negative control	-9.26	6.15	-2.53

Effect of Hydroclathrus sp. on Diabetic Mice after Chronic Treatment

The extract at a low dose (50 mg/kg) significantly decreased the blood glucose level (from 500.88±32.48 mg/dl to 473.4±58.58 mg/dl at seven days and to 485.88±42.69 mg/dl at 14 days) in comparison with 50% DMTEt (580.56±8.23). Treatment with a high dose of the algal extract (100 mg/kg) caused a maximum reduction in blood glucose (from 573.36±12.62 mg/dl to 460.68±26.79 mg/dl at seven days and to 479.52±22.47 mg/dl at 14 days; $P < 0.05$) compared to alloxan group.

The table shows the Reduction % on Chronic Test. There was a noticeable decrease in blood glucose levels (BGL) over seven days after taking glibenclamide, with a further reduction of 6.666% from days 7 to 14. There was an 11.95% reduction in the blood glucose level (BGL) after 14 days. The oral treatments of ethanolic extract of *Hydroclathrus sp.* at the dose of 100 and 50 mg/kg body weight showed a significant ($p<0.05$) reduction of blood glucose levels by about 16.37 % and 3.00%, respectively, on the 14th day of the experiment compared to glibenclamide (11.95%) group. The potency of the algal extract at a dosage of 100mg/kg body weight exhibited an increased impact compared to the reference drug.

The ANOVA analysis revealed a statistically significant difference between the diabetes control group and the group that received the standard medications (**Table 6**).

Table 6. Effect of 50% Ethanolic Extract of *Hydroclathrus sp*. on the Blood Glucose Level in Alloxan-Induced Diabetic Mice after Prolonged Treatment.

Group	Treatment (mg/kg)	BGL (mg/dl)		
		Day 1	Day 7	Day 14
i.	NC:Normal control	129.12±5.09	104.28±3.6	122.28±5.33
ii.	DCTH (10 ml/kg)	540.12±15.62[a]	544.92±21.05[a]	518.16±9.16[a]
iii.	DTE (100 mg/kg)	573.36±12.62[a]	460.68±26.79[a]	479.52±22.47[a]

iv.	DTE (50 mg/kg)	500.88±32.48[a]	473.4±58.58[a]	485.88±42.69[a]
v.	DTG (0.66 mg/kg): positive control	429.96±59.12[a]	405.6±63.16[ab]	378.6±38.35[ab]
vi.	DTEt 50% (1ml/kg): Negative control	517.44±10.55[a]	522±10.28[a]	580.56±8.23[a]

Notes: [a]Significant values at $p<0.05$ compared to the Group-I. [b]Significant values at $p<0.05$ compared to the Group-II

Table 7. Diabetic Reduction Percentage on Chronic Test.

Group	**Treatment (mg/kg)**	**Reduction Percentage (%)**		
		Day 1 to 7	Day 7 to 14	Day 1 to 14
i.	NC: Normal control	19.23	-17.26	5.29
ii.	DCTH (10 ml/kg)	-0.88	4.91	4.06
iii.	DTE (100 mg/kg)	19.65	-4.08	16.36
iv.	DTE (50 mg/kg)	5.48	-2.63	2.99
v.	DTG (0.66 mg/kg): positive control	5.66	6.65	11.94
vi.	DTEt 50% (1ml/kg): Negative control	-0.97	-11.11	-12.19

Effect on Body Weight of Mice

In diabetic mice, continuous reduction in body weight was observed, as shown in **Figure 2**. A lack of carbohydrates causes diabetic mice to lose weight because they break down too many fats and structure proteins for energy (26). After two weeks of treatment with extract (100 mg/kg body weight) and positive control (Glibenclamide), diabetic mice showed improved body weight and hyperglycemic status stabilization. On the other hand, the body weight of the group IV extracts (50 mg/kg body weight) was volatile. Compared to the diabetes group, the standard control group of mice had higher body weights. The diabetic mice in Group VI, who received 50% ethanol treatment, failed to regain their initial weight following 14 days of treatment.

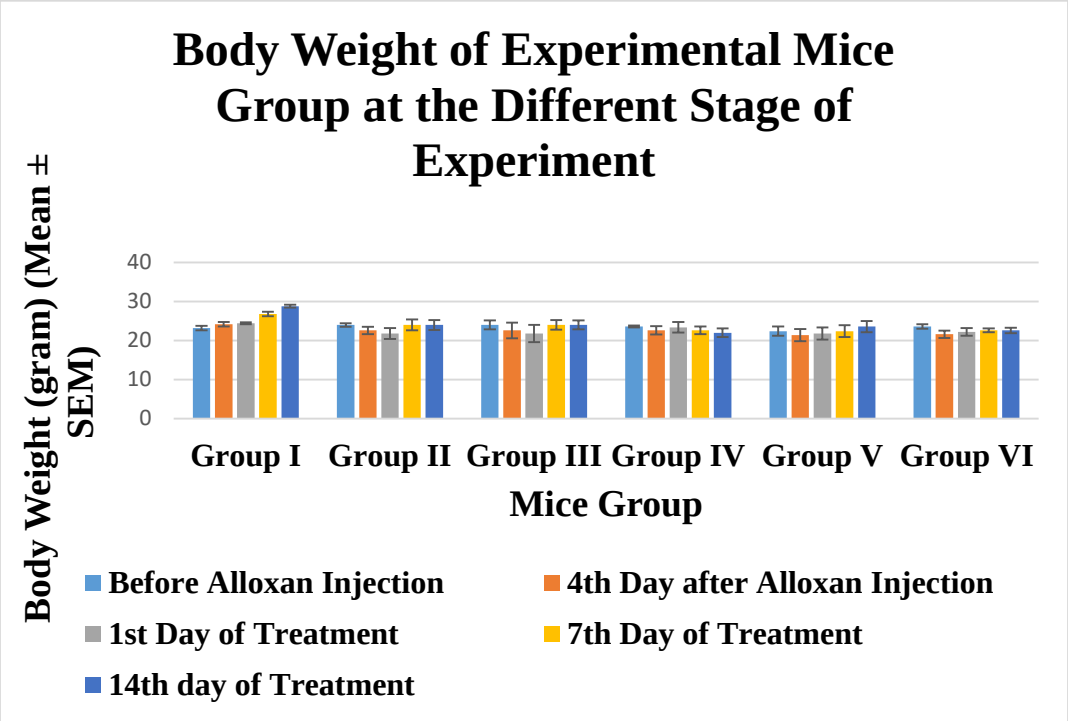

Figure 2. Body Weight of Experimental Mice Group at the Different Stages of Treatment.

Biochemical Test Analysis

Lipid Profile Test Analysis

The relationship between diabetes and lipid profile in mice has been extensively studied, revealing a complex interplay between glucose metabolism and lipid homeostasis. Diabetic mice often exhibit dyslipidemia, characterised by elevated levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol, accompanied by a decrease in high-density lipoprotein cholesterol. These alterations in lipid profile are attributed to insulin resistance and impaired insulin signalling, which lead to increased hepatic lipid synthesis and reduced lipid clearance. Additionally, hyperglycemia in diabetes contributes to oxidative stress and inflammation, further influencing lipid metabolism. Several studies support these findings. Understanding the intricate relationship between diabetes and lipid metabolism in murine models provides valuable insights into the pathophysiology of these conditions and may inform the development of targeted therapeutic interventions (14, 26). **Figure 3** shows the effect of 50% ethanolic extract of *Hydroclathrus sp.* on serum TG, Cholesterol, HDL, VLDL, and LDL. The serum Triglyceride (TG) was significantly increased ($p<0.05$) in Group II, Group IV, and Group II compared to Group I. The serum TG level remained normal upon administration of ethanolic extract of Hydroclathrus sp at a dose of 100mg/kg of body weight and glibenclamide compared to the standard control (Group I).

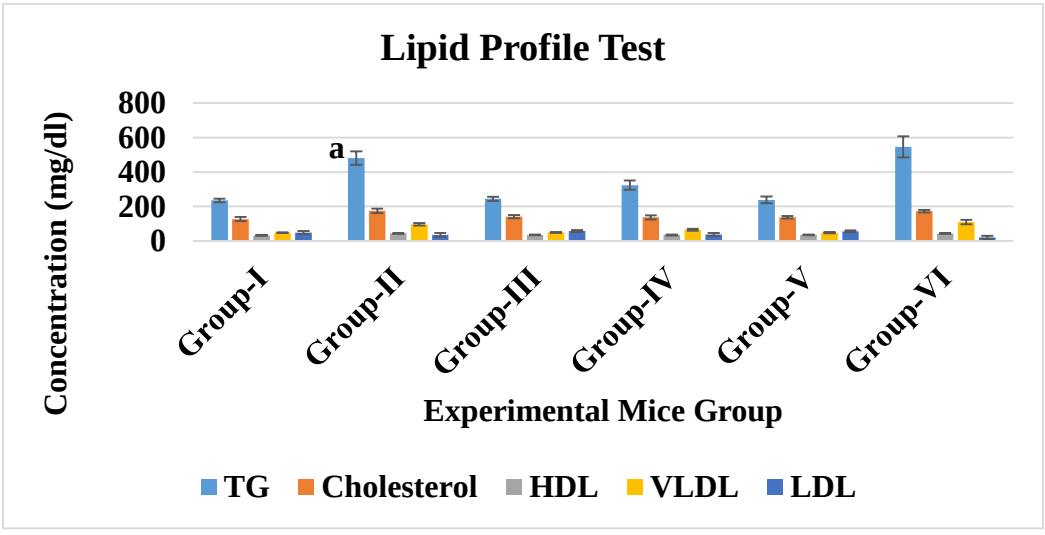

Figure 3. The impact of *Hydroclathrus sp.* extract on the lipid profile of mice with alloxan-induced diabetes. The values represent the mean ± standard deviation for five mice in each group. The bar graph indicates a statistically significant difference ($p<0.05$).

Kidney Function Analysis

Numerous studies have established a close relationship between diabetes and impaired kidney function, often assessed through creatinine levels in murine models. Diabetes-induced hyperglycemia contributes to the development of diabetic nephropathy, a common complication characterized by glomerular dysfunction and progressive renal damage. Elevated blood glucose levels initiate a cascade of events, including oxidative stress, inflammation, and activation of pro-fibrotic pathways, leading to glomerular and tubular damage. As diabetes progresses, the renal filtration barrier becomes compromised, increasing creatinine levels, a widely accepted marker of impaired kidney function. This intricate interplay has been extensively investigated in various mouse models, providing insights into the molecular mechanisms underlying diabetic nephropathy and highlighting the importance of glycemic control in preserving renal function (26). (**Figure 4**) demonstrates the creatinine levels of different experimental groups. No notable difference was found among the groups. The 50% ethanolic extract of *Hydroclathrus sp*. (100 mg/kg of body weight) and glibenclamide normalise the creatinine level as usual control group (Group-I).

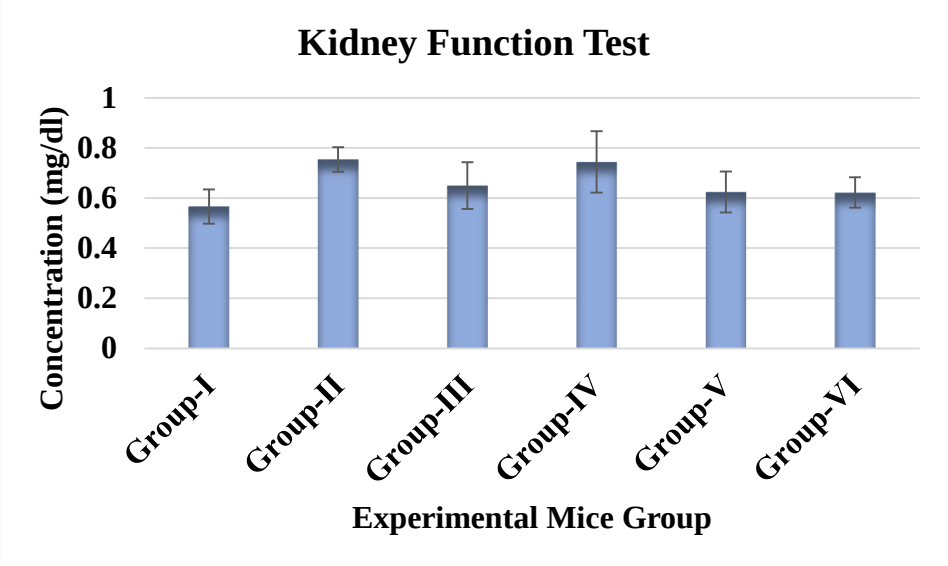

Figure 4. Effect of a 50% ethanolic extract of *Hydroclathrus sp.* on the serum creatinine level of diabetic mice induced by Alloxan. Mean ± S.D. values are used for presentation.

Liver Function Analysis

Several studies have investigated the relationship between diabetes and liver function, explicitly focusing on murine models' serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels. Diabetes has been associated with alterations in liver enzymes, including elevated SGPT and SGOT levels, indicating potential hepatic dysfunction (26). In this study, a significant elevation of SGOT level was observed in Group IV and Group VI at $p<0.05$ compared to Group I. Reduction trend of both SGPT and SGOT level was observed for both 50% ethanolic extract of *Hydroclathrus sp* (100 mg/kg of body weight) and glibenclamide **(Figure 5).**

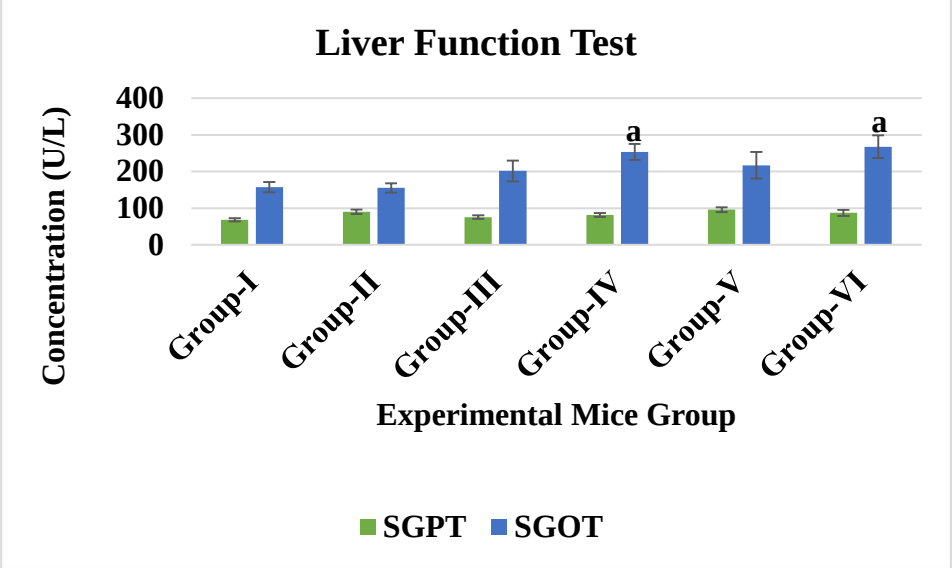

Figure 5. Impact of 50% ethanolic extract of *Hydroclathrus sp* on the SGOT and SGPT levels in mice with alloxan-induced diabetes. The values represent the mean ± standard deviation for five mice in each group. Notably different at $p<0.05$

Discussion

Exploring the potential bioactive compounds, in vitro, and in vivo anti-diabetic efficacy of brown seaweeds *Hydroclathrus sp.* collected from the Bay of Bengal presents intriguing findings that contribute to understanding the potential therapeutic applications of seaweeds in managing diabetes. The phytochemical analysis of brown seaweed *Hydrclathrus sp*. has identified a range of bioactive compounds, including Flavonoids, Steroids, Phenolic compounds, Tannin, Carbohydrates, Amino Acids, Glycosides, Terpenoid, and Quinone (**Table 1**). Phytoconstituents play a crucial role in diabetes management; examples include alkaloids, flavonoids, glycosides, phenols, and many more (28). These compounds are particularly interesting due to their potential relevance in antidiabetic interventions. The presence of flavonoid and phenol in the brown seaweed suggests a natural source for antidiabetic agents. (15). The presence of the chemicals was determined by the investigation using the UV-visible spectroscopy approach.

The in vitro findings suggest that the seaweed may influence key cellular pathways relevant to diabetes management. The inhibitory effects of *Hydroclathrus sp*. on alpha-amylase activity are shown in Table. Starch blockers are another name for amylase inhibitors, which contain chemicals the body can't absorb from food. Amylase and other secondary enzymes in the digestive tract break down starch and other complex carbs before they can be absorbed (26).

Alloxan is an unstable organic compound widely used in studies of experimental diabetes. It is well known to destroy the beta cells of the pancreas and cause hyperglycemia in mice. (29) And it induces necrosis, making it especially harmful to pancreatic beta-cells of the Langerhans islets (26). The result of the study indicated the potential antihyperglycemic effect of the extract. The in vivo studies support the antidiabetic potential of *Hydrclathrus sp.* The in vivo studies involving animal models demonstrated a significant reduction in blood glucose levels following the administration of *Hydroclathrus sp.* extracts. This indicates the potential of these seaweeds to alleviate hyperglycemia, a key characteristic of diabetes. The effects observed were [mention the extent and significance of the impact], supporting the hypothesis that *Hydroclathrus sp.* may be a valuable natural resource for anti-diabetic interventions. The plasma glucose level did not significantly decrease and persistently decreased after the acute therapy. The algal extract showed a higher potency at 100mg/kg body weight than the standard medicine.

It was found that diabetic mice constantly lost body weight throughout the study. Compared to their original body weights, the mice in the normal control group, which did not have diabetes, demonstrated a rise in their body weights. Weight loss or gain in alloxan-induced diabetic mice can be a crucial parameter in evaluating overall health and response to interventions, providing insights into the effectiveness of potential treatments for diabetes in a laboratory setting.

The blood serum triglyceride (TG) levels, Cholesterol, HDL, VLDL, and LDL remained within the normal range following the administration of an ethanolic extract of *Hydroclathrus sp.* at a dosage of 100mg/kg of body weight, as well as glibenclamide, in comparison to the standard control group (group-I). However, the serum triglyceride levels were considerably higher ($p<0.05$) in Groups II, IV, and VI

compared to Group I. These results provide evidence that *Hydroclathrus sp.* extract may help reduce the risk of diabetes-related complications involving lipid metabolism. No significant changes in kidney function tests were seen in any of the groups compared to the Normal control group. However, a modest increase was noted in group II and group IV. However, the administration of a 50% ethanolic extract derived from *Hydroclathrus sp.* at a dosage of 100 mg/kg of body weight demonstrated a comparable impact to glibenclamide in normalising creatinine levels, as observed in the control group of mice (group-I). The liver function test revealed that the levels

of SGOT were significantly elevated in Group iv and Group vi compared to Group I. However, when administered at 100 mg/kg of body weight, *Hydroclathrus sp.* exhibited a more pronounced effect in normalising SGOT levels towards those observed in the standard control group, particularly about glibenclamide. It can be argued that ethanol exhibits significant hepatotoxicity.

Diabetes is treated with insulin, oral medicines, and diet and exercise. These medications can lower blood sugar, but they have drawbacks. (11–13). Regular insulin injections can induce hypoglycemia, weight gain, and other issues. Nausea, diarrhoea, and liver damage can result from oral medicines. (14). Lifestyle adjustments can be difficult and insufficient for some advanced diabetics. These restrictions and adverse effects demonstrate the need for novel diabetic medications. Seaweeds may replace conventional diabetes treatments due to their antidiabetic effects. Hydroclathrus sp. exhibits a distinct phytochemical composition compared to other seaweeds studied for their anti-diabetic effects. The presence of phenolic compounds and flavonoids distinguishes *Hydroclathrus sp.* as a unique source with the potential for novel therapeutic applications. Understanding these unique attributes could contribute to developing targeted and effective anti-diabetic interventions. One of the significant advantages of using natural products like these extracts is that they are often less toxic and have fewer side effects than synthetic drugs. Additionally, natural products tend to be more readily available and affordable, which could make them an attractive option for treating diabetes in low-income populations.

To build upon this study, future research could explore specific compounds from Hydroclathrus sp. to treat diabetes. Additionally, clinical trials to validate the anti-diabetic efficacy in human subjects and further mechanistic studies to elucidate the underlying pathways are imperative for translational potential. The comprehensive analysis of *Hydroclathrus sp.* seaweeds from the Bay of Bengal has revealed promising anti-diabetic properties both in vitro and in vivo. The unique phytochemical composition of *Hydroclathrus sp.* and its consistent effects on diabetes-related parameters underscore its potential as a natural therapeutic agent. This study contributes to the growing knowledge on seaweeds as a source of anti-diabetic compounds. It lays the groundwork for further exploration and development of *Hydroclathrus sp.* in diabetes research and management.

Conclusion

In conclusion, this study has successfully investigated the bioactive compounds within *Hydroclathrus sp.*, a brown seaweed from the Bay of Bengal, Bangladesh, evaluating its pharmacological properties, particularly in antidiabetic effects. The systematic collection, identification, and characterization of bioactive compounds and rigorous pharmacological assessments have revealed promising antidiabetic potential. The in vitro and in vivo studies provided a comprehensive understanding of the efficacy and safety of *Hydroclathrus sp.* compounds. Sustainable harvesting practices, economic considerations, and integration into pharmaceutical and nutraceutical applications were also addressed, emphasizing the potential for responsible exploitation of this marine resource. This research advances our knowledge of *Hydroclathrus sp.* It underscores its significant role in developing novel therapeutic interventions, offering a promising avenue for future studies in marine-based pharmacology and sustainable healthcare solutions. Comprehensive analysis of *Hydroclathrus sp.* seaweeds from the Bay of Bengal has revealed promising anti-diabetic properties both in vitro and in vivo. The properties of brown seaweed *Hydroclathrus sp.* make it a promising candidate for future research in this area. Its abundance in coastal regions makes it an accessible source of antidiabetic compounds for future drug development.

Ethics approval

Animal studies were approved by the Institutional Research Ethics Board of Bangabandhu Sheikh Mujibur Rahman Maritime University (BSMRMU)

Funding

This work was supported by the University Grants Commission (UGC) research grant, Bangabandhu Sheikh Mujibur Rahman Maritime University (BSMRMU), Dhaka, Bangladesh (2021-2022-2023).

Acknowledgments

The authors thank Marine Biotechnology Laboratory, Bangabandhub Sheikh Mujibur Rahman Maritime University, Bangladesh, for providing all the technical and laboratory support needed to conduct this research.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

MNH made the hypothesis, designed the experiments, supervised the work, analyzed the data, and wrote and revised the final version of the manuscript; SA conducted the experiments, analyzed the data, and wrote the initial draft of the manuscript; MA and MTI carried out some experiments, and SS carried out the experiment and did the manuscript editing.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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