Journal of Applied Phycology (2022) 34:3247–3261
https://doi.org/10.1007/s10811-022-02839-3

Anti-hyperglycemic and antioxidant effect of fucoidan extract from *Lessonia trabeculata* in alloxan-induced diabetes rats

Lillyan Teresa Loayza-Gutiérrez[1] · Eder Valdir Apumayta-Suárez[2] · Roberto Abdala[3] · Luis Ángel Aguilar-Mendoza[2] · Jorge Antonio Chávez-Pérez[2] · Juan Decara[4]

Received: 1 June 2022 / Revised and accepted: 9 September 2022 / Published online: 30 September 2022

Abstract

The objective of this research was to evaluate a nutritional strategy based on the consumption of a fucoidan extract from brown algae *Lessonia trabeculata* to control oxidative stress in experimental alloxan-induced insulin-dependent diabetes mellitus rats. Over 30 days, 75, 100, and 125 mg kg^{-1} of body weight of fucoidan doses were administered and both positive and negative control (n = 5 per group). Serum, liver, pancreas, and kidney biochemical indicators of oxidative stress improvement were evaluated. Measures included lipid peroxidation, superoxide dismutase and catalase activity, and antioxidant activity by assessment of free radical scavenging power and histopathological changes. The results showed an increase in the activity of antioxidant enzymes while reducing oxidative damage (lipid peroxidation index) in serum ($p \leq 0.05$) and tissues ($p \leq 0.05$). Further, no liver necrosis was observed in treated groups, unlike the Type 1 diabetes positive control group that presented mild necrosis and moderate congestion. In the pancreas, treated rats presented mild oedema, while the positive control group showed moderate oedema. A significant protective effect against oxidative stress caused by alloxan-induced diabetes was found in this model, therefore it can be concluded that fucoidan extracted from the *Lessonia trabeculata* algae could be considered a good functional compound for the control of oxidative stress in diabetic patients. Because diabetes is such a widespread public health issue, developing fucoidan-based products could be a natural way to improve patients' quality of life.

Keywords Fucoidan · *Lessonia trabeculata* · Phaeophyceae · Alloxan-induced diabetes · Oxidative stress · Antioxidants

Introduction

For centuries a large amount and variety of seaweeds has traditionally been consumed as food for their positive impact on health in different countries, mainly in Asia such as Japan, Korea, and China (Ohno and Critchley 1998). Among seaweed components are protein, carbohydrates, essential fatty acids, dietary fibres, flavonoids, vitamins and minerals, while the proportion of lipids is very low (MacArtain et al. 2007). Due to seaweed being considered natural and safe, a great variety of seaweed extracts have been studied as important sources for a variety of bioactive compounds with potential beneficial health effects (Sun et al. 2018; Murai et al. 2021). One of the most biologically important compounds present in brown algae is fucoidan (Li et al. 2008), which is widely studied for its potential positive effects on health (Fitton et al. 2015; Abdel-Latif et al. 2022). There are countless studies demonstrating the positive effects against certain pathologies such as liver

✉ Roberto Abdala
abdala@uma.es

✉ Juan Decara
juandecara@uma.es

[1] Universidad Científica del Sur, Lima, Peru

[2] Instituto de Investigación de Bioquímica y Biología Molecular (IIBBM), Universidad Nacional Agraria La Molina (UNALM), Lima, Peru

[3] Instituto de Biotecnología y Desarrollo Azul (IBYDA), Departamento de Ecología y Geología, Facultad de Ciencias, Universidad de Málaga, Campus Universitario de Teatinos s/n, 29071 Málaga, Spain

[4] Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Regional Universitario de Málaga, UGC Salud Mental, Av. Carlos Haya 82, 29010 Málaga, Spain

disease (Li et al. 2020), brain injury (Wang et al. 2021), viral infections (Pradhan et al. 2022) inflammatory disease (Sanjeewa et al. 2021), overall having a marked antioxidant activity (El Rashed et al. 2021). Special interest in the antioxidant capability of fucoidan extracts from brown algae has been extensively researched for their use in diabetes treatment (Reys et al. 2022).

Diabetes mellitus, more commonly known as diabetes, is a metabolic disease characterized by chronic hyperglycaemia due to defects in insulin response (Saeedi et al. 2019). Due to the anabolic effect of insulin, its lack of activity can cause different types of abnormalities in the metabolism of carbohydrates, lipids and proteins causing an inadequate response of its target tissues such as skeletal muscles, adipose tissue and liver. Depending on the causes and risk factors diabetes can differ into two types: Type 1 diabetes (T1D), or insulin-dependent diabetes, and Type 2 diabetes (T2D), or insulin resistance. T1D is characterized by an alteration of the pancreas' beta cells, which cannot produce insulin, whereas in T2D, the production of insulin remains normal but cannot be used optimally by the target tissues. Both kinds of diabetes are endocrinological diseases of multifactorial or genetic origin, characterized by abnormally high levels of glucose in the blood being fasted or in a postprandial state currently being a major global public health problem (Kharroubi and Darwish 2015). Many of the symptoms are not limited to organs such as the liver, kidneys, and eyes, but also involve the increase in free radicals, oxidative stress and many metabolic stress factors (Asmat et al. 2016) that worsen the disease pattern and further complicate the development of the pathogenesis of diabetes (Kasznicki et al. 2012).

Several reports suggest that oxidative stress plays an important role in the development of complications in diabetes in the pancreas (Eguchi et al. 2021), liver (Harrison 2006) and kidney (Dave and Kalia 2007). These complications included alteration in the antioxidant system by measuring superoxide dismutase (SOD), catalase activity (CAT), malondialdehyde (MDA), reduced glutathione (GSH), and oxidized proteins levels (Darenskaya et al. 2021). Oxidation/reduction (redox) imbalance can lead to a worsening of the diabetic pathology already present and also contribute to its cause.

It has long been known that seaweed extracts can positively influence some of the most important symptoms of diabetes such as lowering blood glucose (Sharifuddin et al. 2015) reducing blood lipids (Ara et al. 2002) and improving the activity of antioxidant enzymes (Kim et al. 2009). This bioactivity is due to certain bioactive compounds such as polyphenols, carrageenan, fucoidans, a long-chain sulphated polysaccharide found in high concentrations in various species of brown algae (Li et al. 2008). Fucoidans are present in algal cell walls, and contain large percentages of L-fucose and sulfate ester groups, with small proportions of uronic acids, galactose, xylose, and mannose. Fucoidans have in vitro antioxidant effects (Wang et al. 2010), via the inhibitory effect on the formation of hydroxyl (HO^-) and superoxide (O_2^-) radicals (Rocha de Souza et al. 2007). In vivo studies also show the fucoidan's beneficial effects on diabetes symptoms (Wang et al. 2015). For the present study, we used fucoidan extracted from the brown alga *Lessonia trabeculata*, a species of kelp that is abundant and of great economic importance on the coasts of Peru. The concentration of fucoidan present in *L. trabeculata* is similar, on average, to that found in other algae of the class Phaeophyceae (Ponce and Stortz 2020).

We hypothesize that the fucoidan extract obtained from *L. trabeculata* exhibits hypoglycaemic effects and antioxidant properties in a validated animal model of T1D. Thus, the present study was primarily focused on the extraction of fucoidan from *L. trabeculata* (LtFc) collected on the Peruvian coast with a method that optimizes the preservation and structural integrity of molecules. Once the fucoidan extract was obtained it was studied for potential to act as an ameliorative agent of oxidative stress while improving diabetic hyperglycaemia. For this purpose we used an animal model of alloxan-induced diabetes (AID), a common model of insulin-dependent diabetes caused by the selective damage of beta cells in the pancreas (Ighodaro et al. 2017), which we treat with different doses of parenteral LtFc. Based on previously cited evidence, we evaluated antioxidant activity by measuring lipid peroxidation levels, SOD, CAT, and the antioxidant activity potential of LtFc. In addition, glycaemic control and histopathological changes in the liver, pancreas and kidney in these in vivo models were analysed.

Materials and methods

Extraction and chemical characterization of fucoidan

Fucoidans extraction from *Lessonia trabeculata*

The seaweed material corresponds to specimens of the brown alga *Lessonia trabeculata* (Villouta and Santelices 1986), which was collected in the San Nicolás de Marcona bay (15°15′21.0″S, 75°14′31.0″W), Nazca, Ica, Peru, in the winter of 2018 and identified as between juvenile and adult stage according to the size and by the width of the fronds. All processes for fucoidan extraction were carried out in the Instituto de Investigación de Bioquímica y Biología Molecular (IIBBM) of the Universidad Nacional Agraria La Molina (UNALM), Lima, Peru, following the method of Ale et al. (2012). The material was washed with saline water and the fronds, stipes and holdfast were allowed to dry separately,

first at room temperature. in the laboratory for 15 days with periodic removals and then in an oven until completely dry at a temperature of 40 °C. Subsequently, grinding was carried out with a manual mill, and then by a sieve with a 500-µm mesh to finally obtain the algae flour. The *L. trabeculata* samples were identified as specimens corresponding to a stage between juvenile and adult by size, approximately 2 min, and by the width of the fronds.

The first stage, or pre-treatment, consisted of removing pigments and phenolic compounds that can interfere with fractionation and purification. A mixture of MeOH/$CHCl_3$/H_2O (4:2:1) was added to all the dry, ground and 500 µm sieved material and placed on a hot plate at 50 °C for 12 h with constant stirring. After some time, the solvent was filtered and discarded, repeating this process until a filtered mixture of solvents was obtained that was as colourless as possible to ensure the elimination of the pigments.

The second stage was the extraction of the fucoidan from the mixture. 0.07 M HCl solution was added to the pre-treated sample and it was placed on a hot plate at 90 °C for 3 h with constant agitation. After this incubation, the mixture was left to sediment. The supernatant liquid (SL1) was decanted and the sediment still suspended was centrifuged at 1800 × *g* for 10 min, obtaining another supernatant (DL1). The obtained sediment (SD1) underwent a second extraction process in a similar way, from which a supernatant liquid 2 (SL2), decantated liquid 2 (DL2), and another sediment 2 (SD2) were obtained. The mixture of SL1, DL1, SL2 and DL2 was treated with 1 M $CaCl_2$ to precipitate the alginates while fucoidans remained in the supernatant. The supernatant was treated with 2:1 v/v of 70% EtOH and then centrifuged at 800 × *g*. The final obtained sediment, LtFc, was washed with EtOH and dried at 40 °C. The yield of LtFc was calculated by the difference between the initial weight of dried and ground alga and the final extract powder, while the LtFc fucoidan percentage was calculated using the following formula:

$$\%\ Fucoidan = \frac{\%carbohydrates + \%sulphates}{100 - humidity} \times 100$$

Phytochemical screening of fucoidan extract

Total carbohydrates as fucose content of fucoidans were analysed with the phenol–sulfuric acid (DuBois et al. 1956), respectively, using fucose (Sigma Chemical Co., USA) as standard. Total protein concentration was determined using the Lowry method (Lowry et al. 1951) with bovine serum albumin (BSA, Sigma) as standard. The sulfate group content was determined using the barium chloride-gelatin method (Dodgson and Price 1962). The determination of uronic acids as galacturonic acid was carried out by direct titration with a standard sodium hydroxide solution on the exhaustively electrodialyzed sample and by the m-hydroxyphenyl sulfuric method (Blumenkrantz and Asboe-Hansen 1973). For the determination of total polyphenols, the Folin-Ciocalteu method modified was used (Cicco et al. 2009). A mixture of phosphotungstic acid and phosphomolybdic acid, fform a yellow colour to a blue colour consisting of oxides tungsten and molybdenum, and using a standard solution of gallic acid as a reference. The total flavonoid content of the dried extract was determined by the aluminium chloride ($AlCl_3$) colorimetric method (Chang et al. 2020) using rutin for a calibration curve and the result was expressed as mg rutin equivalent per g of dry weight.

Molecular weight determination

To determine molecular weight (MW), a 1% fucoidan solution was aliquoted in triplicate of 10, 20 and 30 µL and brought to a final volume of 500 µL with water. Then, 500 µL of 0.05% $K_3[Fe(CN)_6]$ and 500 µL of 0.065% in 0.053% Na_2CO_3 solution were added, homogenizing the mixture and heating in a water bath at 100 °C for 15 min. Once cooled to room temperature, 2.5 mL of a solution of 1.5 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 1 g of sodium dodecyl sulfate (SDS) in 1 L of 0.05 of H_2SO_4 were added. Once homogenized, the resulting solution was measured at 690 nm. In the same way, the calibration curve was prepared with a stock solution of galactose 1 µM. The determination of the MW (number average) applies the following equation:

$$MW = \frac{m \times \%CH \times P \times V_a \times 10}{A \times V_t} \times MW_{ms} \frac{180}{162}$$

where; *m*: mass of polysaccharide in mg; *% CH*: percentage of total carbohydrates of the polysaccharide; *P*: the slope of the curve of absorbance vs sugar concentration; V_a: volume of the aliquot of the polysaccharide solution; *A*: absorbance; V_t: total volume of the solution (0.5 mL); MW_{ms}: average MW of the monosaccharide unit; 180/162: the factor that corrects the fact that the MW of the monosaccharide unit in the polysaccharide is 18 mass units less than the sugar used as a standard according to the method proposed by Park and Johnson (1949).

In vivo study

Animals and ethics statement

The study was carried out in 25 male Wistar rats provided *by* Bioterio of Universidad Nacional Agraria La Molina weighing approximately 220–250 g, which were placed in standard conditions of animal experimentation including individual cages per animal and maintaining an average ambient

temperature of 22–25° C, humidity 75% with a 12-h photoperiod. All animals received standard rodent chow pellets (2.9 kcal g^{-1}) and water ad libitum during the experiment. Handle and care of animals was made by the Declaration of the World Medical Association on the Use of Animals in Biomedical Research (Reaffirmed by the 203rd WMA Council Session, Buenos Aires, Argentina, April 2016: www.wma.net/policies-post/wma-statement-on-animal-use-in-biomedical-research/), doing all possible efforts to minimize animal suffering and reducing the number of rats used per experimental group. The pertinent authorized protocols for animal research are included in the approved Project; "Desarrollo e Implementación de Procesos Tecnológicos de Validación Analítica y Bioactiva para fucoidano de algas pardas como suplementos nutricionales para humanos" (143-PNICP-PIAP-2015, INNOVATE-PERU), 1 January 2015.

Experimental groups and chronic treatment

Five experimental groups (n = 5) for 30-day chronic LtFc treatment were established for the study; a diabetes positive control (T1D), 75 mg kg^{-1}, 100 mg kg^{-1} and 125 mg kg^{-1} of body weight (BW) and non-diabetic counterpart negative control (NDC). For three doses of treatments, LtFc solution was prepared to dissolve it in saline solution (0.9% NaCl) and parenteral daily administered by gavage after 8 h of fasting to the corresponding group in a volume of 1 mL kg^{-1} of BW for 30 days. The doses were selected based on previous works and covered the range of protective effects against oxidative stress (Heeba and Morsy 2015). For diabetes positive control and late treated groups, the induction of T1D in rats was made with a mixture of 225 mg kg^{-1} BW nicotinamide (98% purity, Merck: cas98920) and 100 mg kg^{-1} BW alloxan (98% purity, Merck: cas2244113) dissolved in saline solution as intraperitoneal (IP) injected to the rats in a 12-h fasting state. NIC was used to partially protect pancreatic beta cells from the cytotoxic effect of alloxan (Abdel-Rahman et al. 1992). All treatments started after the induction of T1D on day 7. For NDC negative control a vehicle saline solution was administered to the rats in the same way as the treatments. The BW of the animals was daily recorded.

Glycaemia measurement

Seven days after T1D induction, the blood glucose concentration (mg dL^{-1}) was determined in the animals deprived of food for 18 h. Tail blood samples were collected by small cut them glucose concentration was determined using a standard glucose oxidase method by Accutrend glucometer (Roche Diagnostics, Germany). The same method was used to determine glycaemia at the end of treatment.

Sample collection

At the end of chronic treatment, all animals were sacrificed by ketamine IP overdose (320 mg kg^{-1} BW). Subsequently, the blood was extracted by cardiac puncture and collected in Vacutainer tubes with EDTA-2Na as an anticoagulant (Becton Dickinson, Madrid). It was allowed to stand at room temperature for 30 min and then centrifuged at 1000 × *g* for 5 min at 4 ºC. The plasma was collected in cryovials and stored at -80 °C until later use.

Immediately after collecting the blood, perfusion was performed through the aorta artery, using saline solution. After perfusion, the pancreas, liver, and kidneys were quickly dissected, weighed, and a piece of the tissue was fixed in 4% formaldehyde for histopathological studies. The rest of the tissues were homogenised in 0.05 M phosphate-buffered saline (PBS) pH 7.4, centrifuged at 1250 × *g* for 5 min and the supernatant was separated to later carry out biochemical essays.

Antioxidant capability determination

Malondialdehyde quantification To determine lipoperoxidation, the thiobarbituric acid (TBA) method was used (Buege and Aust 1978). The plasma or tissue homogenate was mixed with 20% trichloroacetic acid in HCl, to deproteinize and eliminate interferences. Then, it was centrifuged and the supernatant was separated. The supernatant was mixed with TBA (98% purity, Sigma-Aldrich: CAS504176) and incubated in a hot water bath at 97 °C for 30 min. The colour change was quantified at 535 nm. To quantify the MDA levels in the samples, a calibration curve was made with MDA (98% purity, Sigma-Aldrich: CAS4764174) standard solutions (0.042–0.83 µM). The final results of MDA activity were expressed as IU mL^{-1} min^{-1}.

Superoxide dismutase activity quantification The SOD activity quantification was performed according to Marklund and Marklund (1974). The delipidated plasma sample (30 mL chloroform and 50 mL cold methanol per 100 mL of plasma) was mixed with Tris–HCl buffer pH 8.2 and pyrogallol solution (98% purity, Sigma-Aldrich: CAS87661). After 10 s of reaction, the absorbance at 420 nm was read. At the same time a blank test with distilled water was carried out. The percentage of oxidation will be determined by dividing the absorbances of the sample and the blank, and then the percentage of inhibition of auto-oxidation and the enzymatic activity in IU mL^{-1} min^{-1} will be calculated. The de-lipidation step was not required for tissue homogenates.

$$\text{Formula}: \text{Enzymatic activity(EA)IU mL}^{-1}\,\text{min}^{-1} = (\%\text{inhibition of } a\text{utooxidation})/5$$

Table 1 Phytochemical screening of fucoidan extract from *Lessonia trabeculata*

Phytochemical screening	Percentage average (%)	mg kg $^{-1}$ administered per dose		
		75	100	125
Carbohydrates				
Fucose	41.29 ± 3.62	30.97	41.29	51.61
Uronic acids				
Galacturonic acid	32.49 ± 14.89	24.37	32.49	40.61
Sulfates	7.85 ± 0.15	5.89	7.85	9.81
Protein	0.24 ± 0.12	0.18	0.24	0.30
Total Fucoidan content	81.87 ± 6.22	61.40	81.87	102.33
	mg (100 g)$^{-1}$ of dry weight	mg kg^{-1} administered per dose		
Flavonoids	1.52 ± 0.33	1.14	1.52	1.91
Polyphenols	11.53 ± 1.28	8.67	11.56	14.45
Fucoidan yield	1.71 ± 0.13 g (100g)$^{-1}$ of dry algae			

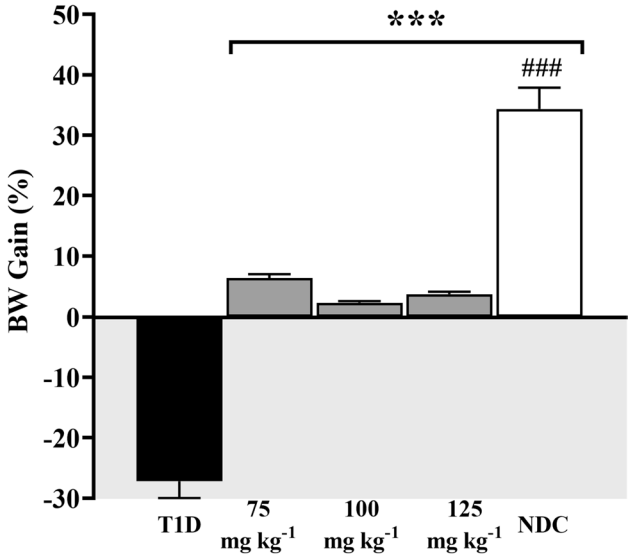

Fig. 1 Effect of chronic fucoidan extract from *Lessonia trabeculata* (LtFc) by gavage administration at the end of 30 days of treatments on body weight (BW) gain in male Wistar rats. Bars are means ± standard error of the mean (SEM) ($n = 5$ animals per group). Data were analysed by one-way ANOVA and Tukey's post hoc multiple comparisons test. (***) $p < 0.001$ indicates significant differences compared with the Type 1 diabetes rats (T1D) group. (###) $p < 0.001$ indicates significant differences compared with all treated groups (75 mg kg^{-1}, 100 mg kg^{-1} and 125 mg kg^{-1})

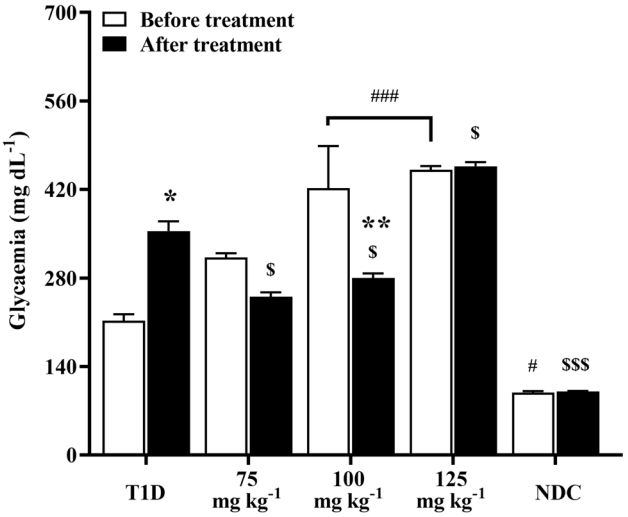

Fig. 2 Effects of administration of fucoidan extract from *Lessonia trabeculata* (LtFc) before and after gavage chronic administration on the blood levels of glucose (glycaemia) in male Wistar rats. Data are expressed as means ± standard error of the mean SEM ($n = 5$ animals/group) analysed by Two-way ANOVA (time and treatment) and Tukey's post hoc multiple comparisons test. (*) $p < 0.05$ and (**) $p < 0.01$ significant differences compared with Before treated group. (#) $p < 0.05$ and (###) $p < 0.001$ significant differences compared T1D Before treatment group. ($) $p < 0.05$ and ($$$) $p < 0.001$ significant differences compared T1D after treatment group

where: % inhibition of autooxidation = 100 -% oxidation

$$\% \text{ oxidation} = (\Delta \text{ODsample}/\Delta \text{ODblank}) \times 100$$

Catalase activity quantification CAT activity quantification was performed, according to Aebi (1984). Briefly, 950 µL of a 50 mM PBS pH 7 was added to a 1 cm diameter cuvette and then 40 µL of the sample (plasma or tissue homogenate) was added. It was mixed at 25 °C and read at 240 nm (Blank). Then, 10 µL of 6% H_2O_2 in PBS was immediately added. After a minute of reaction, the mix was read again at 240 nm. The average change in hydrogen peroxide absorbance per minute was calculated. CAT activity was expressed in IU mL^{-1} min^{-1}.

Antioxidant activity quantification The determination was carried out by the method of Re et al. (1999). 50 µL of the sample (plasma) was mixed with 950 µL of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS) solution (ABTS Solution ready-to-use, ref 11,684,302,001, Roche Diagnostics, Germany). Then, it was homogenized and allowed to react for 10 min in darkness. After a time,

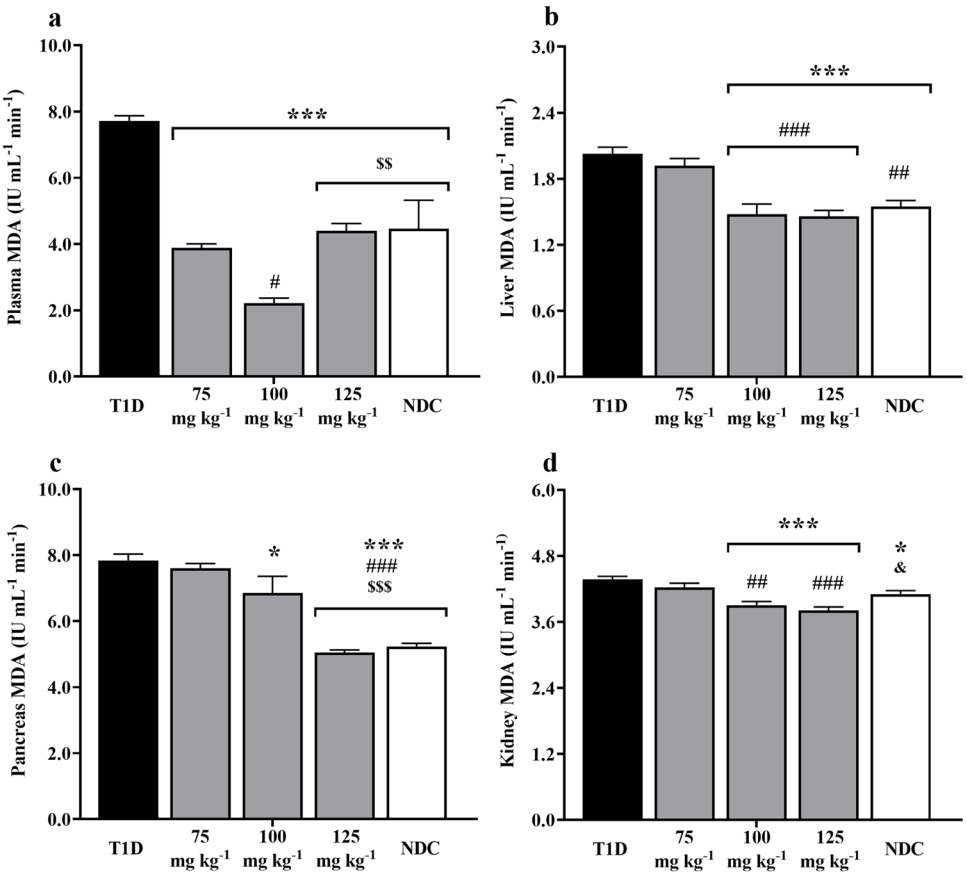

Fig. 3 Effect of chronic fucoidan extract from *Lessonia trabeculata* (LtFc) treatment by gavage administration on tissues malondialdehyde (MDA) activity in male Wistar rats. Bars are means ± standard error of the mean SEM ($n = 5$ animals per group). Data were analysed by Two-way ANOVA and Tukey's post hoc test. (***) $p < 0.001$ denotes significant differences compared with the Type 1 diabetes (T1D) group. (#) $p < 0.05$, (##) $p < 0.01$ and (###) $p < 0.001$ denotes significant differences compared with 75 mg kg^{-1} group. ($$) $p < 0.01$ denotes significant differences compared with the 100 mg kg^{-1} group

the absorbance of the solutions was at 732 nm. The blank sample was prepared with 50 µL of distilled water and 950 µL of ABTS. This solution was also measured at 732 nm. To determine the antioxidant capacity, a standard curve of the reference antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (98% purity, Sigma-Aldrich: CAS53188071) equivalent antioxidant capacity (TEAC) was performed. For the quantification of antioxidant activity in tissues, a variation of the technique according to Joyeux et al. (1995) was carried out.

Histopathological evaluation

The liver, pancreas, and kidney tissue samples were fixed in 10% formaldehyde in 0.1 M PBS for 24 hfolllowed by dehydration, clarification, and impregnation steps. Then, they were embedded in 60% paraffin and cut at 4 m with a horizontal microtome and stained with hematoxylin–eosin. Slides were placed on a binocular optical microscope (Leica DM500 and EC3 digital camera, Leica Microsystems) to determine the main tissue lesions: necrosis, degenerative changes, and congestion. Pancreatic islet counting was performed using the Gomori staining technique (Trichrome Stain AB solution, Sigma-Aldrich: RefHT10516). Size of islets: small: < 50 µm, medium 50—250 µm, large: > 250 µm (Carter et al. 2009). Five rats per group and two sections of the pancreas, and kidney tissue samples per animal were analysed.

Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM). A size of 5 samples per treated group (n = 5) was taken into account for the statistical analysis of the different studies. Statistical analysis was performed using version 8 of GraphPad Prism software (GraphPad Software Inc). One-way or two-way Analysis of Variance (ANOVA) was assessed followed by Tukey's post hoc multiple comparisons test for all results. The post hoc tests only were conducted if the F value in ANOVA reached a $p < 0.05$ while homogeneity of variance was not statistically significant. The results were considered statistically significant at $p < 0.05$.

Results

A yield of 1.71 ± 0.13 (n = 5) g of fucoidan extract was obtained for every 100 g of dried and ground alga. Fucoidan content was 81.87 ± 6.22% and results of the phytochemical

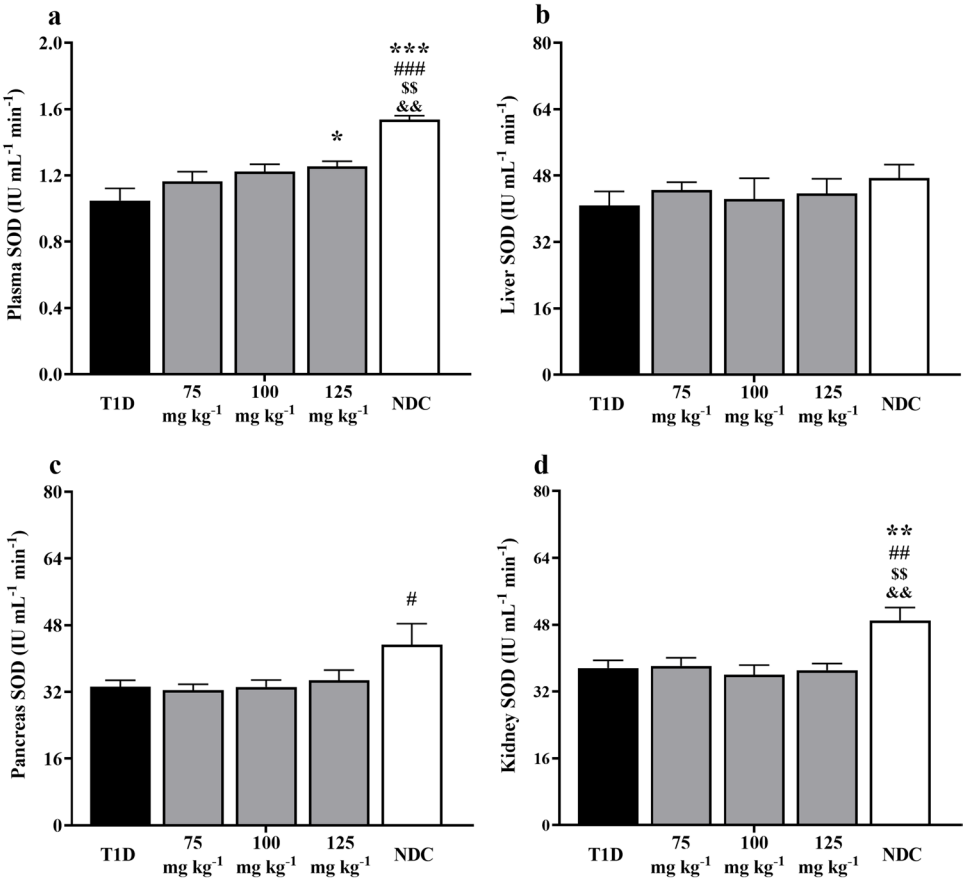

Fig. 4 Effect of chronic fucoidan extract from *Lessonia trabeculata* (LtFc) treatment by gavage administration on tissues superoxide dismutase (SOD) activity in male Wistar rats. Bars are means ± standard error of the mean SEM ($n = 5$ animals per group). Data were analysed by Two-way ANOVA and Tukey's post hoc test. (**) $p < 0.01$ and (***) $p < 0.001$, denotes significant differences compared with the Type 1 diabetes (T1D) group. (#) $p < 0.05$, (##) $p < 0.01$ and (###) $p < 0.001$ denotes significant differences compared with 75 mg kg^{-1} group. ($$) $p < 0.01$ denotes significant differences compared with the 100 mg kg^{-1} group. (&&) $p < 0.01$ denotes significant differences compared with the 125 mg kg^{-1} group

screening of extract showed an abundant presence of carbohydrates, uronic acids and sulfates. The MW calculated for the fucoidan was 10.04 ± 0.02 kDa. Table 1 shows a summary of phytochemical screening of fucoidan and the chemical composition for administered doses.

We determined the prevention of weight loss in the T1D rat model with fucoidan extract from *L. trabeculata*. Throughout the experiment, as expected, T1D positive control rats lost weight at an average of 27.21 ± 2.77% while the NDC gained weight at an average of 34.34 ± 3.51% ($p < 0.001$). As shown in Fig. 1, all the treated groups have a slight tendency to gain weight with treatment. If the BW gain after each treatment is compared to T1D, the changes are highly significant ($p < 0.001$). No significant differences were found between the three treatments.

Throughout the experiment, blood glucose levels constantly increased after induction to diabetes in the T1D positive control group while NDC negative control group remained stable. On one side, the glucose levels of both 75 and 100 mg kg^{-1} treatment groups also changed through the experiments on the first day of T1D induction ($p < 0.05$ and $p < 0.01$ respectively), and on the other hand they reverted significantly the hyperglycaemia regarding the T1D ($p < 0.05$) (Fig. 2).

To determine the capability of LtFc to reduce diabetic oxidative stress we study the MDA activity in plasma, liver, pancreas and kidney tissues. It can show the diminished concentration of MDA after chronic treatments compared with T1D for all LtFc doses ($p < 0.001$) in plasma (Fig. 3a). The dose of 125 mg kg^{-1} was able to equal the activity of MDA in the NDC group. If the different doses are compared, 100 mg kg^{-1} was the most effective ($p < 0.05$). A positive result was obtained in the analyses of the liver (Fig. 3b) when both 100 and 125 mg kg^{-1} doses decreased the MDA activity ($p < 0.01$) matching it with the levels present in the NDC group. Similar results to the liver were obtained in the pancreas (Fig. 3c) and kidney (Fig. 3d) when MDA activity was diminished by 100 and 125 mg kg^{-1} doses, staying unchanged with 75 mg kg^{-1} dose.

To evaluate the capability of removing the superoxide and peroxides from select tissues, the activity of SOD and CAT was measured in the select tissues. According to the T1D model, the SOD enzyme showed lower activity in the positive control compared to the NDC group, except in the liver where no significant differences were found. None of the three treatment doses was able to improve SOD activity in the analysed tissues (Fig. 4a–d).

The activity of CAT enzyme activity has detected an increase in T1D regarding 100 mg kg^{-1} ($p < 0.05$), 125 mg kg^{-1} and NDC groups ($p < 0.001$) in plasma (Fig. 5a). However, in both the liver and pancreas, the

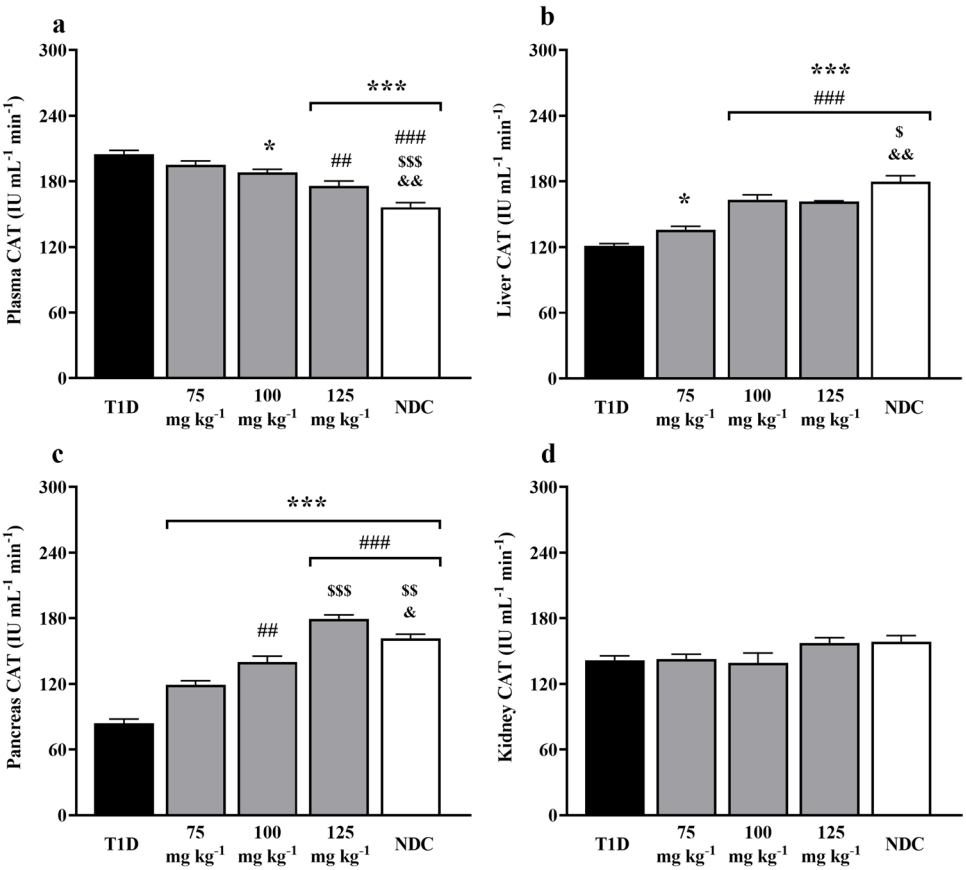

Fig. 5 Effect of chronic fucoidan extract from *Lessonia trabeculata* (LtFc) treatment by gavage administration on tissues catalase enzyme (CAT) activity in male Wistar rats. Bars are means ± standard error of the mean SEM ($n = 5$ animals per group). Data were analysed by Two-way ANOVA and Tukey's post hoc test. (*) $p < 0.05$ and (***) $p < 0.001$ denotes significant differences compared with the Type 1 diabetes (T1D) group. (##) $p < 0.01$ and (###) $p < 0.001$ denotes significant differences compared with the 75 mg kg^{-1} group. ($) $p < 0.05$, ($$) $p < 0.01$ and ($$$) $p < 0.001$ denotes significant differences compared with 100 mg kg^{-1} group. (&) $p < 0.05$ and (&&) $p < 0.01$ denotes significant differences compared with the 125 mg kg^{-1} group

increased activity of CAT was observed with treatments and NDC groups compared with T1D (Fig. 5b and c). When this same CAT activity was analysed in the kidney, no significant differences were found between any of the groups (Fig. 5d).

To quantify antioxidant activity, the percentage of ABTS inhibition was also measured at different doses of treatments. In the plasma sample, the percentage of inhibition of ABTS was equalled to the NDC with both 75 and 100 mg kg^{-1} being higher than T1D ($p < 0.001$) (Fig. 6a). No effects were found in the 125 mg kg^{-1} dose group. Results in the liver show a great improvement in ABTS inhibition where all treatments induced higher percentages of inhibition ($p < 0.001$) matching it to the NDC group (Fig. 6b). Comparable results were observed in the rest of the tissues, except the dose of 100 mg kg^{-1} in the pancreas (Fig. 6c) and 125 mg kg^{-1} in the kidney (Fig. 6d) which had no effects on ABTS inhibition.

The histopathological study of the liver, pancreas and kidney shows a reduction in tissue damage after LtFc treatments. As expected, the NDC negative control did not show congestion, necrosis or necrosis in any of the tested tissues. In turn, the T1D positive control group showed moderate congestion and mild necrosis and oedema in both liver (Fig. 7a–b) and pancreas (Fig. 8a–e) tissues respectively. None of the LtFc treatment groups showed liver necrosis compared with the T1D group. In addition, 100 and 125 mg kg^{-1} doses managed to moderate and reverse the liver congestion respectively. The kidney congestion also was improved with 100 mg kg^{-1} (moderate to mild) and reverted with 125 mg kg^{-1} treatment (Fig. 9a–e).

Microscopic observation per 4 × field allowed quantification of islets of Langerhans. We classified them depending on their size and number according to treatment. The negative control group had the highest number of islets (10) while in the T1D group the number decreased to 2. The 75 and 125 mg kg^{-1} dose treatments only showed a small increase in the number of islets (2 to 3) while the 125 mg kg^{-1} group remained unchanged. Besides, the size of all islets of Langerhans only was could differentiate in NDC negative control (4 small and 6 medium), being all small in the T1D and treatments groups. Also, as can be deduced from the observation and counting by size of the islets of Langerhans according to their size in the pancreas, the NDC group presented larger regions with respect to the treatments and the negative control ($p < 0.001$). The histopathological changes produced in the liver, pancreas and kidney are summarized in Table 2.

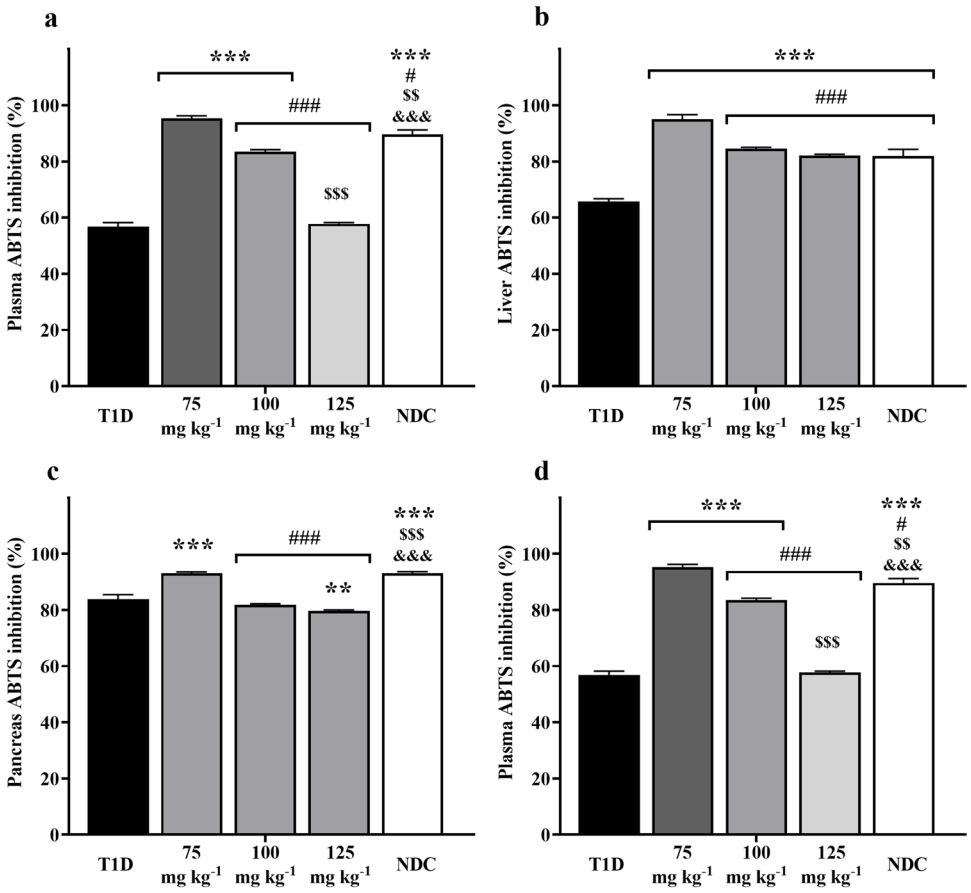

Fig. 6 Effect of chronic fucoidan extract from *Lessonia trabeculata* (LtFc) treatment by gavage administration on tissues 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) inhibition in males Wistar rats. Bars are means ± standard error of the mean SEM ($n = 5$ animals per group). Data were analysed by Two-way ANOVA and Tukey's post hoc test. (**) $p < 0.01$ and (***) $p < 0.001$ denotes significant differences compared with the Type 1 diabetes (T1D) group. (#) $p < 0.05$ and (###) $p < 0.001$ denotes significant differences compared with the 75 mg kg^{-1} group. ($$) $p < 0.01$ and ($$$) $p < 0.001$ denotes significant differences compared with the 100 mg kg^{-1} group. (&&&) $p < 0.001$ denotes significant differences compared with the 125 mg kg^{-1} group

Discussion

Marine macroalgae, in particular the Phaeophyceae such as *Lessonia* spp, contain fucoidan. This includes a group of certain fucose-containing sulfated polysaccharides but also includes branched sulfated galactofucans with fucose or fuco-oligosaccharide, and/or glucuronic acid, xylose or glucose substitutions. This complex of sulphated polysaccharides, homopolysaccharides, and heteropolysaccharides, is known as fucans and they are the metabolic product of a wide variety of significant biological activities (Ale et al. 2012). Fucans can also contain galactose, glucuronic acid, mannose and xylose (Rocha de Souza et al. 2007), and they can be used as by-products in the food and cosmetic industry (Polat et al. 2021). Fucan structural complexity varies according to the degree of branching, substituents, sulfation and type of bonds and the structure depends on the type of polysaccharide. Although the biological activity of fucoidan varies with the species, environment, MW, composition, structure or extraction method (Ale et al. 2011). Currently, fucoidan is the subject of an extensive study on its various therapeutic effects (Luthuli et al. 2019; Wang et al. 2019). Our results on MW are consistent with the main literature, but perhaps both the extraction yield and the chemical composition of fucoidan may be a bit low compared to previous work (Qu et al. 2014). Nevertheless, the fucose content was relatively more elevated that cited for *Lessonia* spp (Qu et al. 2014). For reproducible and trusted biological activities, flavonoids, polyphenols and proteins should be quantified to determine the quality grade of fucoidans. Although these extract components have biological activity, they are present in low concentrations; around 11% for flavonoids, less than 2% for polyphenols and 0.2% for proteins. Thus, it is probable that these secondary metabolites exist in the fucoidan extract of LtFc influence the antioxidant activity. Even some, such as the polyphenols extracted from *L. trabeculata*, could be beneficial for the intestinal microflora, as was verified in diabetic rats, in which the dysbiosis of the microbial ecology in diabetic rats could be regulated (Yuan et al. 2019). In addition, fucoidan is present to greater proportion, more than 80%, in the extract when compared to others secondary metabolites. For that reason, we could hypothesise that the main beneficial effect of extracted from LtFc on health would be due to the presence of fucoidan. In this regard, the proportions of fucoidan found in our extract were consistent with the Phaeophyceae described in the previous reports (Ponce and Stortz 2020).

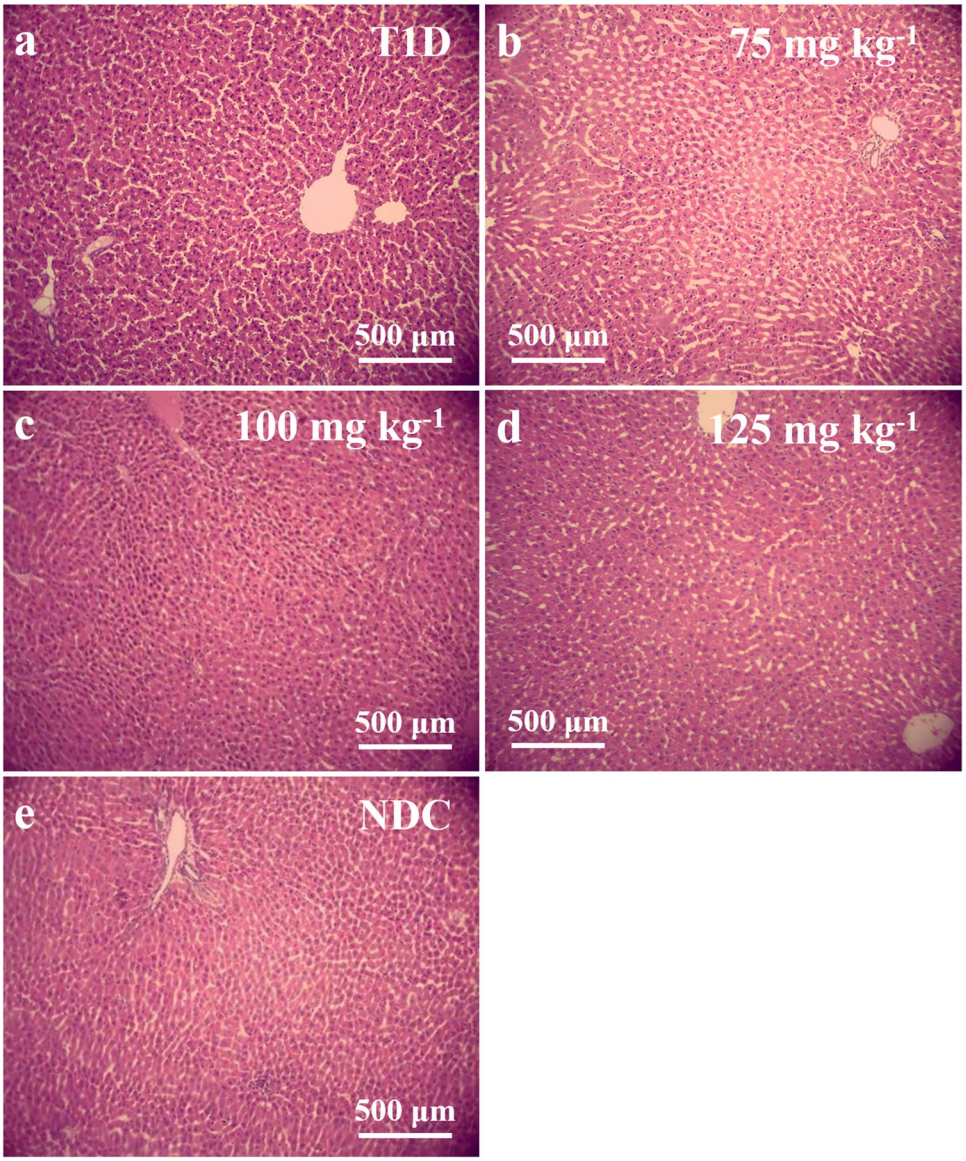

Fig. 7 Representative microscopic images (4x) of liver sections with haematoxylin–eosin staining. (**a**) positive control T1D section showed level of congestion 2 (moderate) and necrosis 1 (Mild); (**b**) 75 mg kg^{-1} fucoidan extract from *Lessonia trabeculata* (LtFc) treatment showed congestion level 2 (moderate) without necrosis; (**c**) 100 mg kg^{-1} LtFc treatment showed congestion level 1 (mild) without necrosis; (**d**) 125 mg kg^{-1} LtFc treatment did not show congestion without necrosis; (**e**) negative control NDC section did not show congestion or necrosis

Hyperglycaemia is one of the main causes of diabetes complications. For this reason there are numerous studies to control this elevation by natural edible compounds due to an absence of side effects. There is previous evidence that fucoidan extract from different seaweed fulfils this premise (Abe et al. 2013) being also easy to obtain and use as a nutraceutical supplement (Fitton et al. 2019). Among other things; fucoidan has been extensively studied in animal models of metabolic syndrome (Yokota et al. 2016; Shang et al. 2017), improver of insulin resistance and fatty liver (Heeba and Morsy 2015) and as a hypoglycaemic agent (Jiang et al. 2015; Shan et al. 2016). In this way, our positive results on the insulin-dependent diabetes animal model confirm this activity when the LtFc is chronically administered.

In diabetic individuals one of the important signs of T1D is weight loss due to the inefficient use of glucose despite the existing hyperglycaemia, which would cause the body to consume lipid deposits and muscle proteins to obtain energy. In the present study, the variation in BW was evaluated finding that all treatment doses with LtFc were able to avoid weight loss. These findings are consistent with a study conducted by Cui et al. (2014).

There is controversy about the relationship between late complications in diabetes and oxidative stress. Although, has been studied a relationship between the increase in ROS production and the decrease in antioxidant systems in diabetic patients (Jiang et al. 2015). Studies also have shown the presence of products derived from lipid peroxidation in the blood and tissues of diabetic subjects which can interact with DNA and damage it (Hoeldtke et al. 2009). Therefore the control of this oxidative stress in diabetes is vitally important, and there is clinical evidence that medical conditions

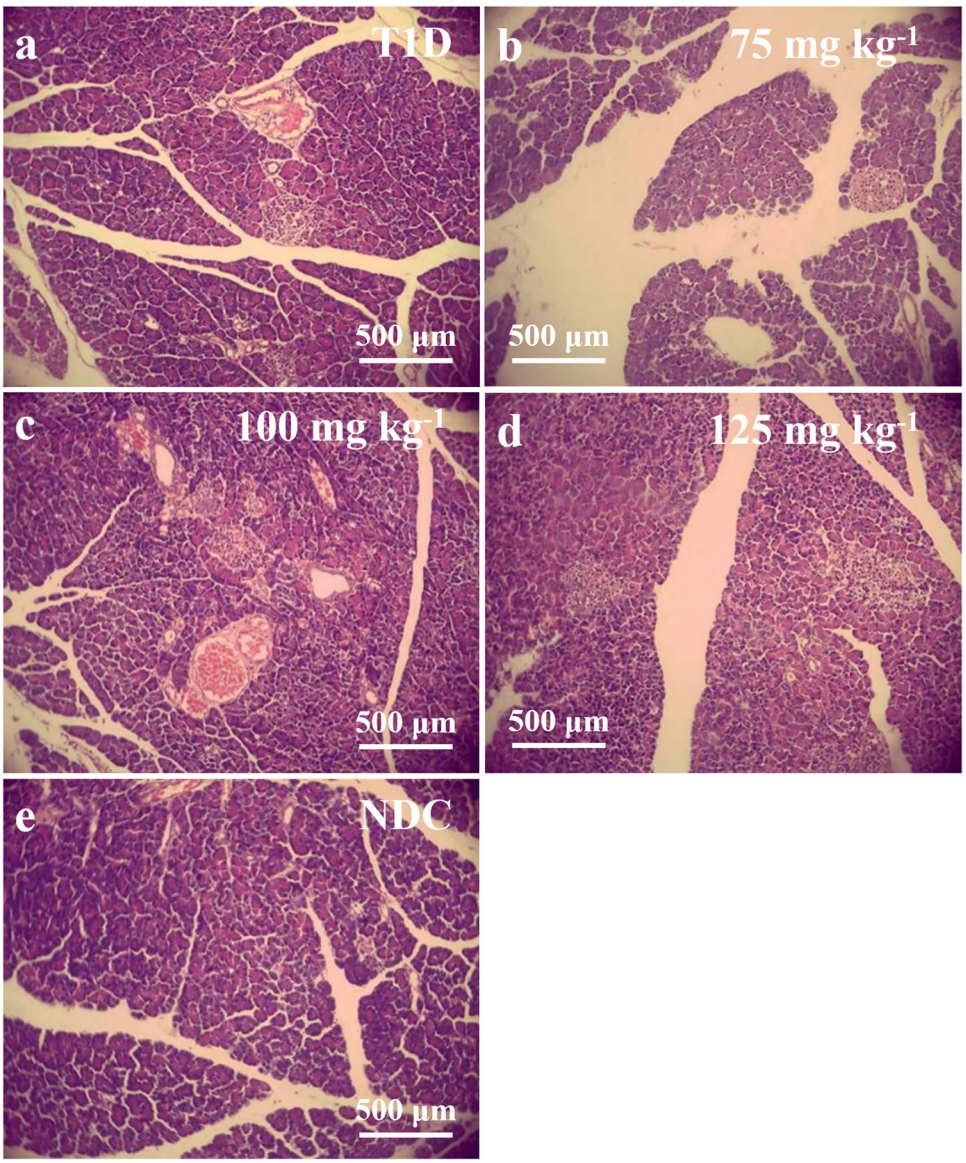

Fig. 8 Representative microscopic images (4x) of pancreas sections with hematoxylin–eosin staining. (**a**) positive control T1D section showed level 2 of congestion and oedema (moderate); (**b**) 75 mg kg^{-1} fucoidan extract from *Lessonia trabeculata* (LtFc) treatment showed congestion level 0 and level 1 of oedema (Mild); (**c**) 100 mg kg^{-1} LtFc treatment showed congestion level 2 (moderate) and level 1 of oedema (mild); (**d**) 125 mg kg^{-1} LtFc treatment did not showed congestion although it did present oedema level 1 (mild); (**e**) negative control NDC section did not show congestion or oedema

can improve with antioxidant supplementation (Golbidi et al. 2011). We also studied the protective activity of fucoidan against oxidative stress present in plasma, liver, pancreas and kidney in AID rats. AID is a validated and standard model, especially for the study of natural therapeutic products such as algae extracts against T1D (Ighodaro et al. 2017). Alloxan has a molecular similarity to the glucose structure and is taken up by the beta cell via the glucose transporter 2 (GLUT-2), generating hydroxyl radicals, thus facilitating the toxic and diabetogenic action (Lenzen 2008). For this, the usual markers of oxidative damage and antioxidant response altered in the animal model of T1D were analysed in comparison with its NDC and then compared with their improvement by the LtFc treatments. The relationship between lipid peroxidation and the development of complications in diabetes is known, in addition to a considerable increase in the lipid peroxidation, CAT, SOD and reduced glutathione in diabetic patients. Lipoperoxidation, is a validated indicator of oxidative damage, as measured by the concentration of MDA in plasma, liver, pancreas and kidneys. We found a significant improvement in lipoperoxidation with treatments in the four tissues analysed. These levels of improvement were comparable to NDC with the highest dose of LtFc treatment. A similar decrease in lipoperoxidation was also observed by others (Li et al. 2008; Wang et al 2010) supporting our conclusion that fucoidan prevents lipids oxidation.

Oxidative damage to macromolecules, which cannot be counteracted by antioxidant defence systems, induces various diseases, such as diabetes, atherosclerosis, inflammatory processes, Alzheimer's disease, Parkinson's disease, and various types of cancer, among others (Pham-Huy et al. 2008). Since diabetes is affected

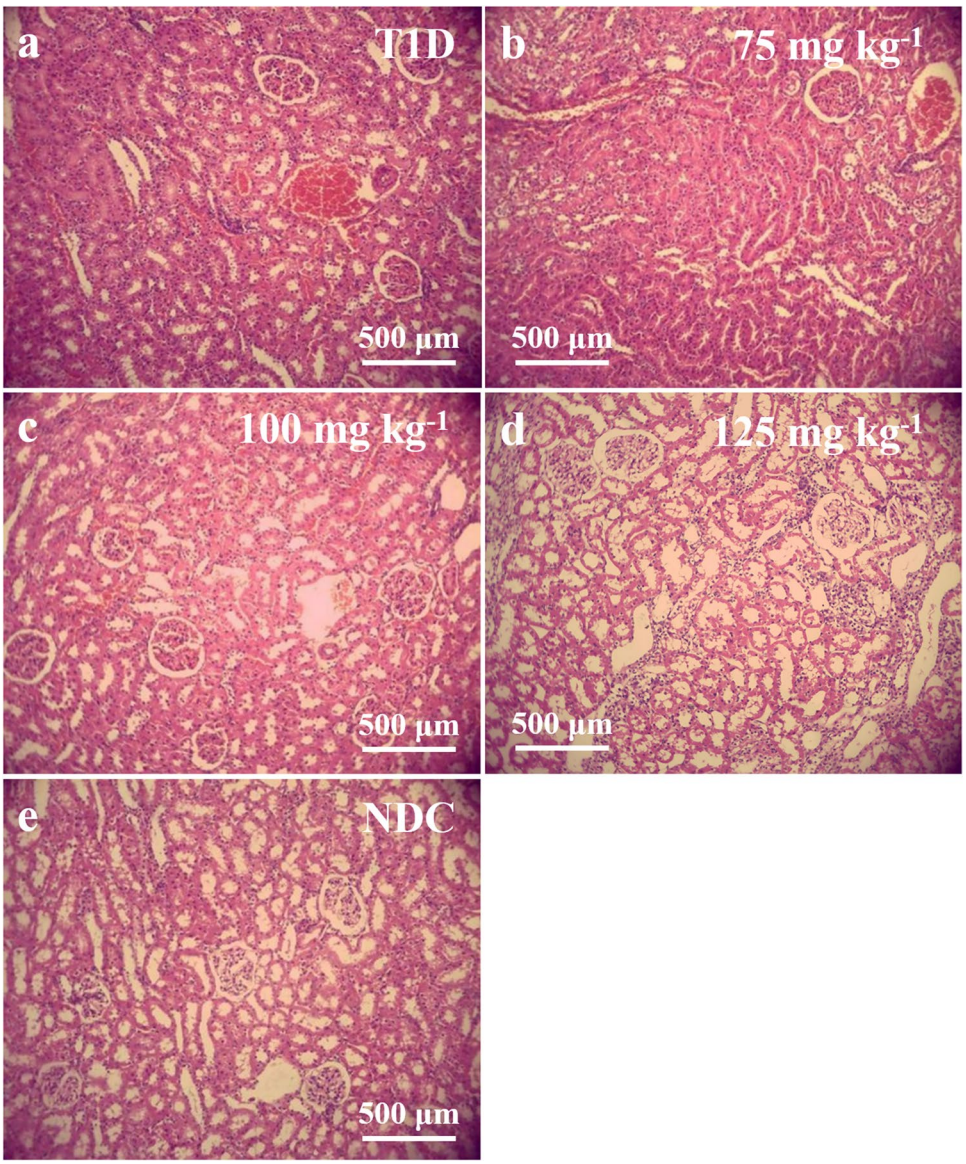

Fig. 9 Representative microscopic images of kidney sections with haematoxylin–eosin staining. Both positive control T1D (**a**) and 75 mg kg^{-1} fucoidan extract from *Lessonia trabeculata* (LtFc) dose (**b**) sections showed level 2 of congestion (moderate); (**c**) 100 mg kg^{-1} LtFc treatment showed congestion level 1 (mild); 125 mg kg^{-1} LtFc dose (**d**) and negative control NDC (**e**) did not show congestion

Table 2 Average values of histopathological evaluation variables after treatment with fucoidan extract from *Lessonia trabeculata* (LtFc) by microscopic observation per field (4x) of five rats per group (n = 5) and two sections for each tissue sample. For variables such as congestion and necrosis or oedema; 1 = mild, 2 = moderate, and 3 = severe. For the pancreas, the columns show the average number and size (small, medium and large) of islets of Langerhans. Type 1 diabetes (T1D); Non-diabetes counteract (NDC)

TISSUE GROUPS	LIVER				PANCREAS					KIDNEY
	Congestion	Necrosis	Congestion	Oedema	Islets					Congestion
					Small	Medium	Large	p mm^{-2}	Size (µm)	
T1D	2	1	2	1	2	0	0	2	41.15 ± 2.5	2
75 mg kg^{-1}	2	0	0	1	3	0	0	3	39.33 ± 4.48	2
100 mg kg^{-1}	1	0	2	1	2	0	0	2	42.00 ± 2.12	1
125 mg kg^{-1}	0	0	0	1	3	0	0	3	39.33 ± 1.44	0
NDC	0	0	0	0	4	6	0	10	112 ± 22.39	0

by this damage to macromolecules, we also measured other indicators of the antioxidant system that could be altered, such as both SOD and CAT activity. Specifically, SOD is an O^- scavenger catalysing its dismutation into hydrogen peroxide (H_2O_2) and oxygen (O_2). The enzymatic activity of SOD measured in plasma, pancreas and kidney after LtFc treatments and non-treated rats was diminished concerning in all diabetic rats compared with NDC. Surprisingly, this alteration was not found in the liver. Although there were increases in the means of SOD activity in plasma regarding diabetic rats with the treatments, these were only statically significant with 125 mg kg^{-1} dose. Previous in vitro work of Rocha de Souza et al. (2007) reported the protective effect of fucoidan extract against superoxide anion by SOD. In this way, the LtFc treatment should have been a good and nice approach to increase SOD activity without administering it exogenously as Di Naso et al. (2011) proposed.

Concerning CAT activity, this enzyme is involved in the protective decomposition of H_2O_2 generated during cellular metabolism. The CAT has a greater affinity for the high concentrations of H_2O_2 formed in hyperglycaemic conditions and its activity is increased in diabetes (Darenskaya et al. 2021). The increase of CAT activity joins an increase in protein synthesis is an adaptation mechanism against damage by H_2O_2. The literature does not report data on the effect of fucoidan on the activity of CAT in tissues but our results also agree with a CAT increase in the T1D and treatments but only in plasma where fucoidan was able to decrease its activity. However, in the pancreas and liver, two hyperglycaemic-sensitive organs, the level of CAT increased with the treatments in the same way as NDC. The expected increase was not seen in organs mostly affected by hyperglycemia, but the levels were normalized concerning the healthy control. No changes were observed in the kidney, maybe for its function of glucose reabsorption and full recovery of filtered glucose eliminating it from the urine.

The antioxidant activity also was completed by assessing the free radical scavenging power ABTS in each studied tissue. The radical cation $ABTS^+$ has reduced thanks to the antioxidant capacity of the antioxidant components present in the tissue. The advantage of using the ABTS is that it evaluates the activity of compounds of a hydrophilic and lipophilic nature. As predicted, the ABTS percentage inhibition showed reduced in the untreated AID model for all tissues analysed in respected non-diabetic animals. Treatments with LtFc are also capable of increasing the ABTS inhibition percentage. The results obtained would agree with the statement that a breakdown of the redox equilibrium is due to the generation of free radicals. Jointly as can be observed by the results of the MDA, SOD and CAT analyses, LtFc treatments produced in minor or major levels improve antioxidant defensive systems.

The histopathological study of the liver, pancreas, and kidney in the rats after treatment concluded that there are mild and moderate degenerative changes. Necrosis is a form of cell death caused by drugs or toxins such as alloxan. This toxicity would be producing this alteration in the liver, pancreas and kidney of AID rats. On the other hand, oedema is the increase in cell volume, it can occur by a physiological process, such as a response to oxidative stress or in response to cell injury or alteration of the cell membrane (DeLeve et al. 2009). Although the duration of chronic treatment could have been a limitation for the appearance of severe degenerative changes, the treatments with higher doses did improve these alterations. In the three analysed tissues of the treated rats, the congestion was recovered concerning untreated AID rats with the high LtFc dose. Necrosis in the liver and oedema in the pancreas also were recovered and improved respectively by LtFc. In the histological study of the pancreas of animals injected with alloxan, the appearance of the islet of Langerhans necrosis with the disappearance of beta cells was also found. Despite improved oxidative damage in the pancreas, islet loss could not be reversed for treatments with LtFc.

Our findings provide more evidence of the therapeutic effect of fucoidan (Fitton et al. 2019; Luthuli et al. 2019) overall, against oxidative damage (Wang et al. 2010, 2015; Qu et al. 2014) and diabetes (Jiang et al. 2015). The main results in the present works demonstrate the protector effect on oxidative stress in a diabetes animal model both at the plasmatic and tissue level. In addition, key negative effects of diabetes such as loss of weight and hyperglycemia also improved oxidative stress related to insulin-dependent diabetes. We can conclude that LtFc supplementation has potential to partially address chronic complications of diabetes. Dietary clinical studies are warranted.

Author contributions Conception and design of the work: Lillyan Teresa Loayza-Gutiérrez, Luis Ángel Aguilar-Mendoza and Jorge Antonio Chávez-Pérez; Data collection: Lillyan Teresa Loayza-Gutiérrez and Juan Decara; Methodology: Lillyan Teresa Loayza-Gutiérrez, Eder Valdir Apumayta-Suárez and Roberto Abdala; Analysis and interpretation of the data: Lillyan Teresa Loayza-Gutiérrez, Eder Valdir Apumayta-Suárez, Luis Ángel Aguilar-Mendoza, Roberto Abdala, Jorge Antonio Chávez-Pérez and Juan Decara; Statistical analysis: Lillyan Teresa Loayza-Gutiérrez, Eder Valdir Apumayta-Suárez. and Juan Decara; Drafting the manuscript: Juan Decara and Roberto Abdala; Critical revision of the manuscript: Roberto Abdala, Luis Ángel Aguilar-Mendoza, Jorge Antonio Chávez-Pérez and Juan Decara. All authors reviewed the manuscript.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. Funding for open access charge: Universidad de Málaga / CBUA This work was supported by grants from

the following institutions: L.T.L-G., E.V.A.S., L.A.A-M. and J.A.C-P. are supported by Project "*Desarrollo e Implementación de Procesos Tecnológicos de Validación Analítica y Bioactiva para fucoidano de algas pardas como suplementos nutricionales para humanos*", *Convenio* N°143-PNICP-PIAP-2015, INNOVATE-PERU. J.D. hold a "Miguel Servet" (CP21/00021) research contract from the *Ministerio de Ciencia e Innovación*, *Instituto de Salud Carlos* III (ISCIII) co-funded by European Social Fund (ESF), "Investing in your future", *Gobierno de España*.

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

Declarations

Competing interests The authors declare no conflicts of interest.

References

Abdel-Latif HMR, Dawood MAO, Alagawany M, Faggio C, Nowosad J, Kucharczyk D (2022) Health benefits and potential applications of fucoidan (FCD) extracted from brown seaweeds in aquaculture: An updated review. Fish Shellfish Immunol 122:115–130

Abdel-Rahman MS, Elrakhawy FI, Iskander FA (1992) Protection of B cells against the effect of alloxan. Toxicol Lett 63:155–164

Abe S, Hiramatsu K, Ichikawa O, Kawamoto H, Kasagi T, Miki Y, Kimura T, Ikeda T (2013) Safety evaluation of excessive ingestion of mozuku fucoidan in human. J Food Sci 78:T648-651

Aebi H (1984) Catalase in vitro. Meth Enzymol 105:121–126

Ale MT, Mikkelsen JD, Meyer AS (2011) Important determinants for fucoidan bioactivity: a critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. Mar Drugs 9:2106–2130

Ale MT, Mikkelsen JD, Meyer AS (2012) Designed optimization of a single-step extraction of fucose-containing sulfated polysaccharides from *Sargassum* sp. J Appl Phycol 24:715–723

Ara J, Sultana V, Qasim R, Ahmad VU (2002) Hypolipidaemic activity of seaweed from Karachi coast. Phytother Res 16:479–483

Asmat U, Abad K, Ismail K (2016) Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J 24:547–553

Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54:484–489

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Meth Enzymol 52:302–310

Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS (2009) A practical guide to rodent islet isolation and assessment. Biol Proced Online 11:3–31

Chang CC, Yang MH, Wen HM, Chern JC (2020) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10:178–182

Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio V (2009) A reproducible, rapid and inexpensive Folin-Ciocalteu micromethod in determining phenolics of plant methanol extracts. Microchem J 91:107–110

Cui W, Zheng Y, Zhang Q, Wang J, Wang L, Yang W, Guo C, Gao W, Wang X, Luo D (2014) Low-molecular-weight fucoidan protects endothelial function and ameliorates basal hypertension in diabetic Goto-Kakizaki rats. Lab Invest 94:382–393

Darenskaya MA, Kolesnikova LI, Kolesnikov SI (2021) Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. Bull Exp Biol Med 171:179–189

Dave GS, Kalia K (2007) Hyperglycemia induced oxidative stress in type-1 and type-2 diabetic patients with and without nephropathy. Cell Mol Biol 53:68–78

DeLeve LD, Valla DC, Garcia-Tsao G, American Association for the Study Liver D (2009) Vascular disorders of the liver. Hepatology 49:1729–1764

Di Naso FC, Simoes Dias A, Porawski M, Marroni NA (2011) Exogenous superoxide dismutase: action on liver oxidative stress in animals with streptozotocin-induced diabetes. Exp Diabetes Res 2011:754132

Dodgson KS, Price RG (1962) A note on the determination of the ester sulphate content of sulphated polysaccharides. Biochem J 84:106–110

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Eguchi N, Vaziri ND, Dafoe DC, Ichii H (2021) The role of oxidative stress in pancreatic β-cell dysfunction in diabetes. Int J Mol Sci 22:1509

El Rashed Z, Lupidi G, Grasselli E, Canesi L, Khalifeh H, Demori I (2021) Antioxidant and antisteatotic activities of fucoidan fractions from marine and terrestrial sources. Molecules 26:4467

Fitton HJ, Stringer DS, Park AY, Karpiniec SN (2015) Therapies from fucoidan: An update. Mar Drugs 13:5920–5946

Fitton HJ, Stringer DS, Park AY, Karpiniec SN (2019) Therapies from fucoidan: New developments. Mar Drugs 17:571

Golbidi S, Ebadi SA, Laher I (2011) Antioxidants in the treatment of diabetes. Curr Diabetes Rev 7:106–125

Harrison SA (2006) Liver disease in patients with diabetes mellitus. J Clin Gastroenterol 40:68–76

Heeba GH, Morsy MA (2015) Fucoidan ameliorates steatohepatitis and insulin resistance by suppressing oxidative stress and inflammatory cytokines in experimental non-alcoholic fatty liver disease. Environ Toxicol Pharmacol 40:907–914

Hoeldtke RD, Bryner KD, Corum LL, Hobbs GR, Van Dyke K (2009) Lipid peroxidation in early type 1 diabetes mellitus is unassociated with oxidative damage to DNA. Metabolism 58:731–734

Ighodaro OM, Adeosun AM, Akinloye OA (2017) Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (Kaunas) 53:365–374

Jiang X, Yu J, Ma Z, Zhang H, Xie F (2015) Effects of fucoidan on insulin stimulation and pancreatic protection via the cAMP signaling pathway in vivo and in vitro. Mol Med Rep 12:4501–4507

Joyeux M, Lobstein A, Anton R, Mortier F (1995) Comparative antilipoperoxidant, antinecrotic and scavenging properties of terpenes and biflavones from *Ginkgo* and some flavonoids. Planta Med 61:126–129

Kasznicki J, Kosmalski M, Sliwinska A, Mrowicka SM, Majsterek I, Drzewoski I (2012) Evaluation of oxidative stress markers in pathogenesis of diabetic neuropathy. Mol Biol Rep 39:8669–8678

Kharroubi AT, Darwish HM (2015) Diabetes mellitus: The epidemic of the century. World J Diabetes 6:850–867

Kim AR, Shin TS, Lee MS, Park JY, Park KE, Yoon NY, Kim JS, Choi JS, Jang BC, Byun DS, Park NK, Kim HR (2009) Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties. J Agric Food Chem 57:3483–3489

Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51:216–226

Li B, Lu F, Wei X, Zhao R (2008) Fucoidan: structure and bioactivity. Molecules 13:1671–1695

Li J, Guo C, Wu J (2020) Fucoidan: Biological activity in liver diseases. Am J Chin Med 48:1617–1632

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Luthuli S, Wu S, Cheng Y, Zheng X, Wu M, Tong H (2019) Therapeutic effects of fucoidan: A review on recent studies. Mar Drugs 17:487

MacArtain P, Gill CI, Brooks M, Campbell R, Rowland IR (2007) Nutritional value of edible seaweeds. Nutr Rev 65:535–543

Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474

Murai U, Yamagishi K, Kishida R, Iso H (2021) Impact of seaweed intake on health. Eur J Clin Nutr 75:877–889

Ohno M, Critchley AT (1998) Seaweed Resources of the World. Kanagawa International Fisheries Training Center, Japan International Cooperative Agency

Park J, Johnson MA (1949) Submicrodetermination of glucose. J Biol Chem 181:149–151

Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci 4:89–96

Polat S, Trif M, Rusu A, Simat V, Cagalj M, Alak G, Meral R, Ozogul Y, Polat A, Ozogul F (2021) Recent advances in industrial applications of seaweeds. Crit Rev Food Sci Nutr https://doi.org/10.1080/10408398.2021.2010646

Ponce NMA, Stortz CA (2020) A comprehensive and comparative analysis of the fucoidan compositional data across the Phaeophyceae. Front Plant Sci 11:556312

Pradhan B, Nayak R, Patra S, Bhuyan PP, Behera PK, Mandal AK, Behera C, Ki JS, Adhikary SP, MubarakAli D, Jena M (2022) A state-of-the-art review on fucoidan as an antiviral agent to combat viral infections. Carbohydr Polym 291:119551

Qu G, Liu X, Wang D, Yuan Y, Han L (2014) Isolation and characterization of fucoidans from five brown algae and evaluation of their antioxidant activity. J Ocean Univ China 13:851–856

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

Reys LL, Silva SS, Soares da Costa D, Reis RL, Silva TH (2022) Fucoidan-based hydrogels particles as versatile carriers for diabetes treatment strategies. J Biomater Sci Polym Ed https://doi.org/10.1080/09205063.2022.2088533

Rocha de Souza MC, Marques CT, Guerra Dore CM, Ferreira da Silva FR, Oliveira Rocha HA, Leite EL (2007) Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. J Appl Phycol 19:153–160

Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R, Committee IDFDA (2019) Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract 157:107843

Sanjeewa KKA, Herath K, Yang HW, Choi CS, Jeon YJ (2021) Anti-inflammatory mechanisms of fucoidans to treat inflammatory diseases: A review. Mar Drugs 19:678

Shan X, Liu X, Hao J, Cai C, Fan F, Dun Y, Zhao X, Liu X, Li C, Yu G (2016) In vitro and in vivo hypoglycemic effects of brown algal fucoidans. Int J Biol Macromol 82:249–255

Shang Q, Song G, Zhang M, Shi J, Xu C, Hao J, Li G, Yu G (2017) Dietary fucoidan improves metabolic syndrome in association with increased *Akkermansia* population in the gut microbiota of high-fat diet-fed mice. J Funct Foods 28:138–146

Sharifuddin Y, Chin YX, Lim PE, Phang SM (2015) Potential bioactive compounds from seaweed for diabetes management. Mar Drugs 13:5447–5491

Sun Z, Dai Z, Zhang W, Fan S, Liu H, Liu R, Zhao T (2018) Antiobesity, antidiabetic, antioxidative, and antihyperlipidemic activities of bioactive seaweed substances. In: Qin Y (ed) Bioactive Seaweeds for Food Applications. Academic Press, London, pp 239–253

Villouta E, Santelices B (1986) *Lessonia trabeculata* sp. nov. (Laminariales, Phaeophyta), a new kelp from Chile. Phycologia 25:81–86

Wang J, Zhang Q, Zhang Z, Song H, Li P (2010) Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. Int J Biol Macromol 46:6–12

Wang Y, Nie M, Lu Y, Wang R, Li J, Yang B, Xia M, Zhang H, Li X (2015) Fucoidan exerts protective effects against diabetic nephropathy related to spontaneous diabetes through the NF-κB signaling pathway in vivo and in vitro. Int J Mol Med 35:1067–1073

Wang Y, Xing M, Cao Q, Ji A, Liang H, Song S (2019) Biological activities of fucoidan and the factors mediating its therapeutic effects: A review of recent studies. Mar Drugs 17:183

Wang Y, Wang Q, Han X, Ma Y, Zhang Z, Zhao L, Guan F, Ma S (2021) Fucoidan: a promising agent for brain injury and neurodegenerative disease intervention. Food Funct 12:3820–3830

Yokota T, Nomura K, Nagashima M, Kamimura N (2016) Fucoidan alleviates high-fat diet-induced dyslipidemia and atherosclerosis in ApoE(shl) mice deficient in apolipoprotein E expression. J Nutr Biochem 32:46–54

Yuan Y, Zheng Y, Zhou J, Geng Y, Zou P, Li Y, Zhang C (2019) Polyphenol-rich extracts from brown macroalgae *Lessonia trabeculata* attenuate hyperglycemia and modulate gut microbiota in high-fat diet and streptozotocin-induced diabetic rats. J Agric Food Chem 67:12472–12480