Effects of the marine microalgae *Isochrysis galbana* and *Nannochloropsis oculata* in diabetic rats


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1. Introduction

Diabetes mellitus is a chronic degenerative disease with high morbidity and mortality rates caused by its complications. These are primarily caused by macro- and microvascular repercussions, including impaired endothelial and gastrointestinal function, increased inflammatory mediator levels, and kidney, retinal, and peripheral nervous system damage (Stratton et al., 2000). Glycemic control of the diabetic subject is a vital part of disease symptom treatment and/or prevention, and nutrition can play an important role in this control.

Food has physiological effects in the organism, which is why proper eating habits, an individualized diet and functional food elements such as dietary fiber and polyunsaturated fatty acids are widely used in medical and nutritional treatment of diabetes mellitus. Dietary fiber stimulates growth and/or activity of beneficial bacteria in the gastrointestinal tract. When fiber is fermented by these microorganisms, it produces short-chain fatty acids with beneficial effects in the intestine. Polyunsaturated fatty acids are an energy source and bind to cell membranes where they become precursors of the chemical mediators of certain processes, such as immunological response. This is especially the case for omega-3 eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, whose biological activities include reduction of the inflammatory response (Al-Nouri, Al-Khalifa, &

Functional food use has become increasingly common, which has driven research into the physiologic effects of high biological value components of natural origin. Marine microalgae have generated interest among health researchers as an alternative source of therapeutic and biological compounds such as vitamins, proteins with essential amino acids, polysaccharides, monounsaturated and polyunsaturated fatty acids, nucleic acids, minerals, and photosynthetic pigments such as carotenoids and chlorophylls (Raja, Hemaiswarya, Kumar, Sridhar, & Rengasamy, 2008; Ratih & Se-Kwon, 2011; Sung-Myun et al., 2012). The microalgae are a group of microscopic photosynthetic organisms capable of converting solar energy into biomass. Its biochemical composition provides this biomass nutritional value, although this can vary by species and culture conditions. For example, cell wall carbohydrates differ between microalgae (diatoms have the highest percentages of arachidonic acid), and low quantities of nitrogen in culture media diminish protein concentration (Brown, Jeffrey, Volkman, & Dunstan, 1997).

Seaweeds have long been used as an aquaculture feed source for mollusks and bivalves and are increasingly popular as healthy foods in the food industry. Some of the most widely used algae in the food industry are Spirulina platensis (antioxidant activity, weight control and hypocholesterolemic effects), Chlorella vulgaris and Dunaliella salina (protein content and pigments) and Porphyridium sp. (dietary fiber and sulfated polysaccharide, benefit gastrointestinal physiology and lipid metabolism) (Dvir, Stark, Cayaoth, Madar, & Arad, 2009; Gantar & Svircev, 2008; Salman et al., 2007). Other microalgae are also rich sources of these functional compounds, but more research is needed before their ingredients can be used in foods for healthy subjects and/or in functional foods. Examples include the marine microalgae Isochrysis galbana and Nannochloropsis oculata, known to have high soluble and insoluble polysaccharide and protein contents as well as significant percentages of polyunsaturated fatty acids. Isochrysis galbana contains high amounts of docosahexaenoic acid (DHA) whereas N. oculata has a higher percentage of eicosapentaenoic acid (EPA) (Brown, Jeffrey, Volkman, & Dunstan, 1997; Rebolloso-Fuentes, Navarro-Pérez, García-Camachó, Ramos-Miras, & Guil-Guerrero, 2001). In diabetic subjects, these polysaccharides and fatty acids may aid in regulating glucose, lipids, lipoproteins and nitrogen compounds in endocrine metabolism, as well as increasing beneficial gastrointestinal bacteria, thus could contributing to modulation of mucosa dysfunction and intestinal epithelium maintenance. The present study aimed to evaluate the potential use of the microalgae I. galbana and N. oculata in nutritional treatment of diabetes mellitus using a rat model.

2. Materials and methods

2.1. Microalgae strains and preparation

Microalgae concentration and biochemical composition were controlled for by using I. galbana (T-ISO; UTEX Culture LB 2307) and N. oculata (NNO-1 UTEX Culture LB 2164) from the strain culture collection at the Northwest Center for Biological Research (Centro de Investigaciones Biológicas del Noroeste – CIBNOR, La Paz, Baja California Sur, Mexico). Strains were cultivated in f/2 medium at 21 °C, 30 ppm NaCl, pH 8.2, and under 2 × 75 W fluorescent lights. Samples were collected on the fifth day of the growth cycle for I. galbana and on the sixth day for N. oculata. These were centrifuged in a continuous flow (JC-F-Z, Beckman, Brea, CA, USA) at 3588 g and 20 °C, and the precipitated microalgae centrifuged again at 897 g and 20 °C for 10 min (GS-6R Allegra, Beckman, Palo alto, CA, USA). Recovered biomass was freeze-dried (VirTis Model 10-145 MR-BA, SP Scientific, Stone Ridge, NY, USA) and stored separately by species at −20 ºC until use.

2.2. Preliminary bioassay and lethal dose (LD 50)

A preliminary trial was run for each microalga species to determine the LD 50, trial conditions and microalga dose to be administered using an oral dosing cannula/needle (VWR®, 20068-642, West Chester, PA, USA). Experimental animals were 16-week-old, male Sprague-Dawley rats (200 ± 8 g initial weight) kept at 25 °C and allowed free access to purified drinking water and feed (Rodent Laboratory, Chow 5001, PMI Nutrition Int'l, LLC, Brentwood, MO, USA). The animals were divided into seven groups, five per group. Groups 1–6 besides food were provided with a single dose of microalgae. Groups 1, 2, and 3 were administered single doses (5 μg, 5 mg, or 50 mg, respectively) of I. galbana dissolved in 0.5 ml purified drinking water; Groups 4, 5, and 6 were administered single doses (5 μg, 5 mg, or 50 mg, respectively) of N. oculata dissolved in 0.5 ml purified drinking water; and Group 7 was a control group, administered 1 ml purified drinking water. After administration, the animals were kept under observation for one week to identify any signs of toxicity, other symptoms or death.

2.3. Induction of hyperglycemia

Hyperglycemia was induced by applying 150 mg/kg alloxan monohydrate (A7413, Sigma–Aldrich®, St. Louis, MO, USA). After 72 h, a blood test confirmed hyperglycemia (>200 mg/dl) (Szkudelski, 2001), after which microalgae were orally administered following the experimental design.

2.4. Experimental design

Of the seven groups (n = 5), three were healthy and four consisted of animals with induced hyperglycemia. The healthy groups included a control group, one treated with I. galbana, and another with N. oculata. The four diabetic groups included an untreated group, one treated with I. galbana, one treated with N. oculata and one treated with glibenclamide (Sigma–Aldrich®, St. Louis, MO, USA). Microalgae dose was 50 mg/day and hypoglycemicant dose was 600 μg/kg/day (Saravanan & Pari, 2007). Both were administered orally for eight weeks using a cannula/needle (VWR®, 20068-642, West Chester, PA, USA).
2.5. Weight measurement

Body weight (g) was measured weekly using a triple-arm scale (730-SW, Ohaus, Pine Brook, NJ, USA) with a basket to hold the animals.

2.6. Blood glucose, lipids, lipoproteins and nitrogen compounds

Measurements were taken of glucose, cholesterol, triacylglycerols, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and nitrogen compounds (creatinine, uric acid and urea). Glucose was measured weekly for 8 weeks (Accu-Check Active®, Roche Diagnostics®, Indianapolis, IN, USA; range = 10–600 mg/dl). At the end of the experimental period, a 12-h fast was imposed on the animals after which they were killed. Lipids and lipoproteins were then measured with commercial kits (Spinreact®, Barcelona, Spain): triacylglycerols (1001312); enzymatic for cholesterol (1001090); LDL-D kit (41023); and HDL-cholesterol precipitating reagent (1001095). Nitrogen compounds were also measured using commercial kits (Spinreact®, Barcelona, Spain): creatinine (1001010); uric acid (1001325); and urea (1001111).

2.7. Histopathology

At the end of the biological assay, the intestine was extracted and placed in a sterile container. Intestinal contents were extracted under aseptic conditions for microbial community analysis. The intestine was placed in 10% neutral buffered formalin (HTS01128, Sigma–Aldrich®, St. Louis, MO, USA), sectioned for histological analysis, stained with hematoxylin-eosin and examined using light microscopy. Diagnosis of intestinal abnormalities was based on histological examinations of biopsies.

2.8. Lactic acid bacteria (LAB) enumeration and identification

Lactic acid bacteria were enumerated in a 1 g stool sample. Briefly, 9 ml of 0.85% sodium chloride (Promega HS271, Madison, WI, USA) were added to the sample and mixed. Three additional decimal dilutions (1:10, 1:100, and 1:1000, v/v) were done. A 0.1 ml sample was taken from each dilution, placed in an agar plate containing MRS medium (Man, Rogose and Sharpe, BD Difco Laboratories®, Sparks Maryland, MD, USA), and cultured using the spread plate method with incubation at 35 °C/48 ± 2 h in a candle jar, under an approximately 15% oxygen atmosphere (Rengpipat, Rueangruklkhit, & Piwatiratitivorakul, 2008). Three replicate plates were prepared for each dilution. Colonies were counted after incubation. Gram positives, which are catalase negative and oxidase positive, were isolated, purified, subcultured on MRS agar and treated as presumptive LAB. The isolates were characterized and identified using API 50CH and API 50CHL medium (bioMérieux®, Marcy-l’Étoile, France) following manufacturer’s instructions.

2.9. Enterobacteriaceae

Enterobacteriaceae were identified from a 1 g stool sample. Briefly, 9 ml of 0.85% sodium chloride (Promega HS271, Madison, WI, USA) were added to the sample and mixed. Samples from this solution were placed on five different media: eosin methylene blue agar (EMB); MacConkey; brilliant green agar (BG); Salmonella-Shigella agar (SS); and Hektoen enteric agar. After incubation at 35 °C for 24 h, representative colonies were isolated, purified, and subcultured on selective agar. They were then subjected to Gram staining and a series of biochemical analyses: catalase (Cura Pack Degasa®, Morelos State, Mexico); oxidase (BD Difco Laboratories®, Sparks, MD, USA); motility-indole-ornithine medium (MIO); lysine iron agar (LIA); triple sugar iron (TSI); citrate; glucose oxidation/fermentation (O/F); esculin hydrolysis; methyl red and Voges Proskauer test (MR–VP); and fermentation of sugars such as glucose, xylose, lactose and saccharose. All media were used following manufacturers’ instructions (BD Bioxon®, Mexico State, Mexico).

2.10. Statistical analysis

Differences between treatments were identified with an analysis of variance (ANOVA; SPSS-PC, version 16.0). In cases where the standard deviation differed between groups, U Mann–Whitney and Kruskal–Wallis multiple comparison non-parametric tests were applied with a 95% (p < 0.05) confidence level. Finally, a cluster analysis was applied using the Ward method.

2.11. Ethical considerations

The experimental protocol was approved by the Technical Council of The Aquaculture Research Program at CIBNOR based on laboratory animal care and use guidelines and in accordance with applicable federal technical specifications for laboratory animal production, care and use (Mexican Official Standard NOM-062-ZOO-1999).

3. Results and discussion

Microalgae have long been used as mussel feed in aquaculture systems. However, they are of increasing commercial interest for use in humans based on the potential biological and therapeutic value of molecules from their cellular components (Raja et al., 2008). Studies have been done for I. galbana and N. oculata as ingredients in foods such as biscuits and spaghetti (Gouveia et al., 2008; Guí-Guerrero, Navarro-Juárez, López-Martínez, Campra-Madrid, & Rebolloso-Fuentes, 2004), but research using them in biological systems is limited.

3.1. Lethal dose

Hazards and risks of the studied microalgae species were assessed with a toxicity test. The tested microalgae concentrations showed no adverse effects in the experimental animals, including possible alterations such as food intake, unusual body growth, reduced activity, diarrhea, bleeding
and death. This coincides with the reported absence of adverse effects in organisms administered *N. oculata* (Markovits, Conejeros, López, & Lutz, 1992), and in albino rats administered *I. galbana* (Herrero, Abalde, & Fabregas, 1993).

### 3.2 Body weight

Animals in the control group, microalgae healthy groups and glibenclamide diabetic group exhibited constant weekly weight gain. From an average initial body weight of \(218 \pm 10\) g, they grew to \(300\) g at eight weeks with differences (\(p < 0.05\)) in body weight between weeks. In contrast, body weight remained unchanged (\(p > 0.05\)) between weeks in the untreated diabetic group and *I. galbana* diabetic group. The *N. oculata* diabetic group exhibited weight loss over time, with no significant decreases between week one (\(200 \pm 16\) g) and week eight (\(186 \pm 28.8\) g). Rats in this group had diarrhea, bloated gut area, and changes in hair and skin color. At the end of the experimental period, all groups treated with *I. galbana* and *N. oculata*, both healthy and diabetic, had a lower (\(p < 0.05\)) final weight than the control group. Both the *I. galbana* and *N. oculata* diabetic groups had lower (\(p < 0.05\)) weights than the glibenclamide treated diabetic group. Final weight in the untreated diabetic group did not differ (\(p > 0.05\)) from that of the *I. galbana* diabetic group, but was higher (\(p < 0.05\)) than in the *N. oculata* diabetic group (Fig. 1).

The lower body mass of the hyperglycemic and healthy rats administered *I. galbana* or *N. oculata* compared to those that did not receive a microalgae treatment suggests the effect of their polysaccharides, which are represented from 45–97% of microalgae carbohydrate content. These polymers are shaped by different monomers (e.g. glucose, galactose, xylose, fucose and arabinose), the beta-glucosidic bonds of which may allow them to act as dietary fiber in the gastrointestinal tract (Brown et al., 1997; Zeng, Zheng, & Chen, 2004). Dietary fiber affects satiety, energy, and body composition, reducing appetite and energy intake up to 10%, consequently leading to weight reduction. Microalgae polysaccharides have the characteristics of dietary elements and may therefore form colloids in the intestine, reducing intestinal transit time and digestion. This would lower absorption of certain nutrients, particularly carbohydrates and lipids, thus reducing available dietary calories (Howarth, Saltzman, & Roberts, 2009; Niewold, Schroyen, Geens, Verheest, & Courtin, 2012).

### 3.3 Glucose

Initial average blood glucose level in the healthy groups was \(126.47 \pm 12\) mg/dl, and \(542.62 \pm 103\) mg/dl in alloxan-induced diabetic groups. In a comparison of week 1 glucose levels to week 8 levels, the control group exhibited no changes (\(p > 0.05\)), whereas in the *I. galbana* or *N. oculata* healthy groups levels declined (\(p < 0.05\)) from \(132.5\) mg/dl to \(83.80\) mg/dl. Two rats in the untreated diabetic group died due to high glucose levels (up to 600 mg/dl), whereas the remaining animals exhibited increasing glucose levels during the experimental period, but with no statistical differences. All three treated diabetic groups exhibited lower (\(p < 0.05\)) glucose levels at week 8: *I. galbana* diabetic group \(\sim 300 \pm 197.4\) mg/dl, *N. oculata* diabetic group \(\sim 487 \pm 56.07\) mg/dl; glibenclamide diabetic group \(\sim 100 \pm 2\) mg/dl. The *N. oculata* diabetic and untreated diabetic groups had higher (\(p < 0.05\)) glucose levels than the glibenclamide diabetic and control groups. In contrast, levels in the *I. galbana* diabetic group did not differ from those of the glibenclamide diabetic and control groups. However, *I. galbana* was apparently so effective at lowering glucose levels that three rats in this treatment had levels below 140 mg/dl, and one exhibited symptoms of hypoglycemia (glucose \(\sim 40\) mg/dl, lethargy) in weeks seven and eight. Levels in the *N. oculata* diabetic group did not differ from those of the untreated and *I. galbana* diabetic groups, whereas the latter two differed at the end of the study (\(p < 0.05\)) (Fig. 2).

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**Fig. 1 – Bodyweight (g) in control and diabetic groups:** ■ control group; ● *Isochrysis galbana* diabetic group; ○ *Nannochloropsis oculata* diabetic group; v glibenclamide diabetic group; and △ untreated diabetic group. Values are expressed as mean ± SE. \(^*^p < 0.05\) vs. *I. galbana* treated diabetic rats. \(^**^p < 0.05\) vs. *N. oculata* treated diabetic rats.

**Fig. 2 – Blood glucose levels (mg/dl) in control and diabetic groups:** ■ control group; ● *Isochrysis galbana* diabetic group; ○ *Nannochloropsis oculata* diabetic group; v glibenclamide diabetic group; and △ untreated diabetic group. Values are expressed as mean ± SE. \(^*^p < 0.05\) vs. *I. galbana* treated diabetic rats. \(^**^p < 0.05\) vs. *N. oculata* treated diabetic rats.
These results agree with previous reports of serum glucose level control with ingestion of *I. galbana* being related to its cell wall composition and polysaccharides content, and absence of an effect with *N. oculata* (Guil-Guerrero et al., 2004; Zeng et al., 2004).

### 3.4. Lipids and lipoproteins

Blood lipids levels in the *I. galbana* healthy group were the lowest on average (54 mg/dl), followed by those of the *N. oculata* healthy group (61 mg/dl) and the control group (66 mg/dl). Of the diabetic groups, the lowest average level was in the *I. galbana* group (70 mg/dl), followed by the *N. oculata* group (73 mg/dl), the glibenclamide group (77 mg/dl) and the untreated group (84 mg/dl). The untreated diabetic group had higher (*p* < 0.05) levels than the microalgae healthy groups, and the glibenclamide diabetic group had higher (*p* < 0.05) levels than the *I. galbana* healthy group (Fig. 3A).

Average triacylglycerol levels were approximately the same (18 mg/dl) in the control group and the microalgae healthy groups, while all the diabetic groups had higher average levels than the control group. However, there were some exceptions: three rats in the *I. galbana* diabetic group had triacylglycerol levels equal to or less than the control group; and two rats in the *I. galbana* healthy group had levels lower than the control group. Finally, the *N. oculata* diabetic group differed from the glibenclamide diabetic group (Fig. 3B).

None of the groups differed (*p* > 0.05) in terms of VLDL, although the highest level (60.64 mg/dl) was observed in the rats treated with *N. oculata*. The groups treated with either microalgae (healthy and diabetic) had higher LDL levels, but differences (*p* < 0.05) were only present between the *N. oculata* diabetic group and the *I. galbana* healthy and control groups. Both microalgae treatments decreased HDL levels (*p* < 0.05) in the hyperglycemic and healthy rats to values below those of the control and untreated diabetic groups (Fig. 3C and D). The overall trend of lower cholesterol and triacylglycerol levels in the *I. galbana* diabetic group may be due to reductions in intestinal absorption caused by this microalga’s polysaccharides (Howarth et al., 2009; Niewold et al., 2012). The LDL and HDL results in rats fed either microalga contradicts previous studies in which inclusion of EPA and DHA fatty acids is reported to help reduce blood LDL and HDL levels (Komprda, 2012). For instance, Conquer and Holub (1996) reported that microalgal DHA increases DHA blood levels in healthy vegetarians and higher EPA ingestion decreases blood LDL and increases HDL. Van-Beelen et al. (2009) suggested that omega-3 fatty acids from linolenic acid lower blood LDL levels, producing an effect similar to fish oil as a protective mechanism in heart disease, atherosclerosis, cancer and diabetes.

The higher LDL and lower HDL levels observed in the present study may have been caused by the presence of palmitic and palmitoleic fatty acids. These are present in *I. galbana* and *N. oculata* in amounts equal to or greater than...
Eicosapentaenoic and docosahexaenoic acids (Rebollos–Fuentes et al., 2001; Sukenink & Wahnon, 1991). Palmitic acid is a monounsaturated fat known to increase LDL levels; studies in volunteers have shown that palmitic acid elevates cholesterol and LDL levels. Moreover, palmitoleic acid, a palmitic acid derivative, constitutes a portion of endogenously formed triacylglycerols and blood lipoproteins. It can also behave as a saturated fat and raise serum LDL levels (Denke & Grundy, 1992; Zock, de Vries, & Katan, 1994).

3.5. Nitrogen compounds

No differences (p > 0.05) in blood nitrogen compound levels between treatments were observed. Uric acid values were higher in the untreated diabetic (2.1 mg/dl) and N. oculata diabetic groups (1.9 mg/dl) than in the I. galbana diabetic (1.5 mg/dl), glibenclamide diabetic (1.2 mg/dl), I. galbana healthy (1.3 mg/dl), N. oculata healthy (1.1 mg/dl) and control groups. Behavior was similar in the urea values with the highest levels in the N. oculata diabetic (59.5 mg/dl) and untreated diabetic groups (61.3 mg/dl) groups, and lower levels in the I. galbana diabetic (54.5 mg/dl), glibenclamide diabetic (50.7 mg/dl), N. oculata healthy (49.1 mg/dl), I. galbana healthy (48.9 mg/dl) and control (48.6 mg/dl) groups. Creatinine values did not differ (p > 0.05) between the groups (0.45 mg/dl).

The lack of change in nitrogen compound levels in the microalgae groups suggests that I. galbana and N. oculata have no negative impact on kidney function since higher creatinine, uric acid and urea levels are reported to be a negative effect of chronic hyperglycemia on glomerular filtration, also associated with increased cardiovascular mortality (Jerums, Premaratne, Panagiotopoulous, & Maclsaac, 2010).

3.6. Histopathology

Intestinal tissue sections (38 × 0.6 × 0.6 cm) showed the control and I. galbana healthy groups to be have intestines lined by intestinal cylindrical epithelium, forming glands; lamina propria with a transmucosal lymphoplasmacytic inflammatory infiltrate; and regular lymphocytes with no histopathological indicators of metaplasia, dysplasia or malignancy. However, three rats in the I. galbana healthy group manifested mild inflammation with hyperplastic lymphoid follicles (Fig. 4A). Sections from the N. oculata healthy group indicated the presence of intestinal atrophy in four rats, two of which had transmural necrosis. Only one animal in this group had normal bowel histology, although its bowel periphery was surrounded by mature adipocytes exhibiting a rejected nucleus at the margin.

Sections from the untreated diabetic group showed an epithelium with severe atrophy covering large areas, flattened villi, inflammation, and lamina propria with lymphoplasmacytic inflammatory infiltrate and moderate neutrophilic infiltration but negative for malignancy (Fig. 4B). Tissues from the glibenclamide diabetic group exhibited intestines lined by columnar epithelium, evidence of inflammation and slight decreases in epithelium thickness in some areas with changes from autolysis, and moderate lymphoplasmacytic and neutrophilic infiltrate with no malignancy. Sections from the I. galbana diabetic group had lamina propria showing moderate inflammatory infiltrate of lymphocytes and neutrophils, accumulation of increasingly large lymphoid germinal centers, fragments of interstitial edema and superficial capillary congestion without metaplasia, dysplasia or malignancy (Fig. 4C). All the sections from the N. oculata diabetic group showed epithelium lined by extensive necrotic areas (Figs. 4D and 5).

The lymphocytes and plasmatic cell infiltration, as well as different degrees of epithelial modification and inflammation exhibited in diabetic groups, support that diabetes mellitus per se modifies gastrointestinal architecture, confirming previous reports that hyperglycemia causes changes in gastrointestinal tract (Olaussen et al., 2008). Other studies indicate that hyperglycemia affects cellular immunity by impairing activity in natural killer cells, monocytes and polymorphonuclear leukocytes, which can induce inflammation, susceptibility to bacterial pathogens and diverse gastrointestinal symptoms (Booth, Stalker, Lefer, & Scalia, 2001).

In contrast to the other diabetic groups, the I. galbana diabetic group exhibited some features of superficial chronic low-inflammation characterized by mild lymphoplasmacytic infiltrate, variable neutrophilic infiltrate (only one animal), no changes in epithelium, no polymorphonuclear infiltrate, no ulceration and focal damage distribution (Bouma & Strober, 2003). Indeed, animals in the I. galbana healthy group had histological characteristics similar to the control, suggesting that use of this microalgae in healthy subjects has no negative gastrointestinal effects. In contrast, both the N. oculata healthy and diabetic groups exhibited severe gastrointestinal damage.

The differences observed between the gastrointestinal outcomes in the I. galbana and N. oculata groups may have resulted from each microalga’s structure. The cell wall of N. oculata is more rigid and thicker than that of I. galbana (Guil-Guerrero et al., 2004), which may produce difficulty in digestion that could affect the epithelium, LAB counts and nutrient absorption, as well as modifying feces composition (e.g. diarrhea). Intake of carrageenan from several red microalgae (particularly Euchema spinosum) has been reported to produce effects similar to those observed here with N. oculata, including modification of gut microbiota through increases in Gram negative microorganisms, unusual immunological response, epithelium toxicity, severe inflammatory responses, and even extreme symptoms such as diarrhea containing blood and mucus (Aceituno & Panés, 2005).

3.7. Lactic acid bacteria (LAB)

Enumeration of Lactic acid bacteria (6.3 log CFU/g) did not differ between the control, I. galbana healthy, N. oculata healthy, untreated diabetic and glibenclamide diabetic groups. The N. oculata diabetic group had the lowest (p < 0.05) enumeration (4.9 log CFU/g) and the I. galbana diabetic group the highest (p < 0.05) enumeration (7.1 log CFU/g). Lactococcus lactis was the most frequent LAB (90%) in the control, I. galbana healthy, N. oculata healthy and I. galbana diabetic group. This species was followed by Lactobacillus brevis (70%) and Lactobacillus fermentum (30%) in the glibenclamide diabetic, N. oculata diabetic and untreated diabetic groups. Yeasts were present in the N. oculata healthy, N. oculata diabetic and glibenclamide diabetic groups.
The thinner cell wall of *I. galbana* compared to *N. oculata* may prove advantageous because it could allow microalga nutrient and component absorption while also providing functional activities in the gastrointestinal tract. The functionality, or prebiotic effect, of the polysaccharides in *I. galbana* would occur by increasing the growth and activities of beneficial bacteria such as *L. lactis* while reducing enterobacteria and pathogen growth and activity (Monteagudo-Mera et al., 2012). Lactococcus lactis is a microorganism commonly found in nature, usually on plant and animal surfaces and in the intestine (Bahey-El-Din, Gahan, & Griffin, 2010). Bernbom et al. (2006) reported that nisin produced by *L. lactis* affects rat microbiota, which is similar to that of humans, increasing bifidobacteria cells in fecal samples. In early-weaned pigs, this LAB has also been described as metabolically active in the intestinal tract, with the capacity to express and secrete active cytokines; as a probiotic capable of increasing villi height; as a growth stimulant; and as reducing

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**Fig. 4** – Small intestine histological sections (10×) comparing the control group with diabetic groups: (A) Control, villi intact, normal histology; (B) Untreated diabetic group, flattened villi, lymphocyte proliferation; (C) Isochrysis galbana diabetic group, muscular wall viability, local damage in segments; (D) Nannochloropsis oculata diabetic group, total necrosis of intestinal wall.

**Fig. 5** – Macroscopic images of small intestine of diabetic rats treated with microalgae: (A) Isochrysis galbana diabetic group, intestine normal with signs of meteorism; (B) Nannochloropsis oculata diabetic group, necrotic segments.
coli counts by producing antimicrobial metabolites (Kang et al., 2010).

Lactobacillus fermentum and L. brevis have probiotic properties and have been isolated from plants, and human and animal gastrointestinal tracts, especially in infant feces (Mackie, Sghir, & Gaskins, 1999; Park et al., 2005). Lactobacillus fermentum is also known to have immune-enhancing activities and hypocholesterolemic potential (Zeng, Pan, & Zhou, 2011), meanwhile, L. brevis has been suggested as a tool to treat H. pylori because it may induce a decline in gastric ornithine decarboxylase activity and polyamine levels (Linsalata et al., 2004). However, low counts of these LAB in diabetic group treated with N. oculata, diminished competition for epithelial binding sites and colonization of pathogens.

3.8 Enterobacteriaceae

The dominant genera in the rat feces were Salmonella and Providencia betic groups. Smaller proportions of isolated from the glibenclamide diabetic and healthy groups, between the untreated diabetic group and N. oculata healthy and diabetic groups. Bacterial clustering did not differ between the control and I. galbana healthy groups, between the N. oculata healthy and diabetic groups, and between the I. galbana diabetic and glibenclamide diabetic groups (Fig. 6).

Type and proportion of enterobacteria isolated from the I. galbana groups were similar to previous reports in which E. coli, Enterobacter and Klebsiella were predominant in the intestinal tract while Providencia, Hafnia, Morganella, Citrobacter, Edwardsiella and Yersinia were present in lower concentrations (Mackie et al., 1999; Thompson-Chagoyán, Maldonado-Lozano, & Hernández, 2004). The Enterobacteriaceae species balance, absence of pathogens and increased LAB in the I. galbana groups suggest that the I. galbana polysaccharides are fermented by LAB, they produce short-chain fatty acids, modify pH, positively affect intestinal mucosa, improve innate immune system function by augmenting the physical barrier, increase disease resistance and enhance lactobacillus and bifidobacteria growth (Kuda, Enomoto, & Yano, 2009). Meanwhile, the decrease in gastric mucosa and intestinal permeability as part of development of chronic inflammatory condition in diabetic rats treated with N. oculata, increased the risk of colonization by pathogens such as Salmonella (Van Vliet, Harmsen, de Bont, & Tissing, 2010).

4. Conclusion

The findings suggest that consumption of the microalga I. galbana could be beneficial in the diabetes mellitus rat model, it promoted body weight loss in healthy animals and helped to maintain weight in diabetic animals while lowering glucose and cholesterol values and raising lactic acid bacteria counts. In contrast, consumption of the microalga N. oculata provided none of these potential benefits and produced negative effects throughout the gastrointestinal tract. Further research will be needed to evaluate the possible uses of these marine microalgae in functional foods and as nutraceuticals.

Conflict of Interest

The authors declare no conflict of interest in this study.

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