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Beneficial Effects of Algerian Green Alga *Ulva lactuca* and Its Hydroethanolic Extract on Insulin Resistance and Cholesterol Reverse Transport in High-Fat/Streptozotocin Diabetic Rats

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ABSTRACT: The aim of this study was to evaluate the impact of the green algae *Ulva lactuca* and its hydroethanolic extract on insulin resistance and cholesterol reverse transport in type 2 diabetic (T2D) rats. Rats had T2D induced by a high-fat diet (HFD) for 5 weeks followed by intraperitoneal injection of streptozotocin. Diabetic rats were divided into three groups and were fed a HFD in the presence or absence of 1% alga (HFD-Alg) or 1% of its hydroethanolic extract (HFD-Ext), for 4 weeks. The control group consumed 20% casein combined with 5% lipids. Hyperglycemia, insulin resistance, hypercholesterolemia, and hypertriglyceridemia were noted in HFD rats *vs* control rats. Whole alga and its extract decreased these parameters *vs* the HFD. Moreover, fecal total cholesterol and triacylglycerols levels were lowered in HFD group *vs* C group, but were increased with HFD-Alg *vs* HFD. Compared with the Control, the HFD group had decreased lecithin:cholesterol acyltransferase (LCAT) activity, apolipoprotein A-I (ApoA-I), high-density lipoprotein (HDL_3)-phospholipids (PL), and HDL_2-cholesteryl ester (CE) levels, but increased HDL_3-unesterified cholesterol (UC) levels. Furthermore, compared with the HFD group, the HFD-Alg and HFD-Ext groups had increased LCAT activity, ApoA-I, HDL_3-PL, and HDL_2-CE levels and decreased HDL_3-UC levels. In addition, in the HFD-Ext group, LCAT activity and ApoA-1 levels were decreased *vs* the HFD-Alg whereas HDL_3-UC levels were increased. In conclusion, these results indicate that *U. lactuca* and its hydroethanolic extract have curative effect on T2D. Therefore, this alga could be considered a functional food supplement for the treatment and prevention of diabetes.

Keywords: cholesterol acyltransferase, high-fat diet, hydroethanolic extract, type 2 diabetes, *Ulva lactuca*

INTRODUCTION

Diabetes mellitus comprised of a group of metabolic disorders of the endocrine system characterized by hyperglycaemia (Sharifuddin et al., 2015). It occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin produced. The disease is becoming a major public health problem globally. The incidence of diabetes is considered high worldwide. Indeed, in 2014, it was estimated that 230 million people have diabetes in the world (AbouZid et al., 2014). In 2017, a later study estimated the worldwide incidence of diabetes to be 451 million and that this number will increase to 693 million by 2045 (Cho et al., 2018).

Poor diets, unhealthy lifestyles, and genetic factors are the main causes of the development of diabetes, which can lead to many vascular complications, such as blindness, renal failure, cardiovascular diseases, and limb amputations. Of all types of diabetes, the type 2 diabetes (T2D) is the most important. It comprises three abnormalities: relative insulin deficiency, insulin resistance involving myocytes and adipocytes, and hepatic insulin resistance resulting in increased gluconeogenesis and impaired glycogen synthesis. The relationships between diabetes and diet composition have been demonstrated in several human and animal studies. A previous study reported that the type of fats consumed may affect insulin resistance in animals (Roza et al., 2016). Furthermore, consumption of a high-fat diet (HFD) decreases insulin sensitivity *in vivo* due to reduced glucose uptake by peripheral tissues and increased hepatic glucose production (Panigrahi et al., 2016). In addition, another study showed that saturated fat induces insulin resistance and provokes lipolysis in adipose tissues, which leads to an increase in hepatic triglycerides (Luukkonen et al., 2018). Moreover, in mice, a HFD induces glucose intolerance

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and increases sensitivity to β cell apoptosis (Acosta-Montaño and García-González, 2018).

Because of the side effects of synthetic molecules used to treat diabetes, it is necessary to find molecules derived from natural compounds. In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods. Marine algae are one of the richest sources of structurally diverse natural products. Indeed, an increasing number of novel compounds have been isolated from marine algae as bioactive ingredients including polyunsaturated fatty acids, polyphenols, flavonoids, sterols, proteins, sulfated polysaccharides, and vitamins (Lee et al., 2016; Manikkam et al., 2016; Ruocco et al., 2016; Suleria et al., 2016). Many of these compounds possess different biological properties such as antidiabetic, antioxidant, antibacterial, antiviral, and anti-inflammatory activities (Choochote et al., 2014; Zhao et al., 2015; Fernando et al., 2016). Moreover, these compounds have been reported to have preventive and protective effects against neuro-inflammation (Barbalace et al., 2019) and are associated with lower risk of cardiovascular diseases (Cardoso et al., 2015; Murai et al., 2019; Roy, 2019). In the study of diabetes, all tested micro or macroalgae such as *Caulerpa lentillifera*, *Spirulina versicolor*, and *Ulva lactuca* shown antihyperglycemic effects in diabetic rats (AbouZid et al., 2014). In addition, Sørensen et al. (2019) showed that Nordic seaweed improves, glycosylated hemoglobin (HbA1c), glucose, and lipid profile, in diabetic mice. Moreover, ethanolic extract of *Ulva reticulata* induces a significant reduction in fasting blood glucose levels and an increase in plasma insulin activity (Ashok Kumar and Sharunetha, 2018). However, Gotama et al. (2018) demonstrated that ethanolic extracts of *Sargassum hystrix* significantly decreases blood glucose levels but has no effect on lipid parameters in diabetic rats. A further study showed that the red alga *Gelidium amansii* decreases plasma glucose levels, homeostasis model assessment (HOMA)-insulin resistance (IR) indexes and lipid levels and increases insulinemia in diabetic rats fed a high fat diet (Yang et al., 2015). In the same context, Ramirez-Higuera et al. (2014) showed that supplementation of a high-caloric diet with the edible green seaweed *Ulva linza* lowers total cholesterol (TC) and triacylglycerol (TAG) concentrations in serum. However, few studies have investigated the effect of *U. lactuca*. Among those that exist, Kammoun et al. (2018) demonstrated ethanolic extracts of *U. lactuca* have cholesterol-lowering effects in mice fed a hypercholesterolemic diet, however, the mechanism of action remains unknown. Therefore, the aim of our study was to investigate the effect of the green alga *U. lactuca* and its hydroethanolic extract on hyperglycaemia, hyperlipidemia and cholesterol reverse transport in HFD/streptozotocin (STZ)-induced type 2 diabetic rats.

MATERIALS AND METHODS

Plant preparation

The green alga *U. lactuca* was collected in November 2013, on the beach of Bousfer, on the west coast of Oran, Algeria. After cleaning with sea water and tap water to remove all epiphytes and excess salt, the alga was dried at 60°C for 8 h. The dried alga was ground into a fine powder and stored in glass bottles in a dark and dry place.

Extract preparation

Alga extracts were prepared according to the method described by Merghem et al. (1995) with some modifications. Briefly, 100 g of *U. lactuca* powder was diluted in a hydroethanolic mixture (50:50, v/v) and stirred for 48 h at an ambient temperature. Maceral was filtered using Whatman filter paper and the solvent was removed by evaporation using a rotary evaporator (Buchi R-100, BÜCHI Labortechnik AG, Flawil, Switzerland). The extract was lyophilized and stored at 4°C until use. The extraction yield was approximately 28%.

Determination of total polyphenol and flavonoid contents in algae extract

Total phenol contents were determined using Folin-Ciocalteu reagent by spectrophotometry (absorbance 765 nm), according to the method of Singleton et al. (1999) using gallic acid (Sigma Aldrich Chimie, Lyon, France) as the standard. Total phenol contents were expressed as mg of gallic acid equivalent (GAE)/g dry weight. Total flavonoid contents were assayed according to the method of Hertog et al. (1992), using quercitin (Sigma Aldrich Chimie) as the standard. The absorbance of the samples and standards were measured at 430 nm. Total flavonoid contents were expressed as mg quercitin equivalent (QE) /g dry weight.

Animals and diets

Male wistar rats (n=30) (Pasteur Institute of Algeria, Algiers, Algeria) weighing 260±25 g were maintained in a stable temperature (22～23°C) and humidity (60%), with a 12-h light/dark cycle (light 07:00～19:00 h). This study was approved by our Institutional Ethical Committee for Animal Research (agreement number 45/DGLPAG/DVA. SDA.14). The General Guidelines for the Use of Living Animals in Scientific Investigations Council of European Communities were followed (Council of European Communities, 1986). T2D was induced by a HFD (53% calories from lamb fat) followed by a single intraperitoneal injection of STZ (35 mg/kg body weight) following the method described by Benaicheta et al. (2016). The control group received the vehicle (i.e., citrate buffer).

Animals with blood glucose levels above 11 mM (1.98 g/L) were considered diabetic and were divided into 3

groups of six rats, and were fed the experimental diet (HFD) alone or supplemented with 1% algae powder (HFD-Alg) or 1% algae extract (HFD-Ext) for 28 days. The control group (C) was fed a standard diet. All diet compositions are shown in Table 1. Body weight and glycemia (assessed using a one-touch glycometer; Accu-Chek Active, Roche Ltd., Basel, Switzerland) were recorded weekly and food consumption was recorded daily. One week before the end of the experiment, rats were placed into individual metabolism cages to collect feces and urines.

Blood and organ samples

At the end of the experiment, rats were fasted overnight and anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight). Blood was obtained from the abdominal aorta and collected into dry tubes. Serum was obtained by centrifugation at 1,000 *g* for 20 min at 4°C. Livers were removed, rinsed with 0.9 % NaCl, dried, and then immediately weighed. Aliquots of serum and 50～100 mg of tissue were stored at −70°C until analysis.

Table 1. Composition of the standard and experimental diets (unit: g/kg)

Composition	Standard diet	Experimental diet		
	C	HFD	HFD-Alg	HFD-Ext
Energy content (kcal/100 g diet)	381	506	502	502
Casein[1)]	200	200	200	200
Sucrose	50	50	50	50
Corn starch	590	340	330	330
Cellulose[1)]	50	50	50	50
Vitamin mix[2)]	20	20	20	20
Mineral mix[3)]	40	40	40	40
Lipids[4)] (olive, nut, and sunflower)	50	—	—	—
Fat of the lamb	—	300	300	300
Algae powder	—	—	10	—
Algae extract powder	—	—	—	10

C, control; HFD, high-fat diet; HFD-Alg, high-fat diet supplemented with 1% algae; HFD-Ext, high-fat diet supplemented with 1% algae extract.

[1)]Prolabo.

[2)]UAR 200: vitamin mixture provides the following amounts (mg/kg diet): retinol, 12; cholecalciferol, 0.125; thiamine, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; ascorbic acid, 1,600; DL-α-tocopherol, 340; menadione, 80; nicotinic acid, 200; *p*-aminobenzoic acid, 100; folic acid, 10; biotin, 0.6.

[3)]UAR 205B: the salt mixture provides the following amounts (mg/kg diet): $CaHPO_4$, 17,200; KCl, 4,000; NaCl, 400; MgO, 420; $MgSO_4$, 2,000; Fe_2O_3, 120; $Fe_2SO_4 \cdot 7H_2O$, 200; trace elements, 400; $MnSO_4 \cdot H_2O$, 98; $CuSO_4 \cdot 5H_2O$, 20; $ZnSO_4$, 80; $CoSO_4 \cdot 7H_2O$, 0.16; KI, 0.32.

[4)]Lipid mixture provides the following amounts (g/kg diet): sunflower oil, 10; olive oil, 39; walnut oil, 1 (with n-6/n-3=7). Olive and sunflower oils from Cevital (Kouba, Algeria) and walnut oil from Cauvin (Saint Gilles, France).

Analysis of serum, liver, and fecal lipid concentrations

Total fecal lipids were determined by the method described of Delsal (1944). Serum and liver TAG, TC, and phospholipids (PL) concentrations were determined by enzymatic colorimetric methods using kits (GPO-POD, SPINREACT, Girona, Spain).

Lipoprotein separation

Serum very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)-high-density lipoprotein $(HDL)_1$ were isolated by precipitation using $MgCl_2$ and phosphotungstate (Sigma Aldrich Chimie) (Burstein et al., 1970). HDL_2 and HDL_3 subfractions were separated by precipitation using $MgCl_2$ and dextran sulfate (Sigma Aldrich Chimie) (Burstein et al., 1989). Contents of TC and PL in HDL subfractions were determined by the enzymatic colorimetric method described previously. Unesterified cholesterol (UC) contents were determined by enzymatic colorimetric kits (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany). Esterified cholesterol (EC) contents were calculated by the difference between TC and UC contents. HDL_2-cholesteryl esters (CE) contents were estimated as 1.67 times HDL_2-EC contents.

Serum glucose, insulinemia, HbA1c, HOMA-IR, and HOMA-β determination

Serum glucose was measured using glucose oxidase kits (SPINREACT). By accounting for the variability of serum glucose levels in rats, HbA1c rates were used as indexes of glycemic control. HbA1c was measured by chromatography using cation-exchange resin micro-columns (Biosystem JL AB, Ludvika, Sweden). Serum insulin levels were determined using kits (SPI-Bio, Hamburg, Germany) and IR indexes were estimated by the HOMA-IR. The latter was calculated according to the formula (Matthews et al., 1985):

$$\text{HOMA-IR index} = \frac{\text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/mL)}}{22.3}$$

HOMA-β, which estimates β-cell function, was calculated using the following equation (Matthews et al., 1985):

$$\text{HOMA-}\beta\text{ index} = 20 \times \left(\frac{\text{fasting insulin (}\mu\text{U/mL)}}{\text{asting glucose (mmol/L)}}\right) - 3.5$$

Determination of lecithin:cholesterol acyltransferase (LCAT) activity

LCAT activity was determined using fresh serum (Albers

Table 2. Body weight and food intake

	C	HFD	HFD-Alg	HFD-Ext
Body weight after 4 weeks (g)	340.32±23.31	243.53±14.05*	326.45±34.88#	265.54±42.87†‡
Weight gain (g)	48.10±24.42	−64.90±13.90*	33.14±19.52#	−68.50±19.80†‡
Food intake (g/d/rat)	24.21±0.62	17.20±0.50*	23.10±1.34#	22.04±0.53†‡

Values are mean±SEM of 6 rats per group.
Statistical analysis was performed using least significant difference test.
*HFD *vs.* C, #HFD *vs.* HFD-Alg, †HFD *vs.* HFD-Ext, and ‡HFD-Alg *vs.* HFD-Ext were considered significantly different at $P<0.05$. C, control; HFD, high-fat diet; HFD-Alg, high-fat diet supplemented with 1% algae; HFD-Ext, high-fat diet supplemented with 1% algae extract.

et al., 1986). LCAT is the key enzyme that converts UC to EC, after 4 h of incubation at 37°C, from a fatty acid and lecithin. LCAT activity was calculated using the following formula:

$$\text{LCAT (nmol/mL/h) activity} = \frac{UCt_{0\,h} - UCt_{4\,h}}{4\text{ h incubation}}$$

Serum ApoA-I was determined using the turbidimetric assay kits (SPINREACT).

Statistical analysis

Values are presented as means±standard error of the mean (SEM) of six rats per group. Statistical evaluation of the data was carried out using STATISTICA version 5.1 (StatSoft, Inc., Tulsa, OK, USA). After analysis of variance (ANOVA), comparison of the means was performed using least significant difference (LSD) test. Differences were considered significant at $P<0.05$.

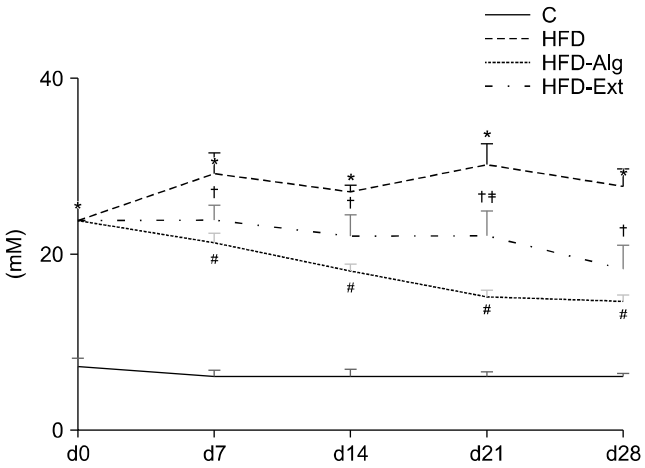

Fig. 1. Glycaemia evolution. Values are mean±SEM of 6 rats per group. Statistical analysis was performed using least significant difference test. *HFD *vs.* C, #HFD *vs.* HFD-Alg, †HFD *vs.* HFD-Ext, and ‡HFD-Alg *vs.* HFD-Ext were considered significantly different at $P<0.05$. C, control; HFD, high-fat diet; HFD-Alg, high-fat diet supplemented with 1% algae; HFD-Ext, high-fat diet supplemented with 1% algae extract.

RESULTS

Weight loss was observed at day 28 in the HFD group compared to the C group. However, weight gain was recorded in the HFD-Alg and HFD-Ext groups *vs* the HFD group. Indeed, body weight significantly increased by 34% and 9%, respectively, and was 19% greater in the HFD-Alg group *vs* the HFD-Ext group. Moreover, compared with the control group, daily food intake, expressed in g/d/rat, significantly decreased by 29% in the HFD group, while it increased by 25% and 22% in the HFD-Alg and the HFD-Ext groups, respectively, *vs* the HFD group (Table 2).

Glycaemia

At day 7, day 14, day 21, and day 28 of the experiment, glycaemia increased by 75, 77, 79, and 77%, respectively in the HFD group *vs* C group. In contrast, in the HFD-Alg and HFD-Ext groups, the glycaemia decreased from day 7 until the end of the experiment (Fig. 1).

Serum glucose, insulinemia, HbA1C, HOMA-IR, and HOMA-β

Compared with the C group, in the HFD group, serum glucose, HbA1c, and HOMA-IR index were 5-, 3-, and 3-fold higher, respectively. Moreover, insulinemia and HOMA-β index were 2- and 45-fold lower, respectively. However, in the HFD-Alg and HFD-Ext groups, these parameters were significantly ($P<0.05$) lower *vs* the HFD group. In addition, serum insulin and HOMA-β indexes were 3- and 19-fold higher respectively, in the HFD-Alg group *vs* HFD group, whereas HOMA-β was 9-fold lower in the HFD-Ext group *vs* HFD group. Moreover, in the HFD-Ext group, serum glucose levels were 1-fold higher and HOMA-β indexes 2-fold lower *vs* the HFD-Alg group (Table 3).

Serum, liver, and fecal lipid concentrations

Compared with the C group, in the HFD group, TC and TAG concentrations increased in the serum (4- and 6-fold, respectively) and liver (7- and 4-fold, respectively). However, these values were significantly decreased in the HFD-Alg and HFD-Ext groups *vs* the HFD group ($P<$

Table 3. Serum glucose, insulinemia, glycosylated hemoglobin (HbA1c), homeostasis model assessment (HOMA)-insulin resistance (IR), and HOMA-β cell function

	C	HFD	HFD-Alg	HFD-Ext
Serum glucose (mM)	4.82±1.54	24.18±1.64*	6.82±0.68#	9.59±0.88†‡
Serum insulin (ng/mL)	0.12±0.05	0.05±0.01*	0.14±0.04#	0.11±0.01
HbA1c (%)	7.03±0.31	21.60±9.76*	6.96±0.85#	7.37±1.14†
HOMA-IR	2.59±0.03	8.67±0.20*	4.28±0.17#	5.73±0.31†
HOMA-β	1.81±0.10	0.04±0.03*	0.97±0.09#	0.37±0.09†‡

Values are mean±SEM of 6 rats per group.
Statistical analysis was performed using least significant difference test.
*HFD *vs.* C, #HFD *vs.* HFD-Alg, †HFD *vs.* HFD-Ext, and ‡HFD-Alg *vs.* HFD-Ext were considered significantly different at $P<0.05$. C, control; HFD, high-fat diet; HFD-Alg, high-fat diet supplemented with 1% algae; HFD-Ext, high-fat diet supplemented with 1% algae extract.

0.05). Fecal total lipids and TC levels were significantly lower (2-fold) in the HFD group *vs* the C group. Inversely, in the HFD-Alg and the HFD-Ext groups, total lipids were 3-fold and 2-fold higher respectively, *vs* HFD group. Furthermore, TC levels were 1-fold higher in the HFD-Alg group *vs* the HFD group, and 1-fold lower in the HFD-Ext group *vs* the HFD-Alg group (Table 4).

LCAT activity, HDL_3-apo, HDL_3-PL, HDL_3-UC, and HDL_2-CE

The activity of LCAT, the enzyme that induces the esterification of HDL_3 cholesterol, decreased by 39% in the HFD group *vs* the C group. This decrease was concomitant with a decrease in levels of HDL_3-PL (substrate of LCAT) and HDL_2-EC (reaction product) and an increase in levels of HDL_3-UC (acyl acceptor). However, compared with the HFD group, the HFD-Alg and HFD-Ext groups showed increases in serum ApoA-I (activator cofactor of the enzyme: 39% and 18%, respectively) levels, which increased LCAT activity (31% and 13%, respectively). This resulted in significant increases in HDL_2-EC (97% and 96%, respectively) levels and decreases in HDL_3-UC (63%

Table 4. Serum, liver and fecal lipid concentrations

	C	HFD	HFD-Alg	HFD-Ext
Serum				
TC (mmol/L)	1.10±0.23	4.16±0.96*	1.66±0.66#	2.35±0.84†
TAG (mmol/L)	0.70±0.58	4.29±0.62*	0.89±0.36#	1.35±0.44†
Liver				
TC (mmol/g liver)	1.10±0.49	8.03±3.18*	1.17±0.14#	1.79±0.75†
TAG (mmol/g)	0.88±0.81	3.51±1.19*	1.61±0.46#	1.98±0.81†
Feces				
Total lipids (mg/d per rat)	67.50±5.00	36.00±5.77*	79.00±2.94#	69.00±2.31†
TC (mg/d per rat)	4.44±0.66	2.67±0.54*	3.05±1.32#	2.85±0.15†

Values are mean±SEM of 6 rats per group.
Statistical analysis was performed using least significant difference test.
*HFD *vs.* C, #HFD *vs.* HFD-Alg, †HFD *vs.* HFD-Ext, and ‡HFD-Alg *vs.* HFD-Ext were considered significantly different at $P<0.05$. C, control; HFD, high-fat diet; HFD-Alg, high-fat diet supplemented with 1% algae; HFD-Ext, high-fat diet supplemented with 1% algae extract; TC, total cholesterol; TAG, triacylglycerol.

Table 5. Serum lecithin:cholesterol acyltransferase (LCAT) activity, apolipoprotein A-I (ApoA-I), high-density lipoprotein $(HDL)_3$-phospholipids (PL), HDL_3-unesterified cholesterol (UC), and HDL_2-cholesteryl ester (CE) contents

	C	HFD	HFD-Alg	HFD-Ext
LCAT (nmol/mL/h)	14.65±3.69	8.88±1.83*	12.87±2.79#	10.17±0.97†‡
ApoA-1 (g/L)	0.91±0.06	0.46±0.03*	0.76±0.08#	0.56±0.05†‡
HDL_3-PL (mM)	0.42±0.04	0.30±0.03*	0.35±0.01	0.36±0.04
HDL_3-UC (mM)	0.22±0.01	2.95±0.34*	1.10±0.36#	2.16±0.06†‡
HDL_2-CE (mM)	3.14±0.45	0.10±0.03*	3.08±0.31#	2.11±0.30†

Values are mean±SEM of 6 rats per group.
Statistical analysis was performed using least significant difference test.
*HFD *vs.* C, #HFD *vs.* HFD-Alg, †HFD *vs.* HFD-Ext, and ‡HFD-Alg *vs.* HFD-Ext were considered significantly different at $P<0.05$. C, control; HFD, high-fat diet; HFD-Alg, high-fat diet supplemented with 1% algae; HFD-Ext, high-fat diet supplemented with 1% algae extract.

and 27%, respectively) levels (Table 5).

DISCUSSION

The objective of this study was to investigate the effect of the green alga *U. lactuca* and its hydroethanolic extract on hyperglycaemia, hyperlipidaemia, and LCAT activity, in HFD-induced type 2 diabetic rats. To mimic the developmental process of human T2D, rats were fed a HFD for 5 weeks. At the end of this period, in HFD group *vs* the C group, there was a significant increase in body weight and a moderate but significant increase in blood glucose levels (7.38±0.16 mM *vs* 5.99±0.44 mM, $P<0.05$). Rats were then administered a low-dose of STZ, which induced a more pronounced increase in blood glucose levels (HFD group: 27.41±3.05 mM, C group: 7.38±0.88 mM, $P<0.05$). The HFD induces insulin resistance (Chun et al., 2010), impairs glucose transport by decreasing expression of glucose transporter 4 and, therefore, inhibits glucose absorption by skeletal muscle (Yu et al., 2016). Moreover, saturated fatty acids (SFA) increase hepatic triglycerides, IR, and harmful ceramides (von Frankenberg et al., 2017; Luukkonen et al., 2018).

STZ, an antibiotic derived from *Streptomyces achromogene*, causes β-cell damages by alkylation or breakage of DNA consequential increases in poly-ADP-ribose synthetase activity (Oztürk et al., 1996). These changes are characterized by permanent hyperglycemia, observed 24 h after injection, due to decreases in pancreatic insulin content. This state is accompanied by polyuria and glycosuria (Ghasemi et al., 2014). A previous study showed that 35 mg/kg of STZ is the optimal dose to induce T2D (Srinivasan et al., 2005). Based on observed hyperglycemia in diabetic rats compared with normal rats, we can conclude that the diabetes was successfully induced in these rats.

The results of our study showed that the diabetic state involved loss of a body weight *vs* controls. This weight loss was coincided with lower food intake, which is consistent with results reported by Magalhães et al. (2019). This can be explained by the capacity of the HFD to release hormones such as cholecystokinin, peptide YY, and glucagon-like peptide (GLP)-1 that promote satiety (Ohlsson et al., 2014). This was not observed when the HFD was supplemented with whole algae or its extract. Indeed, we observed an increase in food intake and consequential body weight in the HFD-Alg and HFD-Ext groups *vs* the HFD group. This may be explained by alga or its extract inhibiting the satietogenic effect of the HFD.

The diabetic state was accompanied by increased glycaemia in the HFD group *vs* the C group, continuing from day 7 until the end of the experiment. Compared with the HFD, HFD-Alg, and HFD-Ext diets were accompanied by a reduction in glycaemia throughout the experiments. These results are in agreement with a previous study (AbouZid et al., 2014) in which *U. lactuca* was shown to have potential antihyperglycemic effect in nicotinamide-STZ-induced T2D rats. These authors reported that all tested micro or macro-algae (including *C. lentillifera*, *S. versicolor*, and *U. lactuca*) have varying degrees of antihyperglycemic effects without significantly increasing serum insulin concentrations. The results of our study showed that hydroethanolic extracts combined with a HFD-Ext reduced glycaemia *vs* a HFD alone, but to a lesser extent than whole alga. This is probably due to the content polyphenols and flavonoids (20±2.43 mg GAE/g dry matter and 1.50±0.96 mg QE/g dry matter, respectively) in the extract, which act by regulating expression of genes involved in insulin signaling and gut microbiota in T2D mice (Lin et al., 2018; Yan et al., 2019).

HbA1c is proportional to blood glucose levels, and reflects the glycemic balance. Thus, HbA1c is considered the best predictor of diabetes progression (Sherwani et al., 2016). In this study, induction of T2D caused an increaseof HbA1c in the HFD group compared with the C group. However, HbA1c decreased concomitantly with glycaemia in diabetic rats in the HFD-Alg and HFD-Ext groups compared with the HFD group, possibly due to the action of polyphenols and flavonoids contained in the alga. Vuppalapati et al. (2016) reported that flavonoids from the red seaweed *Acanthophora spicifera* control hyperglycemia by reducing blood glucose levels in diabetic rats and that the administration of the flavonoid-rich fraction reduces HbA1c levels.

In the present study, there were, significant decreases in insulinemia and HOMA-β indexes in the HFD group *vs* the C group. HOMA-β indexes estimates β-cell function whereas increases in HOMA-IR indicate alterations in insulin sensitivity due to increased free fatty acids in blood that interfere with insulin in tissues. In a previous study, Lichtenstein and Schwab (2000) showed that a HFD containing high amount of saturated fatty acids decreases insulin sensitivity and, consequently, affects glucose homeostasis. This could involve inactivation of GLP-1, an incretin hormone with antidiabetic effects, by dipeptidyl peptidase-IV (Tahara et al., 2009; Domínguez-Vías et al., 2020). Consumption of the HFD supplemented with whole alga or its extract increased serum insulin concentrations and HOMA-β indexes compared with the HFD. Moreover, decreases in HOMA-IR were observed. These results are in agreement with a study by Yang et al. (2015) in which the red alga *G. amansii* decreased plasma glucose levels and HOMA-IR indexes and increased insulinemia in diabetic rats fed a HFD. This may be due to water-soluble fibers that delay carbohydrate absorption in the gastrointestinal tract, thus suppressing increases in postprandial blood glucose levels. Moreover, the whole

alga or its extract could inhibit the peptidase involved in glucose homeostasis. Furthermore, we noted that blood glucose levels and HOMA-IR indexes were higher and HOMA-β indexes were lower in the HFD-Ext group *vs* the HFD-Alg group. This result contradicts with those of Akbarzadeh et al. (2018), which showed that hydroalcoholic extracts of the brown alga *Sargassum oligocystum* in STZ-induced diabetic rats significantly decreased fasting blood glucose levels and HOMA-IR indexes and increased HOMA-β indexes without significantly impacting insulinemia. The authors suggested that focoidans (a type of sulfated heteropolysaccharides) are involved in the regulation of carbohydrate homeostasis. Furthermore, that fucoidans, polyphenols and flavonoids from a variety of other *Sargassum* species, may have anti α-glucosidal activity that can be used in the treatment of diabetes.

Dyslipidemia is an important factor of T2D. Consumption of a HFD, particularly including excess SFA, is known to increase chylomicron synthesis, which increases TC and TAG levels, therefore contributing to the etiology of cardiovascular diseases (CVD) (Karam et al., 2018). In our study, the diabetic state was accompanied by increased serum TC and TAG in the HFD group compared with the C group. This increase was concomitant with their increase in liver production. Consumption of whole alga or its extract decreased serum and liver TC levels and increased it fecal excretion compared with the HFD alone. The significant reduction in liver cholesterol content was probably due to stimulation of bile acid synthesis, which is the major route of cholesterol excretion. Furthermore, decreases in serum and liver TAG levels occurred in parallel indicating decreased TAG synthesis. These results are in agreement with those of Yang et al. (2015), which demonstrated that the red alga *G. amansii* feeding reduces plasma TC and TAG concentrations in diabetic rats. Moreover, Kumar et al. (2015) reported that supplementing rats fed a high cholesterol diet and HFD with the tropical seaweed *C. lentillifera* protected against CVD by reducing plasma TC and TAG concentrations. However, the hydroethanolic extract was less effective than the whole alga. Previous studies have demonstrated that some algal fibers (e.g., sodium alginate, funoran, porphyrin, and carrageenan) that weren't present in the extract interact with dietary cholesterol to facilitate its excretion (Pereira, 2018), probably by promoting 7-α hydroxylase activity. Moreover, it has been reported that polysaccharides from *U. lactuca* can reduce plasma and liver lipids in rats fed a HFD (Tair et al., 2018). This active process of highly sulphated polysaccharides from green alga can be attributed to the improved metabolism and breakdown of cholesterol. In addition, it can regulate the transcription of the peroxisome proliferator-activated receptor gene, leading to a decrease in lipogenesis and an increase in lipolysis, thus accelerating metabolism and decomposition of TAG (Qi and Sheng, 2015).

LCAT is a plasmatic enzyme capable of esterifying cholesterol molecules present in circulating plasma lipoproteins, a function critical for HDL biogenesis and reverse cholesterol transport (Carmo et al., 2017). Cholesterol esterification catalyzed by LCAT reduces UC in plasma. Therefore, LCAT plays a key role in the incorporation of free cholesterol into HDL and its transfer from VLDL to LDL, which is returned to liver cells (Karam et al., 2018). In the present study, LCAT activity and serum ApoA-I (enzyme activator), HDL_3-PL and HDL_2-EC contents were significantly decreased in the HFD group *vs* the C group, whereas HDL_3-UC contents were increased. The decrease in LCAT activity and its activator cofactor (ApoA-I) during T2D may be due to non-enzymatic glycation (Ossoli et al., 2016). In response to consumption of whole alga or its extract, an increase in ApoA-I induced LCAT activity, causing significant accumulation of CE in HDL_2 and leading to uptake of cholesterol by the liver for metabolism and biliary excretion. The increase in ApoA-I content and LCAT activity could be due an increase of their synthesis. Whole alga and its extract may protect this enzyme and its activator cofactor from non-enzymatic glycation. However, the mechanisms by which *U. lactuca* acts require further research.

In conclusion, our study indicates that consumption of the green alga *U. lactuca* and its hydroethanolic extract mitigate insulin resistance, which plays a fundamental role in the pathogenesis of T2D and may help regenerate damaged pancreatic β-cells. In addition, *U. lactuca* and its hydroethanolic extract may have anti-atherosclerotic effects by improving reverse cholesterol transport. Such results may have major therapeutic promise for helping to prevent onset of complications in diabetic patient.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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