Antioxidant potential of *Himanthalia elongata* for protection against ischemia-reperfusion injury in the small bowel

Mariola Belda-Antolí, PhD,[a] Carolina Padrón-Sanz, PhD,[a] Dolores Cejalvo-Lapeña, PhD,[a] Beatriz Prieto-Moure, PhD,[a] Jose Miguel Lloris-Cejalvo, BSc,[a] *and* **Jose Miguel Lloris-Carsí, MD, PhD,[b]** *Valencia, Spain*

Background. *Seaweed has been associated with the prevention and/or treatment of various diseases related to oxidative stress because of its antioxidant activity. We investigated the protective potential of extract of* Himanthalia elongata *against ischemia-reperfusion (I/R) injury in the intestine of rats.*
Methods. *Seventy-two (72) male Wistar albino rats were randomly assigned into 12 groups as follows: sham, I/R only, I/R plus vehicle at 3 time points, and I/R plus extract at 3 time points. The degree of intestinal injury was determined by oxidative stress using lipid peroxidation, superoxide dismutase, catalase, and glutathione peroxidase after mesenteric ischemia-reperfusion. A histological study was also performed.*
Results. *The algae extract helps to maintain normal enzymatic levels because, for all the studied parameters, groups treated with the extract showed significant differences ($P < .05$) compared with the I/R groups, and there were no differences compared with the sham group. The histological study showed that damage to the intestinal mucosa was less severe in animals treated with extract of* H elongata *after up to 24 hours of reperfusion compared with the I/R group.*
Conclusion. *These results suggest that the extract of* H elongata *can protect intestinal tissue against ischemia-reperfusion injury. (Surgery 2017;162:577-85.)*

From the Faculty of Medicine,[a] Catholic University of Valencia; and Faculty of Medicine,[b] University of Valencia, Valencia, Spain

Ischemia-reperfusion (I/R) injury occurs when the blood supply returns (reperfusion) to a tissue that has temporarily been deprived of blood supply (ischemia). This triggers an intense inflammatory response.[1] I/R causes high morbidity and mortality in both surgical and trauma patients and is associated with organ transplantation, strangulated bowel, vascular surgery, and shock.[2] Oxidative stress mediators such as reactive oxygen species (ROS) can cause lipid peroxidation and protein oxidation and play a crucial role in I/R damage.[1,3] Of the internal organs, the intestine is probably the most sensitive to I/R injury. It quickly responds by increasing its permeability. Later, this response is translated in morphologic and histological changes that reveal the degree of damage.[4]

To ensure protection against oxidative injury, cells have evolved complex cellular defense mechanisms and use exogenous antioxidants to eliminate ROS.[5] Thus, commercial antioxidants are in high demand—many of them are synthesized with this purpose in mind, including butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and propyl gallate (PG). However, these commercial antioxidants are toxic and carcinogenic in animal models.[6-9] Therefore, safety concerns related to synthetic antioxidants have led to growing interest in

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Reprint requests: Mariola Belda-Antolí, PhD, Faculty of Medicine, Catholic University of Valencia, C/Quevedo, 2, Valencia 46001, Spain. E-mail: mabelan@mail.ucv.es.

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the development of safer and more economical natural supplements. Examples include phenolic compounds that can act as antioxidants by chelating metal ions, preventing radical formation, and improving the endogenous antioxidant system.[10]

Seaweed is often used as a biopharmaceutical with extensive medical applications.[11,12] Brown algae play an important role in this research. They are the only organism on Earth that produces phlorotannins, which are polyphenols that exhibit important biological activities.[13-15] These polyphenols play a vital role in algae, as they help to protect from UV rays, feeding herbivores, and oxidative stress originating from high concentrations of oxygen that lead to the formation of free radicals and other strong oxidizing agents.[16-18]

The brown algal species *Himanthalia elongata* has a high antioxidant activity],[11,19] and the amount of total polyphenols contained is higher than that shown in other studies with different algae.[20-22] Therefore, the main aim of this study was to investigate the protective role that an extract of *H elongata* has in an intestinal ischemia-reperfusion injury model in rats.

METHODS

Sample pre-treatment. *H elongata* seaweed was obtained from Algamar (Pontevedra, Spain) and dried and cut into 100-g aliquots. The algae was triturated and sieved, and a 125-μm fraction was selected as representative of a perfectly homogeneous sample. Upon completion of this process, the algae powder was stored in airtight bags in a desiccator until further use.

Preparation of seaweed extracts. Six grams of dried seaweed were added to a 30-ml Erlenmeyer flask with an ethanol-water mix (60:40) to begin the extraction process using an orbital incubator (Sartorius Stedim Biotech, Germany) at 60°C for 2 hours. Samples were centrifuged at 2,000 *g* for 20 minutes and then filtered with 0.45-μm nylon filters. Prior to its application to animals, the extract was dried using a rotary evaporator (Buchi R-210) and resuspended in 10 ml of 10% ethanol. The resulting extract was analyzed for antioxidant activity by the DPPH method, with an activity of 76% obtained.[16]

Animals. Male Wistar albino rats (n = 72; 200–300 g) were used in this study. Animals were kept under optimum conditions (22–24°C, 50%–70% humidity, and a 12 h/12 h light/dark cycle) and were allowed free access to food and water. The animals underwent an acclimatization period of 7 days before being used in the experiments and were treated according to the animal welfare standards provided in the Guide for the Care and Use of Laboratory Animals as applicable to European Union regulations. This study was approved by the Animal Welfare Committee of the Catholic University of Valencia in compliance with applicable legislation (Royal Decree 53/2013) and FDA recommendations.

Experimental groups. The effect of the algae extract on the I/R injury was studied in 2 stages. In the first part, we induced 60 minutes of ischemia and 3 hours of reperfusion (sham, I/R, excipient, extract). In the second part of the study, we investigated the effects of the extract after 60 minutes of ischemia and 24 hours of reperfusion. However, to minimize the use of animals, the excipient and sham groups were not studied after we noticed that conditions in the excipient and sham groups do not affect the I/R injury.

Twelve groups of animals were established in this study (n = 6 animals) and distributed as follows:

- Sham group: These animals underwent laparotomy without I/R injury.
- Ischemia-Reperfusion group (I/R): These animals underwent laparotomy + 60 minutes of ischemia followed by 3 hours or 24 hours of reperfusion.
- Excipient groups (solution used to resuspend seaweed extract)
  - Excipient t.1 (Exc t 1): These rats were treated with 0.5 ml of ethanol 10%, intraperitoneally, 24 hours before surgery + ischemia 60 minutes + reperfusion 3 hours.
  - Excipient t.2 (Exc t 2): These rats were treated with 0.5 ml of ethanol 10%, intraperitoneally, at the moment of ischemia + ischemia 60 minutes + reperfusion 3 hours.
  - Excipient t.3 (Exc t 3): These rats were treated with 0.5 ml of ethanol 10%, intraperitoneally, at the moment of reperfusion + ischemia 60 minutes + reperfusion 3 hours.
- Treated groups (Extract *H elongata*):
  - Extract t.1: These rats were treated with 0.5 ml of extract algae (830 mg/kg), intraperitoneally, 24 hours before surgery + ischemia 60 minutes + reperfusion 3 hours or 24 hours.
  - Extract t.2: These rats were treated with 0.5 ml of extract algae (830 mg/kg), intraperitoneally, at the moment of ischemia + ischemia 60 minutes + reperfusion 3 hours or 24 hours.
  - Extract t.3: These rats were treated with 0.5 ml of extract algae (830 mg/kg), intraperitoneally, at the moment of reperfusion + ischemia 60 minutes + reperfusion 3 hours or 24 hours.

Surgical procedures. After fasting overnight, the rats were anesthetized by an intraperinoneal injection of ketamine 80 mg/kg (Merial Laboratory,

Barcelona, Spain) and xylazine 10 mg/kg (Calier Laboratory, Barcelona, Spain). Animals were allowed to breathe spontaneously during the surgery. A heating lamp was used to preserve the body temperature at approximately 37°C. The abdomen was shaved and soaked with betadine before aseptic surgery using sterile instruments. After a midline laparotomy, the superior mesenteric artery (SMA) was exposed. The SMA was gently isolated and occluded immediately distal to the aorta with collateral interruption for 60 minutes with atraumatic microvascular clamps (Scanlan International, St. Paul, MN). After 60 minutes of ischemia, the clamp was removed and the reperfusion period began.

Subsequently, in the animals that underwent I/R for 24 h, the abdominal incisions were closed with continuous 2/0 silk (B. Braun Surgical SA, Rubi, Spain). All animals were anesthetized and euthanized 24 hours after reperfusion. Tissue samples were collected after 3 hours or 24 hours of reperfusion to evaluate the I/R-induced intestinal injury. The segments of jejunum (2 cm of the third intestinal loop) were removed, frozen in liquid nitrogen, and stored at –80°C for further biochemical analysis of lipid peroxidation (level of malondialdehyde [MDA]), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity.

Sample processing. After selecting the intestine segment, we eliminated the connective tissue and blood using phosphate buffered saline (PBS) containing protease inhibitors. This prevented our proteins of interest from being degraded. These were placed in 15-ml tubes in which the already clean sample was added. The mechanical disintegration of the tissue was then continued by grinding the sample with Ultraturrax (IKA T10 basic) for 1 minute at maximum speed in the cold (thus avoiding protein degradation). The samples had been previously minced with a scalpel. After addition of 20 μl of detergent, Triton X-100 (Sigma-Aldrich, St. Louis, MO) was added to denature the cell membrane without affecting the denaturation of the membrane proteins. Finally, the samples were centrifuged at 10,000 *g* for 10 minutes at 4°C to achieve homogenization. We then collected the supernatant of interest and stored it at −80°C.

Biochemical analysis. *Measurement of malondialdehyde levels.* The malondialdehyde (MDA) levels reflected gastrointestinal system lipid peroxidation levels in tissue samples. The MDA was measured according to a previously established procedure.[23] Briefly, the reaction mixture contained 50 μl of the tissue sample, 150 μl of 60% perchloric acid (Fisher, United Kingdom), and 150 μl thiobarbituric acid (Sigma Aldrich, Germany). This mixture was incubated for 55 minutes at 95°C with agitation (300 rpm). Then, samples were cooled to −20°C for 10 minutes. After this time, 100 μl of 20% trichloroacetic acid (Sigma Aldrich, Germany) and 250 μl of n-butanol (Acros Organics, Spain) were added to the mixture; afterward, the mixture was vigorously shaken. After centrifugation at 13,000 *g* for 6 minutes, the absorbance of the organic layer was measured at 532 nm (Nanodrop 2000c, Thermo Scientific). The MDA levels were expressed as nmol/mg.

Measurement of superoxide dismutase. This enzyme is a metalloprotein found inside the cells. It acts as the first defense against ROS, and it catalyzes the dismutation of O_2^- to H_2O_2. The superoxide dismutase (SOD) activity was evaluated via inhibition of a tetrazolium salt reduction via a superoxide anion generated by xanthine oxidase and hypoxanthine using a commercial assay kit (SOD Assay kit, #706002, Cayman Chemical).

Measurement of catalase. Catalase (CAT) is a hemoprotein located in the peroxisomes. Its function is to catalyze the decomposition of the hydrogen peroxide formed previously by SOD in water and molecular oxygen. A commercial catalase assay kit (Catalase Assay kit, #707002, Cayman Chemical) was used to determine the catalase activity level. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The resulting formaldehyde was measured colorimetrically in tissue homogenate. The result is expressed in nmol/min/mg.

Measurement of glutathione peroxidase. Glutathione peroxidase (GPx) is a selenoprotein located in the cytosol and mitochondrial matrix. It requires the presence of glutathione (GSH) as a reducing agent to catalyze the reduction of hydrogen peroxide in water and alcohol. Cayman's glutathione peroxidase assay kit (Glutathione Peroxidase Assay kit, #703102, Cayman Chemical) measures GPx activity indirectly by a coupled reaction with glutathione reductase. The oxidized glutathione produced upon reduction of hydroperoxide by GPx is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm.

Histology. The intestine was fixed by immersion in 10% formaldehyde, and tissues were embedded in paraffin blocks, sectioned in 5-μm slices, placed on glass microscope slides, and stained with hematoxylin and eosin. The sections were examined with 10x objectives, and the length of the villi was

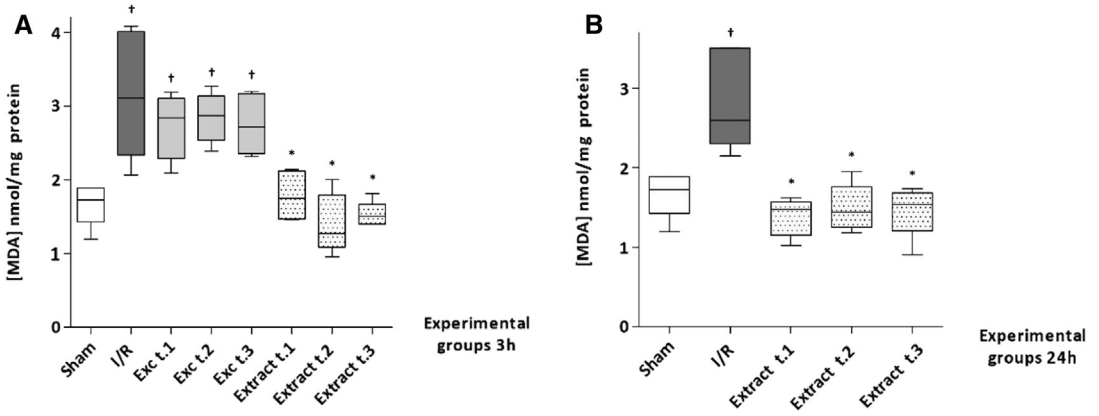

Fig 1. MDA levels in the different study groups. (*A*) (60 min ischemia, reperfusion 3 h) and (*B*) (60 min ischemia, reperfusion 24 h). Values expressed in nmol/mg protein. Mean ± standard deviation ($n = 6$). *Significant differences with respect to I/R group, Mann-Whitney U test, SPSS ($P < .05$). †Significant differences with respect to sham group.

Table. Lipid peroxidation and enzyme activity values for the experimental groups

Experimental groups $(\overline{X}\pm\sigma)$	*MDA (nmol/mg protein)*	*SOD (U/mg protein)*	*CAT (nmol/min/mg protein)*	*GPx (nmol/min/mg protein)*
Sham	1.67 ± 0.28	2.44 ± 0.58	49.97 ± 6.14	0.60 ± 0.12
I/R 3h	3.16 ± 0.86†	6.07 ± 0.94†	29.00 ± 4.67†	1.34 ± 0.29†
I/R 24h	2.84 ± 0.63†	8.01 ± 1.12†	15.16 ± 1.83†	1.53 ± 0.21†
Exc t.1	2.73 ± 0.44†	6.11 ± 2.48†	27.10 ± 4.58†	1.22 ± 0.20†
Exc t.2	2.84 ± 0.33†	4.96 ± 1.05†	33.17 ± 7.81†	1.16 ± 0.14†
Exc t.3	2.76 ± 0.41†	6.03 ± 1.53†	30.12 ± 4.00†	1.07 ± 0.24†
Extract t.1 (3h)	1.79 ± 0.33*	2.11 ± 0.46*	48.43 ± 12.48*	0.52 ± 0.09*
Extract t.2 (3h)	1.41 ± 0.40*	1.79 ± 0.38*	42.58 ± 4.74*	0.50 ± 0.09*
Extract t.3 (3h)	1.528 ± 0.17*	2.04 ± 0.30*	39.95 ± 6.65*	0.60 ± 0.09*
Extract t.1 (24h)	1.38 ± 0.24*	2.23 ± 0.49*	39.75 ± 7.27*,†	0.58 ± 0.09*
Extract t.2 (24h)	1.49 ± 0.29*	2.31 ± 0.67*	35.56 ± 5.01*,†	0.55 ± 0.12*
Extract t.3 (24h)	1.46 ± 0.32*	2.08 ± 0.35*	35.01 ± 6.77*,†	0.53 ± 0.17*

*($P < .05$) significant differences with respect to I/R group.
†($P < .05$) significant differences with respect to sham group.
Mean ± standard deviation ($n = 6$).

measured. The intestinal lesions were graded according to 5 levels of I/R injury using the scoring system of Chiu et al.[24]: Grade 0, normal mucosa; Grade 1, subepithelial space development at the tip of the villus often with edema and vascular congestion; Grade 2, lifting of the epithelial layer from the lamina propria and moderate extension of subepithelial space with fragmentation of tips of villi and hemorrhage; Grade 3, some denuded tips of villi and massive lifting of epithelial layer with fragmentation and loss of upper third of villi; Grade 4, dilated and exposed capillaries and lost villi but crypts present; Grade 5, hemorrhage, ulceration, disintegrated lamina propria and complete mucosal necrosis. We examined 10 different fields within each group.

Statistical analysis. For the statistical results of MDA, SOD, CAT, GPx, and villus length, we used SPSS statistics software (Version 20.0). Data are expressed as the mean ± standard error. Differences among the groups were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney U-post hoc test with the minimum level of significance set at $P < .05$. For the grade of injury, the Pearson chi square test was realized ($P < .05$).

RESULTS

Effect of treatment with *H elongata* on malondialdehyde levels in intestinal tissues. Lipid peroxidation of the membrane increased in animals from the I/R group ($P < .05$ vs sham). However, the groups treated with the extract of algae showed peroxidation levels similar to the sham group (animals not subjected to I/R). On the other hand, the results reveal that there were no significant differences between the times of the extract

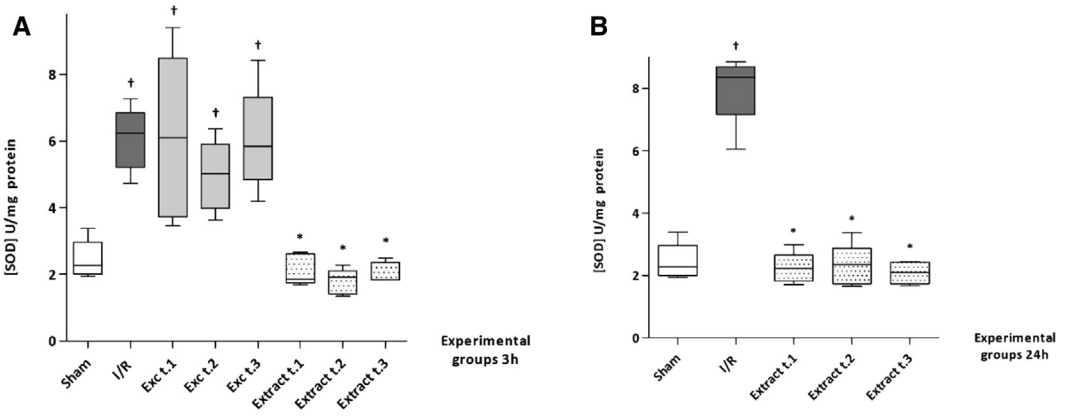

Fig 2. SOD activity in the different study groups. (*A*) (60 min ischemia, reperfusion 3 h) and (*B*) (60 min ischemia, reperfusion 24 h). Values expressed in U/mg protein. Mean ± standard deviation (n = 6). *Significant differences with respect to I/R group, Mann-Whitney U test, SPSS (P <.05). †Significant differences with respect to sham group.

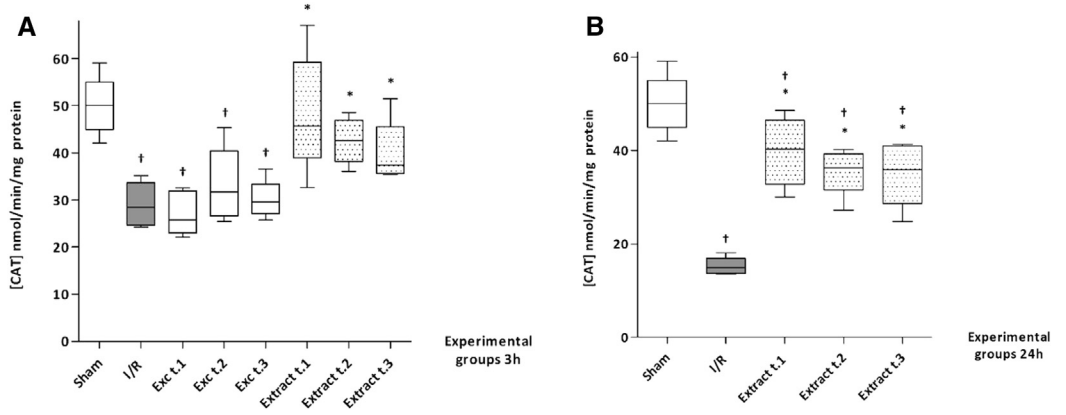

Fig 3. CAT activity in the different study groups. (*A*) (60 min ischemia, reperfusion 3 h) and (*B*) (60 min ischemia, reperfusion 24 h). Values expressed in nmol/min/mg protein. Mean ± standard deviation (n = 6). *Significant differences with respect to I/R group, Mann-Whitney U test, SPSS (P <.05). †Significant differences with respect to sham group.

administration (Extract t.1, Extract t.2, and Extract t.3). The groups treated with the excipient showed no significant differences compared to the I/R group. These results are shown in Fig 1 and Table.

Effect of treatment with *H elongata* on SOD activity. The results shown in Fig 2 and Table represent the effect of extract of *H elongata* on SOD activity. Compared to the sham group, the level of intestine SOD in the I/R group was significantly increased (P < .05). However, the level of this enzyme was significantly lower in the treated group with extract of algae compared to the I/R group (P < .05). On the other hand, the results show that the different administration times (Extract t.1, Extract t.2, and Extract t.3) did not show significant differences. Finally, the groups treated with the excipient had no differences when compared to the I/R group.

Effect of pretreatment with *H elongata* on catalase activity. The effects of *H elongata* extract on catalase activity are shown in Fig 3 and Table. The antioxidant enzyme activity was significantly lower in the I/R group than in the other groups (P < .05), although CAT activity in the group treated with extract of algae was significantly higher than that in the I/R group. This trend was not observed in the excipient groups (Exc t.1 P = .465, Exc t.2 P = .347, and Exc t.3 P = .465). There was no significant difference in CAT enzyme activity among the treatment groups and the control groups. In addition, the administration times of the algae extract (Extract t.1, Extract t.2, and Extract t.3) showed no significant differences.

Effect of treatment with *H elongata* on glutathione peroxidase activity. The I/R group saw a significant elevation in GPx activity (P = .009) compared with the sham group. Conversely, there was no significant GPx activity in the algae extract groups compared to the sham group, but the I/R group had notable differences. The results show

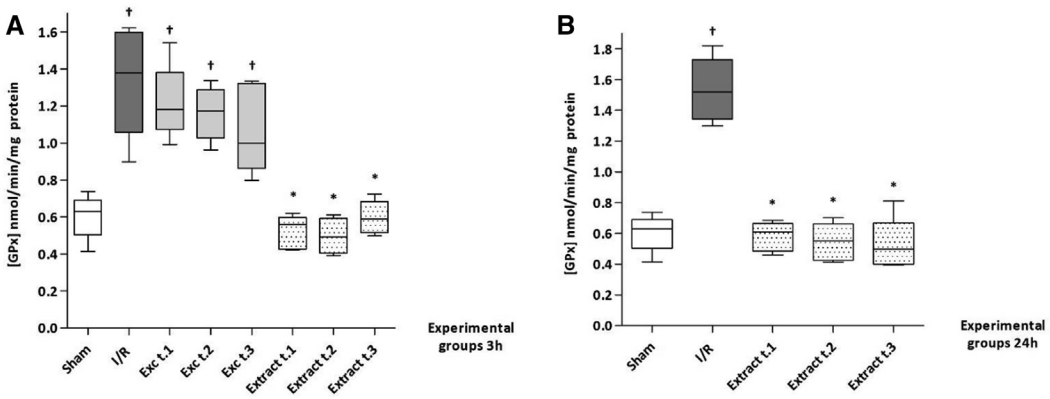

Fig 4. GPx activity in the different study groups. (*A*) (60 min ischemia, reperfusion 3 h) and (*B*) (60 min ischemia, reperfusion 24 h). Values expressed in nmol/min/mg protein. Mean ± standard deviation ($n = 6$). †Significant differences with respect to sham group. *Significant differences with respect to I/R group, Mann-Whitney U test, SPSS ($P < .05$).

that there are no differences in the time of administration because Extract t.1, Extract t.2, and Extract t.3 groups at 3 hours or 24 hours reperfusion showed no significant differences. However, the excipient group showed otherwise: there are no significant differences with the I/R group but there are significant differences with the sham group. These results are shown in Fig 4 and Table.

Histopathologic studies. The injury to the intestinal mucosa was quantified using a modified version of the scoring grade described by Chiu et al.,[24] and the results are presented in Fig 5, *B*. Histopathological analysis of the sham group presented a normal mucosal pattern (G0), in which the villi were packed, tall, well-spaced, and of equal thickness. The crypts were also packed with thin lamina propria. Animals of the I/R group had severe mucosal damage compared to the sham group. The I/R group showed loss, atrophy, and fragmentation of the villi. Most fields showed mucosal damage in category G3 with a few in G4 grade. However, in treated groups, the intestinal mucosa was restored, and the injury was much less severe relative to animals in the I/R group—mainly the group treated with extract of *H elongata* for 24 hours of reperfusion (Extract t.1 [24 h]). The mucosa showed a fairly normal pattern with mostly grades 0 and some grades 1 and 2. The differences were significantly better ($P < .001$) in extract of *H elongata* 24 hours in the reperfusion group than in the I/R group.

On the other hand, the villus length (Fig 5, *C*) shows that all groups have significant differences with respect to I/R. The excipient group was an outlier because there are no significant differences with the I/R group but there are significant differences with the sham group. The most effective experimental group is Extract t.1 (24 hours) because it offers a prevention of damage against I/R.

DISCUSSION

Intestinal ischemia is usually produced by a mesenteric thromboembolism and is a clinical situation that affects a significant number of patients. Other situations, such as surgical interventions leading to decreased mesenteric flow, may lead to I/R injury syndrome. This can cause alterations at the molecular and tissue levels. These alterations include an increase in free radical levels—mainly in the process of reperfusion.[25] Antioxidants have been used by several researchers to prevent the formation of these radicals.[26]

Well-known antioxidants such as Vitamin C and quercetin protect against post-ischemic and post-reperfusion injury in rats.[27,28] This suggests that rich natural sources of antioxidants would be useful for conditions that produce I/R injury.

Recently, polyphenols from algae have been shown to be potent antioxidants. Previous studies have shown the antioxidant potential of algae *H elongata*.[29] One study used a methanolic extract of this algae with high antioxidant activity.[30] Another study developed an ethanol extract of *H elongata* with a high amount of total polyphenols and high antioxidant activity.[19] This group studied the effect of the extract on the damage caused by intestinal ischemia reperfusion in rats because the polyphenols inhibit ROS.

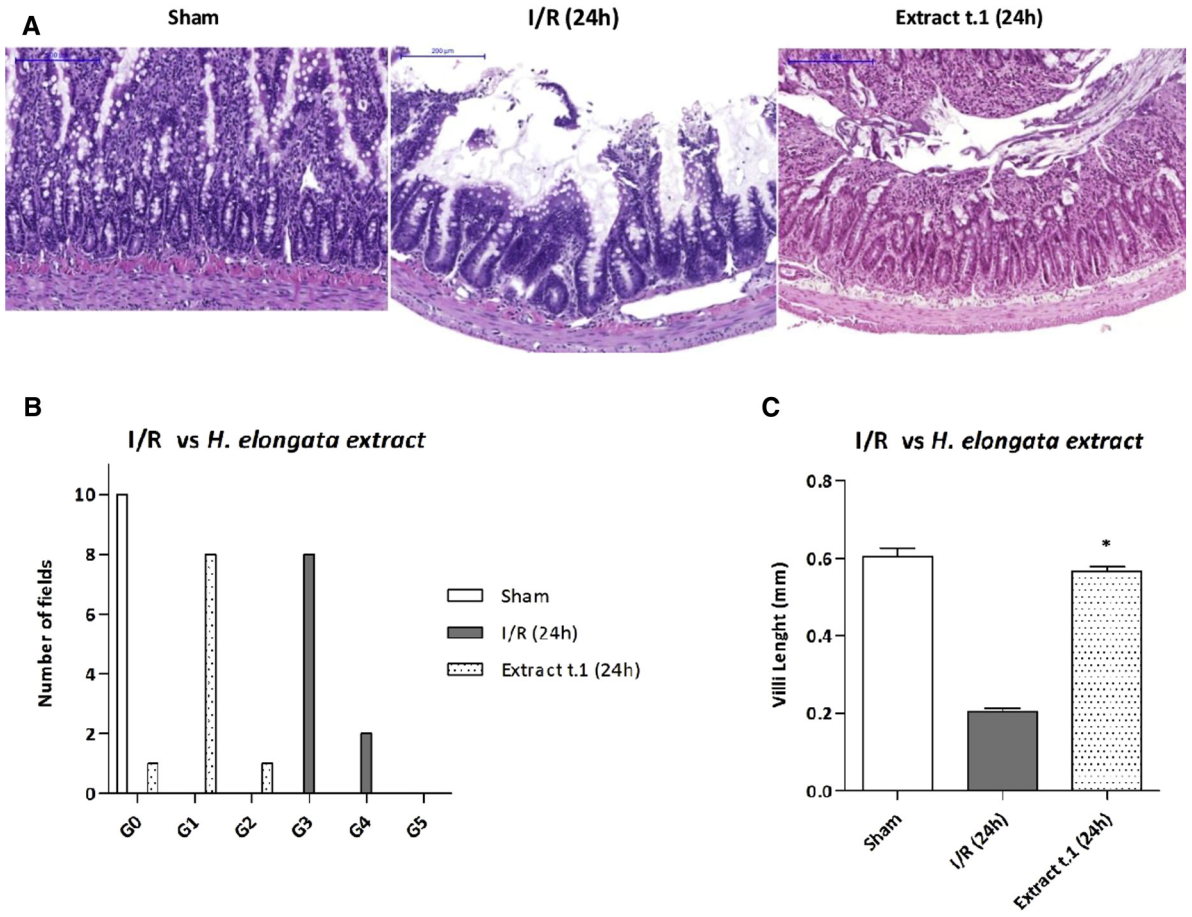

Fig 5. Histopathologic changes in the intestinal mucosa of sham group, animals group with IR or group treated with the best of administration time (Extract t.1 [24 h]). (*A*) Tissue sections of small intestinal mucosa stained with H&E shows extensive fragmentation and loss of villi in the I/R group. In contrast, the villous architecture was protected in the extract *H elongata group*. (*B*) In the I/R group, the intestinal villi showed important changes with fragmentation of the upper part of the villi as well as loss of crypts. (*C*) Moreover, there was a significant decrease in the length of the villi in the I/R group compared with the *H elongata* extract and sham groups. *($P < .05$) significant differences with respect to I/R group.

Lipid peroxidation is an important consequence of free radical generation. It can lead to the formation of toxic aldehydes such as MDA.[31] Increased concentrations of MDA indicate the degree of lipid peroxidation, verifying the oxidative damage in tissue.[3,23,32] The extract from *H elongata* reduced MDA levels versus the I/R group. These results could corroborate some recent studies in which the trends were similar to those obtained in our work. Ozturk et al.[33] recently reported that lithospermic acid reduced MDA levels compared to those detected in the I/R group.

Antioxidant enzymes (CAT, SOD, and GPx) assist cells in repairing damaged membranes caused by oxidative damage.[34] The primary gastric mucosa antioxidant enzyme is SOD—an antioxidant enzyme found in the cytosol, mitochondria, and extracellular matrix.[35] It catalyzes the conversion of excess superoxide anions and converts them to H_2O_2, which is then removed by glutathione peroxidase and catalase.[36] The SOD thus prevents the gastrointestinal damage induced by I/R.[37,38] GPx plays a fundamental role in the elimination of hydrogen peroxide and lipid hydroperoxide in the gastric mucosa.[39,40] The increased activities of SOD and GPx in the intestines of I/R group rats may also be an adaptive mechanism by the cells to detoxify the reactive species to minimize tissue damage.[41]

We noted a significant decrease in SOD activity upon administration of extract regardless of the time of administration relative to the I/R group. These results may indicate that the extract has potent free radical scavenging and antioxidant

properties during the progression of I/R injury. Our results are consistent with a previous study in which levels increased relative to the control I/R group and were decreased by applying the appropriate treatment in each trial.[42]

GPx activity was also reduced upon administration of the extract regardless of the time of administration compared to the I/R group. Akinrinmade et al.[26] recently reported that an extract from *Parquetina nigrescens* reduced GPx activity relative to the I/R group, although the values are still higher than those in the sham group. On the other hand, the decreased CAT activity during ischemia is due to the formation of an inactive complex—there was no proteolysis, decreased enzyme synthesis, or inactivation during reperfusion.[43]

In our study, CAT activity was significantly higher in the treated group regardless of the time of administration. Our results are consistent with a previous study where dexmedetomidine prevented the production of reactive oxygen species during mesenteric I/R injury in rats because CAT activity increases when the substance is administered. These results indicate that dexmedetomidine has potent free radical scavenging and antioxidant properties during the progression of I/R injury.[34]

Histologically, the structural improvement in damaged tissue was confirmed in the groups treated with algae extract because the integrity of the intestinal mucosa remained unchanged in this group. Severe histological lesions (grades 3–4) occurred after 60 minutes of ischemia and 3/24 h of jejunal reperfusion. This was when the ROS levels were striking. However, in the groups treated with the extract of *H elongata*, the damage was less severe (grades 0-1-2). These results suggest that the extract could reduce I/R injury through its antioxidant properties.

In conclusion, we showed that the extract of *H elongata* possessed considerable potential to reduce I/R injury in the intestines of rats. The biochemical assays revealed that the intestinal mucosa could stimulate its antioxidant defenses to protect against ROS attacks generated by the acute I/R injury. Moreover, the histological data shown here corroborated that the administration of the extract of *H elongata* protected the intestinal mucosa against I/R damage. Thus, the application of *H elongata* extract is very promising and could be investigated in the prevention of the ischemic processes of mesenteric thromboembolism as well as in surgical interventions.

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