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ORIGINAL

Egg White Hydrolysate as a new bioactive food ingredient in the prevention of gastrointestinal effects induced by aluminum exposure in rats

Hidrolizado de clara de huevo como nuevo ingrediente alimentario bioactivo en la prevención de los efectos gastrointestinales inducidos por la exposición al aluminio en ratas

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Abstract

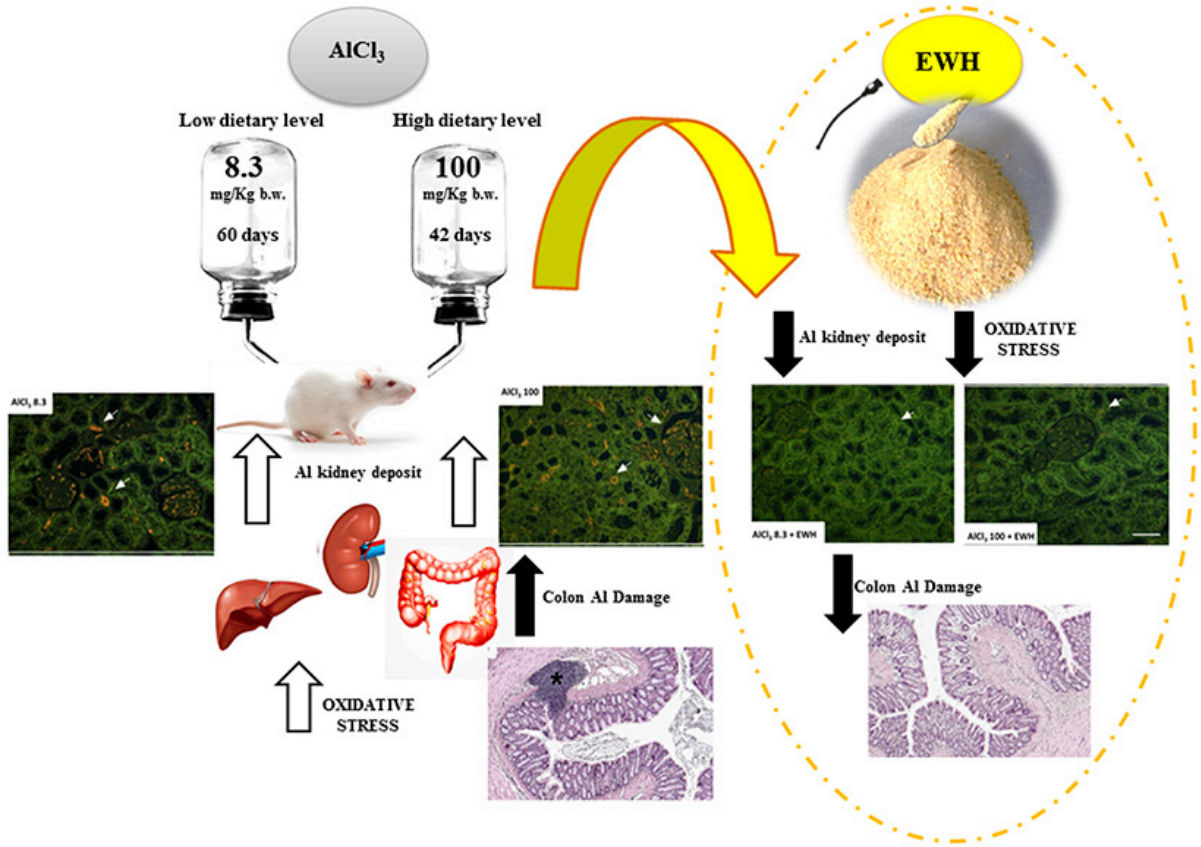
Objective: We investigated the effects of an egg-white hydrolysate (EWH) on the gastrointestinal tract and organs related to aluminum (Al) metabolism after Al exposure at both low and high-human dietary levels.
Methods: Male Wistar rats were orally treated to both low and high dietary doses of Al. Group 1) Aluminum-low dietary level (AlCl3 at 8.3 mg/kg b.w. for 60 days), co-treated or not with EWH (1 g/kg/day); Group 2) Aluminum-high dietary level (AlCl3 at 100 mg/kg b.w. for 42 days), co-treated or not with EWH.
Results: Both Al treatments increased oxidative damage in the liver and kidney. The highest Al dose impaired colon morphology, inducing inflammation and mucosal ulcerations. EWH prevented the raised oxidative stress level and colon damage and seems to reduce the presence of Al at the tissue level.
Conclusions: Our results appoint the EWH as a promisor food ingredient to prevent adverse effects produced by Al exposure in human health.

Keywords: Environmental contaminant, human health, bioactive ingredient, egg-derived peptides.

Resumen

Objetivos: Se investigaron los efectos de un hidrolizado de clara de huevo (EWH) en el tracto gastrointestinal y en los órganos relacionados con el metabolismo del aluminio (Al) tras la exposición a niveles dietéticos bajos y altos de Al.
Metodología: Las ratas Wistar macho fueron tratadas por vía oral con dosis dietéticas bajas y altas de Al. Grupo 1) Nivel dietético bajo de aluminio (AlCl3 a 8,3 mg/kg de peso durante 60 días), tratado o no con EWH (1 g/kg/día); Grupo 2) Nivel dietético alto de aluminio (AlCl3 a 100 mg/kg de peso durante 42 días), tratado o no con EWH.
Resultado: Ambos tratamientos con Al aumentaron el daño oxidativo en el hígado y el riñón. La dosis más alta de Al deterioró la morfología del colon, induciendo inflamación y ulceraciones en la mucosa. El EWH evitó el aumento del nivel de estrés oxidativo y el daño en el colon y parece reducir la presencia de Al a nivel tisular.
Conclusión: Nuestros resultados designan al EWH como un ingrediente alimentario prometedor para prevenir los efectos adversos producidos por la exposición al Al en la salud humana.

Palabras clave: Contaminante ambiental, salud humana, ingrediente bioactivo, péptidos derivados del huevo.



Introduction

Human is continuously exposed to aluminum (Al), a hazardous environmental contaminant without physiological function, and diet is an important route by which humans are exposed to this non-essential metal[1,2]. After reaching the gastrointestinal tract, the absorption and, consequently, distribution and excretion of Al from the human body are under continuous investigation. However, it seems that the bioavailability of Al is dependent on the surrounding gastrointestinal medium[3,4]. Once in the body, Al is deposited in bone and brain[5], kidney[6], liver[7], heart, and in reproductive organs of rats[8] producing deleterious effects.

Al is a toxin; therefore, the increased presence in the body of this metal may have consequences for human health and is linked to the development of hematological disorders[9,10], osteopenia[11], neurological disorders[12], macrophagic myofascitis[13], cardiovascular dysfunction[14], reproductive disorders[15] and breast cancer[16]. However, we still do not understand the real consequences of Al in the body and the predominant toxic mechanism.

Recently, the oxidative and inflammatory actions of Al have been suggested[17,18]. Moreover, pro-oxidant capacity of Al has been recognized and seems to be that a Fenton promotion cycle catalyzed by the formation of the radical Al-superoxide is underlying[19].

Nevertheless, the systemic and long-term effects of Al exposure in human health are not well understood. Recently, by creating an animal model of dietary exposure to Al, we have demonstrated that Al even at a considered low-level of exposure could represent a risk for human health and, more than once, the adverse effects were similar to the observed after a high-dietary level of Al exposure[17].

Considering the burgeoning human exposure to Al, the development of therapies to prevent or minimize these consequences is of great importance. In previous works, the dietary administration of this egg white hydrolysate (EWH) prevented in part the development of memory loss, behavioral impairment and cardiovascular dysfunction observed after Al exposure in rats[20,21]. Moreover, the beneficial effects of EWH against cardiometabolic dysfunction observed in various obesity experimental models[22,23] as well as in the prevention of systemic toxicity induced by heavy metals in rats[24,25] have been

also demonstrated. These effects seem to be associated with the potent anti-inflammatory and antioxidant properties of EWH[26,27,28], which could minimize the impact of the growing environmental presence of Al in the human health. In this regard, the effect of these bioactive peptides produced after an enzymatic treatment of egg white with pepsin for 8h[29], could counteract the toxic consequences of Al exposure.

In this context, the gastrointestinal tract plays an important role in the absorption and metabolism of Al into the body. Therefore, the consequences of Al exposure in the gastrointestinal epithelium and, whether EWH could achieve a protective role on gastrointestinal tract are unknown. In the current study, our purpose was to investigate the effects of Al exposure on the gastrointestinal tract and on other related digestive organs implicated in the Al metabolism, and to elucidate the protective role of EWH on these effects.

Materials and Methods

1. Preparation of Egg White Hydrolysate

EWH was obtained after enzymatic treatment of pasteurized egg white with pepsin for 8h, frozen, and lyophilized until used, as described[22]. The peptide profile and the degree of hydrolysis of EWH were checked by RP-HPLC and some bioactive peptide sequences previously identified were analyzed by HPLC-MS/MS (FRADHPFL, RADHPFL, YAEERYPIL, YRGGLEPINF, ESIINF, RDILNQ, IVF, YQIGL, SALAM, FSL)[29].

2. Animals treatment

Male Wistar rats (90 days-old, 350 ± 10.5 g) were obtained from the Charles River Animal Laboratory (Barcelona, Spain), housed under constant room temperature, humidity, and 12:12h light-dark, with water and food ad libitum. The experiments presented in this study were developed in accordance with the Brazilian Societies of Experimental Biology and the European legislation on the use of experimental animals (EU Directive 2010/63/EU; R.D. 53/2013). This work has Brazilian and Spanish ethical approvals (CEUA, Universidade Federal do Pampa, Brazil - 017/2018; Universidad Rey Juan Carlos, Spain - 39/2014).

Rats were randomized into two groups:

Group 1. Aluminum low dietary level - rats were divided into 4 subgroups (n=8/each) (1a-d) and received once a day for 60 days: a) Control - ultrapure water (Milli-Q, Merck Millipore Corporation. © 2012 EMD Millipore, Billerica, MA); b) Aluminum – Al at 8.3 mg/kg b.w., dose similar to human dietary Al intake[17]; c) Hydrolysate - ultrapure water plus EWH at 1 g/kg by gavage[27]; d) Hydrolysate-Aluminum - Al at 8.3 mg/kg b.w. plus EWH. In Group 1 rats received water and Al in their drinking water for 60 days, to simulate human exposure by diet[17].

Group 2. Aluminum high dietary level - rats were divided into 4 subgroups (n=8/each) (2a-d) and received daily for 42 days: a) Control – ultrapure water; b) Aluminum – Al at 100 mg/kg b.w., dose considered as a super-dietary Al intake[30]; c) Hydrolysate - ultrapure water plus EWH at 1 g/kg by gavage; d) Hydrolysate-Aluminum - Al at 100 mg/kg b.w. plus EWH. In Group 2 rats received Al by gavage and ultrapure water as drinking water for 42 days[30]. The stock solutions of Al (AlCl3.6 H2O at 0.034 M, Group 1; 8.3 mg/kg/b.w. and, 0.331 M, Group 2; 100 mg/kg/b.w.) was prepared in ultrapure water.

The body weights and the consumption of food and liquid intakes were measured once a week. After the exposure period, rats were euthanized, and the kidney, liver, and colon were removed and, being one side processed for imaging analysis and the other prepared for biochemical determinations [homogenized in 50 mM Tris HCl, pH 7.4, (1/10, w/v) centrifuged and, supernatants were frozen at -80°C].

3. Reactive species levels

Reactive oxygen species levels in the liver and kidney were measured by the spectrofluorometric method of[31] with modifications[17]. The fluorescence intensity was recorded for 60 min at 15 min intervals (520 nm emission, 480 nm excitation - SpectraMax M5 Molecular Devices, CA, USA), and the reactive species levels were expressed as units of fluorescence.

4. Lipid peroxidation levels

Lipid peroxidation levels in liver and kidney were determined as malondialdehyde (MDA) levels according to the colorimetric method of[32], with modifications[17]. The lipid peroxidation levels were measured at 532 nm (SpectraMax M5 Molecular Devices, CA, USA) and expressed as nanomoles of MDA per mg of protein.

5. Ferric Reducing/Antioxidant Power Assay

The total antioxidant capacity in the liver and kidney was measured by Ferric Reducing/Antioxidant Power (FRAP) assay, according to[33], with modifications[17]. The FRAP levels in tissues were measured at 593 nm (SpectraMax M5 Molecular Devices, CA, USA), normalized using a dose-response curve of Trolox (50-1000 µM –vitamin E analog) and expressed respected to Trolox equivalents.

6. Histological analysis

The liver, kidney, and colon were histopathological analyzed. For that, tissues were fixed for 2 days in 10% formaldehyde, washed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin/eosin. 10 randomly regions of each tissue were blinded evaluated under the 10X objective using a Zeiss Axioskop 2 microscope (Zeiss, Jena, Germany), and the images were analyzed using AxioVision 4.6. A

semi-quantitative scoring system was used to analyze and morphologically classify the colon, following the protocol described by[34]. Briefly, for the morphological classification, it was considered the following features: 1. epithelium damage (vary from 0, normal to 3, severe epithelial lifting), 2. inflammatory cells infiltration (vary from 0, absence to 3, severe infiltration involving the muscle), 3. extent of muscle thickening (vary from 0, normal to 2, severe), 4. edema (vary from 0, no edema to 2, diffuse edema). The results were expressed as a sum of scores for each analyzed feature.

7. Lumogallion staining for the presence of aluminum

The presence of Al was verified in formalin-fixed tissues using the specific lumogallion staining method[12,35]. Briefly, tissues were rehydrated and placed for 45 minutes into either 1 mM lumogallion (TCI Europe N.V. Belgium) buffered in 50 mM PIPES, pH 7.4 or the PIPES-buffer alone for auto-fluorescence analyses. After several washes with PIPES-buffer, slides were rinsed in ultrapure water, mounted using an aqueous mounting medium, and stored at 4ºC overnight before imaging. Tissues were imaged using a Zeiss Axioskop 2 microscope and, the fluorescence intensity calculated with NIH Image J software version 1.46r (http://rsbweb.nih.gov/ij/), using the same imaging parameters.

8. Statistical analysis

Data are expressed as mean ± SEM and analyzed using Graphpad Prism6 (GraphPad Software, Inc., LaJolla, CA, USA). Results were analyzed using two-way ANOVA; when ANOVA showed a significant treatment effect, Bonferroni's post hoc test was used to compare individual means. Values were considered statistically different when $P < 0.05$.

Table I: Effects of EWH on body weight (g), absolute (g or mg) and relative (g/100g or mg/100g) weights of organs and, water and food intake of rats exposure to AlCl3 for 60 days (8.3 mg/kg b.w. *per day* – Group 1), co-treated or not with EWH.

Parameters	Experimental groups			
	Control (n=8)	$AlCl_3$ (n=8)	Hydrolysate (n=8)	Hydrolysate-Aluminum (n=8)
Initial body weight (g)	365.6 ± 10.32	409.7 ± 9.57	385.6 ± 14.71	397.7 ± 8.64
Final body weight (g)	434.1 ± 13.11	468 ± 10.58	467.6 ± 21.03	448.4 ± 13.98
Water intake (ml/day)	36.10 ± 0.80	36.16 ± 0.67	37.73 ± 0.95	35.72 ± 0.38
Food intake (mg/day)	21.46 ± 0.28	22.55 ± 0.38	22.27 ± 0.59	22.26 ± 0.69
Faeces (g)	1.57 ± 0.21	2.48 ± 0.50	1.89 ± 0.41	1.82 ± 0.26
Urine (ml)	24.92 ± 1.41	25.00 ± 3.28	27.25 ± 3.59	19.38 ± 1.98
Liver (g)	11.93 ± 0.50	12.78 ± 0.59	12.18 ± 0.78	11.06 ± 0.56
Liver (g/100g)	2.75 ± 0.13	2.73 ± 0.13	2.59 ± 0.09	2.46 ± 0.06
Kidney (g)	1.15 ± 0.04	1.29 ± 0.08	1.14 ± 0.03	1.19 ± 0.07
Kidney (g/100g)	0.26 ± 0.01	0.58 ± 0.32	0.24 ± 0.01	0.26 ± 0.01
Epididymal fat (g)	11.13 ± 1.20	13.61 ± 1.37	12.98 ± 1.48	11.69 ± 0.75
Brown fat (g)	0.53 ± 0.03	0.51 ± 0.02	0.51 ± 0.05	0.47 ± 0.05
Subcutaneous fat (g)	8.82 ± 0.89	10.34 ± 1.01	11.27 ± 2.01	10.27 ± 0.76
Retroperitoneal fat (g)	12.72 ± 1.38	13.89 ± 1.40	16.61 ± 2.04	13.30 ± 1.28
Tibia height (cm)	3.81 ± 0.12	3.98 ± 0.02	3.98 ± 0.1	3.97 ± 0.08
Soleus (g)	0.13 ± 0.04	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.01

Data are expressed as means ± SEM. The relative organ weight was calculated by use of the formula: organ weight/body weight x 100. Units: g: gram, mg: milligram, cm: centimeters, ml: milliliters; 1 way ANOVA (P>0.05).

Table II: Effects of EWH on body weight (g), absolute (g or mg) and relative (g/100g or mg/100g) weights of organs and, water and food intake of rats exposure to AlCl3 for 42 days (100 mg/kg b.w. *per day* – Group 2), co-treated or not with EWH.

Parameters	Experimental groups			
	Control (n=8)	$AlCl_3$ (n=8)	Hydrolysate (n=8)	Hydrolysate-Aluminum (n=8)
Initial body weight (g)	284.4 ± 12.58	283 ± 9.64	297 ± 9.75	309.5 ± 13.57
Final body weight (g)	386.9 ± 8.59	408.3 ± 12.48	413 ± 7.16	408.5 ± 12.33
Water intake (ml/day)	31.49 ± 1.30	31.78 ± 1.27	32.45 ± 1.72	30.62 ± 0.76
Food intake (mg/day)	20.72 ± 0.56	21.48 ± 0.67	21.91 ± ¬0.81	21.58 ± 0.27
Faeces (g)	2.90 ± 0.20	2.94 ± 0.42	2.21 ± 0.36	2.68 ± 0.32
Urine (ml)	10.96 ± 3.55	17.37 ± 6.07	16.11 ± 5.33	9.87 ± 3.84
Liver (g)	10.54 ± 0.39	11.60 ± 0.41	11.38 ± 0.30	11.43 ± 0.41
Liver (g/100g)	2.73 ± 0.03	2.80 ± 0.09	2.79 ± 0.04	2.80 ± 0.10
Kidney (g)	1.18 ± 0.08	1.17 ± 0.04	1.19 ± 0.02	1.21 ± 0.04
Kidney (g/100g)	0.30 ± 0.02	0.28 ± 0.01	0.29 ± 0.01	0.29 ± 0.01
Epididymal fat (g)	8.00 ± 0.89	9.01 ± 0.45	8.51 ± 0.45	10.70 ± 1.23
Brown fat (g)	0.58 ± 0.03	0.41 ± 0.01	0.53 ± 0.05	0.55 ± 0.03
Subcutaneous fat (g)	9.62 ± 0.95	7.38 ± 0.88	9.39 ± 0.83	9.68 ± 1.40
Retroperitoneal fat (g)	10.03 ± 0.94	10.42 ± 0.66	10.42 ± 1.26	11.61 ± 1.65
Tibia height (cm)	3.75 ± 0.04	3.97 ± 0.04	3.87 ± 0.05	3.90 ± 0.05
Soleus (g)	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.01

Data are expressed as means ± SEM. The relative organ weight was calculated by use of the formula: organ weight/body weight x 100. Units: g: gram, mg: milligram, cm: centimeters, ml: milliliters; 1 way ANOVA (P>0.05).

Figure 1: Effects of EWH on oxidative stress assays in Al-exposed rats. Reactive oxygen species (ROS), lipid peroxidation and total antioxidant capacity in liver and kidney of rats exposed to AlCl3 at low doses (8.3 mg/kg b.w. for 60 days) co-treated or not with EWH. Results are expressed as mean ± SEM, n=8, * P < 0.05 compared with their corresponding controls, # P < 0.05 compared with AlCl3 group (two-way ANOVA and Bonferroni as post-hoc test).

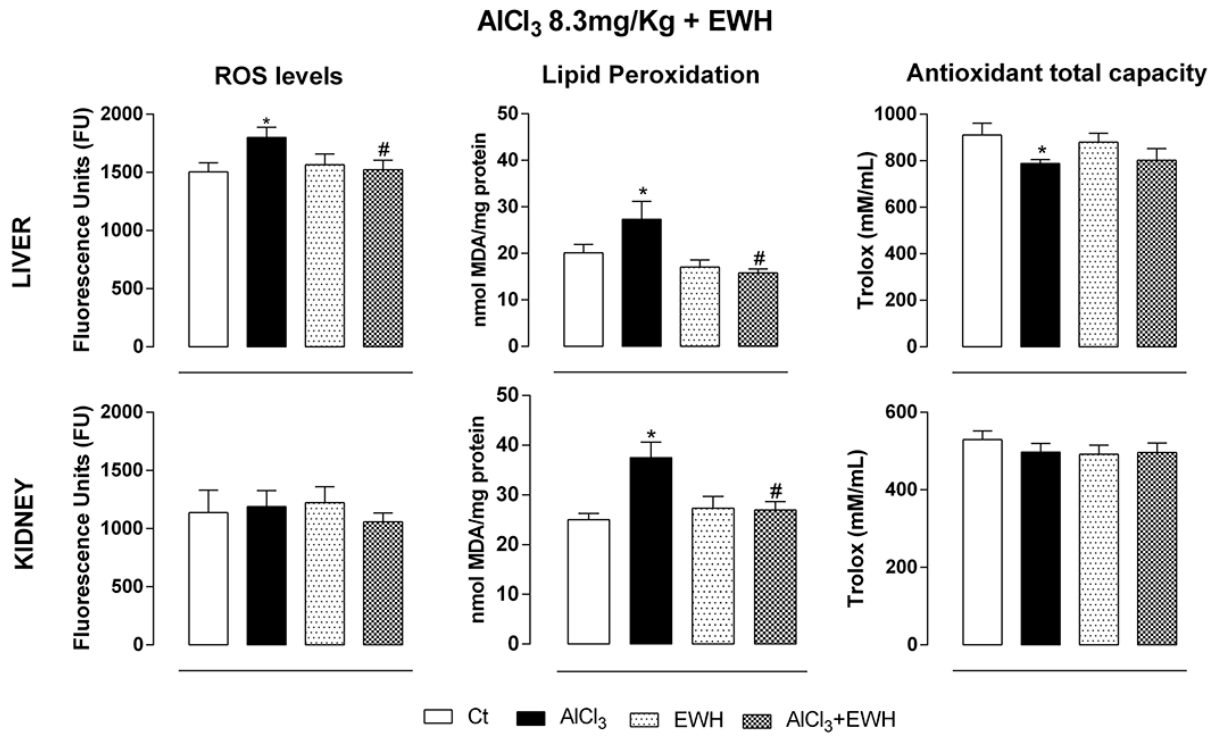


Figure 2: Effects of EWH on oxidative stress assays in Al-exposed rats. Reactive oxygen species (ROS), lipid peroxidation and total antioxidant capacity in liver and kidney of rats exposed to AlCl3 at high doses (100 mg/kg b.w. for 42 days) co-treated or not with EWH. Results are expressed as mean ± SEM, n=8, * P < 0.05 compared with their corresponding controls, # P < 0.05 compared with AlCl3 group (two-way ANOVA and Bonferroni as post-hoc test).

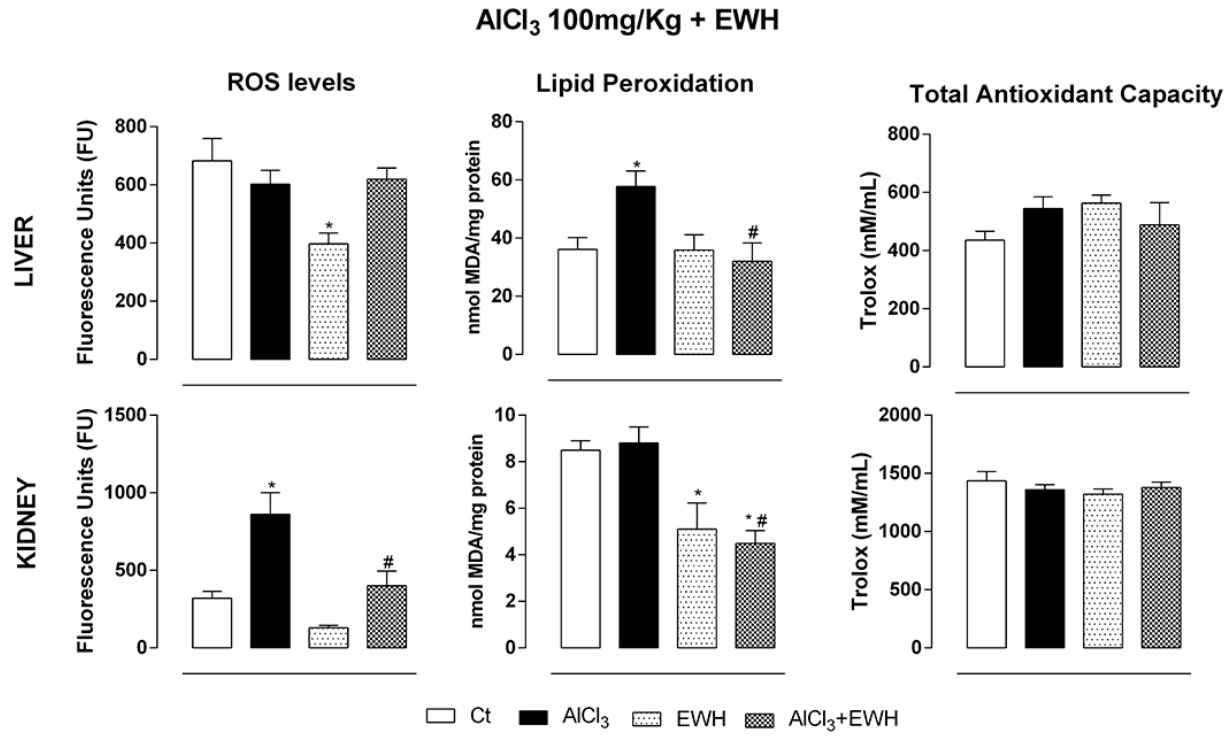


Figure 3: Effect of EWH on liver histology in Al-treated rats. Representative images showing normal histology in all groups: control (Ct), EWH, Al-exposed rats at 8.3 or 100 mg/kg b.w., and Al-exposed rats and co-treated with EWH. Scale bar: 100 µm.

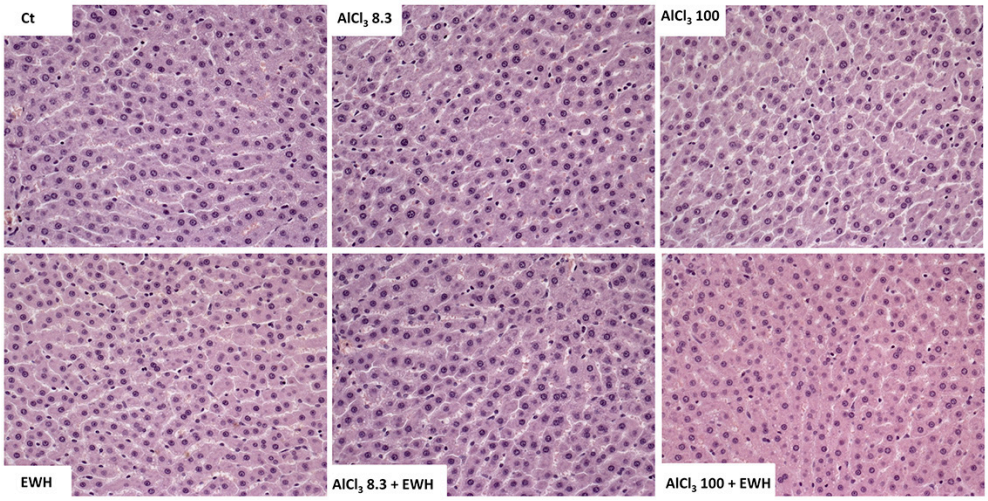


Figure 4: Effect of EWH on kidney histology in Al-treated rats. Representative images showing normal histology in all groups: control (Ct), EWH, Al-exposed rats at 8.3 or 100 mg/kg b.w., and Al-exposed rats and co-treated with EWH. Scale bar: 100 µm.

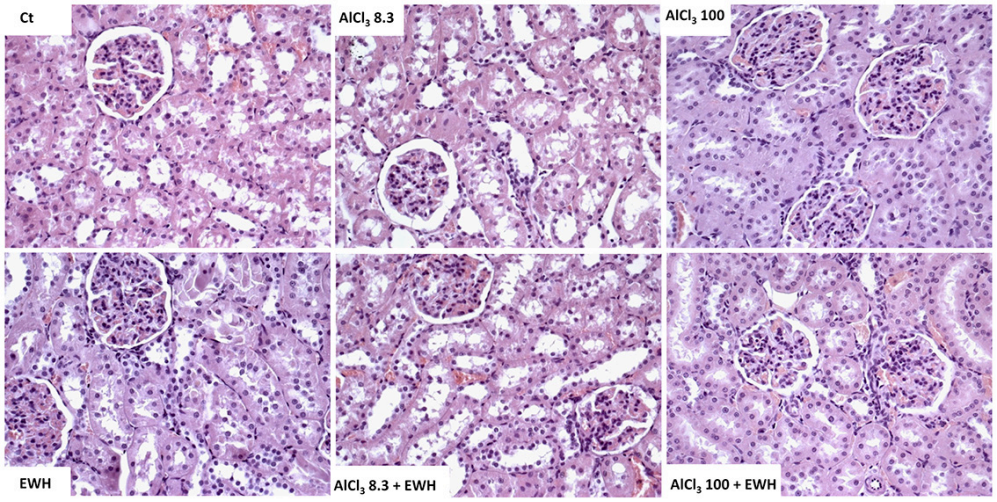


Figure 5: Effect of EWH on colon histology in Al-treated rats. Normal histology in colon of control (Ct) and EWH groups. Colon sections of Al-exposed rats at 100 mg/kg b.w. indicating the presence of epithelial damage and mucosal ulcerations (arrows) and Peyer's patches (*). The co-treatment with EWH prevents the impairment of colon histoarchitecture after Al exposure at 100 mg/kg. Histological damage score after the analysis of the average of 5 fields per rat. Scale bar: 100 µm.

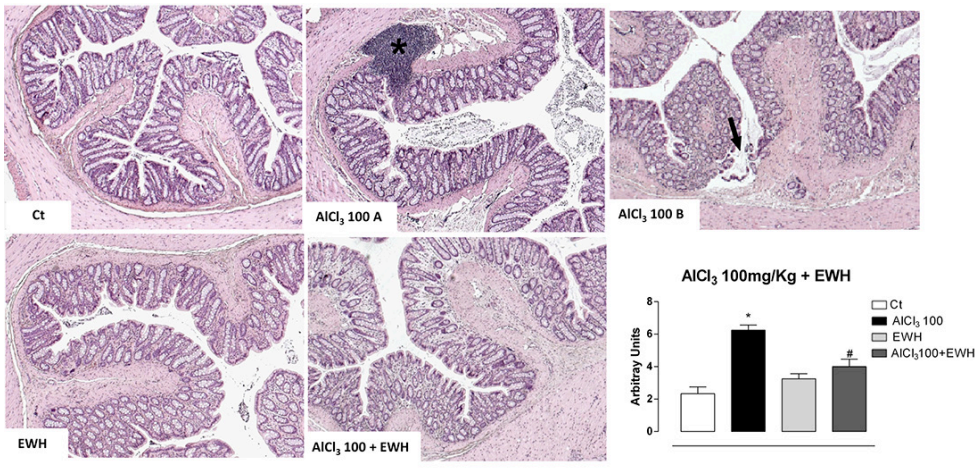


Figure 6: Effect of EWH on the presence of aluminum (orange) in liver. Representative images indicating the presence of Al: lumogallion fluorescence in control (Ct), EWH treated rats and, animals treated with AlCl3 at 8.3 or 100 mg/kg, co-treated or not with EWH. Arrows indicate the presence of aluminum. Fluorescence intensity after the analysis of the average of 6 fields per rat. Scale bar: 50 µm.

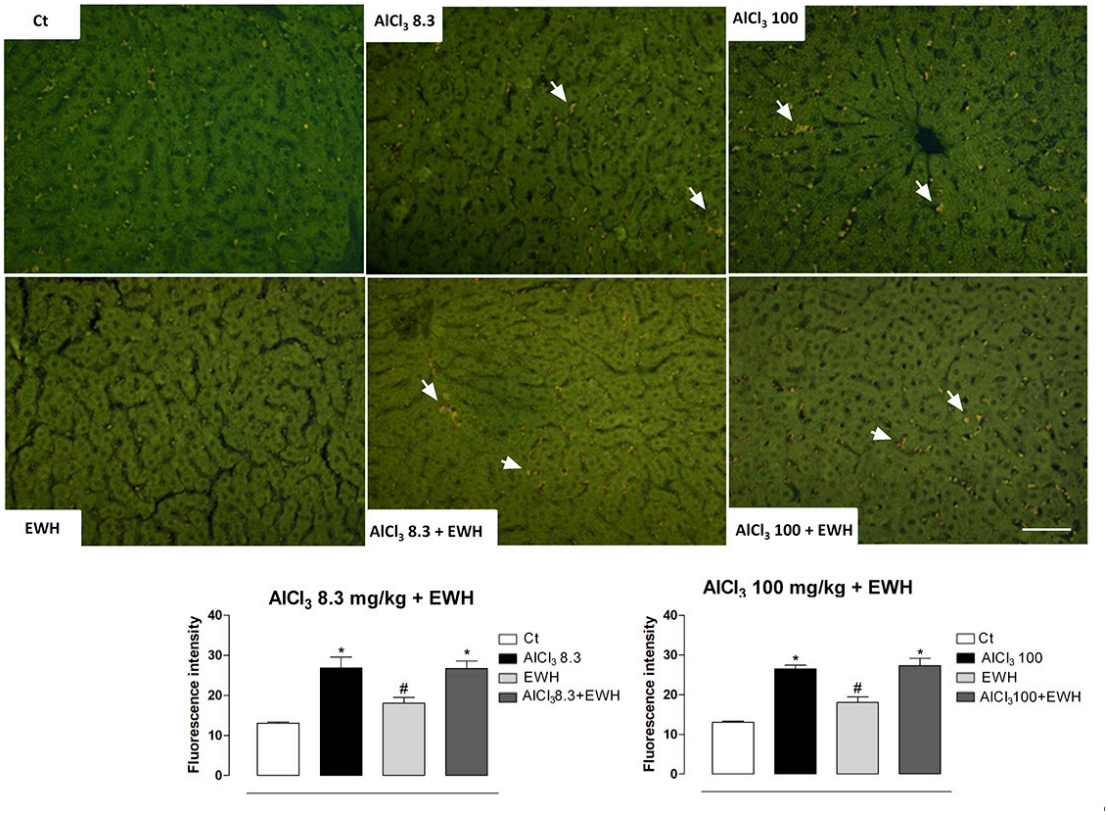
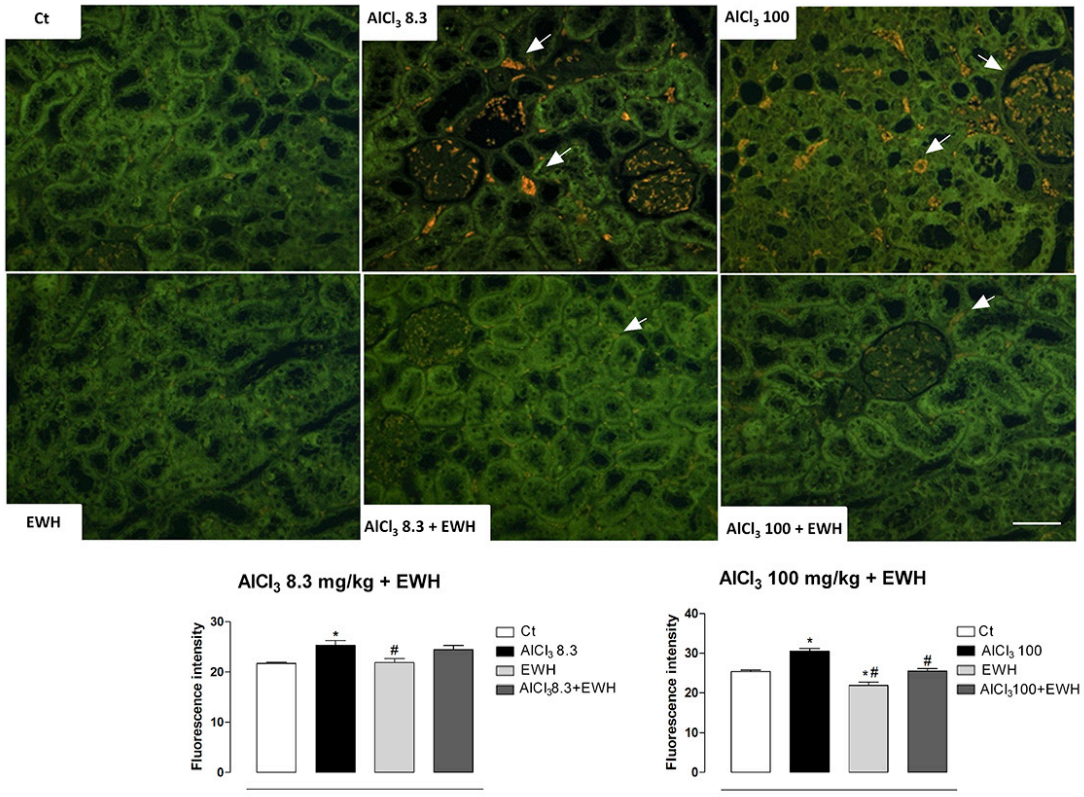


Figure 7: Effect of EWH on the presence of aluminum (orange) in kidney. Representative images indicating the presence of Al: lumogallion fluorescence in control (Ct), EWH treated rats and, animals treated with AlCl3 at 8.3 or 100 mg/kg, co-treated or not with EWH. Arrows indicate the presence of aluminum. Fluorescence intensity after the analysis of the average of 6 fields per rat. Scale bar: 50 µm.



Results

1. Body weight, feed, and fluid consumption

Neither the Al intakes or EWH treatments modified the body and organs weights of rats; the fluid (water or Al) and food consumptions were similar between groups (**Tables I** and **II**).

2. Oxidative stress

Al treatment at low doses of 8.3 mg/kg b.w. raised ROS levels and decreased total antioxidant capacity in the liver and raised lipid peroxidation in both liver and kidney (**Figure 1**). These effects were almost totally prevented by the concomitant oral uptake of EWH (**Figure 1**). Rats treated with Al at the highest dose of 100 mg/kg b.w. showed increased ROS levels in the kidney and lipid peroxidation in the liver, which was prevented by the concomitant intake of EWH (**Figure 2**). Moreover, the dietary supplementation with EWH decreased ROS levels in liver and lipid peroxidation in the kidney of animals not exposed to Al as well as decreased levels of lipid peroxidation in the kidney of animals co-exposed to Al and EWH (**Figure 2**).

3.Histopathology analysis of liver, kidney, and colon

Histology showed normal histoarchitecture of liver and kidney in all experimental groups (**Figures 3** and **4**). On the opposite, Al at a high dietary level strongly impaired the histology of the colon (**Figure 5**). Specifically, Al-induced zonal destruction of epithelium surface and mucosal ulcerations involving submucosa, promoted inflammatory cell infiltrations and the presence of Peyer's patches (**Figure 5**). However, rats that were exposed to both Al and EWH showed colon histology similar to the control and EWH groups (**Figure 5**).

4. Presence of aluminum in liver and kidney

The presence of Al in the liver and kidney was verified by lumogallion and means of fluorescence microscopy. Tissues not incubated with lumogallion showed green autofluorescence (data not shown) and, tissues from control (Ct) and EWH rats showed unspecific fluorescence (**Figures 6** and **7**). Liver and kidney sections from Al-treated rats showed specific bright orange fluorescence when incubated with lumogallion, indicating the presence of Al. Rats in the Al + EWH groups showed weakly orange fluorescence, in which the oral uptake of EWH significantly prevented the presence of Al in the kidney of rats exposed to the highest dose of the metal (**Figures 6** and **7**).

Discussion

Al is everywhere and, besides the efforts to reduce its impact, humans are continuous highly exposed and, a load of Al in the human body, increasing. Oral ingestion is the most important way of human exposure to Al. Therefore, the gastrointestinal tract is the first and primary Al contact pathway, which, when ingested, seems to alter the immune function and microflora permeability[36,37]. Al is a neurotoxin, leading to encephalopathy in renal dialysis patients[38] and appointed as an environmental factor in Alzheimer's Disease[39,40]. Over the past few decades, the involvement of Al in human diseases has considerably increased. Al is now suggested as have a role in human reproductive dysfunction and infertility[41], diabetes mellitus[42], peripheral neuropathy[43], hypertension[44] bone and hematological diseases[9,11]. The human toxic effects of Al depend upon the achievement of a threshold or burden[45].

Our research group have developed an animal model of Al exposure, and we have observed that the equivalent amount of Al ingested by the dietary source is sufficient to induce toxic effects, suggesting the achievement of this "toxic" threshold[17]. Herein, we have demonstrated that beyond the increased oxidative stress in target organs of Al metabolism, Al strongly impairs colon morphology, inducing inflammation, and mucosal ulcerations. Interestingly, these alterations already start in rats exposed to Al at human equivalent dietary level.

Moreover, due to numerous applications and sources, it seems that most of the population are exceeding the maximum limits imposed by regulatory agencies[1,2]. In this sense, strategies and therapies that aim to minimize or prevent the effects of human exposure to numerous environmental contaminants are needed. Food-derived compounds could be an important alternative to maximize the benefits of natural ingredients and seem to be a potential remedy[46,47].

In the current study, we have addressed the effects of dietary supplementation with EWH in rats exposed to human equivalent dietary Al intakes. Our results appoint the EWH as an effective functional food ingredient in the prevention of long-term effects of Al exposure. Specifically, the co-ingestion of EWH was able to prevent colon inflammation and epithelial damages, reduce inflammatory cell infiltrations, and the number of Peyer's patches after Al exposure at a high dietary level and, the increased oxidative damage in liver and kidney of Al-exposed rats. Besides, the ingestion of EWH per se seems to reduce the presence of Al in liver and kidney of control rats not exposed to Al and prevents the increased presence of Al in the kidney of rats exposed to Al at the highest dose, suggesting the putative beneficial effect of a functional food ingredient that could be added to the human diet.

In previous works the potential effects of EWH have been attributed to its antioxidant and anti-inflammatory abilities. The pepsin hydrolysis of egg white releases bioactive peptides with several biological properties[29]. Some

peptide sequences were identified and some of them demonstrated angiotensin-converting enzyme inhibitory activity[29], vascular-relaxing function[48] and/or, antioxidant capacity[26,49] and *in vivo* blood pressure lowering effect[50].

In the present study, rats exposed to both Al and EWH showed reduced oxidative stress in the liver and kidney, and the EWH was able to prevent the increased gastrointestinal inflammation and histopathological damages observed after Al exposure at a super-dietary level. The important inflammation and damage in the colon of Al-treated rats and the efficacy of EWH to prevent may suggest a protective action of these bioactive peptides in the gastrointestinal tract. The gastrointestinal tract is the first site of contact with the oral uptake Al, influencing its bioavailability and absorption[36]. It is known that the surrounding gastrointestinal medium and several dietary compounds can modulate Al absorption. Citrate and other short-chain carboxylic acids such as acetate, oxalate, lactate, malate, tartrate, gluconate, ascorbate, and carbonate seem to increase Al absorption in the gastrointestinal tract and, on the contrary, compounds containing silicone may decrease the absorption of Al facilitating its excretion from the human body[51,52,53].

Recently, by using the intestinal model of Caco-2 cells, it was shown that the intestinal cellular uptake of Al occurs preferably in the particle form[54]. Of interest, our group, by using the same model of differentiated cells, has demonstrated that the EWH released peptides FRADHPFL, RADHP and YPI are susceptible to intestinal transepithelial transport through the monolayer[28]. Therefore, we could also postulate a competition between these small peptides and Al transporters influencing the Al absorption into the small intestinal epithelial cell.

Nevertheless, there are numerous mechanisms and pathways by which EWH could be acting to prevent or minimize the effects of Al that must be extensively studied. In the cardiometabolic disease, the beneficial effects of EWH against metabolic complications in Zucker obese rats were related to changes of gut microbiota, specifically obese rats receiving EWH in drinking water for 12 weeks show microbiota pattern similar to those of the control lean rats[55]. Recently, it was demonstrated the influence of gut microbiota in the Al absorption and systemic effects. The specific probiotic bacteria *L. plantarum* CCFM639 seems to increase fecal Al excretion, decrease intestinal Al absorption and Al accumulation in kidney, liver, and brain, alleviating increased oxidative stress after Al exposure in mice[56,57]. In the current study, EWH was able to reduce the tissular presence of Al in the kidney, and the increased oxidative stress as well as prevent inflammation and colon histopathological damages in rats after Al exposure at a human super dietary level. Therefore, it is also possible to postulate that EWH could interfere and modulate the gut microbiota, which must be further investigated and is the focus of our follow studies.

Conclusion

Our data suggest the use of pepsin EWH as an alternative bioactive food ingredient in the prevention of Al-related complications as an attempt to reduce the impact of the increased human exposure to Al. The EWH supplementation was able to prevent the increased oxidative stress in the liver and kidney, markedly reduced colon histopathological damages and the presence of Al in the kidney of rats exposed to Al at doses that "mimics" high human exposure to this metal. While there are several lines to explain the role of pepsin EWH in the protection against Al toxicity, its antioxidant and anti-inflammatory properties may play an important role. However, it is likely that the underlying mechanisms of egg-derived peptides to reduce the effects of Al are more extensive and must be better understood.

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Author Contributions

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All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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