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Potential dietary toxicity assessment of alum-processed jellyfish

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As a fishery commodity for more than a thousand years, edible jellyfish is an important seafood worldwide. In order to form the crunchy and crispy texture, jellyfish is traditionally processed by curing with salt and hydrated aluminum potassium sulfate. Therefore, potential bioaccumulation of aluminum after dietary consumption of alum-processed jellyfish has received great attention. Here, the concentration of Al in alum-processed jellyfish was measured by inductively coupled plasma mass spectrometry. Furthermore, the safety assessment of alum-processed jellyfish, especially its effects on Al accumulation in different organs were evaluated in mouse. The results indicated alum-processed jellyfish contain high concentrations of Al (549.90±4.66 mg/kg), which could be released into the in vitro human gastric digestion fluid achieving a concentration of 50.92±4.26 μg Al/L. Although no significant changes in the mortality rate, body weight, behavioral patterns, and neurotoxicity signs were observed after 30 days with intragastric administration of jellyfish slurry (up to 34.7 g/kg.bw), consumption of desalted jellyfish slurry significantly increased the Al accumulation in liver when given at medium (17.4 g/kg.bw) and high (34.7 g/kg.bw) doses. Moreover, the relative liver weights in the medium and high dose consumption group were lower than that in the control group. Furthermore, we show for the first time that organic acids such as citric acid may be a useful way to lower the Al concentration in jellyfish.

Keywords: Aluminums, jellyfish, hepatotoxicity, simulated gastric fluid**.**

INTRODUCTION

Edible jellyfish is a popular seafood consumed in the world. The global catch of edible jellyfish is estimated to be more than 300,000 tons annually [1] and is valued as a multi-million dollar business with an increasing demand [2]. However, traditional processing of jellyfish involves the use of alum, which raises a food safety concern due to the possible bioaccumulation of aluminum in humans. Furthermore, marine food resources can accumulate high levels of trace elements and heavy metals in their edible tissues, such as mercury (Hg), cadmium (Cd), plumbum (Pb), arsenic (As), zinc (Zn), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), nickel (Ni) and stannum (Sn) [3], which could interfere with human physiology, leading to a negative impact on renal, endocrine, gastrointestinal, cardiovascular and nervous systems [4].

Edible jellyfish belong to the order *Rhizostomeae*, in the class of *Scyphomedusae*, with symmetrical soft bodies consisting of a gelatinous umbrella-shaped bell and trailing tentacles.[5]. Jellyfish has been commercially exploited for food consumption worldwide for more than a thousand years, especially in Asian countries including China, Japan, Philippines, Vietnam, Thailand, Malaysia, Indonesia, Singapore and Myanmar [6-8]. Jellyfish is often served as a salad or appetizer in these countries due to its firm texture. A range of studies have reported that jellyfish possesses immuno-stimulatory, anti-fatigue, anti-oxidation properties and promotes weight loss and skin beauty [9-12]. Jellyfish has positive effects on hypertension, asthma and gastric ulcer[13].

Owing to its perishability, jellyfish is traditionally treated with a dehydration processing using a mixture of salt (NaCl) and alum ($KAl(SO_4)_2 \cdot 12H_2O$) to reduce the water content and decrease the pH [14]. This traditional processing procedure can extend the shelf life to up to 6-12 months at ambient temperature. Meanwhile, alum treatment also creates a crunchy texture as aluminum is a firming agent interacting with the carboxyl groups on the side chains of collagen [15], which constitutes more than 60% of tissue protein in jellyfish[16].

Although alum treatment has its merits for improving the durability and texture of jellyfish, it was also demonstrated to increase aluminum levels in processed jellyfish [7]. Therefore, possible bioaccumulation of aluminum after consumption of alum-processed jellyfish has become a food safety concern. Indeed, the Centre for Food Safety (CFS),

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the food safety authority of the Hong Kong government, has issued an advice to the public to limit the consumption of jellyfish due to confirmed reproductive toxicity and developmental toxicity of aluminum compounds in experimental animals. A number of studies also suggested that long-term exposure of aluminum shows toxicity to plants, animals and humans [17-19].For example, aluminum can evoke several changes in neurons, which are similar to degenerative lesions observed in patients suffering from Alzheimer's disease [20].

Here we examined the Al content in alum-processed jellyfish and its release in an *in vitro* human gastric digestion system. Furthermore, the accumulation of Al and other trace elements and heavy metals (including Co, Mn, Zn, Cu, Pb, Mg, As, Hg, Fe, Cd, Sn and Ni) in different organs *in vivo* after consumption of alum-processed jellyfish was also determined using inductively coupled plasma mass spectrometry (ICP-MS). In addition, a possible economic and rapid processing technology to lower the Al residues in jellyfish was investigated.

MATERIAL AND METHODS

Materials

Male SPF KM mice, weighed 33 g to 36 g (aged 8 to10 weeks), were purchased from Xiamen University Laboratory, Xiamen, PR China. The animals were group housed in polysulfonate cages (five animals per sex). The rooms are controlled for temperature (20-22 ˚C), relative humidity (45-65 %) and lighting (12 h light/dark cycle). The animals were allowed to acclimatize for one week before the initiation of experiments with food and water available *ad libitum*. All of the animal experiments were performed according to the ethics rules approved by the Xiamen University Ethics Committee (Xiamen, China) (Permit Number: XMUMC2012-12-9).

The alum-processed jellyfish was purchased from the aquatic products market of Mawei in Fuzhou, PR China and immediately stored at -20 ˚C until use.

Ethylenediamine tetraacetic acid solution, acetic acid, citric acid, and peroxide of hydrogen were analytical grade and purchased from Sigma Chemicals (St. Louis, MO, USA). Nitric acid and perchloric acid were ultrapure grade and purchased from Sigma Chemicals (St. Louis, MO, USA). Studied elements including Co, Zn, Cu, Pb, Mg, As, Hg, Fe, Cr, Mn, Sn, Ni and Hg were purchased from Sigma Chemicals (St. Louis, MO, USA). Standard stock solutions (1 g/L) were prepared by dissolution of corresponding spectrum metal and inorganic compounds with 1.2 mol/L HNO_3. Working solutions were obtained by diluting of these standard solutions with 1.2 mol/L HNO_3. Milli-Q ultrapure (Elix UV5 and MilliQ, Millipore, USA) water was used in the whole experiment.

Methods

Preparation of jellyfish slurry

Jellyfish was prepared according to a traditional method as previously reported [7]. Briefly, alum-processed jellyfish, 15 cm in diameter for the part of the umbrella, were split into 5 equal wedges from the center and then were soaked in tap water (material: water ratio is 1:20) for 8 hours. During the procedure, the water was changed every two hours. Then, they were wiped with a paper towel for several seconds, cut into strips and ground into slurry by a miller. The slurry was sealed in zip-lock bags and stored in a 4 ˚C refrigerator.

Chemical composition analysis

Content of the total carbohydrate and total protein in the jellyfish slurry were determined calorimetrically using phenol-sulfuric acid[21]and bicinchoninic acid protein assay[22] as described previously. Standard methods published by the Association of the Official Analytical Chemists (1995) were used to measure the content of moisture, crude fat and ash[23].

In vitro human gastric digestion model

The experimental setup of the *in vitro* human gastric digestion model used was modified from the previous report [24]. The components of gastric juices used were prepared as follows: 2 g of NaCl and 7 mL of HCl (Merck, 37%), with the addition of 3.33 g of pepsin (Sigma, P7125) were diluted to 1 L and the pH was adjusted to 1.2 using 1.0 M HCl. 0.1 g of jellyfish slurry was digested under simulated 40 mL gastric juices with continuous shaking at approximately 60 rpm at 37 ˚C for 2 h to mimic the conditions in the stomach. After the digestion, the mixtures were centrifuged at 10,000 g for 10 min at 4 ˚C in order to separate the digestion fluids (aqueous phase) from the particulate residue. The quantification of Al in samples was performed through the ICP-MS before/after digestion. The concentration of Al solubilized in gastric juices was calculated as the Al (mg) released from jellyfish slurry (kg).

Intragastric administration of jellyfish slurry

The animal experiments were performed according to the ethics rules approved by the Xiamen University Ethics Committee (Xiamen, China) (Permit Number: XMUMC2012-12-9).Forty mice (7-week-old male) were randomly divided into four groups. Group A: Control mice received injections of saline (10 mL/kg.bw·d); group B: positive control mice received 34 mg (Al)/kg.bw by the means of $AlCl_3$ solutions; group C (low dose): mice treated

with jellyfish slurry at 1.74 g/kg.bw; group D (mid dose): mice treated with jellyfish slurry at 3.47 g/kg.bw; group E (high dose): mice treated with jellyfish slurry at 34.7 g/kg.bw. Jellyfish slurry was administered to the mice by intragastric administration. All mice were allowed free access to diets and water throughout the test period. Signs of toxicity (general status and behavioral characteristics) were observed daily. Mice were weighed every five days. After 30 days, blood was collected from the eyeballs of mice before executed using cervical dislocation, and then the brains, liver, kidney and brain were dissected and weighed. Samples of organs were quickly frozen in liquid nitrogen, and then stored at -80 ˚C to perform the measurements of trace elements and metal concentrations.

Trace element and heavy metals quantification by ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700) with external calibration approach was applied to quantify Al in mouse diet, jellyfish slurry and gastric juices. The sample digestion method performed, was as described in the previous report [10, 25]. For solid samples (mouse diet, jellyfish slurry), a portion of sample was accurately weighed and digested on a block heater with 500 μL of nitric acid 3 % and 500 μL of hydrogen peroxide at 120 ˚C for 3 min followed by 150 ˚C for 3 min, then increased the digestion temperature to 180 ˚C for 18 min. After partial evaporation, samples were cooled down and diluted to 5 or 10 mL with ultrapure water. For liquid sample (gastric juices and blood), 50 μL of gastric juices were wet digested with 500 μL of concentrated nitric acid at 65 ˚C for 1 h in a plastic digestion vessel on a block heater. Reagent blanks were prepared in the same manner. The sample solutions were stored in polyethylene vials below 8 ˚C and analyzed within 5 days.

Similarly, the presence and content of the following metals in mice blood and tissue including Al, Co, Mn, Zn, Cu, Pb, Mg, As, Hg, Fe, Cd, Sn and Ni were determined by ICP-MS (Agilent 7700)with an external calibration approach. Concentrations were expressed in μg or mg per wet weight of tissue samples (μg/kg or mg/kg). Calibration and verification of instrument performance were realized using multi-element solutions, respectively tune F and tune A (Thermo®). The internal standard used was rhodium (Agilent Technologies, USA) to correct for matrix effects and instrumental drift. Calibration ranges preparation was carried out using a multi-element calibrator solution (SCP Science® Plasma Cal).

The limits of detection (LOD) and quantification (LOQ) of each analyte were calculated through the analyte concentration that corresponded to three and ten times the standard deviation of ten independent blank measurements respectively. The correlation coefficient of the standard curves ranged from R^2=0.9994 to R^2=0.9999, with the calibration curves displaying great linearity in the concentration range from 1 to 100 ng/g. The result is then divided by the slope of the calibration curve run on the same day of the experiment. Recovery (%), were in accordance with the performance criteria required by the European Commission (EC, 401/2006).

Organic acids treatment on alum-treated jellyfish

Different organic acids used to remove the Al in jellyfish was based on the protocol reported previously [26]. 10 g alum-treated jellyfish were soaked in 200 mL organic acids solutions, including ethylenediamine tetraacetic acid (EDTA) solution (0.01-0.2 g/L), acetic acid (AA) solution (0.1-2 g/L) and citric acid (CA) solution (0.1-2 g/L), for 100 min, and during the procedure, the solution was changed every 40 min. Jellyfish soaked in tap water was used as control.

After equilibration, the solutions were removed and jellyfish slurry was prepared as described above. The Al content was measured using ICP-MS as described above.

Statistical analysis

All experiments were performed in triplicate. All data were presented as means ± SD and all statistics were performed using GraphPad Prism 5.0 software. Differences between means were analyzed by student's t-test or one-way ANOVA followed by Dunnett's multiple comparison. Differences were considered to be statistical significant at $p< 0.05$.

RESULTS

Chemical compositions

Edible jellyfish has been considered as natural diet food for its low calories and richness in protein and minerals for a long time. Consistent with previous results[7, 27], nutritional composition analysis of alum-processed jellyfish demonstrated samples mainly consists of water and protein. The jellyfish samples had very high moisture content(82.7-86.8 %) and protein content (14.3-15.8%), but low crude fat content (0.38-1.5 %). Besides, the ash content and the carbohydrate content of jellyfish sample were 0.71±0.34% and 0.82±0.21 %, respectively. These results suggested jellyfish may be a good source of protein content.

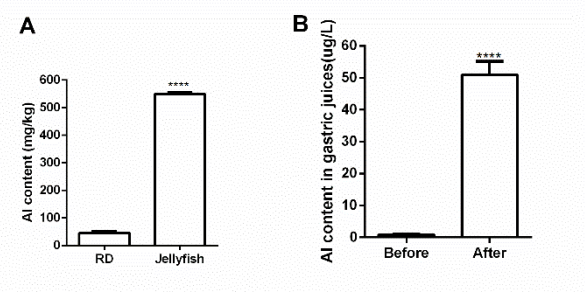

Fig. 1. (A) Aluminum content (mg/kg) in normal laboratory rodent diet (RD) and jellyfish measured by ICP-MS. (B) Aluminum content (μg/L) in gastric juices before and after digested measured by ICP-MS. Data are represented as mean ±SD (n=3), statistical significance was assessed by student's t-test. ****P< 0.01 compared with the aluminum content in normal laboratory rodent diet.

Al residue in alum-processed jellyfish is released in simulated gastric juices in vitro

Next, we analyzed Al content in alum-processed jellyfish by ICP-MS and compared with normal laboratory rodent diet. The results showed that alum-processed jellyfish contain significantly higher (~10-fold) amount of aluminum than normal laboratory rodent diet (549.90±4.66mg/kg and 45.90±5.46 mg/kg, respectively) (Figure 1.A), suggesting alum-processed jellyfish is a Al-rich food, which is consistent to a previous report [10]. Moreover, after 2 hours digestion in the *in vitro* human gastric juices, Al content in gastric juices was significantly increased to 50.92±4.26 μg Al/L (Figure 1.B), indicating that Al in jellyfish slurry might be released after human gastric digestion and absorbed by the digestive system.

Alum-processed jellyfish affect liver-to-body weight index (%) of mice

The appearance and general behavioral patterns of mice were observed daily after jellyfish administration. No toxic symptoms or mortality were observed in all groups. All tested mice lived up to 30 days after the administration of jellyfish slurry at a dose of 34.7 g/kg.bw (Table. 1). Furthermore, the animals in both control group and jellyfish slurry-treated groups displayed no significant changes in behavior, skin appearance, hair loss, breathing, neurotoxicity signs, and postural abnormalities.

The effect of jellyfish slurry on the body weight was determined by recording the body weight at the beginning of the experiment (day 0) and after feeding for 30 d. As summarized in table 1, the control animals gained a mean of approximately3.51±0.98 govern the four-week experimental period. Over the same period, the weight gains for jellyfish slurry treated mice was 3.76±0.97, 4.16±0.71 and 4.68±0.93for animals receiving1.74 g/kg.bw, 3.47 g/kg.bw and 34.7 g/kg.bw of jellyfish slurry, respectively. These values did not differ significantly from the control group.

Although no significant changes in body weight were found, the analysis of organ-to-body weight index (%) of several organs including brain, kidney and liver showed an interesting result. The organ-to-body weight index of liver in mice receiving medium-dose and high-dose of jellyfish slurry significantly lower from the control group(Figure 2), indicating alum-processed jellyfish may affect the functions of liver [7].

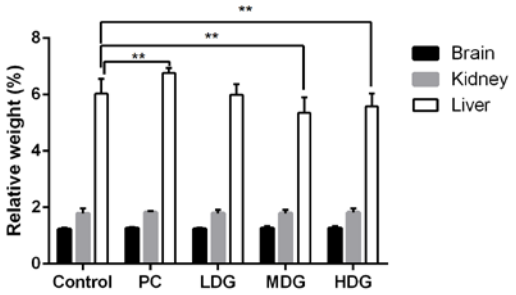

Fig. 2. Organ-to-body weight index (%) of mice exposed to different doses of jellyfish slurry. Organ body index (%) was calculated as (organ weight/body weight) ×100. PC: $AlCl_3$-treated positive control group; LDG: low dose group; MDG: medium dose group; HDG: high dose group. Each value is represented as mean ±SD (n=10), statistical significance was assessed by one-way ANOVA (Dunnett's multiple comparison test). **P< 0.01 compared with control group.

Table 1. The effects of jellyfish slurry on mice survive and body weights

	Number of mice (day 1)	Number of mice (day 30)	Body weight day 1 (g)	Body weight gain (g)
Control	10	10	34.52±4.66	3.51±0.98
Positive control group	10	10	35.14±2.23	-1.27±2.40*
Low dose group	10	10	34.31±5.24	3.76±0.97
Mid dose group	10	10	36.65±2.94	4.16±0.71
High dose group	10	10	34.56±4.35	4.68±0.93

All values are presented as mean ± SD.

Alum-processed jellyfish up-regulates aluminum accumulation in liver and kidney

We next investigated the effects of alum-processed jellyfish on Al accumulation in blood,

liver, kidney, brain and bone of mice. Accumulation of Al was found to be increased in all tested organs in $AlCl_3$ treated mice compared to the control group. Meanwhile, around 2-fold and 3-fold increase in Al accumulation in the liver were observed in mice after receiving intragastic administration of jellyfish slurry at medium- and high-doses, respectively, while a significant increase of Al accumulation was also found in the kidney. The Al concentrations in the liver and the kidney were positively correlated to the amount of jellyfish consumption, suggesting the direct contribution of jellyfish to hepatic and renal Al accumulation.

We also quantified several other trace elements and heavy metals including Co, Mn, Zn, Cu, Pb, Mg, As, Hg, Fe, Cr, Sn and Ni, in blood, liver, kidney, brain and bone of mice by ICP-MS. Values obtained are summarized in table 2. The results demonstrated that only Zn and Cu accumulation were found to increase upon medium-dose and high-dose jellyfish slurry consumption. However, compared to the dramatic change of Al accumulation in the liver (~2 folds in medium-dose group and ~3 folds in high-dose group), the changes of Zn and Cu in all other organs were relatively smaller, suggesting Al should be the major concern with regards to the food safety of alum-processed jellyfish. Interestingly, several heavy metals such as Pb, As and Hg showed a significant decrease in the organs, indicating that the jellyfish may also affect the metabolism of other heavy metals. Finally, metals such as Cd, Mn, Sn and Ni were found to be present at very low levels, beyond detection limits.

Effect of organic acids treatment on removal of Al in jellyfish

To test the effects of organic acids on lowering Al in jellyfish, EDTA, citric acid, and acetate acid solutions were used to soak jellyfish. As shown in Figure 3, the amount of Al existing in the jellyfish decreased significantly after treatment with all these three organic acids. Among the three organic acids, citric acid and acetate acid demonstrated stronger abilities in lowering the Al residues in jellyfish. Treatment with a 0.5 g/L citric acid solution significantly decreased(~80 %) the Al content in jellyfish compared to controls, while a higher concentration of citric acid (up to 2 g/L) can lower the amount of Al further (Figure 3.B).

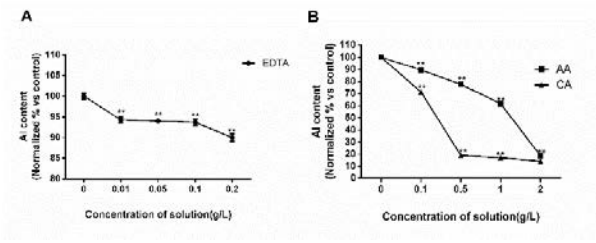

Fig. 3. Effect of different concentration (g/L) of A. EDTA, B. acetic acid (AA) and critic acid (CA) on Al content in jellyfish normalized (%) compared with the content in control. Data are represented as mean ±SD (n=3), statistical significance was assessed by one-way ANOVA (Dunnett's multiple comparison test). $^{**}P< 0.01$ compared with control group.

DISCUSSION

The determination of harmful trace elements and heavy metals accumulation is an important part in health risk assessment in the food industry [28]. This could provide important information and help formulate guidelines for food consumption to authorities, industry and consumers. To date, the systematic study of the bioaccumulations of Al, Co, Mn, Zn, Cu, Pb, Mg, As, Hg, Fe, Cr, Sn and Ni after jellyfish consumption are lacking.

Previous studies have shown that alum-processed jellyfish contain a significantly higher amount of aluminum than fresh jellyfish, suggesting that the curing of jellyfish with alum leads to high concentrations of alum in jellyfish[7, 10].Our results support these findings - alum-processed jellyfish contains higher amount of Al (549.90±4.66 mg/kg) than regular rodent diet (45.90±5.46 mg/kg). Furthermore, it was demonstrated that the Al in alum-processed jellyfish could be released into the gastric digestive liquids after 2 hours digestion through the *in vitro* human digestion model. The *in vitro* human digestion model has been demonstrated to offer an appealing alternative to human and animal studies [29], which can mimic the human gastrointestinal conditions to study the structural changes, digestibility, and release of food components[30]. Therefore, the results here highlights and provide compelling data that the Al in alum-processed jellyfish is available for absorption in the intestinal epithelium, and the consumption of these jellyfish may possess a risk for Al accumulation in human.

Notably, chronic exposure to Al has be linked with several pathological processes, including neurodegenerative diseases [31, 32], microcytic anaemia [33] osteoporosis [34] and liver damage [35].

Table 2. Mean concentrations (W./W.) and standard deviations (Mean ± SD) of trace elements and heavy metals in the matrices of mouse from different groups.

Elements	Matrix	Control group	Positive control group	Low dose group	Mid dose group	High dose group	Recovery (%)
Al (μg/kg)	Blood	831.59±46.13	868.37±46.22**	601.82±49.84*	540.75±39.55**	511.89±19.58**	97.9
	Liver	985.94±47.76	4284.40±52.24**	1058.94±53.02	2009.46±22.62**	3073.53±20.35**	
	Kidney	1191.40±66.97	2332.59±18.61**	896.69±31.66**	1201.32±59.13	2068.56±81.97**	
	Brain	909.72 ± 13.76	1323.48 ±34.65**	946.92±40.62	872.60±34.65	736.61±52.68**	
	Bone	1075.34±23.28	1323.84±11.02**	904.67±41.18**	863.83±27.50**	772.60±7.28**	
Mg (mg/kg)	Blood	37.37±2.42	38.98±2.95	39.16±1.70	41.24±2.10	37.58±5.28	93.4
	Liver	244.80±13.38	250.76±20.28	247.02±7.56	260.11±13.32	243.51±15.25	
	Kidney	202.30±6.57	224.06±14.28	223.34±21.11	207.39±9.84	212.07±9.43	
	Brain	181.03±1.22	189.83±9.28	184.46±4.22	185.25±3.13	184.67±5.21	
Fe (mg/kg)	Blood	47.32±17.78	42.46±15.58	48.99±18.86	46.55±14.57	46.89±11.88	94.5
	Liver	159.53±93.24	148.05±46.20	110.04±14.27	122.50±16.67	103.76±9.02*	
	Kidney	80.84±10.46	89.52±15.58	73.31±12.16	73.32±7.67	80.10±36.62	
	Brain	22.32±2.17	25.38±3.22	24.09±3.61	24.10±2.26	25.911±3.37	
Zn (μg/kg)	Blood	4837.76±460.82	4677.46±200.28	4387.48±468.90	4397.00±193.60	5974.27±174.44**	88.9
	Liver	25007.68±1368.17	28800.45±891.55	25363.65±574.35	26849.24±1722.90*	24531.27±1650.36	
	Kidney	17275.69±741.20	18855.75±198.08	19164.63±1672.67*	17564.32±912.47	18633.05±778.22	
	Brain	15274.10±120.98	15733.63±131.51	15722.63±284.35	15681.93±316.17	16306.40±621.46	
Pb (μg/kg)	Blood	4.358±0.61	4.26±0.61	4.835±0.83	4.413±0.39	4.653±0.55	92.1
	Liver	6.589±0.84	6.68±0.64	5.729±0.97	6.130±0.50	6.643±0.23	
	Kidney	22.021±7.51	21.17±8.83	18.408±5.61	15.975±3.87*	13.980±7.47**	
	Brain	3.641±0.33	4.01±0.01	3.919±0.28	4.114±0.29	4.122±0.41	
As (μg/kg)	Blood	44.62±6.97	42.76±1.68	39.50±4.66	34.81±2.05	40.33±4.76	91.0
	Liver	339.97±43.07	384.05±36.20**	357.08±23.59	341.53±40.32	368.92±30.69	
	Kidney	194.00±3.54	189.12±4.58	192.96±7.98	184.89±9.54	175.31±1.74*	
	Brain	15.58±0.62	15.48±2.22	14.83±1.42	13.48±0.27	14.94±2.20	
Cu (μg/kg)	Blood	701.35±43.00	796.56±12.22	625.01±33.46	746.56±147.49	756.29±45.59	92.9
	Liver	3578.38±483.30	3661.91±488.61	3702.30±165.16	3722.70±463.12	4031.51±158.04*	
	Kidney	3702.83±26.59	3671.53±55.84	3878.53±90.11	3861.42±92.01	3854.05±80.55	
	Brain	4019.69±119.58	4045.11±78.34	4199.50±349.95	4078.69±256.51	3798.73±121.23	
Hg (μg/kg)	Blood	0.47±0.05	0.45±0.44	0.370±0.13	0.163±0.03	0.126±0.03	91.7
	Liver	2.62±0.15	2.79±0.52	3.675±0.23	3.805±0.17	3.830±0.03	
	Kidney	31.40±2.43	32.52±2.35	30.712±1.61	21.483±1.37**	22.019±1.41**	
	Brain	0.76±0.06	0.82±0.026	0.52±0.03**	0.49±0.02	0.25±0.03	

The values are presented as Mean ± SD. Statistically significant differences are analyzed by one-way ANOVA. *P< 0.05; **P< 0.01; n= 10.

Although current evidence suggests that dietary exposure to aluminum may not pose a risk for developing Alzheimer's disease, the food safety authorities of some countries have listed aluminum residues in alum-processed jellyfish as a significant food safety concern, and have encouraged the public to limit their consumption of jellyfish.

Our *in vivo* studies found that the medium- and high-dose consumptions of jellyfish significantly lowered the relative liver weights and increased the hepatic Al accumulation in mice. There has been numerous reports on the adverse effects of Al on the liver [17, 36]. For example, it has been reported that the treatment with $AlCl_3$ solutions (25-36 mg Al^{3+}/kg.bw) in mice or rats resulted in liver damage by cell death or apoptosis of hepatocytes [37, 38]. Therefore, our results indicate that the liver functions may be impaired due to the Al accumulation after consumption of traditional alum-processed jellyfish.

Previous studies also found that the bone, brain and kidney are important accumulation sites of Al in the body[17]. Our results are also consistent with these findings. Among tested organs, the highest Al accumulation is found in the kidney, followed by the bone. Furthermore, after jellyfish slurry treatment, a significant increase in Al accumulation was also found in the kidney. It was also found that Al concentration in these organs was positive-correlated to the amount of jellyfish consumption, indicating a possible direct link between the jellyfish consumption and Al accumulation.

Furthermore, it is widely known that seafood consumption possess a higher risk of bioaccumulation of heavy metals, such as Hg, Cd, Pb, As, Zn, Mg, Fe, Cu, Pb, Mn, Ni and Sn [3]. Among all the analyzed metals, Cd and Ni are recognized as human carcinogens [39]. Despite being weak mutagens, these metals also have the ability to enhance the mutagenicity and carcinogenicity of directly acting genotoxic agents such as ultraviolet or ionizing radiation [39]. Although it has been reported that jellyfish contained abundant inorganic and organic constituents including Zn, Mg, Ca, Al, Sr, Si and alkali metals [10], our data indicated that jellyfish consumption had little influence on the accumulation of these ions in mice compared to Al. Heavy metal contamination also appears not to be a major concern in the safety evaluation of jellyfish since the concentration of

several heavy metals even show a significant decrease after jellyfish consumption. Our data support the hypothesis that the excessive consumption of alum-processed jellyfish will increase the risk of Al poisoning for the consumers. The recommended dietary allowance of jellyfish should be considered based on the Al metabolism *in vivo*.

Several studies have documented that chelating agents such as EDTA and citric acid (CA) can effectively extract heavy metals from waste water and soil [33, 40]. We also applied these organic acids in the desalination process in order to decrease the Al concentration in jellyfish. The results indicate that organic acids would be effective chelators to reduce the content of Al in jellyfish. Future research should focus on the cost, toxicity and degradability of these chelatorsin the development of novel jellyfish food processing technologies[41].

CONCLUSION

Consumption of jellyfish slurry worsens the Al accumulation in liver and affects the relative liver weights at medium-dose (17.4 g/kg.bw) and high-dose (34.7 g/kg.bw) in mice, indicating a possible toxic effect of over-dose consumption of alum-processed jellyfish. Although no significant changes in mortality rates were observed after 30 days feeding with processed jellyfish, however, considering the adverse effects on liver reported here, the long-term effects of aluminum-rich jellyfish consumption warrants further investigation. Citric acid solutions may reduce Al in alum-processed jellyfish and increase its safety.

The present study provides novel and important data on the safety assessment of alum-processed jellyfish consumption, consistent with the recommendations of food authorities in some countries to reduce the consumption of alum-processed jellyfish. Moreover, this study highlights the urgent need to develop novel processing food technologies of jellyfish, which lowers the aluminum bioaccumulation in jellyfish.

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ABBREVIATIONS

Al- Aluminums; As- Arsenic; Cd- Cadmium; Co- Cobalt; Cu- Copper; Cr- Chromium; Fe- Iron; Hg- Mercury; Mg- Magnesium; Mn- Manganese; Ni – Nickel; Pb- Plumbum; Sn- Stannum; Zn- Zinc; ICP-MS - inductively coupled plasma-mass spectroscopy system

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