https://doi.org/10.31925/farmacia.2026.2.17 **ORIGINAL ARTICLE**

PROTECTIVE ROLE OF THE GREEN ALGA *ULVA LACTUCA* AGAINST OXIDATIVE STRESS IN TYPE 2 DIABETES: *IN VITRO* AND *IN VIVO* EVIDENCE

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Abstract

This study investigates the phytochemical composition, antioxidant activity, and cytoprotective potential of *Ulva lactuca* hydroethanolic extracts. The extract was subjected to standard phytochemical screening, confirming the presence of bioactive compounds including polyphenols, flavonoids, tannins, and terpenoids. Quantitative analysis indicated high levels of carbohydrates and proteins, with strong antioxidant potential (IC_{50} = 0.26 mg/mL). Experimental type 2 diabetes was induced in adult rats through a high-fat diet (HFD) followed by a single intraperitoneal injection of streptozotocin (35 mg/kg). Oxidative stress markers (lipid peroxidation, hydroperoxides, protein carbonyls), antioxidant enzyme activities, paraoxonase-1 (PON1), and non-enzymatic antioxidants were assessed in serum, red blood cells, lipoproteins, and tissues. Treatment with either whole algae or its extract significantly reduced oxidative stress markers and restored antioxidant enzyme activities, thiol content, and levels of vitamins C and E. Notably, the extract showed greater efficacy in kidneys and red blood cells, while whole algae enhanced PON1 activity and preserved vitamin status. These findings demonstrate the potent antioxidant and protective effects of *U. lactuca* and support its potential role in mitigating oxidative damage and complications associated with type 2 diabetes.

Rezumat

Acest studiu investighează compoziția fitochimică, activitatea antioxidantă și potențialul citoprotector ale extractelor hidroetanolice de *Ulva lactuca*. Extractul a fost supus unei analize fitochimice standard, care a confirmat prezența unor compuși bioactivi, printre care polifenoli, flavonoide, taninuri și terpenoide. Analiza cantitativă a evidențiat niveluri ridicate de carbohidrați și proteine, însoțite de un efect antioxidant semnificativ (IC_{50} = 0,26 mg/mL). Diabetul zaharat de tip 2 a fost indus experimental la șobolani adulți prin administrarea unei diete bogate în grăsimi (HFD), urmată de o singură injecție intraperitoneală de streptozotocină (35 mg/kg). Markerii stresului oxidativ (peroxidarea lipidică, hidroperoxizii, carbonilii proteici), activitățile enzimelor antioxidante, paraoxonaza-1 (PON1) și antioxidanții neenzimatici au fost evaluați în ser, eritrocite, lipoproteine și țesuturi. Tratamentul cu produsul vegetal sau cu extractul acesteia a redus semnificativ markerii stresului oxidativ și a restabilit activitățile enzimelor antioxidante, conținutul de tioli, precum și nivelurile vitaminelor C și E. Este de remarcat faptul că extractul a manifestat o eficacitate superioară la nivelul rinichilor și al eritrocitelor, în timp ce produsul vegetal a amplificat activitatea PON1.

Keywords: *Ulva lactuca*, type 2 diabetes, high-fat diet, oxidative stress, antioxidant activity

Introduction

The long-term metabolic disease known as diabetes mellitus (DM) affects the body's capacity to metabolize proteins, fats, and carbohydrates. The cause is typically either insufficient insulin synthesis or decreased insulin sensitivity. This malfunction can lead to a number of serious health issues, such as nerve damage (neuropathy), kidney problems (nephropathy), eye problems (retinopathy), and cardiovascular abnormalities (1). According to data from the International Diabetes Federation, there are currently 537 million people with diabetes worldwide; by 2030, that number is predicted to increase to 643 million (2, 3). Although the overall prevalence is currently higher in high-income nations, the number of cases is rising

faster in low- and middle-income nations, mostly due to poor eating habits, urbanization, and inactivity (4).

Among the different types of diabetes, type 2 diabetes mellitus (T2DM) accounts for approximately 95% of cases. The main drivers of T2DM are progressive insulin resistance and subsequent β-cell dysfunction, leading to impaired insulin secretion and utilisation. Several risk factors, including obesity, unhealthy diet, lack of physical activity, and genetic factors, play a significant role in its development and progression (5). Metformin and sulfonylureas, as well as other classes such as thiazolidinediones, DPP-4 inhibitors, and GLP-1 receptor agonists, are the main treatments currently available. Nevertheless, many of these treatments have drawbacks in terms of tolerability and efficacy, as well as the potential for unintended side effects. Furthermore, low-income people frequently cannot afford synthetic antidiabetic medications due to their high costs. These difficulties highlight the urgent need for more accessible, affordable, and secure alternative treatment approaches for long-term care (6).

Recently, there has been significant interest in medicinal plants due to their potential to treat a range of chronic conditions, including diabetes. Due to their accessibility, affordability, and safety, traditional herbal therapies are widely used worldwide. Roughly 25% of current pharmaceutical drugs are derived from plants, and about 80% of the world's population relies on plant-based medicines. As a result, there is now more scientific interest in using contemporary techniques to validate traditional medical practices. Organizations such as the World Health Organization (WHO) have emphasized the importance of studying medicinal plants to understand their pharmacological properties. The role of natural antioxidants in the management and prevention of diabetes is an area that is increasingly being studied (7). Hyperglycemia-induced oxidative stress plays a major role in the development of complications associated with diabetes. The auto-oxidation of glucose produces excessive free radicals, which can overwhelm the body's inherent antioxidant defenses, leading to damage in both microvascular and macrovascular systems. Natural antioxidants, especially those from dietary sources, have demonstrated protective benefits against oxidative damage, reduced inflammation, and enhanced insulin sensitivity (8).

Marine resources have shown promise as sources of new bioactive compounds, especially marine algae. Numerous phytochemicals, such as polyphenols, flavonoids, sterols, vitamins, polysaccharides, and polyunsaturated fatty acids, are abundant in algae. These substances exhibit a variety of biological properties, including antiviral, antibacterial, anti-inflammatory, antidiabetic, and antioxidant activities (9-11). *Ulva lactuca*, a green macroalga in the *Chlorophyta*, is unique among marine algae for its wide distribution, high nutritional value, and long history of human consumption. Numerous bioactive substances, including sinapic acid, quercetin, cinnamic acid, apigenin, flavonoids, and unsaturated fatty acids like palmitic acid, eicosenoic acid, and linoleic acid, have been found in *U. lactuca*, according to phytochemical analysis. These substances support its strong antioxidant properties (12). The medicinal benefits of *U. lactuca*, including its hypoglycemic, hypolipidemic, and anti-inflammatory properties, have been demonstrated in numerous *in vivo* studies. It has been demonstrated that its oligosaccharides improve glucose metabolism and control oxidative stress *via* microRNA regulation. In particular, *U. lactuca* increases glutathione, superoxide dismutase, catalase, and total antioxidant capacity while lowering detrimental markers like malondialdehyde (MDA) and advanced glycation end products (AGEs) (13, 14). Therefore, the present study aimed to evaluate the effects of *Ulva lactuca* powder and its hydroethanolic extract on oxidative stress biomarkers in erythrocytes, serum, tissues, and lipoprotein fractions in diabetic rats. This research could pave the way for the development of safe, affordable, and effective plant-based interventions for diabetes management.

Materials and Methods

Plant collection and extract preparation

The green alga *Ulva lactuca* was collected on the west coast of Oran, Algeria (Latitude: 35° 41' 28.00" North, Longitude: 0° 38' 30.01" East) in February 2023. The *Algae* were washed with tap water, sun-dried to evaporate the moisture, and ground using a grinder machine. The hydroethanolic extract was prepared according to the procedure described by (15). *U. lactuca* (100 g) powder was macerated in a hydroethanolic mixture (70% ethanol:water (v/v), 1:10 w/v ratio, and macerated for 48 h at room temperature. The solution was filtered by using a Whatman filter paper (0.45 µm). Afterwards, the solvent was evaporated by a rotary evaporator (Buchi R-100, Büchi Labortechnik AG, Flawil, Switzerland). The evaporated extract was lyophilized using a Freez dryer (Christ, loc-1, Alpha1-4) and stored at 4°C for further analysis. The extraction yield was approximately 28%.

Animals

Male Wistar (n = 24, 260 ± 25 g) rats were purchased from Pasteur Institute, Algiers, Algeria. The study was conducted according to the European Council Communities guideline (16), and rats were kept at 23°C and 60% humidity, with 12 h dark and 12 h light conditions. Standard diets were provided to the rats during the experiments.

Phytochemical analysis of U. lactuca

Preliminary phytochemical screening was performed using standard methods to determine total carbohydrates, proteins, and lipids (17). To determine total

phenolic content, the spectrophotometric Folin-Ciocâlteu method was used. In a solution containing 1.25 mL of Folin-Ciocâlteu, 1 mL of 2% sodium carbonate (Na_2CO_3), and 0.25 mL of diluted extract (or gallic acid standard substance and its dilutions), incubated at room temperature for 90 min, the absorbance is measured at 765 nm. The results are expressed as mg gallic acid equivalent/g of dry plant material (mg GAE/g) by reference to the gallic acid calibration curve (18). Moreover, the total flavonoid content was measured according to the $AlCl_3$ standard method (19). Add 0.3 mL of sodium nitrite ($NaNO_2$) to 1 mL of extract (or quercetin solution and its dilutions). After 5 minutes, add 0.3 mL of Aluminium trichloride ($AlCl_3$), and after 6 minutes, add 2 mL of sodium hydroxide (NaOH) to bring the volume up to 10 mL. Absorbance was measured at 510 nm. Results are expressed as mg quercetin equivalent/g, referencing the quercetin calibration curve. The amount of condensed tannins was quantified following the vanillin method (20). A 0.5 mL volume of the extract or standard (catechin) is added to a 3 mL mixture of 4% vanillin and methanol (w/v), 1.5 mL of hydrochloric acid, and the mixture is homogenized. The mixture is left to stand at room temperature for 15 min. The absorbance of each extract is calculated at 500 nm using a UV-Vis spectrophotometer. The total condensed tannin content is calculated as mg catechin equivalent (mg EC/g) using the equation obtained from the calibration curve. In addition, antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (21). A volume of 1.95 mL of DPPH methanolic solution (2.5 mg DPPH/100 mL methanol) was mixed with 50 µL of various doses of extract or standard (ascorbic acid) (0 - 1000 µg/mL). Concurrently, 50 µL of methanol and 1.95 mL of DPPH methanolic solution are combined to create a negative control. After 30 minutes of dark, room-temperature incubation, absorbance is measured at 517 nm against a blank prepared for each concentration.

Diabetic animal model preparation

Adult rats (n = 18) were given a HFD (30% lamb fat) for 4 weeks, then mixed with their basal diet, and the control group (n = 6) received the vehicle (citrate buffer) only. Algae doses: 100 mg/kg body weight/day (whole powder or hydroethanolic extract, based on prior study (10), administered orally *via* gavage, daily for four weeks. Afterwards, a single intraperitoneal injection of streptozotocin (STZ) (Sigma, St Louis, USA) (35 mg/kg body weight) was given to develop T2DM. Blood glucose levels were measured before STZ injection (0 h) and 48 h after STZ injection (T48h). Glucose levels were measured using a glucometer with Accu-Chek Active strips (Roche Diagnostics, Germany), and 200 mg/dL of glucose in the blood (11 mM) was considered a T2DM model. The blood sample was taken by single amputation from the tail vein (22). In the four-week chronic experiment, the experimental animals were divided into four groups: (i) C - control group; (ii) D - diabetic control; (iii) D-Alg - diabetic rats supplemented with algae powder; and (iv) D-Ext - diabetic rats supplemented with a hydroethanolic extract of algae.

Blood collection and organ separation

On the 28th day of the experiment, the rats were fasted for 12 hours and then anaesthetized by intraperitoneal injection of a 10% chloral hydrate solution at a dose of 3 mL/kg body weight. Blood samples were collected from the abdominal aorta into dry tubes and centrifuged at 1000 × g for 15 minutes at 4°C. The serum was carefully separated from the red blood cells (RBCs) after centrifugation. The RBCs were washed three times with 0.9% sodium chloride (NaCl) solution, with each wash followed by centrifugation at 1000 × g for 15 minutes at 4°C. After each wash, the supernatant was discarded. The final RBC pellet was lysed in ice-cold distilled water, centrifuged, and the resulting supernatants were collected and stored at -70°C for future analysis. Following blood collection, organs including the liver, muscle, heart, and kidney were excised, rinsed with fresh 0.9% NaCl solution, blotted dry, and weighed. All collected samples, including serum, RBC lysates, and organs, were stored at -70°C for subsequent biochemical analyses.

Separation of lipoproteins

Serum lipoproteins were separated by precipitation according to the method described by (23). Phosphotungstate (Prolabo, France) and magnesium chloride ($MgCl_2$) were used to precipitate very low-density lipoproteins (VLDL; $d < 1.006$) and LDL-HDL_1 fractions ($1.006 < d < 1.075$). High-density lipoproteins, specifically HDL_2 ($1.085 < d < 1.121$) and HDL_3 ($1.121 < d < 1.210$), were subsequently precipitated using dextran sulfate (Sigma, USA) in combination with $MgCl_2$.

Lipid peroxidation levels

Thiobarbituric acid reactive substances (TBARS) were assessed at multiple levels, including lipoprotein fractions (VLDL, LDL-HDL_1, HDL_2, and HDL_3) and tissues, following the protocols described by (24). TBARS levels (nmol MDA equivalents/mg protein) were calculated using a standard curve of 1,1,3,3-tetramethoxypropane (MDA precursor), with a molar extinction coefficient at 532 nm of 156,000 M^{-1} cm^{-1}, adjusted for sample dilution, TCA volume, and protein content. TBARS levels were calculated using the following formula:

C (nmol MDA equivalents/mg protein) = optical density x 104 x weighing $^{-1}$ x volume TCA x 0.641 x 2.

Hydroperoxides and protein carbonyl levels

Hydroperoxide concentrations in serum, RBCs, tissues, and lipoprotein fractions (VLDL, LDL-HDL_1, HDL_2, and HDL_3) were measured using the method described by (25). Serum protein carbonyl levels were

determined using the 2.4-dinitrophenylhydrazine (DNPH) method.

The evaluation of the antioxidant enzyme activity

Superoxide dismutase (SOD) activity was determined using the method described by (26). Glutathione peroxidase activity (GPx) was measured as described by (27). Catalase activity (CAT) was assessed using the method described by (28). Glutathione reductase activity (GR) was measured according to the method described by (29).

Serum HDL_2 and HDL_3paraoxonase (PON1) activities

PON1 is an enzyme that catalyzes the cleavage of phenyl acetate, resulting in the formation of phenol. Arylesterase activity of PON1 (using phenyl acetate as substrate, measured as phenol formation at 270 nm, at 25°C. Arylesterase activity of PON1 was calculated using the molar absorption coefficient of phenyl (1310 M^{-1} cm^{-1}) (30).

The estimation of non-enzymatic antioxidant activity

Serum and tissue thiol levels were evaluated using the method described by (29). Serum ascorbic acid (vitamin C) and α-tocopherol (vitamin E) concentrations were determined following the protocols of (31).

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Statistical analysis was performed using STATISTICA software (Version 5.1; StatSoft, Tulsa, OK, USA). Following one-way ANOVA, group comparisons were conducted using the least significant difference (LSD) post hoc test. Differences were considered statistically significant at $P < 0.05$. The symbols indicate the following comparisons: *D *vs*. C; #D-Alg *vs*. D; $D-Ext *vs*. D; ¥D-Ext *vs*. D-Alg.

Results and Discussion

Phytochemicals and antioxidant screening of U. lactuca

The preliminary phytochemical screening, primary and secondary metabolite content, and antioxidant activity of the hydroethanolic extract of *Ulva lactuca* algae are presented in Tables I and II. Preliminary analysis indicated that *Ulva lactuca* is a rich source of total polyphenols, flavonoids, tannins, phlorotannins, saponosides, and terpenoids, but no alkaloids were detected (Table I). Preliminary qualitative screening suggested the presence of phlorotannins; however, as these are uncommon in green algae, this requires confirmation by HPLC-MS or similar targeted analysis in future studies. Quantitative analysis of the primary and secondary metabolites revealed substantial levels of carbohydrates (45.0 ± 0.21 g/100 g), total proteins (26.1 ± 0.11 g/100 g), total lipids (2.1 ± 0.001 g/100 g), total phenolic content (20 ± 2.43 mg GAE/g), total flavonoid content (1.50 ± 0.96 mg QE/g), and condensed tannins content (1.47 ± 0.01 mg CE/g). Furthermore, the antioxidant scavenging activity, assessed by IC_{50}, was determined to be 0.26 ± 0.01 mg/mL (Table II).

Table I

Preliminary phytochemical screening of *U. lactuca*

Chemical compound		Reaction
Total polyphenols		++
Flavonoids		+
Tannins		+
Phlorotannins		+++
Saponosides		+
Alkaloids	Mayer reagent	-
	Wagner reagent	-
Terpenoids		+

All tests were performed three times. +++ = appreciable quantity (positive after 5 min.); ++ = moderate quantity (positive after 5 min.); + = trace (positive after 10 min.); - = not detected

Table II

Primary/secondary metabolites and antioxidant activity of *U. lactuca*

Parameters		Quantity
Primary metabolites (g/100 g dry algae)	Carbohydrates	45.0 ± 0.21
	Total proteins	26.1 ± 0.11
	Total lipids	2.1 ± 0.001
Secondary metabolites	TPC (mg GAE/g)	20.0 ± 2.43
	TFC (mg QE/g)	1.50 ± 0.96
	CTC (mg CE/g)	1.47 ± 0.01
Antioxidant activity	IC_{50} (mg/mL)	0.26 ± 0.01

CE: Catechin equivalent; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TFC: Total flavonoid content; TPC: Total phenolic content; CTC: Condensed tannins content; IC_{50}: median inhibitory concentration.

The present study underscores the therapeutic potential of *Ulva lactuca*, a green macroalga with a rich biochemical composition and potent antioxidant properties. Nutrient analysis revealed that *U. lactuca* contains a high carbohydrate content (45%), moderate protein levels (26%), and a low lipid concentration (2%) (Table II). Phytochemical screening indicated a substantial presence of beneficial secondary metabolites, including polyphenols, flavonoids, tannins, phlorotanins, saponosides, and terpenoids, though alkaloids were not detected (Table I). Quantitatively, total phenolic content (TPC), flavonoid content (TFC), and condensed tannins (TCT) measured 20.00 ± 2.43 mg GAE/g, 1.50 ± 0.96 mg QE/g, and 1.47 ± 0.01 mg CE/g, respectively. The high concentration of these bioactive compounds resulted in a strong antioxidant effect, as reflected by an IC_{50} of 0.26 ± 0.01 mg/L (Table II). The metabolic profile of *U. lactuca* is shaped by multiple factors, including species variation, seasonal and environmental conditions, geographical origin, and extraction methodology (32). These variables are known to influence phytochemical yields, particularly phenolic compounds, which are sensitive to plant maturity, genetic traits, and storage duration (33, 34).

Serum/lipoproteins and erythrocytes oxidative stress

As presented in Table III, diabetic rats exhibited significantly elevated levels ($P < 0.05$) of TBARS in serum, RBCs, and lipoproteins, along with increased hydroperoxide concentrations in VLDL and HDL_2 fractions but reduced in HDL_3 in diabetic rats compared to controls. Additionally, it was found that diabetic rats had a serum protein carbonyl content roughly 4 times higher. Surprisingly, treatment with *Ulva lactuca* or its hydroethanolic extract significantly reduced these oxidative stress markers in serum, red blood cells, and lipoproteins to near normal levels. Serum TBARS levels were reduced by 61% and 56% in rats treated with *U. lactuca* and its extract, respectively, compared to the diabetic group. Similarly, hydroperoxide levels in serum decreased by 40% and 25%, and in VLDL and HDL_2 fractions by 24% and 44%, and 25% and 37%, respectively, following *U. lactuca* and extract administration. Serum protein carbonyls were also significantly lowered by 67% and 62% with *U. lactuca* and its extract, respectively. Moreover, *U. lactuca* extract was more effective than the whole algae in reducing TBARS levels in RBCs (13%) and VLDL (23%), as well as VLDL hydroperoxides (27%) in diabetic rats.

Table III

Biomarkers of oxidative stress in serum, erythrocyte, and lipoprotein fractions

Parameters	C	D	D-Alg	D-Ext
TBARS levels				
Serum (nmol/mL)	13.52 ± 3.14	33.41 ± 3.01*	12.99 ± 2.90$^{\#}$	14.78 ± 2.09$^{\$}$
RBC (nmol/mL)	4.76 ± 0.77	12.41 ± 1.00*	5.41 ± 0.21$^{\#}$	4.77 ± 0.77$^{\$¥}$
VLDL (nmol/mL)	00.10 ± 0.00	4.59 ± 0.70*	2.53 ± 0.31$^{\#}$	1.96 ± 0.05$^{\$¥}$
LDL-HDL_1 (nmol/mL)	00.19 ± 0.01	0.26 ± 0.00*	0.15 ± 0.02$^{\#}$	0.21 ± 0.01$^{\$¥}$
HDL_2 (nmol/mL)	0.03 ± 0.00	0.11 ± 0.01*	0.04 ± 0.00$^{\#}$	0.06 ± 0.00$^{\$¥}$
HDL_3 (nmol/mL)	0.07 ± 0.00	0.15 ± 0.01*	0.05 ± 0.00$^{\#}$	0.07 ± 0.03$^{\$¥}$
Hydroperoxide levels				
Serum (nmol/mL)	3.71 ± 0.13	5.20 ± 0.20*	3.12 ± 0.10$^{\#}$	3.90 ± 0.13$^{\$}$
RBC (nmol/mL)	0.09 ± 0.001	0.16 ± 0.03*	0.12 ± 0.03	0.11 ± 0.01$^{\$}$
VLDL (nmol/mL)	13.04 ± 1.20	27.00 ± 2.29*	20.41 ± 1.02$^{\#}$	15.00 ± 2.05$^{\$¥}$
LDL-HDL_1 (nmol/mL)	3.53 ± 0.90	4.51 ± 0.50	4.08 ± 0.12	4.81 ± 0.10
HDL_2 (nmol/mL)	08.12 ± 0.17	11.93 ± 0.20*	9.00 ± 0.14$^{\#}$	7.65 ± 0.13$^{\$}$
HDL_3 (nmol/mL)	09.13 ± 1.00	6.33 ± 0.57*	6.40 ± 0.61	5.21 ± 0.70
Serum carbonyl levels (nmol/mL)	0.10 ± 0.08	0.42 ± 0.07*	0.14 ± 0.01$^{\#}$	0.16 ± 0.02$^{\$}$

TBARS: Thiobarbituric Acid Reactive Substances; RBC: red blood cell; VLDL: Very Low-Density Lipoproteins; LDL: Low-Density Lipoproteins; HDL: High-Density Lipoproteins; *D *vs*. C, $^{\#}$D-Alg *vs*. D, $^{\$}$D -Ext *vs*. D, $^{¥}$D-Ext *vs*. D-Alg.

Concerning T2DM, oxidative stress is recognized as a critical contributor to disease progression. Diabetic conditions elevate the production of reactive oxygen species (ROS), which can damage DNA, lipids, and proteins. In our study, untreated rats fed a HFD showed significantly increased levels of TBARS-a key marker of lipid peroxidation-in serum, erythrocytes, lipoproteins, and multiple tissues (liver, muscle, heart, and kidney). These findings support existing evidence linking hyperlipidic diets to oxidative stress and compromised antioxidant defenses. Promisingly, supplementation with *U. lactuca*, either in its entirety or as an extract, markedly lowered TBARS levels, suggesting a reduction in lipid peroxidation (Table III). This protective mechanism may be linked to enhanced glycemic management, as suggested by previous studies showing reductions in blood glucose, HbA1c, and AGEs following *U. lactuca* supplementation in similar models (10, 13, 14). In the present work, the observed reductions in oxidative stress markers are consistent with improved antioxidant defenses. These results are consistent with earlier research that emphasized the importance of flavonoids in mitigating oxidative damage in diabetic models (35).

Biomarkers of oxidative stress in different tissues

As shown in Table IV, diabetic rats exhibited significantly increased levels of TBARS and protein carbonyls in tissues, including the liver, muscle, heart, and kidneys ($P < 0.05$) compared to control rats. Additionally, hydroperoxide levels were elevated in the liver, muscle, and heart. Treatment with *Ulva lactuca* or its hydroethanolic extract significantly reduced TBARS levels in the liver (by 46% and 54%, respectively), muscle (by 54% and 59%, respectively), and heart (by 30% and 50%, respectively). Similarly, hydroperoxide concentrations in the muscle decreased by 51% and 59% following treatment with *U. lactuca* and its extract, respectively. Protein carbonyl levels were also significantly reduced in the liver (by 38% and 14%, respectively) and heart (by 39% and 19%, respectively) after treatment. Notably, *U. lactuca* treatment had a stronger effect than the extract in reducing kidney protein carbonyl levels. Although Hydroperoxide levels in kidneys remained comparable to controls, they were significantly modulated by treatments. Furthermore, the *U. lactuca* extract demonstrated greater efficacy than the whole algae in reducing kidney hydroperoxide levels.

Table IV

Tissue biomarkers of oxidative stress

Parameters	C	D	D-Alg	D-Ext
TBARS (nmol/mg protein)				
Liver	7.63 ± 0.70	19.15 ± 4.23*	10.38 ± 5.66$^{\#}$	8.87 ± 4.13$^{\$}$
Muscle	5.05 ± 0.85	12.20 ± 3.09*	5.65 ± 0.51$^{\#}$	4.98 ± 1.84$^{\$}$
Heart	0.10 ± 0.01	0.30 ± 0.09*	0.21 ± 0.06$^{\#}$	0.15 ± 0.08$^{\$}$
Kidney	6.54 ± 1.50	13.72 ± 1.04*	12.03 ± 1.24	12.00 ± 1.05
Hydroperoxides (nmol/mg protein)				
Liver	0.16 ± 0.04	0.26 ± 0.01*	0.21 ± 0.04	0.24 ± 0.02
Muscle	1.32 ± 0.13	2.35 ± 0.18*	1.15 ± 0.16$^{\#}$	0.97 ± 0.04$^{\$¥}$
Heart	0.11 ± 0.01	0.21 ± 0.02*	0.17 ± 0.04	0.19 ± 0.04
Kidney	0.83 ± 0.06	0.78 ± 0.30*	1.25 ± 0.13$^{\#}$	0.84 ± 0.05$^{\$¥}$
Protein carbonyls (nmol/mg protein)				
Liver	1.03 ± 0.02	2.43 ± 0.93*	1.50 ± 0.51$^{\#}$	2.10 ± 0.79$^{\$}$
Muscle	0.76 ± 0.03	1.89 ± 0.58*	1.02 ± 0.13$^{\#}$	1.20 ± 0.14
Heart	1.44 ± 0.41	2.69 ± 0.16*	1.65 ± 0.51$^{\#}$	2.17 ± 0.63$^{\$}$
Kidney	0.66 ± 0.03	2.45 ± 0.17*	1.23 ± 0.60$^{\#}$	2.25 ± 0.04$^{¥}$

Apart from TBARS, increased levels of lipid hydroperoxides—precursors to lipid peroxidation—were detected in diabetic rats, except in kidney tissue. Such increases are likely due to diminished activities of GPx and GR, the enzymes tasked with neutralising these compounds. Both the whole algae and their extract effectively reduced hydroperoxide levels in various tissues and serum lipoprotein fractions, with the extract demonstrating relatively stronger effects in some tissues (Figure 1). This indicates the antioxidant properties of phenolic compounds, which can chelate pro-oxidant metal ions, disrupt lipid peroxidation, and regenerate other antioxidants, such as α-tocopherol. The antioxidant enzyme system-comprising SOD, CAT, GPx, and GR-represents the body's first line of defense against ROS. Diabetic rats exhibited a marked reduction in these enzymes at both erythrocyte and tissue levels (Table V), mirroring earlier reports by (36) and (37). Decreased CAT activity may result from superoxide-induced inactivation, while lower GPx levels are likely tied to glutathione depletion, its essential substrate (38).

Supplementation with *U. lactuca* significantly restored these enzyme activities. Interestingly, the whole alga generally showed more pronounced effects than the extract, likely due to the presence of fibers and polysaccharides, which are absent in the extract. Polyphenols, flavonoids, and tannins, all abundant in *U. Lactuca* are known to enhance antioxidant defenses in chronic metabolic disorders, including diabetes. Moreover, studies revealed that ulvans, the sulfated polysaccharides that comprise about 30% of *U. lactuca's* dry weight, support liver antioxidant activity by modulating bile acid metabolism and activating detoxification enzymes (39, 40).

Serum and erythrocyte biomarker of enzymatic Antioxidant defense

In diabetic rats, RBC antioxidant enzyme activities were significantly reduced, with decreases of 28% in SOD, 54% in GPx, 20% in CAT, and 77% in GR compared to control animals. Treatment with whole *Ulva lactuca* markedly restored these antioxidant enzyme activities. In contrast, *U. lactuca* extract selectively enhanced GPx and CAT activities compared to the untreated diabetic group. Notably, GPx activity in extract-treated rats was significantly higher than in those treated with whole *U. lactuca*. PON1 activity was also significantly reduced in the serum, HDL_2, and HDL_3 fractions of diabetic rats by 45%, 60%, and 19%, respectively. Treatment with *U. lactuca* significantly improved PON1 activity in serum and HDL_2 by 56% and 94%, respectively. Furthermore, the enhancement of PON1 activity was greater with whole *U. lactuca* than with its extract (Table V). The improvement in PON1 activity was treatment-dependent, mainly observed in the whole-alga group, whereas the extract group did not show a significant increase in HDL_3-associated PON1.

Markers of enzymatic antioxidant defense in tissues

Enzymatic antioxidant activities in the liver, heart, muscle, and kidney tissues are illustrated in Figure 1. Diabetes was associated with a significant reduction in GPx, GR, and CAT activities across all examined tissues. Additionally, SOD and CAT activities were reduced in all tissues except for SOD in the liver and CAT in the kidney, where their levels remained unchanged. In diabetic rats treated with *Ulva lactuca*, GPx activity in the liver, heart, and muscle showed significant improvement compared to untreated diabetic rats. Furthermore, whole *U. lactuca* administration enhanced CAT activity in the liver and kidney and increased GR activity in all tissues. Conversely, the *U. lactuca* extract specifically improved SOD, GPx, and GR activities in the heart and muscle. Consistent with our findings, Liu *et al*. (13) reported that sulfate oligosaccharides from *U. lactuca* increased glutathione, SOD, and CAT while reducing MDA and AGEs in aged SAMP8 mice. However, GR activity in the

muscle and kidney was significantly lower in the *U. lactuca* extract-treated group compared to the whole algae group, with reductions of 32% and 47%, respectively

Table V

Enzymatic antioxidant defense at the serum and erythrocyte level

Parameters	C	D	D-Alg	D-Ext
SOD (U/g hemoglobin)	6.60 ± 0.30	4.73 ± 0.92*	5.06 ± 1.61$^{\#}$	5.12 ± 0.88
GPx (U/g hemoglobin)N	0.13 ± 0.02	0.06 ± 0.01*	0.11 ± 0.09$^{\#}$	0.20 ± 0.03$^{\$¥}$
CAT (U/g hemoglobin)	32.12 ± 0.36	25.60 ± 0.02*	32.15 ± 2.00$^{\#}$	36.49 ± 1.67$^{\$}$
GR (U/g hemoglobin)	12.76 ± 1.62	2.88 ± 0.34*	3.48 ± 0.53$^{\#}$	2.53 ± 0.39
Paraoxonase Serum (UI/mL serum)	14.88 ± 0.68	8.23 ± 0.60*	12.87 ± 0.75$^{\#}$	8.94 ± 1.36$^{¥}$
HDL_2 (UI/mL serum)	13.11 ± 0.10	5.21 ± 0.10*	10.10 ± 0.44$^{\#}$	5.64 ± 0.08$^{¥}$
HDL_3 (UI/mL serum)	7.23 ± 0.47	5.87 ± 0.56*	6.00 ± 0.66	5.94 ± 0.33

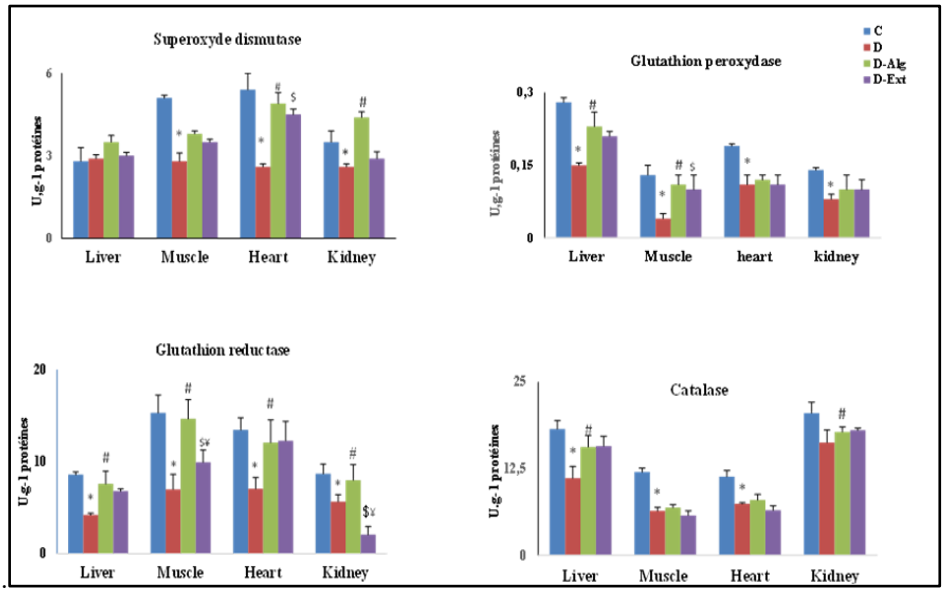

Figure 1.

Activities of antioxidant enzymes in different tissues. *D *vs*. C, #D-Alg *vs*. D, $D -Ext *vs*. D, ¥D-Ext *vs*. D-Alg

Non-enzymatic antioxidant status

In diabetic rats compared to controls, thiol content was significantly reduced by 79% in RBCs, 72% in the liver, 68% in muscle tissue, and 63% in the kidneys. Treatment with *Ulva lactuca* or its extract notably restored thiol levels in RBCs, increasing them by 94% and 155%, respectively. In the kidneys, the extract led to a 202% increase in thiol content compared to untreated diabetic rats. The thiol restoration was partial and tissue-dependent, with significant improvement observed only in the groups that met the statistical criteria.

Additionally, serum concentrations of vitamins C and E were markedly lower in diabetic rats, with reductions of 13-fold and 2-fold, respectively, compared to controls. However, treatment with whole algae significantly elevated ascorbic acid and α-tocopherol levels by 12-fold and 1-fold, respectively, relative to the diabetic group. In contrast, vitamin C levels in the extract-treated group were half of those observed in the whole algae-treated group (Table VI). Non-enzymatic antioxidants also play a crucial role in ROS defense. Diabetic rats showed a significant drop in total thiol levels in RBCs and various tissues, reflecting widespread protein oxidation. Supplementation with either form of *U. lactuca* reversed this decline, with the extract showing superior improvement at the renal level—likely due to the targeted action of isolated phenolics and flavonoids, which help prevent oxidative protein damage (41).

PON1, an HDL-associated enzyme that protects against LDL oxidation, was significantly reduced in diabetic rats. This is consistent with prior findings linking T2DM to low PON1 activity and increased lipid peroxidation (42). Notably, both the whole alga and its extract enhanced PON1 activity, potentially through the modulation of gene expression by polyphenols, thereby reinforcing HDL's antioxidant function. Serum levels of vitamins C and E, key non-enzymatic antioxidants, were also depleted in diabetic rats, reflecting their increased utilization or impaired recycling in the face of chronic oxidative stress. Treatment with *U. lactuca* restored both vitamins, with the whole alga being more effective in replenishing vitamin C-possibly due to the synergistic role of dietary fibers and polysaccharides, absent in the extract, in supporting vitamin retention and activity (43).

Table VI

RBCs, serum and tissue levels of thiols, Vit C and Vit E

Parameters	C	D	D-Alg	D-Ext
Thiols levels				
RBCs (SH nmol/mL)	0.85 ± 0.06	0.18 ± 0.03*	0.35 ± 0.04$^{\#}$	0.46 ± 0.03$^{\$}$
Liver (SH nmol/mg)	2.59 ± 0.98	0.73 ± 0.04*	0.77 ± 0.02	0.83 ± 0.02
Muscle (SH nmol/mg)	0.56 ± 0.18	0.18 ± 0.04*	0.20 ± 0.01	0.31 ± 0.01
Heart (SH nmol/mg)	1.31 ± 0.66	1.00 ± 0.27	1.04 ± 0.30	1.30 ± 0.28
Kidney (SH nmol/mg)	1.09 ± 0.02	0.40 ± 0.01*	0.96 ± 0.02	1.21 ± 0.14$^{\$}$
Vit C (mg/dL)	1.35 ± 0.03	0.10 ± 0.01*	1.25 ± 0.03$^{\#}$	0.78 ± 0.13$^{¥}$
Vit E (mg/dL)	0.60 ± 0.10	0.34 ± 0.22*	0.44 ± 0.12$^{\#}$	0.37 ± 0.14

SH: Sulfhydryl.

Conclusions

This research highlights the impressive antioxidant properties of *Ulva lactuca*, particularly due to its abundance of bioactive compounds, including polyphenols, flavonoids, and tannins. These phenolic substances, recognised for their multiple hydroxyl groups, are essential for neutralising free radicals and reducing oxidative harm. Using either the entire alga or its ethanolic extract effectively reduced oxidative stress by decreasing lipid peroxidation and protein oxidation, while simultaneously enhancing the activity of the body's antioxidant enzymes. Together, these protective mechanisms help maintain tissue integrity and provide a shield against diabetes-related oxidative injury. Further studies are necessary to completely grasp the therapeutic benefits of *U. lactuca.*

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

The animal experiments were conducted in accordance with the European Council Communities guidelines and approved by the Institutional Animal Ethics Committee of the University of Oran 1 Ahmed Ben Bella (Approval No. 2020/EA/UNIV-ORAN1/001, dated 15 January 2020).

AI use disclosure

During the preparation of this manuscript, the author(s) used Google translation for translation from French to English, Grammarly and Quillbot for editing assistance and language improvement, specifically to improve spelling, grammar, word choice, readability, and clarity. The author(s) reviewed and revised all outputs from these tools and take(s) full responsibility for the final content of the work.

Conflict of interest

The authors declare no conflict of interest.

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