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Effects of ID-alG™ on Weight Management and Body Fat Mass in High-Fat-Fed Rats†

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Seaweed extract of *Ascophyllum nodosum*, ID-alG™, was evaluated for its chronic effects on weight management in high-fat-fed Sprague-Dawley rats. ID-alG™ was orally administered daily during 9 weeks at doses of 40 and 400 mg/kg/day with fat-enriched diet (FED) in comparison with two control groups consuming standard diet (negative control) or FED (positive control) and orally treated with vehicle. Body weight, percentage of body fat mass and lipid parameters were measured. After 9 weeks, the oral administration of ID-alG™ at both doses decreased significantly the mean body weight gains (MBWG) of rats submitted to the FED in comparison to the positive control (−6.8% and −11.8%). ID-alG™ at both doses improved significantly the MBWG of rats and decreased significantly the percentage of body fat mass of rats (−9.8% and −19.0%), in comparison to the positive control. In the same way, the triglyceride blood level was also significantly improved for the dose of 400 mg/kg/day (−30.6% vs. +49.9% for the positive control); and the dose of 40 mg/kg/day just lead to a trend. Moreover, in both controls and ID-alG™-treated groups, total cholesterol, LDL and HDL blood levels were not modified. The seaweed extract of *Ascophyllum nodosum*, ID-alG™, demonstrated beneficial effects on weight management of rats submitted to a high-fat diet. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: ID-alG™; Ascophylum nodosum; seaweed; weight management; lipase inhibition; α-amylase inhibition.

INTRODUCTION

Weight management and the development of new ingredients possibly having a beneficial effect on the obesity epidemic are two of today's major challenges. Overweight and obesity problems are clearly linked to an increase in plasma triglycerides and also lead to a modification of the cholesterol profile, which are known to be risk factors incoronary heart disease (Wildman *et al*., 2008). Triglyceride is a glyceride in which the glycerol is esterified with three fatty acids. It is the main constituent of vegetable oil and animal fats. Triglycerides are formed from a single molecule of glycerol, combined with three fatty acids on each of the OH groups, and make up most of the fats digested by humans. That is where the enzyme pancreatic lipase acts on. Ester bonds form between each fatty acid and the glycerol molecule, hydrolysing the bond and 'releasing' the fatty acid (Dubois *et al*., 1994). Whereas the triglyceride form cannot be absorbed by the duodenum, fatty acids, monoglycerides and some diglycerides are absorbed by the duodenum. Triglycerides, as major components of very low density lipoprotein (VLDL) and chylomicrons, play an important role in metabolism as energy sources (Bracco, 1994). They contain more than twice as much energy (9 kcal/g) as carbohydrates and proteins. In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis (Gandotra and Miller, 2008), and, by extension, to the risk of heart disease and stroke (Talmud *et al*., 2004; Alagona, 2009). Therefore promoting weight loss and/or preventing weight regain may lead to improvement in triglyceride metabolism and reduce risk factors.

ID-alG™ is produced from the brown alga *Ascophyllum nodosum* (Fucacea family) using grape extract as a carrier (< 5%). This brown alga is known to contain specific polyphenols, phloglucinol (Pavia and Brock, 2000) and, in polymeric form, phlorotannins (Shibata *et al*., 2004; Audibert *et al*., 2010).

Some evidence for the effect of polyphenol components on digestive enzymes has been reviewed previously (Kandra *et al*., 2004; McDougall and Steward, 2005; McDougall *et al*., 2005; Li *et al*., 2007; Lee *et al*., 2007; Adisakwattana *et al*., 2010; Kawakami *et al*., 2010). Extracts from *Ascophyllum nodosum* were found to inhibit rat intestinal α-glucosidase and to stimulate basal glucose uptake into 3 T3-L1 adipocytes (Zhang *et al*. 2007). This α-glucosidase inhibition was associated with the polyphenolic components of the *Ascophyllum nodosum* extracts (Apostolidis and Lee, 2010) and an enriched polyphenolic fraction was shown to reduce the rise in blood glucose after an oral sucrose tolerance test in diabetic mice (Zhang *et al*., 2007). The crude polyphenol extract and an enriched polyphenolic fraction had decreased blood total cholesterol and glycated serum protein levels compared with untreated diabetic mice, whereas the crude polyphenol extract also normalized the reduction in liver glycogen level that occurred in diabetic animals. Seaweed *Ascophyllum nodosum* extracts containing phlorotannins were also found to be active on differentiation and fatty acid accumulation in differentiating 3 T3-L1 adipocytes (He *et al*., 2009).

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Other extracts from marine algae were also found to inhibit α-glucosidase, α-amylase (Heo *et al.*, 2009) and lipase (Bitou *et al.*, 1999; Ben Rebah *et al.*, 2008).

The inhibition properties of the polyphenolic components of *Ascophyllum nodosum*, on α-glucosidase and α-amylase, were particularly studied in order to determine their potential application on the prevention of hyperglycemia. The relationship between polyphenol extracts characterized by inhibition properties of digestives enzymes and weight management has been studied in polyphenols other than that of *Ascophyllum nodosum*. Some *in vivo* studies were performed on hypercaloric diet models (high-fat diet) showing antiobesity and hypolipidemic effects of polyphenols in relation to their inhibition properties in digestive enzymes (Bose *et al.*, 2008; Han *et al.*, 2003; Uchiyama *et al.*, 2011; Yang *et al.*, 2010). Han *et al.* (2003) showed that the inhibitory effects of the polyphenol fraction of *S. matsudana* leaves on obesity induced by a high-fat diet might be due to the inhibition of carbohydrate and lipid absorption from the small intestine through the inhibition of α-amylase. On mice fed with a high-fat diet, polyphenols of tea suppressed increases in body weight, parametrial adipose tissue mass and liver lipid content, and these healthy effects were related to the inhibition of intestinal lipid absorption (Uchiyama *et al.* 2011).

The aim of this study was to determine the lipase and α-amylase inhibitory properties of ID-alG™ in an *in vitro* model and to investigate the chronic effects of ID-alG™ in female Sprague-Dawley rats, on a high-fat-diet model, using two doses (40 and 400 mg/kg/day) orally administered over a period of 9 weeks. Measurements of body weight, body fat mass, triglycerides, cholesterol, HDL and LDL blood levels were performed at the beginning and the end of the study. The drink and food intakes were recorded three times per week.

MATERIAL AND METHODS

ID-alG™ is a manufactured ingredient produced by Bioserae and obtained from thallus of brown alga, *Ascophyllum nodosum* (*Fucaceae* family) using *Vitis vinifera* grape extract as a carrier (< 5%) (Bioserae confidential process).

***In vitro* models (enzymatic activity).** Pre-tests were performed in order to determine the effect of ID-alG™ on the enzymatic activities of lipase and α-amylase in *in vitro* models.

Measurement of lipase activity. The enzymatic activity of lipase (Lipase *Candida rugosa*, Ref. Sigma L-1754 at a concentration of 1000 units/mL) was determined using pure olive oil as a substrate (1.5 mL) and the released fatty acid was quantified by a titrimetric method adapted from Sigma (EC 3.1.1.3 (1993) *Reagent Chemicals ACS Specification*, 8th edn, 95). The hydrolyse of pure olive oil was performed at 37 °C and at pH 7.2 during 30 min and the released fatty acid was measured by NaOH titration under blue colour reagent (Thymolphthalein, Ref. Sigma T-0626). The control used was olive oil with added lipase and was considered as the positive control (100% lipase activity). The measurement of the effect of ID-alG™ on the lipase activity was calculated from the same assay plus the product ID-alG™ reacting as an inhibitor.

The inhibitory effect of ID-alG™ on the release of fatty acid was determined at 50 mg/L and the percentage of enzymatic inhibition was expressed as:

$$\%\ \text{inhibition} = 100 - \frac{V_{\text{inh}} \times 100}{V_{\text{max}}}$$

where V_{inh} is the volume in millilitres of NaOH used to obtain a light blue colour in the presence of ID-alG™ at 50 mg/L, and V_{max} is the volume in millilitres of NaOH used to obtain a light blue colour without any inhibitor.

Measurement of α-amylase activity. The enzymatic activity of α-amylase (amylase from porcine pancreas, Ref. Sigma A-3176 at a concentration of 1.9 units/mL) was determined with potato starch as substrate (10 mg of potato starch, 1% solution in water previously boiled) and the released maltose was quantified by a colorimetric method adapted from Sigma Procedure (EC 3.2.1.1.; Bernfeld, P. (1955) *Methods in Enzymology* **1**, 149–158). The hydrolysis of starch was performed at 37 °C (body temperature), at pH 6.9 during 3 min, and the reaction was stopped with sodium potassium tartrate (Ref. Sigma S-2377) and 3,5-dinitrosalicylic acid (Ref. Sigma D-0550) colour reagent in a boiling water batch for 15 min and cooled at room temperature. Then, the released maltose was measured by absorbance at 540 nm. The control used was potatoes starch added with the amylase and was considered as the positive control (100% amylase activity). The measurement of the effect of ID-alG™ on the α-amylase activity was calculated from the same assay plus the product ID-alG™ reacting as an inhibitor.

The inhibitory effect of ID-alG™ on the release of maltose was determined at 41 mg/L and the percentage of enzymatic inhibition was expressed as:

$$\%\ \text{inhibition} = 100 - \frac{A_{\text{inh}} \times 100}{A_{\text{max}}}$$

where A_{inh} is the absorbance level at 540 nm in the presence of ID-alG™ at 41 mg/L, and A_{max} is the maximal absorbance level at 540 nm without any inhibitor. For both conditions (A_{inh} and A_{max}) the values of absorbance were determined taking into account sample blanks.

***In vivo* model: high-fat-fed rats.**

Animals and obesity induction. Twenty-four female Sprague-Dawley rats, weighing 170–180 g at the start of the experiment, were obtained from the 'Centre d'élevage HARLAN France' (Gannat, France). Animals were identified and placed two per cage in an air-conditioned room under controlled conditions of temperature (22 ± 2 °C), relative humidity (50 ± 10%), with an inverted 12-h light:dark cycle (light off at 08:00 hours) and they had access to standard diet TD.94045 or the fat-enriched diet TD.06414 (Harlan Teklad, US, Madison), which are described in Table 1. During the quarantine period, rats received the standard diet TD.94045 (Harlan Teklad US, Madison, U.S.A.), with water provided *ad libitum*. After one week of acclimatization, rats were weighed and randomly divided into four groups ($n = 6$): one group received the standard diet TD.94045 for the last 8 weeks (negative control) and three groups received the fat-enriched diet TD.06414 for

Table 1. Composition of the standard and fat-enriched diets (%)

	Standard diet TD.94045	Fat-enriched diet TD.06414
Casein	20.0	26.5
L-cystein	0.3	0.4
Corn starch	39.7	–
Maltodextrin	13.2	16.0
Sucrose	10.0	9.0
Bacon	–	31.0
Soya oil	7.0	3.0
Cellulose	5.0	6.55
Mineral mix, AIN-93 G-MX	3.5	4.8
Dialkaline, calcium phosphate	–	0.34
Vitamin mix, AIN-93 G-VX	1.0	2.1
Choline bitartrate	0.25	0.3
TBHQ (antioxidant)	0.0014	–
Blue food colouring agent	–	0.01

the induction of obesity for the same duration (8 weeks). For two of the three groups receiving the fat-enriched diet TD.06414, ID-alG™ was freshly dissolved each day in spring water (source Cristaline Aurèle, France) prior to oral administration by intragastric gavage, with an administration volume of 10 ml/kg: one group at the dose of 40 mg/kg/day (20 mg/kg in the morning and the same dose in the afternoon), the other group at the dose of 400 mg/kg/day (200 mg/kg in the morning and the same dose in the afternoon). The oral treatments with ID-alG™ began 1 week before the induction of obesity. The dose used for rats corresponded to our recommended daily dosage of ID-alG™ for humans (400 mg per day) during the meal.

The animal care unit is authorized by the French Ministres of Agriculture and Research (Government Authorization No. A 54-547-1), the protocol was approved by the local ethical committee and the animal experiments were performed according to the European guidelines for animal experimentation (European Communities Council Directive no. 86/609/EEC of 24 November 1986), the rules provided by the ASAB Ethical Committee (2006) and the Canadian Council on Animal Care (2003).

Assessment of weight gain management. The food and drink intakes of all the cages of rats were recorded three times per week in order to measure the ingested quantities of diet and water per rat.

On Day 0 (D0), 24 hours before the beginning of the oral treatments and on Day 64 (D64), 24 hours after the last oral treatment, an EM scan was performed on all rats to determine the percentage of body fat mass. Rats were anesthetized by intraperitoneal injections of acepromazine (Calmivet, Vetoquinol, Lure, France) at the dose of 2 mg/kg and ketamine (Ketamine 1000, Virbac, Carros, France) at the dose of 50 mg/kg. Rats were then placed in the EM scan chamber (EM-Scan/TOBEC®, Model SA-3114 Detection Chamber, Swantech International, Gennevilliers, France) and five measurements of the total body electrical conductivity were performed for each rat (with a standard error $< 3.0\%$) in order to determine the percentage of the body fat mass.

Assessment of lipidic parameters. On D0, 24 hours before the beginning of the oral treatments and on D64, 24 hours after the last oral treatment, a blood sample of 1.5 mL was performed on each rat from the caudal vein in a dry tube (Térumo, Leuven, Belgium), without anesthesia.

The blood samples were placed at +4 °C for 20 to 30 min for blood clotting and centrifuged at $1500 \times \mathbf{g}$ for 15 min. Serums were then collected in polypropylene tubes, frozen at −20 °C and stored at −80 °C until performing lipidic status with dosage of triglycerides, total cholesterol, HDL and LDL levels. Dosages were performed by the Laboratory of Medical Analysis Aubert (Vandœuvre-lès-Nancy, France) using a biochemical automate (Kone Prime 60, Thermo Fisher Scientific Inc., Cergy-Pontoise, France) under the responsibility of Doctor M.-C. Dederichs (PharmD, Doctor Biologist and Director of the Laboratory of Medical Analyses).

Statistical analyses. Results are expressed as mean standard error of the mean (SEM). The weight and weight gain of rats, the body fat mass and the blood levels of triglycerides, total cholesterol, HDL and LDL were analysed at the end of the experiment. Statistical analyses of the data were performed using the Kruskall–Wallis test (non-parametrical ANOVA). When significance was observed, the Mann–Whitney *U*-test was used to compare treated groups with the control groups. For all the comparisons, differences were considered to be significant at the level of $p < 0.05$. All statistical analyses were carried out using the StatView®5 statistical package (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Effect of ID-alG™ on lipase and α-amylase activities

For the lipase activity using the titrimetric method with NaOH titration and measured with a solution of ID-alG™ at 50 mg/L on 30 batches, ID-alG™ induced a decrease in the lipase activity of 71.0 ± 2.0% (Table 2).

For the α-amylase activity using the colorimetric method with reading of absorbance at 540 nm and measured with a solution of ID-alG™ at 41 mg/L on 30 batches, ID-alG™ induced a decrease in the α-amylase activity of 68.0 ± 2.0% (Table 2).

Effect of ID-alG™ on a fat-enriched-diet model

Food and drink intakes. The recording of food and drink intakes during the 9 weeks of experiment are presented in Figs 1 and 2, respectively. No statistical analyse was performed on these parameters because of the low number of values per group ($n = 3$).

Nevertheless, the food intakes of the two groups consuming ID-alG™ with the fat-enriched diet was always higher than that of the positive control from the third week but globally lower than that of the negative

Table 2. Effects of ID-alG™ on lipase and α-amylase inhibition activities (%) measured *in vitro*

	Lipase activity	A-amylase activity
Inhibition activity (%)	71.0 ± 2.0[a]	68.0 ± 2.0[a]

[a]Values are expressed as mean ± SEM on 30 batches.

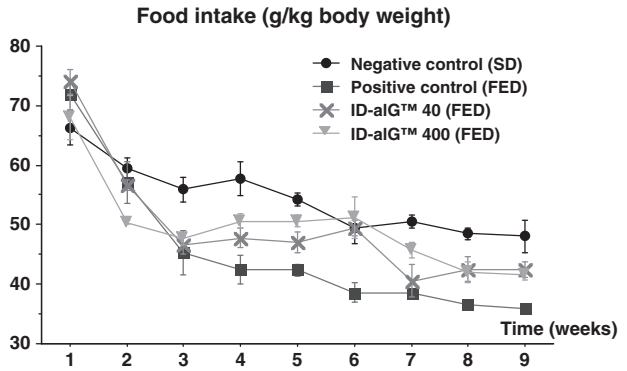

Figure 1. Evolution of weekly food intake of rats (g/kg body weight) on a standard diet (SD) and fat-enriched diet (FED) during the 9 weeks of the experiment and orally treated with ID-alG™ at both doses of 40 and 400 mg/kg/day and vehicle between weeks 2 and 9.

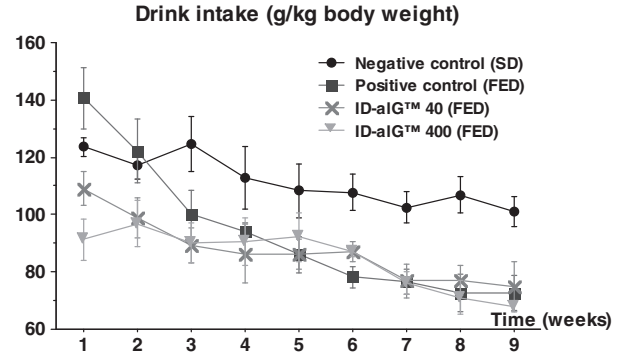

Figure 2. Evolution of weekly drink intake of rats (g/kg body weight) on a standard diet (SD) and fat-enriched diet (FED) during the 9 weeks of the experiment and orally treated with ID-alG™ at both doses of 40 and 400 mg/kg/day and vehicle between weeks 2 and 9.

control. In addition, the drink intakes of the two groups consuming ID-alG™ with the fat-enriched diet were always lower than that of the negative control. Globally, the three groups consuming the fat-enriched diet had an equivalent drink intake except during the first week of the experiment.

Effect on body weight of rats under fat-enriched diet. Figure 3 shows the body weight curves of rats fed with a standard diet (negative control group) or fat-enriched diet during the 9 weeks of the experiment and orally treated daily with the seaweed extract ID-alG™ at doses of 40 and 400 mg/kg/day or the vehicle (positive control group). The Kruskal–Wallis test showed no significant difference between the mean body weights of rats of the four experimental groups on D1 ($H_{(ddl=3)} = 0.28$, $p = 0.96$) and on D8 ($H_{(ddl=3)} = 2.98$, $p = 0.40$). On the other hand, at the end of the 9 weeks of experiment, the Kruskal–Wallis test showed a significant difference between the mean body weights of rats of the four experimental groups on D64 ($H_{(ddl=3)} = 13.18$, $p = 0.004$).

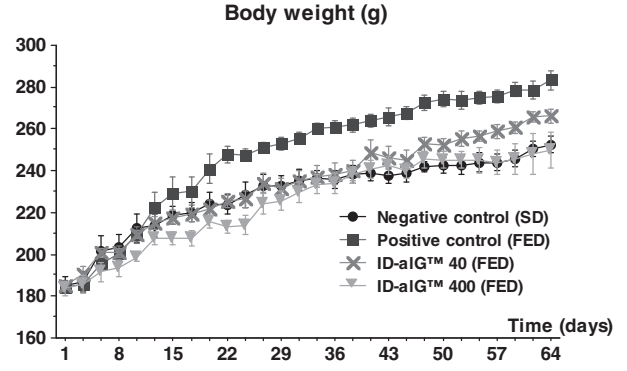

Figure 3. Evolution of body weight of rats (g) on a standard diet (SD) and fat-enriched diet (FED) during the 9 weeks of the experiment and orally treated with ID-alG™ at both doses of 40 and 400 mg/kg and vehicle between Day 8 and Day 64.

At the end of the experiment on D64, the mean body weight (MBW) of rats was significantly increased by the fat-enriched diet (252.5 g ± 3.9 for the negative control group versus 283.3 g ± 4.7 for the positive control group, $p = 0.004$) and the oral consumption of ID-alG™ induced a significant decrease of MBW under the fat-enriched diet in comparison to the positive control: 264.0 g ± 3.3 for the dose of 40 mg/kg/day ($p = 0.016$) and 249.8 g ± 8.6 for the dose of 400 mg/kg/day ($p = 0.025$) (Table 3).

A statistical difference was observed between the mean body weight gains (MBWG) of rats of the four experimental groups between D8 and D64 according to the Kruskal–Wallis test ($H_{(ddl=3)} = 10.01$, $p = 0.02$). As presented in Table 3, the fat-enriched diet induced a significant increase of the MBWG of rats from 49 g ± 7.3 for the negative control group to 82.8 g ± 5.4 for the positive control group ($p = 0.007$). The oral consumption of ID-alG™ significantly decreased the MBWG of rats in comparison to the positive control group in a dose-dependent manner. Between D8 and D64 and in comparison to the positive control group, the MBWG of rats was reduced by 22.0% (64.6 g ± 3.2; $p = 0.029$) and by 31.8 % (56.5 g ± 9.3; $p = 0.05$) for the two groups consuming the fat-enriched diet and orally treated with ID-alG™ at the doses of 40 and 400 mg/kg/day, respectively. In addition, no statistical difference was observed between the negative control group and the two groups consuming the fat-enriched diet and orally treated with ID-alG™ at both doses of 40 and 400 mg/kg/day.

Effect on body fat mass of rats under the fat-enriched diet. Table 3 shows the percentage of body fat mass (BFM) of rats fed with a standard diet (negative control group) or fat-enriched diet during the 9 weeks of the experiment and orally treated daily with the seaweed extract ID-alG™ at doses of 40 and 400 mg/kg/day or the vehicle (positive control group). The Kruskal–Wallis test showed no significant difference between the BFM of rats of the four experimental groups before the start of oral treatments on D0 ($H_{(ddl=3)} = 0.41$, $p = 0.94$) but a statistical difference was observed at the end of the 9 weeks of oral treatments on D64 ($H_{(ddl=3)} = 11.78$, $p = 0.008$). The BFM was statistically different between the negative control (5.40% ± 0.18) and the positive control (6.32% ± 0.14) groups ($p = 0.006$). As for the MBW and the MBWG of rats, the BFM was significantly lower in the two groups fed with the fat-enriched diet and orally treated daily with ID-alG™ at both doses than that of the positive control group: 5.70 % ± 0.06 ($p = 0.005$) and 5.12% ± 0.35 ($p = 0.04$) for the doses of 40 and 400 mg/kg/day, respectively. No statistical difference was observed between the mean BFM of rats of the negative control group and the two groups fed with the fat-enriched diet and orally treated with ID-alG™ at both doses of 40 and 400 mg/kg/day (Table 3).

Table 3. Effects of oral treatments during 9 weeks (D1-D64) with ID-alG™ at both doses of40 and 400 mg/kg/day and vehicle on body weight, mean body weight gain (MBWG) and body fat mass (BFM) of rats (Mean ± SEM) on a fat-enriched diet (FED) or standard diet (SD) during 8 weeks (D8-D64)

Treatment (n = 6)	Dose (mg/kg/day)	BW (g)			MBWG (g)		BFM (%)	
		Day 1	Day 8	Day 64	Day 1–Day 8	Day 8–Day 64	Day 0	Day 64
Negative control (SD)	–	185.3 ± 3.8	203.5 ± 6.3	252.5 ± 3.9	+18.2 ± 4.4	+49.0 ± 7.3	1.70 ± 0.20	5.40 ± 0.18
Positive control (FED)	–	184.7 ± 2.7	200.5 ± 3.3	283.3 ± 4.7[a]	+15.8 ± 3.3	+82.8 ± 5.4[e]	1.67 ± 0.24	6.32 ± 0.14[h]
ID-alG™ 40 (FED)	40	185.2 ± 1.9	200.7 ± 2.4	264.0 ± 3.3[b;c]	+15.5 ± 2.6	+64.6 ± 3.2[f]	1.72 ± 0.13	5.70 ± 0.06[i]
ID-alG™ 400 (FED)	400	183.7 ± 3.4	193.3 ± 3.8	249.8 ± 8.6[d]	+9.7 ± 3.6	+56.5 ± 9.3[g]	1.68 ± 0.23	5.12 ± 0.37[j]

Values are expressed as mean ± SEM of six rats in each group.
BW: [a]$p = 0.004$ and [b]$p = 0.025$ compared with the negative control group on Day 64 using the Mann–Whitney U-test. [c]$P = 0.016$ and [d]$P = 0.025$ compared with positive control group on Day 64 using Mann–Whitney U-test.
MBWG: [e]$p = 0.007$ compared with the negative control group between Day 8 and Day 64 using the Mann–Whitney U-test. [f]$p = 0.029$ and [g]$p = 0.05$ compared with positive control group between Day 8 and Day 64 using Mann–Whitney U-test.
BFM: [h]$p = 0.006$ compared with the negative control group on Day 64 using the Mann–Whitney U-test.. [i]$p = 0.005$ and [j]$p = 0.04$ compared with the positive control group on Day 64 using the Mann–Whitney U-test.

Effect on lipidic parameters. Table 4 presents the evolution of triglyceride blood levels between D0 and D64. The Kruskal-Wallis test showed no significant difference between the mean triglyceride blood levels of rats of the four experimental groups on D0 ($H_{(ddl=3)} = 0.01$, $p = 0.99$) but a significant difference on D64 ($H_{(ddl=3)} = 10.37$, $p = 0.016$). At the dose of 40 mg/kg/day, ID-alG™ did not improve the triglyceride blood level in comparison to the Positive control group. However, the highest dose of 400 mg/kg/day of ID-alG™ induced a significant decrease in the triglyceride blood level in comparison to the positive control group (0.52 g/L ± 0.07 vs. 1.06 g/L ± 0.21, $p = 0.01$).

No statistical difference was observed between the mean total cholesterol, LDL and HDL blood levels of rats of the four experimental groups on D0 and at the end of the treatment period on D64 (data not shown).

DISCUSSION

The present study was designed to determine the chronic effects of orally administered ID-alG™, a seaweed extract of *Ascophyllum nodosum*, on the weight management of rats receiving a fat-enriched diet for inducing obesity. The comparison between the negative control and the positive control groups showed clearly the impact of the fat-enriched diet on the MBWG and the percentage of BFM. The consumption of the fat-enriched diet at 60.0% fat/kcal during 8 weeks induced a significant increase in the MBWG of 69.0% and in the BFM of 17.0% in comparison with the standard diet at 16.0% fat/kcal. Rats receiving the fat-enriched diet at 60.0% fat/kcal and orally treated with ID-alG™ showed significantly lower MBWG and BFM in comparison to the positive control group at both doses of 40 and 400 mg/kg/day of ID-alG™: the MBWG was reduced by 22.0% and 31.8%, respectively, and the BFM was reduced by 9.8% and 19.0%, respectively. Over the same period, the MBWG and the BFM of rats receiving the fat-enriched diet and orally treated with ID-alG™ at both doses of 40 and 400 mg/kg/day were statistically equivalent to those of rats receiving the standard diet, indicating that the oral consumption of ID-alG™ seemed to neutralize the weight gain of rats induced by a fat-enriched diet.

Table 4. Effects of oral treatments during 9 weeks (D1-D64) with ID-alG™ at both doses of40 and 400 mg/kg/day and vehicle on triglyceride blood levels of rats (mean ± SEM) on a fat-enriched diet (FED) or standard diet (SD) during 8 weeks (D8–D64)

Treatment (n = 6)	Dose (mg/kg/day)	Triglyceride blood level (g/L)	
		Day 0	Day 64
Negative control (SD)	–	0.72 ± 0.13	0.67 ± 0.11
Positive control (FED)	–	0.71 ± 0.11	1.06 ± 0.21
ID-alG™ 40 (FED)	40	0.72 ± 0.14	0.98 ± 0.11
ID-alG™ 400 (FED)	400	0.75 ± 0.18	0.52 ± 0.07[a]

Values are expressed as mean ± SEM of six rats in each group. [a]$p = 0.01$ compared with the positive control group on Day 64 using the Mann–Whitney U-test.

The oral administration of ID-alG™ was associated with a significant reduction of the triglyceride blood levels at the dose of 400 mg/kg/day, showing its potential to improve triglyceride metabolism and to reduce risk factors. Hepatic triglyceride accumulation from peripheral dietary sources and from endogenous *de novo* lipogenesis has been quantified in adult Sprague-Dawley rats and shown that hepatic triglyceride accumulation concentrations are acutely influenced by dietary lipid concentrations (Delgado *et al.*, 2009). The results observed on the inhibition of triglyceride blood levels by ID-alG™ are one promising key to help solve overweight problems, as shown in this rat study.

No effect was observed on the cholesterol level in the female Sprague-Dawley rats, which could be due to the rodent model used, as the main criteria of this study was the fat absorption inducing triglycerides and fat mass. Further investigations are essential to determine if ID-alG™ indeed has a direct (lipid metabolism) or an indirect (weight loss) effect on the cholesterol profile, ideally with further clinical trials in humans.

The beneficial effect of ID-alG™ observed on the *in vivo* model on weight management could be linked to its inhibitory properties of digestive enzymes such as lipase and α-amylase. Preliminary results showed that ID-alG™ had an important inhibitory effect on the enzymatic activities of lipase and α-amylase. However,

a dose-dependent investigation would be more appropriate and conducted to test this *in vitro* potential. ID-alG™ contains a level of tannins of 39% eq. phloroglucinol ± 1% (measured according to European Pharmacopeia analytical method (Ph. Eur. 6.3, §2.8.14) based on the complexation of the polyphenols with the higher polymerization degree with powder and expressed as phloroglucinol equivalent). These results are in accordance with previous studies performed on *Ascophylum nodosum* extracts showing enzymatic inhibitory activities of α-glucosidase and α-amylase in correlation with the phenolic components (Apostolidis and Lee, 2010). Marine brown algae such as *Ascophyllum nodosum*, accumulate polyphenols in polymeric form, i.e. phlorotannins (Shibata *et al*., 2004; Audibert *et al*., 2010) and the specificity of the seaweed extract of *Ascophyllum nodosum*, ID-alG™, is its higher content of polyphenols in polymeric form, with 39.0% ± 1.0 of tannins. Polymers showed a strong inhibitory activity against α-amylase, while oligomers had a relatively weak effect suggesting that the inhibition of α-amylase activity would probably depend on the degree of polymerization (Lee *et al*., 2007). The polymerization of polyphenols is also required for enhancement of pancreatic lipase inhibition (Nakai *et al*. 2005).

Our results clearly demonstrate the beneficial effects of chronic oral administration of ID-alG™ in high-fat-fed female Sprague-Drawley rats and they show that ID-alG™ could be helpful to facilitate the inhibition of triglyceride blood levels in situations of promoting weight loss and/or prevention of weight gain. The specific high content of tannins could explain its specific inhibitory activities on α-amylase and lipase, leading to a lower absorption of lipids and carbohydrates resulting from the diet. Further investigations are essential to prove *in vivo* the relevant mechanism of action involved for this ID-alG™ effect on weight management and body fat mass reduction. Moreover, these *in vivo* results have already been confirmed in humans in a monocentric, parallel, double-blind, randomized and placebo controlled clinical trial made on 60 women, characterized with a mean age of 33 years old over a period of 2 months (unpublished results from a clinical study performed by BIO SERAE Laboratoires).

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Conflict of Interest

The authors state there was no conflict of interest.

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