

ARTICLE

# Toxicological and therapeutic evaluation of the algae *Macrocystis pyrifera* (Phaeophyceae) in rodents

Evaluación toxicológica y terapéutica del alga *Macrocystis pyrifera* (Phaeophyceae) en roedores

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**Resumen.-** *Macrocystis pyrifera* es un alga parda gigante con alto contenido nutricional, utilizada como suplemento dietético en algunos animales de granja. Contiene fucoidan, el cual posee efecto farmacológico sobre el metabolismo de lípidos y glucosa. Algunas algas pardas pueden bioacumular tóxicos en su pared celular, representando un problema si no se realizan los ensayos de toxicidad que garanticen la seguridad de su consumo. *M. pyrifera*, no presenta estudios toxicológicos que garanticen su inocuidad. El objetivo del trabajo fue realizar la evaluación toxicológica aguda y subcrónica de *M. pyrifera* en roedores, así como, determinar su posible efecto terapéutico. Frondas de *M. pyrifera* fueron recolectadas en Baja California Sur, México, para su secado y obtención de la harina del alga. El ensayo de toxicidad aguda LD50 se realizó en ratones macho C57BL/6 ( $25 \pm 3$  g) durante 7 días, encontrando una LD50 superior a  $10 \text{ g kg}^{-1}$ . La toxicidad subcrónica se determinó durante 121 días en ratas Sprague Dawley macho adulta con peso ( $98 \pm 2$  g), en este periodo los animales de experimentación no mostraron signos de toxicidad, ni cambios en los parámetros hematológicos, bioquímicos ni histopatológicos de sus órganos. En el balance óxido-reducción, las especies reactivas de oxígeno, la peroxidación lipídica y la proporción de glutatión no fueron diferentes al grupo testigo. Se encontró un efecto terapéutico sobre la regulación del peso corporal y un efecto hipolipidémico (triglicéridos:  $82,27 \pm 5,55 \text{ mg dL}^{-1}$ , colesterol:  $98,61 \pm 6,48 \text{ mg dL}^{-1}$ ). Se concluye que, bajo las condiciones en que se desarrolló este estudio, el consumo de *M. pyrifera* como ingrediente funcional es seguro.

**Palabras clave:** Toxicidad sub-crónica, *Macrocystis pyrifera*, ingesta segura, antiobesogénico, hipolipidémico

**Abstract.-** *Macrocystis pyrifera* is a giant brown seaweed with high nutritional content, used as a dietary supplement in some farm animals. It contains fucoidan which has a pharmacological effect on lipids and glucose metabolism. Some brown seaweeds can bioaccumulate toxic elements in their cell wall, representing a problem if toxicity tests that guarantee the safety of their consumption are not done. *M. pyrifera*, does not present toxicological studies that guarantee its safety. This work aimed to perform the acute and subchronic toxicological evaluation of *M. pyrifera* in rodents as well as to determine its possible therapeutic effect. Fronds of *M. pyrifera* were collected in Baja California Sur, Mexico, for drying and obtaining algae flour. The acute toxicity test LD50 was performed in C57BL / 6 male mice ( $25 \pm 3$  g) for 7 days, finding an LD50 greater than  $10 \text{ g kg}^{-1}$ . Subchronic toxicity was determined for 121 days in adult male Sprague Dawley rats ( $98 \pm 2$  g), during which the experimental animals showed no signs of toxicity, or changes in the hematological, biochemical and histopathological parameters of the organs. The reactive oxygen species, lipid peroxidation or glutathione ratio were not different from the control group. A therapeutic effect on the regulation of body weight and a hypolipidemic effect (triacylglycerides:  $82.27 \pm 5.55 \text{ mg dL}^{-1}$ , cholesterol:  $98.61 \pm 6.48 \text{ mg dL}^{-1}$ ) were found. It is concluded that, under the conditions in which this study was developed, the consumption of *M. pyrifera* as a functional ingredient is safe.

**Key words:** Subchronic toxicity, *Macrocystis pyrifera*, safe intake, antiobesogenic, hypolipidemic

## INTRODUCTION

Brown algae represent an important food resource for human population. Hence, it is very important to evaluate toxicity to ensure the safety of its consumption and take advantage of its beneficial effects and therapeutic properties (Rajapakse & Kim 2011, Makkar *et al.* 2016). *Macrocystis pyrifera* (Linnaeus) C. Agardh 1820, commonly known as giant kelp or giant bladder kelp, is a species of kelp (large brown algae) that can be found forming large aquatic

forests. The thallus can grow up to 45 m in length and last from 4 to 8 years attached to a substrate using a conical support (Hernández-Carmona *et al.* 1991). This alga is found in America, mainly in northern Mexico from Baja California to Alaska, Perú and Chile (Carrillo-Domínguez *et al.* 2002). *M. pyrifera* is also distributed in South Africa, Australia, New Zealand and Sub Antarctic Islands (Macaya & Zuccarello 2010).

From a nutritional point of view, *Macrocystis pyrifera* is a low-calorie product, with a high concentration of minerals ( $Mg^{+2}$ ,  $Ca^{+2}$ ,  $P^{+5}$ ,  $K^{+}$ ,  $I$ ), vitamins, complex polysaccharides and low lipids content (Rodríguez-Montesinos 1991, Jiménez-Escrig & Goñi-Cambrodón 1999). In addition, the species contains molecules with pharmacological activity such as fucoidan, a compound that inhibits lipid accumulation. For this reason, it has been considered that its consumption can be a therapeutic alternative to improve the metabolism of lipid and glucose (Kim *et al.* 2009, Park *et al.* 2011).

This species has been proven as a nutritional supplement in shrimp (*Farfantepenaeus californiensis*) (Casas-Valdez *et al.* 2006), laying hens (Carrillo *et al.* 2008) and goats (Mora-Castro *et al.* 2009). Due to these characteristics, it could be considered an option as a functional food to improve human nutritional status (Mohamed *et al.* 2012).

On the other hand, toxicological studies are very important to consider that the consumption of algae is safe for humans, this because, in the cell walls, kelps contain structural polysaccharides such as alginates, which can act as binding sites for the accumulation of toxic substances that are resuspended in marine currents, such as heavy metals. This situation is important since if consumed, it can cause alterations in oxidative stress and in the REDOX environment of the organism, causing functional and structural changes in tissues (Tapia-Martínez *et al.* 2019). Although *M. pyrifera* is widely consumed, there are no toxicological studies to ensure its consumption as an additive in animal diets or for direct human consumption. Therefore, the objective of this study was to carry out a toxicological study of *M. pyrifera* to determine acute and subchronic toxicity in rodents as well as its possible therapeutic effect.

## MATERIALS AND METHODS

### OBTAINING THE SEAWEED

*Macrocystis pyrifera* was provided by Laboratorio de Macroalgas from Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Instituto Politécnico Nacional (I.P.N), México. It was collected from Tortugas (27°41'30"N, 114°53'45"W) and Asunción bay (27°08'18"N, 114°17'45"W), Baja California Sur, Mexico. *M. pyrifera* fronds were sun dried for 3 days on a cement surface. To obtain the powdered algae, samples were ground in a Hammer Mill (M 20S3, IKA Labortechnik) at 20,000 cycles for 10 min. After that, the powder was passed through a N100 sieve with a mesh size of 150  $\mu$ m.

### RODENTS EXPERIMENT

All the experimental procedures described in this study were carried out in accordance with the guidelines of the laws and codes approved in the Seventh Title of the General Health Law in matter of Research for the Health (DOF 2014) and the NOM-062-ZOO-1999, which show the technical specifications for the production, care and use of laboratory animals. The IPN Internal Bioethics Committee approved this protocol.

The experiments were design as previously reported (Tapia-Martínez *et al.* 2019). The LD50 acute toxicity test was based in accordance with the method provided by the Organization for Economic Cooperation and Development (OECD 2008) and Lorke method (Lorke 1983). Twelve C57BL/6 male mice ( $20 \pm 1$  g) were used. The subchronic toxicity test was based on a WHO guide (WHO 2000) and the OECD Guidelines for Chemical Tests (OECD 1998). 16 male Sprague Dawley rats ( $98 \pm 2$  g) were used. They were housed in a room with controlled lighting (8:00 a.m. to 8:00 p.m.), temperature ( $21 \pm 1$  °C) and relative humidity (40-60%). Food and water intake were *ad libitum*.

### LD50 ACUTE TOXICITY TEST

The acute toxicity LD50 of *M. pyrifera* was determined using 20 male C57BL/6J mice, randomly divided into 4 groups (n= 3). Singly doses were administered by oral gavage route (o.g.r.): 1) 10 g kg<sup>-1</sup> (Purine rat chow® powder suspended in 0.9% saline solution SS), 2) 1 g kg<sup>-1</sup> of *M. pyrifera* (administrated from an 0.008 g mL<sup>-1</sup> mixture of the powder suspended in 0.9% SS), 3) 5 g kg<sup>-1</sup> of *M. pyrifera* (administrated from a 0.016 g mL<sup>-1</sup> mixture of the powder suspended in 0.9% SS), and 4) 10 g kg<sup>-1</sup> of *M. pyrifera* (administrated from a 0.16 g mL<sup>-1</sup> mixture of the powder suspended in 0.9% SS).

Doses greater than 10 g kg<sup>-1</sup> were not possible to administer, because the high alginate composition in the algae formed a colloid that was not possible to use via o.g.r. After the administration, the male C57BL/6J mice were observed every 30 min, during 4 h and then, daily for 7 days. Signs of toxicity were documented according to Lorke scale (Lorke 1983), such as piloerection, ocular irritation, salivation, displacement of motor activity, postural anomalies or death. Animals were euthanized by cervical dislocation.

### SUBCHRONIC TOXICITY TEST

Sixteen male Sprague Dawley rats ( $98 \pm 2$  g) were placed together in racks to maintain visual, auditory and olfactory contact. The rats were randomly divided into two groups (n= 8): 1) control group that received a commercial diet and water *ad libitum*, 2) experimental group that received 20% granules of *M. pyrifer*.

*M. pyrifer* oval granules were prepared by mixing 20% powdered *M. pyrifer* and 80% commercial milled diet (Laboratory Rodent Diet 5001, LabDiet). The oval granules were formed ( $2.5 \times 1 \times 1.6$  cm) by homogenizing the mixture with 10% distilled water, and then were dried at 50 °C for 12 h (Tapia-Martinez *et al.* 2019). All rats were observed daily for 112 days and signs such as piloerection, eye irritation, motor abnormalities, and mortality were recorded.

The rodents were euthanized by decapitation and blood samples were taken immediately from the trunk for hematological and biochemical analyzes. The serum was separated using a clinical centrifuge at 15,000 rpm for 15 min and stored at -70 °C until further analysis. The stomach, duodenum, liver, and kidney were preserved in paraformaldehyde solution 4% in PBS for histological studies, oxidative stress and evaluations of the redox environment.

### HEMATOLOGICAL AND BIOCHEMICAL INDICATORS

Blood drawn from the trunk of each animal was analyzed to determine hematocrit. In order to calculate the hematocrit value, heparinized tubes and capillaries were used. Once loaded with blood, they were sealed at one end and centrifuged for 5 min at 10,000 rpm in a hematocrit centrifuge. Height of the whole blood and red cell pack were measured with a ruler, the hematocrit corresponds to the percentage of the red cell pack with respect to the total. Hemoglobin was determined by a colorimetric assay, using a commercial kit (Randox™, UK). Lymphocyte number ( $\text{mm}^3$ ) was quantified using a Neubauer camera. Serum samples were used to evaluate aspartate aminotransferase (glutamic-oxaloacetic transaminase or GOT), alanine aminotransferase (glutamic-pyruvic transaminase or GPT) using a commercial enzyme assay (Randox™, UK). Total, direct and indirect concentrations of bilirubin, uric acid and creatinine were measured using commercial test kits (Randox™, UK), according to the manufacturer's instructions.

### OXIDATIVE STRESS MARKERS

An approximately 1 cm<sup>2</sup> portion of the stomach, duodenum, liver, and kidney of each rodent was mechanically homogenized using a manual tissue homogenizer in 3 mL of 10 mM phosphate buffer. The protein content of the homogenates was determined by the Bradford method, the oxidative stress values and the markers of the REDOX environment are expressed in milligram of proteins (Bradford 1976). Reactive oxygen species (ROS) and lipid peroxidation (LP) are markers of oxidative stress. On the other hand, reduced glutathione (GSH), oxidized glutathione (GSSG) and the GSH<sup>2</sup>/GSSG ratio are parameters of the REDOX environment.

### REACTIVE OXYGEN SPECIES

For quantification of ROS in the stomach, duodenum, liver, and kidney of rodents, the 2',7'-dichlorofluorescein diacetate method (DCFH-DA) was used, which is de-esterified by the presence of hydrogen peroxide that produces an oxidized molecule (2',7'-dichlorofluorescein or DCF). DCF has an excitation length at 488 nm and a maximum fluorescence emission length at 525 nm. For evaluation, 10  $\mu\text{L}$  of the homogenate was taken and placed in test tubes containing 1940  $\mu\text{L}$  of TRIS: HEPES regulator (18: 1v/v). The diluted fraction was incubated with 50  $\mu\text{L}$  of DCF-DA for 1 h at 37 °C with constant shaking. The reaction was stopped by placing the tubes on ice and the fluorescence reading were immediately taken on a SHIMADZU RF5301 spectrofluorometer, the wavelengths used were 488 nm and 525 nm for excitation and emission, respectively. The results were expressed as DCF  $\text{mg}^{-1}$  protein.

### LIPID PEROXIDATION

Lipid peroxidation in the stomach, duodenum, liver, and kidney was evaluated by the formation of a soluble fluorescent lipid as previously described (Cano-Europa *et al.* 2008). 1 mL of the homogenate was taken and 7 mL of a chloroform/methanol mixture (2:1 v/v) was added. The samples were shaken vigorously for 15 s and kept protected from light under refrigeration for 30 min to allow phase separation. Finally, 2 mL of the chloroform phase were taken to obtain the fluorescence reading on a SHIMADZU RF5301 spectrofluorometer, at an excitation wavelength of 370 nm and emission of 430 nm. The sensitivity of the spectrofluorometer was adjusted to 140 fluorescence units with a quinine solution of 1  $\mu\text{g mL}^{-1}$  in 0.005M H<sub>2</sub>SO<sub>4</sub>. Results were expressed as relative fluorescence units  $\text{mg}^{-1}$  of protein.

### REDOX ENVIRONMENT MARKERS

Reduced glutathione (GSH), oxidized glutathione (GSSG) levels and the GSH<sup>2</sup>/GSSG ratio were evaluated in the stomach, duodenum, liver, and kidney. The homogenates (500  $\mu$ L) were treated with 30% phosphoric acid and centrifuged at 12,000 rpm for 30 min at 4° C. To determine GSH, 100  $\mu$ L were added of 1:10 diluted supernatant with 1.9 mL of FEDTA (100 mM phosphate and 5 mM EDTA). The mixture reacted with 100  $\mu$ L of o-phthaldialdehyde (OPT). To measure the GSSG, 75  $\mu$ L of the supernatant was combined with 35  $\mu$ L of N-ethylmaleimide. After 30 min, 60  $\mu$ L of the mixture was mixed in 1.84 mL of FEDTA and then 100  $\mu$ L of o-phthaldialdehyde was added. Two-chemical species were measured on a SHIMADZU RF5000U spectrofluorometer at 350 nm excitation and 420 nm emission. Results were expressed as  $\mu$ g of GSH or GSSG per mg of protein. The GSH<sup>2</sup>/GSSG ratio was used as an indicator of the redox environment (Cano-Europa *et al.* 2008).

### HISTOLOGICAL STUDY

The stomach, duodenum, liver, and kidney tissues from rodents were dissected and fixed in paraformaldehyde solution 4% in PBS. The fixed tissues were embedded in paraffin and then sectioned into a microtome (LEICA RM 2145, Germany). To evaluate the possible histopathological alterations caused by the ingestion of *M. pyrifer*, 5  $\mu$ m sections were stained with hematoxylin-eosin and examined by an expert pathologist, who did not know the group and treatment, using an optical microscope.

### THERAPEUTICALLY EFFECT OF *M. PYRIFERA*

The possible therapeutic effect of *M. pyrifer* was evaluated for 112 days in male Sprague Dawley rats, which were fed a diet of Purina® Chow Plus with 20% *M. pyrifer* powder. Water intake, food intake, and body weight were measured weekly to assess the effect on body weight regulation. After 112 days, the plasma concentration of glucose, cholesterol, triglycerides, HDL, LDL, VLDL and NEFA were quantified to determine the hypolipidemic and hypoglycemic effect, with the serum samples of rats, following the colorimetric methods available in commercial kits (Randox™, UK). In addition, lipid accumulation was evaluated by total adipose tissue.

During the euthanasia of the rodents, the main adipose tissue reserves (epididymal, mesenteric, retroperitoneal) were dissected and immediately weighed to determine the total adipose tissue with respect to body weight (total adipose tissue  $\times$  100 g of body weight).

### STATISTICAL ANALYSIS

All results are presented as the mean  $\pm$  the standard error. The hematocrit and area under the curve (AUC) of each determination were analyzed by the Mann-Whitney U test. Hemoglobin, lymphocytes, biochemical studies, oxidative stress markers and total adipose tissue were analyzed using Student's t-test. Water intake, energy intake, and body weight were assessed using a Repeated Measure (RM) two-way ANOVA and Student-Newman-Keuls *post hoc* tests. A statistically significant difference was considered if  $P < 0.05$ .

### RESULTS

The *M. pyrifer* acute toxicity test showed no signs of toxicity or death in mice. The LD50 value was higher than 10 g kg<sup>-1</sup>.

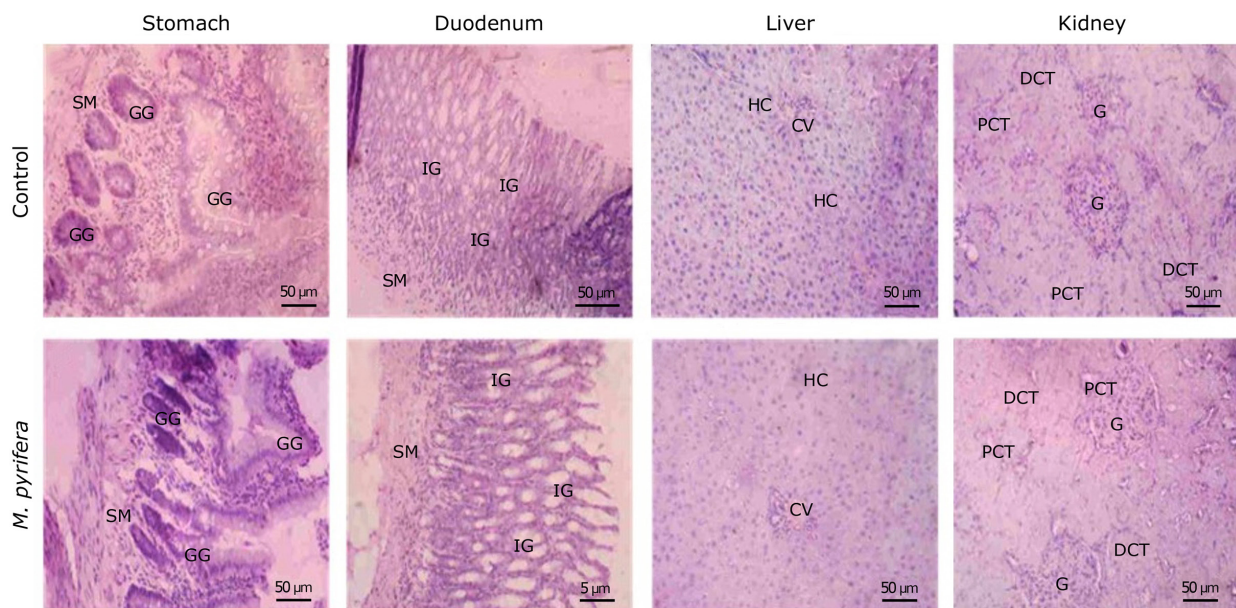
Rats under subchronic toxicity test (20% *M. pyrifer* supplemented diet) did not modify the hematological or biochemical parameters. In addition, they showed lower levels of glucose (10%), triglycerides (22.63%), cholesterol (37.79%), LDL (33.91%) and a higher non-esterified free fatty acid (57.53%) compared to the control group (Table 1).

Histological analysis of the rats fed with a 20% *M. pyrifer* supplemented diet did not show changes in the cytoarchitecture of the evaluated organs. There were no changes in the epithelium, mucosa or gastric glands of the stomach. Duodenum conserved the integrity of the mucosa, submucosa, intestinal glands and villi. Liver preserved the typical cytoarchitecture formed by hepatocyte cords arranged around a centrilobular vein. The kidney, nephrons, glomerulus, proximal and distal convoluted tubules showed the typical morphology (Fig. 1).

**Table 1. Effect of *M. pyrifera* on hematological parameters, lipid profile, hepatic and renal functions in male Sprague Dawley rats / Efecto de *M. pyrifera* sobre parámetros hematológicos, perfil lipídico, funciones hepáticas y renales en ratas Sprague Dawley macho**

Parameter	Group	
	Control	<i>M. pyrifera</i>
Hematocrit (%)	48.13 ± 1.01	50.8 ± 2.9
Hemoglobin (g dL <sup>-1</sup> )	12.84 ± 0.91	14.54 ± 0.56
Lymphocytes (per mm <sup>3</sup> )	11286 ± 565.4	12296 ± 613.8
GOT (IU L <sup>-1</sup> )	83.75 ± 10.9	88.74 ± 11.28
GPT (IU L <sup>-1</sup> )	26.42 ± 0.51	26.85 ± 0.45
Total bilirubin (mg dL <sup>-1</sup> )	0.915 ± 0.09	0.904 ± 0.01
Direct bilirubin (mg dL <sup>-1</sup> )	0.515 ± 0.01	0.501 ± 0.001
Indirect bilirubin (mg dL <sup>-1</sup> )	0.39 ± 0.09	0.29 ± 0.06
Glucose (mg dL <sup>-1</sup> )	92.67 ± 3.47	83.37 ± 2.36*
Triglycerides (mg dL <sup>-1</sup> )	110.53 ± 4.58	82.27 ± 5.55*
Cholesterol (mg dL <sup>-1</sup> )	120.03 ± 8.05	98.61 ± 6.48*
HDL (mg dL <sup>-1</sup> )	15.92 ± 6.34	26.11 ± 3.22
LDL (mg dL <sup>-1</sup> )	61.21 ± 9.80	40.45 ± 7.83*
VLDL (mg dL <sup>-1</sup> )	12.11 ± 3.4	10.19 ± 4.30
Non-esterified free fatty acids (mmol dL <sup>-1</sup> )	0.73 ± 0.08	1.15 ± 0.09*
Uric acid (mg dL <sup>-1</sup> )	2.53 ± 0.15	2.31 ± 0.26
Creatinine (mg dL <sup>-1</sup> )	1.76 ± 0.39	1.01 ± 0.55

Data are expressed as means ± SE and were analyzed by Student's t tests. \*  $P < 0.05$  is significantly different vs the control group. GOT: Glutamic Oxaloacetic Transaminase, GPT: Glutamic Pyruvic Transaminase, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low-Density Lipoprotein



**Figure 1. Subchronic toxicity evaluation of *M. pyrifera* on stomach, duodenum, liver, and kidney of male Sprague Dawley rats. Photomicrography of the control group and 20% *M. pyrifera* supplemented diet. Submucosa (SM), gastric glands (GG), intestinal glands (IG), centrilobular vein (CV), hepatocyte cords (HC), glomerulus (G), proximal convoluted tubules (PCT), distal convoluted tubules (DCT). The horizontal line represents 50 μm / Evaluación de la toxicidad subcrónica de *M. pyrifera* en estómago, duodeno, hígado y riñón de ratas Sprague Dawley macho. Fotomicrografía del grupo control y del de dieta suplementada con 20% de *M. pyrifera*. submucosa (SM), glándulas gástricas (GG), glándulas intestinales (IG), vena centrilobular (CV), cordones de hepatocitos (HC), glomérulo (G), túbulos contorneados proximales (PCT), túbulos contorneados distales (TDC). La línea horizontal representa 50 μm**

In addition, the oxidative stress markers (ROS and lipid peroxidation) and the redox environment were not modified in any of the organs mentioned above (Table 2).

The therapeutic effect of *M. pyrifera* is presented in the Figure 2. Rats in the group that ingested a diet supplemented with 20% *M. pyrifera* had a higher water intake (24.4%), a lower food intake (12.4%) and a decrease in body weight (20.4%) that is most evident when the area under the curve of each graph is analyzed. Also, there is a reduction in total adipose tissue reserves (16.07%).

## DISCUSSION

The acute toxicity study was determined in mice as LD50 > 10 g kg<sup>-1</sup>, which agrees with other toxicity studies of brown algae such as *Sargassum liebmannii* and the fucoidan from *Saccharina japonica* (Kumar *et al.* 2009, Tapia-Martinez *et al.* 2019). According to the value of LD50 in the scale proposed by Loomis & Hayes (1996), *M. pyrifera* can be classified as non-toxic.

The toxicity studies are used to ensure the safety in the consumption of food intended for human intake. In the case of algae, it is important to carry out the toxicological study, because these organisms can bioaccumulate toxins, altering oxidative stress and the REDOX environment which causes cellular damage.

The results obtained in the subchronic toxicity study in rats show that the diet supplemented with 20% *M. pyrifera* is safe because rats showed no signs of toxicity according to the Lorke scale (Lorke 1983). Hematological parameters were also evaluated because blood tissue is one of the first to be affected when a toxic substance is ingested orally.

The visceral organs play an important role in the biotransformation of toxic substances, the liver has a primary role in this detoxification process so it can be affected when a toxic is ingested; Biotransformation products can generate cellular damages that alters the structure and function of hepatocytes (Hoekstra *et al.* 2013). Some markers of liver function are transaminases and bilirubin (Gill *et al.* 2014). The results show that the enzymatic activity of the transaminases (TGO and TGP) and the bilirubin concentration were not modified, suggesting that the consumption of the diet supplemented with 20% *M. pyrifera* does not generate a negative effect on the liver.

Furthermore, kidney function did not change in animals fed the diet supplemented with 20% *M. pyrifera*, indicating that it did not cause kidney toxicity.

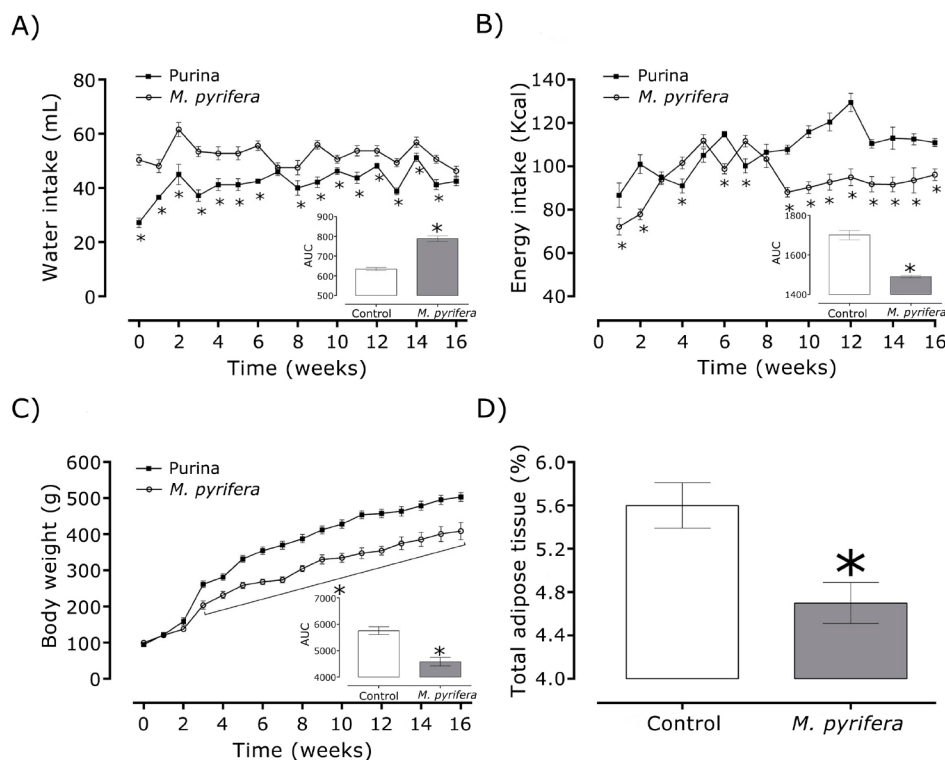
The consumption of toxic substances such as those bioaccumulated by algae can cause cellular damage and an imbalance in oxidative stress and the REDOX environment, increasing the damage to all cellular components including proteins, lipids, and DNA (Halliwell & Gutteridge 1984, Halliwell & Chirico 1993). The results show that the consumption of a diet supplemented with 20% *M. pyrifera* does not modify the reactive oxygen species, lipid peroxidation or the glutathione ratio. This may be due the fact that brown algae contain a large number of compounds with antioxidant properties such as carotenes, chlorophylls, tannins, and polyphenols, which protect the organism against free radicals and prevent oxidative stress (Conner & Grisham 1996).

**Table 2. Oxidative stress markers on stomach, duodenum, liver, and kidney of male Sprague Dawley rats / Marcadores de estrés oxidativo en estómago, duodeno, hígado y riñón de ratas Sprague Dawley macho**

Oxidative stress markers	Groups							
	Stomach		Duodenum		Liver		Kidney	
	Control	<i>M. pyrifera</i>	Control	<i>M. pyrifera</i>	Control	<i>M. pyrifera</i>	Control	<i>M. pyrifera</i>
Lipid peroxidation (RFU mg <sup>-1</sup> protein)	2.2 ± 0.3	1.9 ± 0.4	2.3 ± 0.3	1.8 ± 0.3	4.7 ± 0.8	4.8 ± 0.7	0.7 ± 0.2	0.78 ± 0.11
Reactive oxygen species (ng mL <sup>-1</sup> protein)	160 ± 36	141 ± 31	452 ± 58	323 ± 86	88.3 ± 16.8	87.5 ± 16.3	96.4 ± 28.6	77.2 ± 11.5
GSH (µg GSH mg <sup>-1</sup> protein)	1.78 ± 0.4	1.79 ± 0.3	3.14 ± 0.7	1.95 ± 0.7	7.32 ± 1.4	9.0 ± 1.8	2.1 ± 0.6	2.1 ± 0.4
GSSG (µg GSSG mg <sup>-1</sup> protein)	3.96 ± 0.8	4.4 ± 0.17	4.7 ± 0.95	4.0 ± 0.8	2.01 ± 0.6	2.5 ± 0.7	1.8 ± 0.3	2.5 ± 0.2
GSH <sup>2</sup> /GSSG ratio	0.8 ± 1.2	0.8 ± 0.2	4.5 ± 1.8	3.41 ± 2.6	35.2 ± 9.9	41.1 ± 9.3	1.4 ± 0.7	2.02 ± 0.7

Data are expressed as means ± SE and were analyzed by student's t tests. \* *P* < 0.05 is significantly different vs the control group. RFU: Relative fluorescence units, GSH: Reduced glutathione, GSSG: oxidized glutathione

**Figure 2. Effect of 20% *M. pyrifera* supplemented diet on A) water intake, B) energy intake, C) body weight, and D) total adipose tissue of male Sprague Dawley rats. The bar graphs show the area under the curve (AUC) of each determination. Values are expressed as the mean  $\pm$  SE. Line graphics were analyzed by RM two-way ANOVA and Student-Newman-Keuls *post hoc*, AUC by U Mann-Whitney. \* $P < 0.05$  vs. control group / Efecto de la dieta suplementada con 20% de *M. pyrifera* sobre A) la ingesta de agua, B) ingesta de energía, C) peso corporal y D) del tejido adiposo total de ratas Sprague Dawley macho. Los gráficos de barras muestran el área bajo la curva (AUC) de cada determinación. Los valores se expresan como la media  $\pm$  EE. Los gráficos de líneas se analizaron mediante RM ANOVA de dos vías y Student-Newman-Keuls *post hoc*, AUC por U Mann-Whitney. \* $P < 0,05$  vs el grupo control**



Histological studies showed that 20% *M. pyrifera* supplemented diet did not cause abnormalities in the cytoarchitecture or tissue integrity of the stomach, duodenum, liver, and kidney, reinforcing the evidence that the algae do not cause any toxic effect.

Rats fed with 20% *M. pyrifera* supplemented diet had a higher water intake, probably as a physiological response to the high mineral content of this algae; the increasing water intake improve the osmotic balance (Gojon-Báez *et al.* 1998). The lowest energy intake of rats fed with the 20% *M. pyrifera* supplemented diet can be explained by the high content of soluble fiber and alginate in these algae (Rhein-Knudsen *et al.* 2017), which hydrates the gastrointestinal tract causing stomach distention and satiety (Clark & Slavin 2013). In addition, the animals with supplemented diet had lower body weight gain during the whole treatment compared to the control group, this could be because they had a lower energy intake, having a beneficial effect on the body weight regulation.

The consumption of algae not only has therapeutic effects in the regulation of body weight, but also improved the metabolism of glucose and lipids, decreasing the concentration of glucose, triacylglycerides, cholesterol and LDL, and increasing the concentration of non-esterified

free fatty acids. These benefits may be due to the presence of algal metabolites such as fucoxanthins (Maeda *et al.* 2005, Peng *et al.* 2011) and fucoidan (Zhang *et al.* 2015).

The mentioned metabolites have an inhibitory effect on lipid accumulation by stimulating lipolysis and inhibiting adipocyte differentiation (Miyashita 2009, Park *et al.* 2011, Jeong *et al.* 2013). They also decrease plasma glucose concentrations (Kim *et al.* 2012) by increasing insulin sensitivity (Gammone & D'Orazio 2015).

The lower amount of total adipose tissue in the animals fed with 20% *M. pyrifera* supplemented diet is directly related to the lower body weight presented. In addition, they had a higher concentration of non-esterified fatty acids, which indicates that the consumption of algae has a positive effect on the mobilization and oxidation of fatty acids, decreasing their accumulation in adipose tissue.

These findings allow us to conclude that the ingestion of *M. pyrifera* does not generate any toxic effects in rodents. Moreover, it has therapeutically effects related to the regulation of body weight and lipid metabolism. For these reasons, *M. pyrifera* can be considered as a potential functional food to be included in the human diet.

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