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Research Article

Evaluation of antidiabetic, hypolipidemic and antioxidant activity of seaweed *Halimeda macroloba* in Wistar albino rats

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The seaweed *Halimeda macroloba* Decaisne, 1841 has been used in the pharmaceutical and nutraceutical industries, but no research has described its in-vivo activity. Therefore, the current study is done to explore the antidiabetic, hypolipidemic, and antioxidant properties of the hydroalcoholic extract of *H. macroloba* (HME) in Wistar rats. HME was prepared using 70 % alcohol as the solvent by maceration for 72 h. Nicotinamide (i.p., 110 mg/kg) and streptozotocin (i.p., 45 mg/kg) were administered to induce diabetes, and the diabetes status in the rats was assessed 72 h after induction. Following induction, the rats were randomly divided into seven groups and treated with two doses of HME and conventional medications. Weekly monitoring of body weight and fasting blood glucose, with monthly lipid profiles, was measured, and on the 90th day, antioxidant parameters and HbA1c were measured. Histopathological analysis was performed on the renal, liver, and pancreatic tissues. Significant improvements in body weight and fasting blood glucose, and a reduction in HbA1c, have been observed following treatment with HME (100 mg/kg and 200 mg/kg). Similarly, HME substantially raised HDL levels while lowering LDL, TC, and TG. Comparing the HME groups to the untreated diabetic rats, there was a significant increase in the antioxidant levels of GSH, CAT, and SOD. However, compared to diabetic rats, HME groups had lower levels of MDA. Comparably, HME reversed the histological alterations and restored the liver, kidney, and pancreatic functions. The overall results show that administering HME improved BW, HbA1c, and glycemic management. Additionally, the lipid profiles and antioxidant activities have been considerably enhanced.

[**Keywords**: Antidiabetic, Green macroalgae, *Halimeda macroloba*, Hypolipidemic, Seaweed, Wistar rats]

Introduction

Diabetes is a chronic metabolic condition accompanied by elevated blood glucose levels, according to the World Health Organisation (WHO)[1]. Moreover, Diabetes Mellitus (DM), which further manifests as hyperglycemia, can harm several systems and result in organ failure or malfunction, including, if untreated, the heart, blood vessels, eyes, kidneys, and nerves[2]. Type 2 diabetes, the most prevalent type of health condition, mainly affects adults and is caused by the body that generates insufficient or resistant amounts of insulin. It was once primarily associated with adults, but it is increasingly affecting young ones as well. Type 2 diabetes has been considerably more prevalent over the last three decades in all nations, regardless of wealth. Approximately 422 million individuals globally suffer from diabetes, and the disease is directly responsible for 1.5 million fatalities annually. Over the past few decades, there has been a steady rise in both the number of cases and the incidence of diabetes[1]. According to the 2021 release by the IDF Diabetes Organisation, about 537 million adults worldwide have diabetes. This figure is anticipated to increase to 643 million by 2030 and 783 million by 2045. China now has the greatest prevalence of diabetes among adults aged 20 to 79, followed by India and Pakistan[3]. As of now, a precise drug or total cure for diabetes has not been found[4].

Multiple studies revealed that decreased levels of endogenous antioxidants and increased free radical production led to the death of high lipid peroxidation,

pancreatic β-cells, oxidative stress, and cellular organelle impairment. These events ultimately cause secondary complications such as neuropathies, nephropathies, diabetes-related retinopathies, and cardiovascular diseases[5]. Since diabetes is thought of as a chronic metabolic condition, many conventional antidiabetic medications do not include a single dose because most of the drugs require periodic injections, sometimes for the individual's lifespan. However, a number of these conventional medicinal products are ineffective and have significant adverse consequences[6]. Owing to these drawbacks, research into management techniques utilising medicinal plants which are reportedly less expensive antidiabetic medicines with less documented adverse effects, has been intensified[7]. Extensive research on seaweeds has identified phytochemical compounds that contribute to the regulation and management of diabetes. Incorporating edible seaweeds into the diet has been associated with a reduced risk of developing this condition. Many polyphenolic chemicals widely known for their ability to prevent diabetes have been identified from seaweeds[8]. Among such seaweeds, the green macroalgae *Halimeda macroloba (H. macroloba)* Decaisne, 1841, member of the Chlorophyta phylum, has *in vitro* α – glucosidase and cytotoxic properties[9], antioxidant activity[10], as well as antibacterial activity[11]. However, adequate investigations have not been undertaken to utilise its pharmacological activities and potential mechanism of action. Based on the *in vitro* information already available and prior knowledge, this study aimed to scientifically validate the antidiabetic, hypolipidemic, and antioxidant activities of the seaweed *Halimeda macroloba* in Wistar albino rats.

Materials and Methods

Ethical clearance

Following approval from the Institutional Animal Ethics Committee (IAEC), MGMCRI, SBV University, Puducherry, the study was initiated (CPCSEA regd. No.: 686/GO/Re/S/02/ CPCSEA (2022-2027). Moreover, proposition no. 08/IAEC/ MG/04/2023-I was accepted.

Plant material collection and identification

In November of 2022, the seaweed *H. macroloba* was gathered from the shores of Vedali, Mandapam, Rameswaram, India. With the help of a marine biologist, seaweeds were verified as generated by CAS Marine Biology in Parangipettai, Tamil Nadu, India, and also received a voucher herbarium number, CAS MBMBTL02.

Chemicals and equipment

Streptozotocin and Nicotinamide were procured from Sigma Aldrich Chemicals, Mumbai. Metformin, Acarbose, Atorvastatin, and 70 % alcohol were procured from the MGMCRI pharmacy. A One Touch glucometer was used to estimate blood glucose. All other reagents and chemicals used were of analytical grade.

Hydroalcoholic extract preparation

After being cleaned with tap water to remove any dirt, the gathered seaweed was left to dry in the shade for 6 – 7 weeks at room temperature (24±2 °C). After being completely dried and ground into a coarse powder using a mixer grinder and sieve, the dried seaweed was used for further investigation. After that, it was stored in airtight containers. Using 70 % alcohol as a solvent, the extracts were prepared by macerating for 72 h[(ref. 12)]. The crude extract solution was refined using Whatman No. 42 filter paper. Following lyophilisation, the yield was concentrated to produce an *H. macroloba hydroalcoholic extract* (HME), which was then stored at 20 °C in a sealed container for further analysis. Rats were given the extract orally after it had been dissolved in distilled water.

Animals

Animals consisting of adult male Wistar rats (n = 42), weighing between 150 to 200 g, were procured from Biogen Laboratory Animal Facility, Bangalore (a breeding facility authorised by CCSEA) and kept at the animal house of MGMCRI. Prior to testing, the animals were acclimated for at least 1 week. The animals were kept in clean polypropylene cages under standard laboratory conditions (12/12 light/dark cycle, a temperature of 25±2 °C, and a relative humidity of 65 %). Clean paddy husk is used to make the bedding. The animals were fed with a standard pellet diet, and drinking water was given *ad libitum*. The study animals were handled in accordance with the CCSEA and OECD chemical testing guidelines.

Induction of type 2 diabetes mellitus

In overnight fasted rats, Nicotinamide (NA) was injected intraperitoneally at a dose of 110 mg/kg Body Weight (BW) 15 min prior to the injection of streptozotocin. Following that, 45 mg/kg BW of streptozotocin (STZ) in 0.1 M cold sodium citrate

buffer was given. Blood glucose levels were assessed 72 h after the STZ-NA injection, and rats exhibiting blood glucose levels greater than 250 mg/dl were considered diabetic and utilised for experimentation[13].

Experimental design

The animals were randomly assigned to seven groups, each containing six rats (a total of 42 rats), and given the following treatments: (i) normal saline; (ii) diabetic control; iii) HME at 100 mg/kg body weight (BW); (iv) HME at 200 mg/kg BW; (v) Metformin at 100 mg/kg BW; (vi) Acarbose at 12.5 mg/kg BW; and (vii) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin. Using an oral gavage needle, the extract and medication were reconstituted in distilled water and administered orally every day for 90 days. The extract dosages were chosen based on the pilot research (1/10th and 1/20th of the acute toxicity study of seaweed *H. macroloba* were utilised), and the literature[14]. The rats body weight was measured weekly using an electronic weighing scale for all the animals.

Analysis of blood glucose level and serum lipid profiles

The rats were subjected to a 12-hr fasting period, after which their blood glucose levels and body weight were measured weekly using a one-touch glucometer by collecting blood from the rat's tail vein. The glucometer was validated before use. On the 1st, 30th, 60th, and 90th days, blood was collected from the retro-orbital plexus of the rats under mild anaesthesia using capillary tubes containing heparin to estimate lipid levels. The serum was separated by centrifugation (5 min at 5000 rpm). On the 90th day, whole blood was used to determine the HbA1c levels using the biochemistry autoanalyser method[15,16].

Tissue homogenate assays for enzymatic and non-enzymatic antioxidant characteristics

An aliquot of the liver homogenate was prepared with 20 % TCA (200 µL) and centrifuged at 2000 rpm for 5 min at 4 °C. Subsequently, 50 µL of the resulting supernatant was mixed with 5 mM dinitrothiocyanobenzene (DNTB), along with 100 µL and 50 µL of 1 M phosphate buffer. A modified colourimetric procedure was used to measure reduced glutathione (GSH) levels[17]. According to Shagirtha *et al*.'s instructions, the LPO assay was performed by quantifying TBARSs[18]. The tissue (liver) homogenate's Superoxide Dismutase (SOD) activity was measured using a methodology defined by Kakkar *et al*.[19], and catalase (CAT) activity was measured using a method reported by Sinha[20].

Histopathological analysis

On the 90th day, rats were euthanised using a carbon dioxide chamber, and rat liver, kidneys, and pancreas were collected and fixed in a 10 % formaldehyde solution. Hematoxylin and Eosin (H&E) staining was applied, and the slides were fixed in paraffin. Changes in pathophysiology were seen using a light microscope with a 40x objective and 400x overall magnification[21].

Statistical analysis

All the data were analysed using the SPSS software version 26.0. A one-way Analysis of Variance (ANOVA) and a post hoc Bonferroni's test were used. The mean ± Standard Deviation (SD) is used to express all experimental data. There was acceptance of significant variations at $*p < 0.05$, $**p < 0.001$, $^{\#}p$-value < 0.05 compared to the metformin group, and $^{\$}p$-value < 0.05 compared to the acarbose group.

Results

Effect of the HME (Hydroalcoholic extract of *H. macroloba*) on fasting blood glucose levels and Body Weight (BW) (in grams) in rats with STZ-NA-induced diabetes

Table 1 displays the impact of the HME on the Fasting Blood Glucose levels (FBG) of rats with diabetes caused by STZ. In contrast to the normal glycaemic rat controls, the current study showed that experimental rats' FBG (Table 1) and BW (Table 2) significantly increased and decreased when administered 45 mg/kg BW of STZ and 110 mg/kg Nicotinamide. Furthermore, compared to diabetic rats that were left untreated, FBG significantly decreased after receiving treatments with the HME and conventional medications. Furthermore, rats treated with the extract showed a considerable improvement in BW compared to diabetic rats that were not treated. This shows that HME has better glycaemic control.

Effect of HME on HbA1c in rats with STZ-NA-induced diabetes

Following the induction of diabetes with STZ-NA, there was a significant increase in HbA1c levels in diabetic control rats compared to normal control and standard control rats. A statistically significant ($p < 0.001$) decrease in HbA1c levels were observed with both HME doses and the standard drugs. Similarly, the HME 100 mg/kg BW group showed a significant ($p < 0.05$) decrease compared to both the metformin and acarbose groups, as depicted in

Table 1 — Effect of the HME (Hydroalcoholic extract of *H. macroloba*) on Fasting Blood Glucose (FBG) levels (mg/dl) in rats with STZ – NA induced diabetes

	Normal control	Diabetic control	HME 100 mg	HME 200 mg	Metformin	Acarbose	Atorvastatin+ metformin
Day 0	98.33±6.861	95.33 ±2.422	95.67±3.266	93.83±4.355	90.33±2.160	94.00±5.621	95.17±7.782
Day 3	98.33±7.421	329.83±71.848	320.17±60.234	369.83±80.517	352.00±70.163	347.17±64.648	363.83±74.160
Day 7	97.67±7.174	332.67±72.896	297.17±53.011	347.83±80.817	336.17±71.317	328.50±60.089	342.33±74.484
Day 14	97.00±6.986	336.50±73.913	279.83±51.759	326.33±77.871	322.50±69.209	310.50±57.249	325.00±74.897
Day 21	96.33±7.501	339.83±74.327	267.00±54.490	304.67±75.841	291.00±63.122	293.83±58.297	308.00±79.236
Day 28	96.17±7.731	342.00±76.811	253.00±52.907	282.33±68.269	293.17±65.786	276.50±60.998	290.17±79.215
Day 35	96.00±7.563	344.83±78.111	240.33±51.263	265.67±68.986	261.00±57.002	258.83±58.714	275.50±78.584
Day 42	96.33±7.992	347.33±79.867	226.00±46.277*	249.50±63.677	260.50±63.789	243.17±57.610	258.00±73.569
Day 49	96.50 ±8.216	350.00±80.085	216.67±45.085*	235.67±63.761*	247.00±59.029	224.17±59.267*	247.17±74.405
Day 56	97.17±7.111	351.50±79.515	200.67±39.682*	218.17±63.521*	233.50±57.667*	208.83±53.708 *	232.00±72.878
Day 63	97.83±6.555	354.00±79.388	185.50±38.135**	200.17±61.121*	219.50±57.075*	189.17±50.487 **	216.00±71.395
Day 70	97.17±6.940	356.00±78.509	174.17±34.822**	187.17±60.204**	202.17±55.217**	173.00±48.658**	199.67±69.793
Day 77	97.33±6.743	356.17±76.311	159.67±29.330**	171.33±56.020**	189.50±49.995**	158.83±44.170**	183.00±64.894
Day 84	97.50±7.176	357.17±75.632	144.00±23.715**	162.17±54.271**	173.83±45.963**	144.50±41.230**	167.67±0.649
Day 90	98.00±6.841	358.83±74.540	134.67±22.097**	152.50±49.314**	159.67±44.451**	135.67±38.124**	156.83±58.215

Results are expressed as mean±SD (n = 6). *p-value < 0.05 as compared to diabetic control, **p-value < 0.001 as compared to the diabetic control

Table 2 — Descriptive values regarding the effect of the HME (Hydroalcoholic extract of *H. macroloba*) on Body Weight (BW) (in grams) in rats with STZ-NA-induced diabetes

	Normal control	Diabetic control	HME 100 mg	HME 200 mg	Metformin	Acarbose	Atorvastatin+ metformin
Day 1	248.33±5.16	248.3±2.58	247.83±2.78	248.33±2.58	248.60±2.43	249.41±3.58	248.63± 2.45
Day 7	248.46±5.33	246.50±2.42	249.05±2.40	249.65±2.65	248.88± 2.53	249.78±3.70	249.11 ±2.61
Day 14	249.10±5.41	245.81±3.09	250.05±2.30	249.89±2.91	249.46±2.40	250.31 ±3.57	249.65 ±2.50
Day 21	249.85±5.49	244.61±3.16	250.70±2.26	251.01±3.02	249.85±2.49	250.80±3.70	250.05 ±2.57
Day 28	250.38±5.80	242.83±3.08	251.83±1.92	251.71±2.96	250.38±2.49	251.20±3.62	250.56 ±2.48
Day 35	251.15±5.59	241.48±3.41	252.71±2.23	252.50±3.02	250.80±2.62	251.55±3.64	250.98 ±2.58
Day 42	251.76±5.87	239.90± 3.33	253.76± 2.08	253.40±3.19	251.13±2.76	251.85±3.72	251.26 ±2.59
Day 49	252.71±5.89	237.83±3.68	254.58±2.23	254.08±3.42	251.58±2.69	252.25±3.67	251.58 ±2.52
Day 56	251.81±7.43	236.06±3.45	255.48± 1.98	254.76±3.29	251.93±2.72	252.65±3.75	251.96 ±2.52
Day 63	254.53±5.50	234.85±3.58	256.20±1.83	255.60± 3.22	252.45±2.96	253.01±3.88	252.45 ±2.64
Day 70	255.40±5.32	233.21±3.35	256.86± 2.03	256.18±3.07	252.90±3.00	253.40±3.91	252.80 ±2.68
Day 77	256.40±4.76	231.10±2.86	257.58± 2.01	257.06±2.70	253.31±3.01	253.85±3.95	253.13 ±2.69
Day 84	257.36±4.35	229.76±2.67	258.26± 2.00	257.76±2.58	253.65±2.91	254.38±3.92	253.46 ±2.77
Day 90	258.23±4.02	228.71±2.71	258.78± 2.24	258.56±2.60	254.03±2.93	254.76±3.98	252.98 ±2.70

Results are expressed as mean±SD (n = 6)

Table 3. The decrease in fasting blood glucose levels and HbA1c, and the increase in body weight in rats treated with different doses of HME reveal that *H. macroloba* possesses a significant antidiabetic effect.

Results are expressed as mean ± SD (n = 6). **p-value < 0.001 as compared to the diabetic control, $^{\#}p$-value < 0.05 as compared to metformin group, $^{\$}p$-value < 0.05 as compared to acarbose group

Table 3 — Effect of the HME (Hydroalcoholic extract of *H. macroloba*) on HbA1c% in rats with STZ-NA-induced diabetes

Groups	HbA1C % on day 90
Normal control	4.91±0.62 %
Diabetic control	6.66±0.22 %
HME 100 mg	4.72±0.18 % ** $^{\#\$}$
HME 200 mg	4.03±0.15 % **
Metformin	4.01±0.17 % **
Acarbose	4.13±0.25 % **
Atorvastatin + metformin	4.06±0.18 %

Effect of HME on lipid profiles in rats with STZ-NA-induced diabetes

When comparing untreated diabetic rats to normal control rats, the untreated diabetic induced rat showed a substantial drop in HDL cholesterol and a significant increase in LDL cholesterol, total cholesterol, and serum triglycerides (TGs) (Table 4). Compared to untreated diabetic controls, treatment with HME (100 and 200 mg/kg BW), metformin alone, atorvastatin combined with metformin, and acarbose significantly (p < 0.001) lowered Total Cholesterol (TC), triglycerides (TGs), and LDL

Table 4 — Effect of HME (Hydroalcoholic extract of *H. macroloba*) on lipid profiles (HDL, LDL, TC and TG) in STZ-NA-induced diabetes

	HDL (mg/dl)				LDL (mg/dl)				TC (mg/dl)				TG (mg/dl)			
Groups	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90
Normal control	47.19 ±0.83	47.34 ±0.81	47.50 ±0.84	47.68 ±0.86	47.30 ±0.76	47.37 ±0.77	47.65 ±0.92	47.95 ±1.22	116.56 ±2.85	117.54 ±2.41	116.83 ±2.16	115.88 ±1.74	78.35 ±4.08	86.23 ±21.40	91.15 ±33.09	95.93 ±44.31
Diabetic control	47.68 ±0.63	34.75 ±1.83	28.88 ±0.95	20.42 ±0.88	47.84 ±0.67	68.18 ±2.42	81.97 ±1.83	99.01 ±1.14	116.04 ±2.95	154.83 ±4.03	198.74 ±1.69	256.68 ±3.16	77.25 ±5.45	128.73 ±6.19	157.54 ±3.03	186.21 ±2.30
HME 100 mg	47.89 ±0.64	46.74 ±0.95**	47.13 ±1.02**	47.44 ±1.00**	47.72 ±0.85	47.78 ±0.87**	47.98 ±0.91**	48.16 ±0.97**	116.60 ±3.77	137.68 ±1.62** #	133.84 ±1.98** #	131.07 ±1.33** #	79.92 ±5.99	108.82 ±2.79 *	97.13 ±2.67 **	88.30 ±2.97 **
HME 200 mg	47.22 ±0.83	47.28 ±1.49**	48.11 ±1.66**#	48.52 ±1.76**#	47.86 ±1.14	47.00 ±0.79**	46.50 ±0.59**	45.93 ±0.68**	114.81 ±2.88	125.35 ±0.98**	121.82 ± 1.20**	118.88 ±1.95**	78.41 ±5.35	105.98 ±3.41 *	94.06 ±1.68**	83.28 ±1.95**
Metformin	47.55 ±0.65	45.11 ±1.15	45.50 ±0.99	45.84 ±0.93	47.43 ±0.69	47.03 ±0.73	46.77 ±0.79	46.33 ±0.61	114.46 ±3.11	127.65 ±1.49	124.83 ±1.42	121.99 ±0.92	77.41 ±5.89	108.20 ±3.66	97.42 ±2.35	87.25 ±3.23
Acarbose	48.17 ±0.71	44.92 ±0.62	45.26 ±0.61	45.48 ±0.72	47.19 ±0.83	46.84 ±0.85	46.25 ±0.70	45.63 ±0.53	113.89 ±2.21	125.31 ±1.54	122.20 ±1.65	120.25 ±2.12	79.17 ±5.41	105.71 ±2.81	95.41 ±2.11	86.62 ±3.56
Atorvastatin+ metformin	47.43 ±0.69	45.10 ±0.66	45.33 ±0.63	45.55 ±0.62	47.77 ±1.04	47.43 ±1.13**	47.14 ±1.05**	46.71 ±1.04**	112.61 ±1.24	124.75 ±1.31**	123.50 ±0.67**	121.74 ±1.05**	79.35 ±7.26	106.01 ±2.80 *	95.63 ±2.66 **	84.20 ±2.95**

Results are expressed as mean ± SD (n = 6). **p-value < 0.001 as compared to the diabetic control, #p-value < 0.05 as compared to the atorvastatin + metformin group

Table 5 — Oxidative stress biomarkers in the liver of diabetic rats treated with the HME on day 90

Groups	SOD (U/ml)	Catalase (U/ml)	Reduced Glutathione (GSH) (µg/ml)	TBARS (µg/ml)
Normal control	0.28±0.003	0.22±0.023	0.11±0.002	0.08±0.001
Diabetic control	0.23±0.015	0.18±0.010	0.93±0.039	0.16±0.009
HME 100 mg	0.31±0.003**	0.22±0.005**	0.13±0.003**	0.06±0.003**
HME 200 mg	0.32±0.001**	0.27±0.005**	0.16±0.002**	0.07±0.005**
Metformin	0.28±0.001	0.21±0.002	0.11±0.001	0.08±0.001
Acarbose	0.28±0.003	0.21±0.003	0.11±0.003	0.08±0.004
Atorvastatin+ metformin	0.28±0.003	0.21±0.002	0.11±0.002	0.08±0.002

Results are expressed as mean ± SD (n = 6). **p-value < 0.001 as compared to the diabetic control

cholesterol, while increasing HDL cholesterol. Notably, the HME 200 mg/kg group showed a greater improvement in HDL cholesterol levels ($p < 0.05$) compared to the atorvastatin-metformin combination group. Similarly, the HME 100 mg/kg group demonstrated a more pronounced reduction in TC than the atorvastatin-metformin group, highlighting the potent hypolipidemic activity of HME.

Effect of antioxidant levels in rats with STZ-NA-induced diabetes

It has been found that the HME demonstrated dose-dependent antioxidant activity by markedly increasing the levels of the non-enzymatic antioxidant GSH and the activities of the liver enzymatic antioxidants, such as CAT and SOD. In a diabetic control group, both doses of HME showed antioxidant activity, with higher activity at HME 200 mg/kg BW. In contrast, rats treated with the HME exhibited significantly ($p < 0.001$) reduced levels of MDA, the stable by-product of lipid peroxidation, compared to untreated diabetic rats. In summary, these findings showed that the HME demonstrated notable antioxidant effects *in vivo* in the liver of STZ-NA-induced diabetic rats, indicating that the HME could aid the liver if employed as a hypoglycemic drug to treat diabetes, as shown in Table 5.

Effect of HME on liver histology

A normal hepatic architecture with portal triad, central vein, and hepatocytes was seen in photomicrographs of liver tissue sections taken from normal control rats. Followed by the administration of STZ-NA intraperitoneally, the untreated diabetic control rats experienced significant severe Periportal Inflammation (PI), severe degeneration, and congested vessels with severe Intrahepatic Inflammation (CI) and steatosis. As expected, the 100 mg HME group rats showed healed liver sinusoids with normal hepatocytes and mild congestion, while the 200 mg HME group showed normal liver sinusoids and hepatocytes with less steatosis. Other groups displayed normal hepatocytes, while the metformin group displayed slight hepatic congestion and inflammation, as shown in Figure 1.

Effect of HME on kidney histology

A typical renal architecture, including glomeruli, Bowman's space, renal tubules, and tubular cells was visible in a photomicrograph of kidney tissue sections from normal control rats. After administering STZ-NA, the untreated diabetic rats showed nodular glomerular sclerosis, significant intrarenal bleeding, and severe intrarenal inflammation. In the results obtained for untreated diabetic control rats, it is noteworthy that oral administration of the HME 100 mg/kg BW resulted in mild renal globular and tubular regeneration with mild thickened globular basement membrane, and the 200 mg/kg BW group rats also showed normal glomeruli with lobular regeneration and typical tubular epithelial

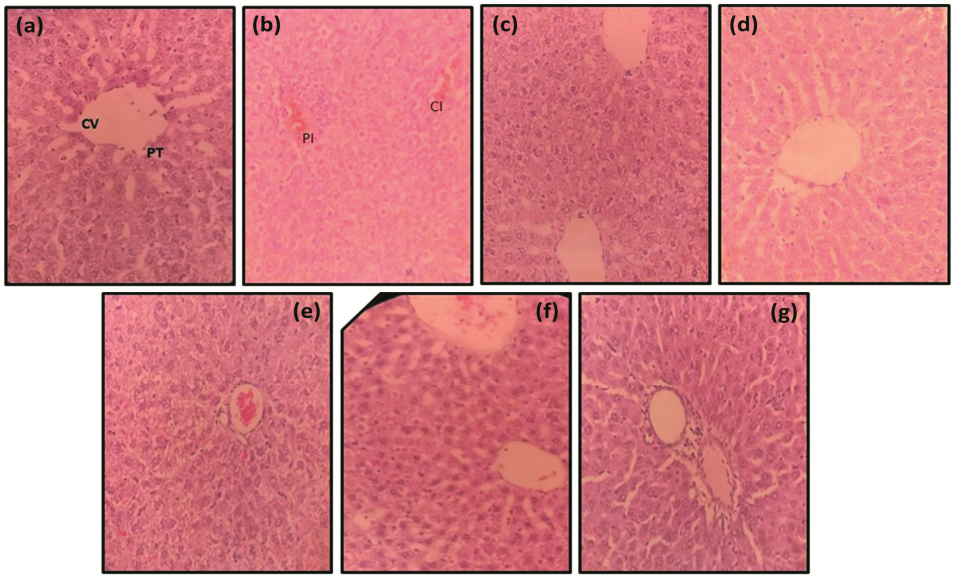

Fig. 1 — Light photomicrographs of Hematoxylin and Eosin (H&E) sections of the liver (40x magnification): a) Normal control, b) Diabetic control, c) 100 mg/kg body weight (BW) of *Halimeda macroloba* extract (HME), d) 200 mg/kg BW of the HME, e) 100 mg/kg BW Metformin, f) 12.5 mg/kg BW Acarbose, and g) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin. PT: Portal Triad, CV: Central Vein, PI: Periportal Inflammation, and CI: Congested vessel with Inflammation

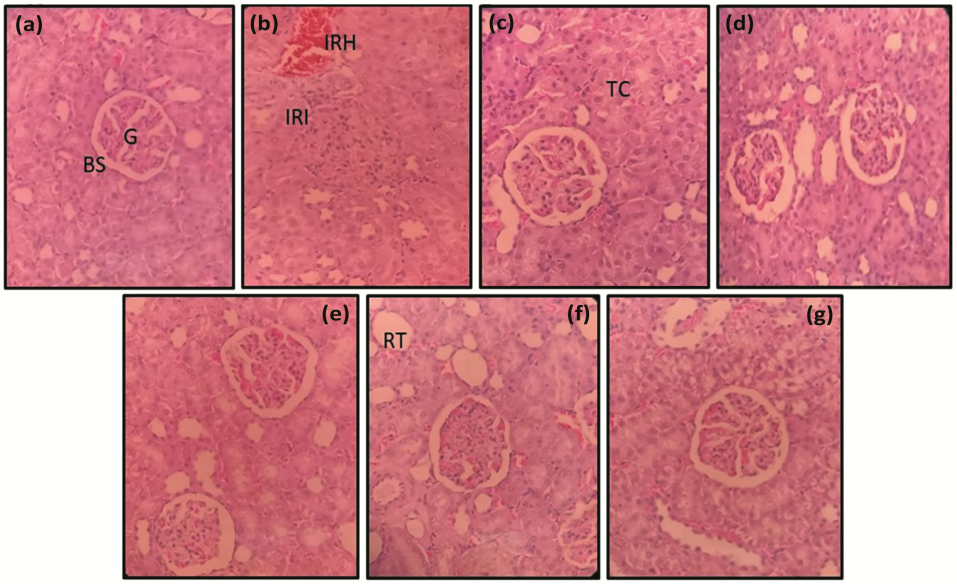

Fig. 2 — Light photomicrographs of Hematoxylin and Eosin (H&E) sections of the kidney (40x magnification): a) Normal control, b) Diabetic control, c) 100 mg/kg body weight (BW) of *Halimeda macroloba* extract (HME), d) 200 mg/kg BW of the HME, e) 100 mg/kg BW Metformin, f) 12.5 mg/kg BW Acarbose, and g) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin. G: Glomeruli, BS: Bowman Space, RTs: Renal Tubules, TC: Tubular Cells, IRH: Intra Renal Haemorrhaging, and IRI: Intra Renal Inflammation

vascularisation. The standard group experienced similar effects; however, glomerular basement thickening was observed in the metformin group, as depicted in Figure 2.

Effect of HME on pancreatic tissue histology

A photomicrograph of pancreatic tissue sections of normal control rats revealed normal, well-perfused pancreatic tissues with active islets of Langerhans, pancreatic acini and well-outlined acinar cells (Fig. 3a). STZ-NA administration results in chronic acinar inflammation and atrophy of islet cells in the diabetic control rats. Whereas both HME-treated groups show normal pancreatic tissue, islets of cells and pancreatic acini with regenerative signs were visualised throughout the parenchyma. Another

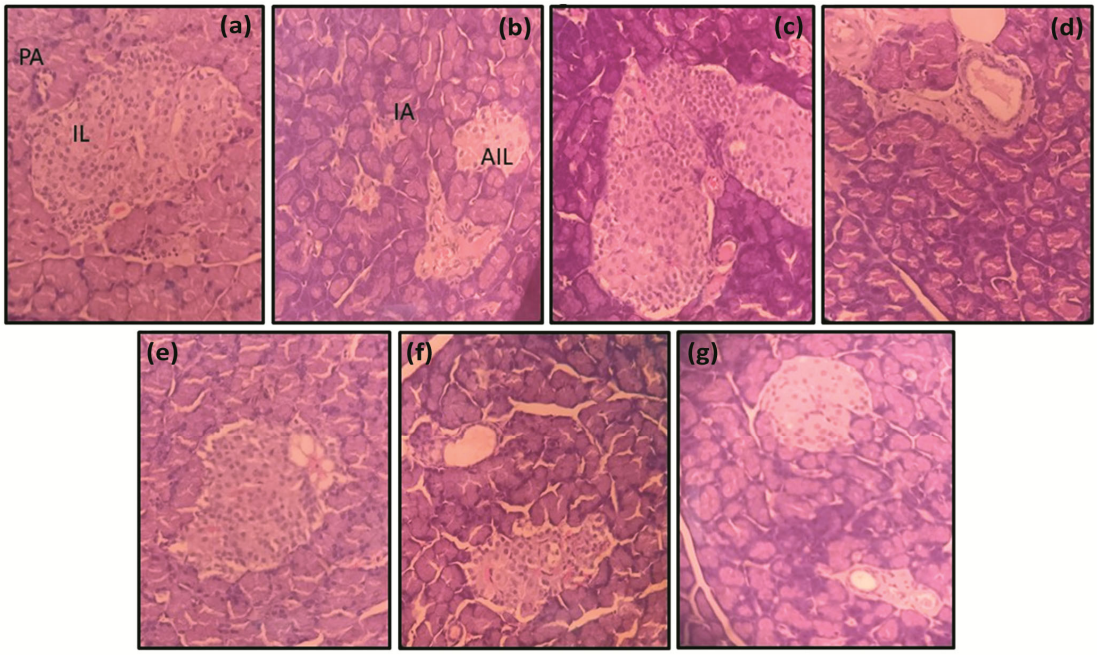

Fig. 3 — Light photomicrographs of Hematoxylin and Eosin (H&E) sections of the pancreas (40x magnification): a) Normal control, b) Diabetic control, c) 100 mg/kg body weight (BW) of *Halimeda macroloba* extract (HME), d) 200 mg/kg BW of the HME, e) 100 mg/kg BW Metformin, f) 12.5 mg/kg BW Acarbose, and g) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin. ILs: Islets of Langerhans, PA: Pancreatic Acini, IA: Inflammation of Acinar cells, and AILs: Atrophy of Islet of Langerhans

standard group also shows normal pancreatic acini and the islet of Langerhans.

Discussion

There are a few outcomes from seaweed-based medications for the treatment of diabetes, in addition to the advantages of seaweed chemicals[22]. The α-glucosidase inhibitory property of *H. macroloba* has been demonstrated *in vitro*[9]. However, to the best of our knowledge, the pharmaceutical or nutraceutical properties of *H. macroloba* have not been examined *in vivo.* Therefore, this study attempts to assess the antidiabetic, hypolipidemic, and antioxidant activities of HME *in vivo* in Wistar rats with STZ-NA-induced diabetes.

According to results from earlier research, the oxidative stress induced by STZ-NA treatment may have caused the elevated fasting blood glucose concentration, HbA1c, and BW loss observed in STZ-NA-intoxicated rats. Increased catabolism of proteins and fats may have contributed to the lower BW, which in turn causes weight loss and a decrease in muscle mass[23,24]. Crucially, current findings showed that in diabetic-induced rats that were treated with HME had a normalising effect on blood glucose, HbA1c, and enhanced body weight, indicating that *H. macroloba* may have antidiabetic activity. These results were in concurrence with previous discoveries, such as the *Sargassum aquifolium* (formerly *Sargassum echinocarpum*) (Phaeophyceae) possesses anti-hyperglycemic properties[25]. In diabetic rats, Lamela *et al*. 1989[(ref. 26)] found that *S. echinocarpum* inhibited α-glucosidase activity and had insulin-mimetic properties. Polyphenols cause glucose transporter 4 to quickly translocate to the cell membrane, which aids in the blood and muscle absorption of glucose as reported by Borriello *et al*.[27].

It has been claimed that insulin resistance causes several metabolic diseases, including hyperlipidemia[28]. The primary cause of hyperlipidemia is decreased insulin action, which raises lipase activity and results in the discharge of free fatty acids from fat cells[29]. The accumulation of hepatic fat due to lipid metabolic disorders is one of the primary problems associated with type 2 diabetes, which causes the liver's detoxifying capacity to be diminished[30]. The HME's effectiveness in reducing hyperlipidemia brought on by STZ-NA treatment is shown in the current investigation. Compared with untreated rats, the HME decreased TG, TC, and LDL cholesterol levels while simultaneously increasing HDL cholesterol levels. The results of current study are consistent with those of Ara *et al.*[31], who demonstrated the effectiveness of seaweed extracts in reducing hyperlipidemia.

Oxidative stress has been attributed to the aetiology of diabetes. Antioxidants are an essential therapeutic strategy for the treatment of diabetes, given the suggested role of oxidative stress in the disease development[32,33]. The ability of plant-based antioxidants to reduce oxidative stress caused by free radicals has been correlated to the propensity of the active ingredients to bond with hydrogen. According to the *in vitro* investigation, the green macroalgae substantially scavenged free radicals and prevented lipid peroxidation[10]. These results demonstrated that the HME may be liable to control oxidative stress in diabetes mellitus and restrict lipid peroxidation of tissues. Several antioxidant enzymes, encompassing CAT, SOD and GSH, are crucial components of the defence mechanism that prevents oxidative damage to cells[34]. GSH, an antioxidant which helps to prevent damage to tissues from oxidative stress, for instance, and has been shown to play significant roles in metabolism and detoxification as a substrate or cofactor for other enzymes[35]. Two important enzymes that scavenge free radicals are SOD and CAT. While CAT catalyses the reduction of H_2O_2, shielding tissues from hydroxyl radical damage, superoxide anions are detoxified by SOD, which transforms them into water and H_2O_2[(ref. 36)]. The present study found that rats with STZ-NA-induced diabetes had higher lipid peroxidation and lower antioxidant enzyme activities. This suggested that the livers of untreated diabetic control rats produced more free radicals, which caused oxidative damage to proteins, lipids, and membranes, and subsequently decreased endogenous antioxidant levels (CAT, SOD, and GSH). It is noteworthy that current research showed that in rats treated with STZ-NA, EESTL increased hepatic GSH and antioxidant enzyme levels, while decreasing hepatic MDA levels.

A cytotoxic substance called STZ caused the production of free radicals, which in turn destroyed the ß cells in the pancreatic islets. This resulted in decreased insulin secretion, decreased glucose utilisation by different tissues, elevated blood glucose levels and insulin resistance[37]. Therefore, the HME extract antioxidant properties (as shown by *in vivo* data) might be mainly involved in the effects of blood glucose normalisation and the enhancement of the pancreas' histology in rats given HME. These antioxidants enhance the sensitivity to insulin, boost insulin production from the islets' β-cell, improve pancreatic function, and lower the rats' blood sugar levels[5].

PI, severe degeneration, CI, and steatosis were observed in the livers of diabetic rats treated only with STZ-NA during histological examination. Remarkably, after HME treatment, the degenerative alterations, severe PI, CI, and steatosis were further enhanced, confirming HME's hepatoprotective action as an excellent antioxidant. Similarly, when compared to the untreated diabetic rats, extract treatment improved the histological abnormalities of the kidneys and pancreas. In conclusion, the histological observations and serum and tissue biochemical tests were found consistent, and this clearly implied the therapeutic significance of the HME in treating diabetes mellitus and the associated disorders.

Likewise, histological impairments of the diabetic control rats' kidneys showed Intrarenal Haemorrhage (IRH), intrarenal inflammation, and nodular glomerular sclerosis. These were reversed in the HME-treated group rats, showing normal renal parenchyma with lobular regeneration and typical tubular epithelial vascularisation. Furthermore, the pancreas of diabetic control rats' showed chronic Acinar Inflammation (AI) and Atrophy of Islets of Langerhans (AILs). These effects were attenuated in the pancreas by extract treatment, and normal pancreatic acini and islet cells, with regenerative signs, were visualised throughout the pancreatic parenchyma, compared with diabetic non-treated rats.

When free radicals are produced during hyperglycemia, cellular macromolecules (lipids, proteins, and carbohydrates) undergo oxidative changes, which sets off a series of inflammatory processes that harm the hepatocytes[38,39]. Consequently, the extract's positive impact on biochemistry and tissue histology may be due to the combined action of the plant extract's antioxidant and glucose normalisation properties.

The current work will therefore be the first to detail the antidiabetic, hypolipidemic, and antioxidant activities of this marine seaweed *in vivo*, which has shown antioxidant, cytotoxic, antidiabetic, and antibacterial effects *in vitro*. The results of the current study have advanced our understanding by providing preclinical proof of *H. macroloba*'s therapeutic effectiveness in reducing oxidative stress, lowering blood sugar, and lowering cholesterol in rats given STZ-NA. Based on the study data, the HME extract appears to help regulate oxidative stress, lipid profile, and glycemic levels. Additionally, it has nearly identical outcomes to conventional medication, which

may be useful in developing novel alternative treatments for diabetes mellitus.

The development of this model leverages the opposing actions of the two substances on β cells, as NAD is generally cytoprotective and STZ is cytotoxic to β cells. Nicotinamide can thereby reduce the harm caused by STZ to β cells. The safety profile of the HME extract was established through an acute toxicity test, wherein a dose of up to 2000 mg/kg BW was found to be safe in our previous study[14]. A dose-dependent response of the extract was assessed for its therapeutic relevance and safety against the standard drugs. The histopathological assessment provided clear, visual evidence of the extract's antidiabetic, hypolipidemic, and antioxidant effects. Future research must examine the potential mechanisms. Gene expression and associated pharmacogenomics may have provided additional insight into the potential mechanisms of action.

Conclusion

In a broader sense, the research presents entirely novel preclinical proof of *Halimeda macroloba*'s antidiabetic, hypolipidemic, antioxidant properties, and its ability to prevent oxidative stress in diabetic rats dosed with STZ-NA. Various kinds of bioactive components with potential impact on the biological activities of marine products were identified during the characterisation of the extract. Therefore, when combined, HME provides an extensive list of bioactive metabolites for the treatment of diabetes and its associated health risks.

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Competing Interests

The authors state no conflict of interest.

Research Ethics

This study was commenced after approval from the institutional animal ethics committee (IAEC) of MGMCRI, SBV University, Puducherry (CPCSEA regd. No.: 686/PO/Re/S/02/CPCSEA, 21-08-2002) and approved proposition no.: 08/IAEC/MG/04/ 2023 – I.

Data Availability

The raw data can be obtained on request from the corresponding author.

Author Contributions

KR: Conceptualisation or design of the work, data collection and analysis; VA: Drafted of the manuscript, critical revisions, and verification of intellectual content; KM: Manuscript editing and reviewing; KJS: Manuscript drafting and revision for important intellectual content; ShV: Statistical analysis and manuscript reviewing; SS: Histopathological analysis; and PD & SuV: Reviewing of the manuscript.

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