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Original Article

Arsenic accumulation in various organs of rats fed pre-treated Akamoku (*Sargassum horneri*)

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Summary

Akamoku (*Sargassum horneri*) is a popular seaweed product in Japan but has a high arsenic content. We investigated arsenic accumulations in various organs of rats fed Akamoku. Sprague-Dawley male rats, 5 weeks old, were fed three kinds of diets for 2 weeks: pre-treated Akamoku diet, non-treated Akamoku diet, and control diet. Arsenic distributions in several organs were determined by atomic absorption spectrophotometry. The pre-treated Akamoku samples were prepared by washing in water, soaking in a boiling saline solution for 1 minute, and then washing with cold water. The samples were then lyophilized and pulverized. After this pre-treatment process, the ratio of the arsenic content retained in the pre-treated Akamoku samples to that in the non-treated Akamoku samples was 0.35. The non-treated samples were washed with water, lyophilized and pulverized. The pre-treated Akamoku diet group showed less accumulation of arsenic in the all organs examined in comparison with those of the non-treated Akamoku diet group. The degree of arsenic accumulation could be classified into three groups: in the spleen, heart and femur, the ratio was approximately 0.35, but in the liver, kidney and testis, the ratios were higher while in the lung and muscle, they were lower. This may be related to the different states of arsenic present in the Akamoku plant.
Keywords: Akamoku; *Sargassum horneri*; arsenic; rat; atomic absorption spectrophotometry; pre-treated Akamoku.

Introduction

Akamoku (*Sargassum horneri*), a brown algae, is a traditional but local seaweed, having a delicious taste as well as high contents of dietary fiber[1] and fucoidan[2]. Akamoku is popularly consumed and utilized as a good source of dietary fibers having viscosity. Thus, Akamoku is becoming an attractive and important seaweed product along the sea coast of Japan. However, Akamoku contains high levels of arsenic[3], as do other brown algae[4-6]. Studies have shown that the accumulation of arsenic in the rat body shows unique behavior[7], but the effects of pre-treatment of Akamoku were not known. The purpose of this study was to investigate the accumulation of arsenic in the organs of rats fed Akamoku that had or had not been pre-treated.

When sold at the local market, Akamoku is often passed through hot saline water to make the seaweed turn from brown to a vivid green. In this study, a similar process was adopted to reduce the arsenic content (pre-treated Akamoku). We compared the retention of arsenic in the organs of rats fed Akamoku with or without this pre-treatment.

Materials and Methods

1. Plant samples

The brown seaweed Akamoku *Sargassum horneri* was harvested at the sea coast of Ohshima Nagasakibana, Fukuoka, Kyushu, Japan. The plants harvested were growing in sea water at a depth of 1 m.

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2. Sample preparation

The harvested fresh Akamoku plants were washed three times with fivefold volume of ultra-pure water, blotted, lyophilized and pulverized (non-treated Akamoku sample). The pre-treated sample was prepared by washing three times with fivefold volume of ultra-pure water, followed by blotting and then boiling with fivefold volume of 2.2% NaCl solution for 1 min, washing with fivefold volume of cold extra-pure water, blotting, lyophilization and pulverization (pre-treated Akamoku sample).

The pulverization was performed with a mixer, Milcer, Japan. Before lyophilization, fresh samples of Akamoku contained 89.1% (average) of water. The dried Akamoku is rich in dietary fiber, accounting for 57% of the dry matter[1].

3. Diet compositions

The diet composition was prepared according to the AIN-M formulation. The control diet contained 5% cellulose. The pre-treated and non-treated Akamoku diets contained 5% of pulverized dried Akamoku plant (Table 1).

4. Animals

Sprague-Dawley male rats, 4 weeks old, were fed the solid diet MF (Oriental Yeast Co. Ltd. Japan) for 1 week and then, for the subsequent 2 weeks, fed (1) the diet containing pulverized pre-treated Akamoku, (2) the diet containing pulverized non-treated Akamoku, or (3) the control diet (AIN-M). The rats (seven rats per group) were kept individually in stainless steel cages under a cycle of 12 hours of light (6:00 to 18:00) and 12 hours of dark (18:00 to 6:00). Water and diet were available *ad libitum*.

The rats were fasted on the 14th day, anesthetized with sodium pentobarbital and euthanized.

5. Ethical Conditions

The animal experiment in the present study was approved by the Ethical Committee of Fukuoka Women's University. The approval number is H-18−2.

6. Determination of arsenic in the samples

The samples of the respective organs were lyophilized, and the dried specimens were decomposed with conc HNO_3 and conc H_2SO_4, in a Kjeldahl flask. After addition of saturated ammonium oxalate, the filtrate was mixed successively with KI, ascorbic acid, sodium borohydride-sodium hydride and HCl. The arsenic hydride generated was determined at 193.7 nm with an atomic absorption spectrophotometer, SOLLAR 969 (Thermo Fisher Scientific) or AA-240 (Varian Technologies Japan).

7. Statistics

Statistical values were expressed as average values ± standard deviations, and arsenic values were determined in duplicate or triplicate measurements. Significance was assessed with the *t*-test.

Results and Discussion

1. Animal growth curves

The growth curves of the rats were not significantly different for the pre-treated Akamoku diet, the non-treated Akamoku diet and the control diet groups (Fig. 1). No difference was observed over the 2 weeks, indicating that Akamoku does not contain any special components affecting body weight. The body weight of the pre-treated Akamoku group on the first day of 140.9 g increased to 235.9 g on the 13th day, that of the non-treated group from 140.9 g to 238.5 g on the 13th day and that of the control group from 143.2 g to 239.1 g. On the 14th day, the body

Table 1. Diet compositions (%)

Component	Pre-treated Akamoku diet	Non-treated Akamoku diet	Control diet
Corn starch	46.57	46.57	46.57
α-Corn starch	15.5	15.5	15.5
Casein	14.0	14.0	14.0
Sucrose	10.0	10.0	10.0
Soybean oil	4.0	4.0	4.0
Cellulose	**0.0**	**0.0**	**5.0**
Pre-treated Akamoku	**5.0**	**0.0**	**0.0**
Non-treated Akamoku	**0.0**	**5.0**	**0.0**
Mineral mixture	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0
L-Cystine	0.18	0.18	0.18
Choline hydrogen tartrate	0.25	0.25	0.25
t-Butylhydroquinone	0.0008	0.0008	0.0008

The mineral mixture and vitamin mixture were prepared according to the AIN-M formulation. The pulverized pre-treated Akamoku and pulverized non-treated Akamoku were prepared as described in the text.

weights decreased due to the fasting on the last day.

Daily food intake was 18.2 ± 1.04 g/day for the non-treated Akamoku group, 17.8 ± 0.85 g/day for the pre-treated Akamoku group, and 19.1 ± 0.30 g/day for the control group; a significant difference of 5% was between the control and the pre-treated Akamoku diet group (Fig 2). The water intake did not significantly differ among the three groups (21.44 ± 1.83 ml/day, for each rat).

2. Organ weights

After 2 weeks of feeding, no modification of organ weights of the three groups were observed due to the Akamoku components, except for the lung (Table 2). A significant difference was found between the lung weight of the control and the pre-treated Akmoku diet group. The lungs of rats fed pre-treated Akamoku were heavier than those of the control, even though the amount of diet eaten was less.

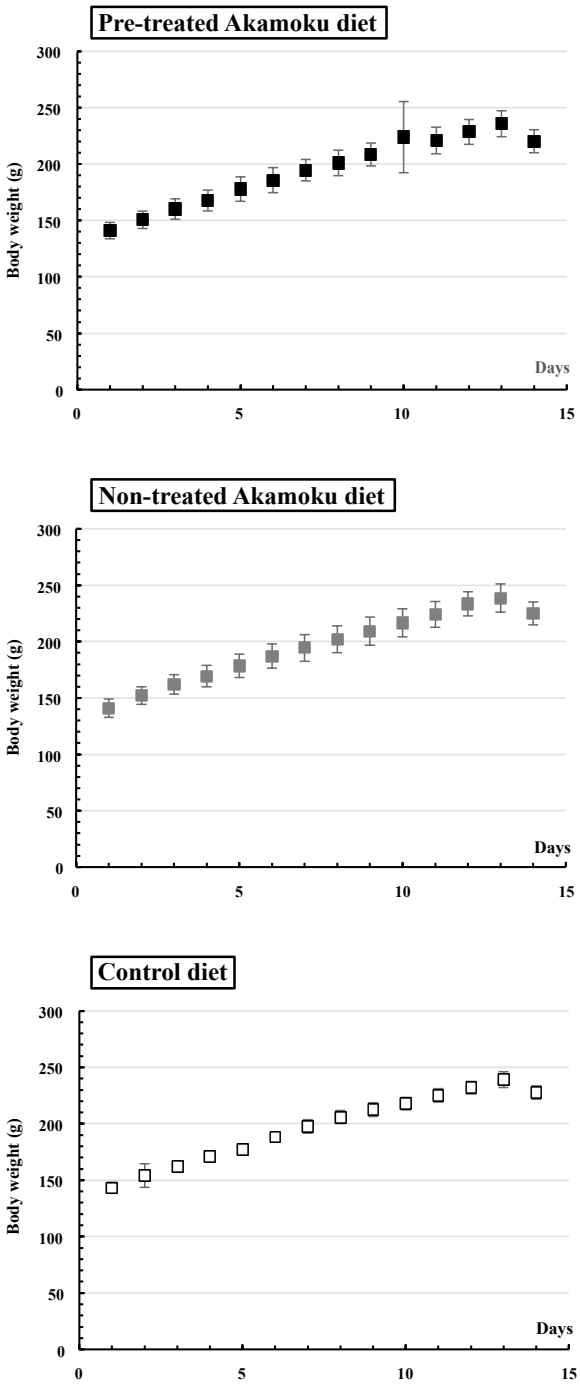

Fig. 1 Growth curves of rats fed pre-treated Akamoku diet, non-treated Akamoku diet and control diet. Bars express the standard deviation. On the 14th day, the body weight decreased due to fasting on the last day.

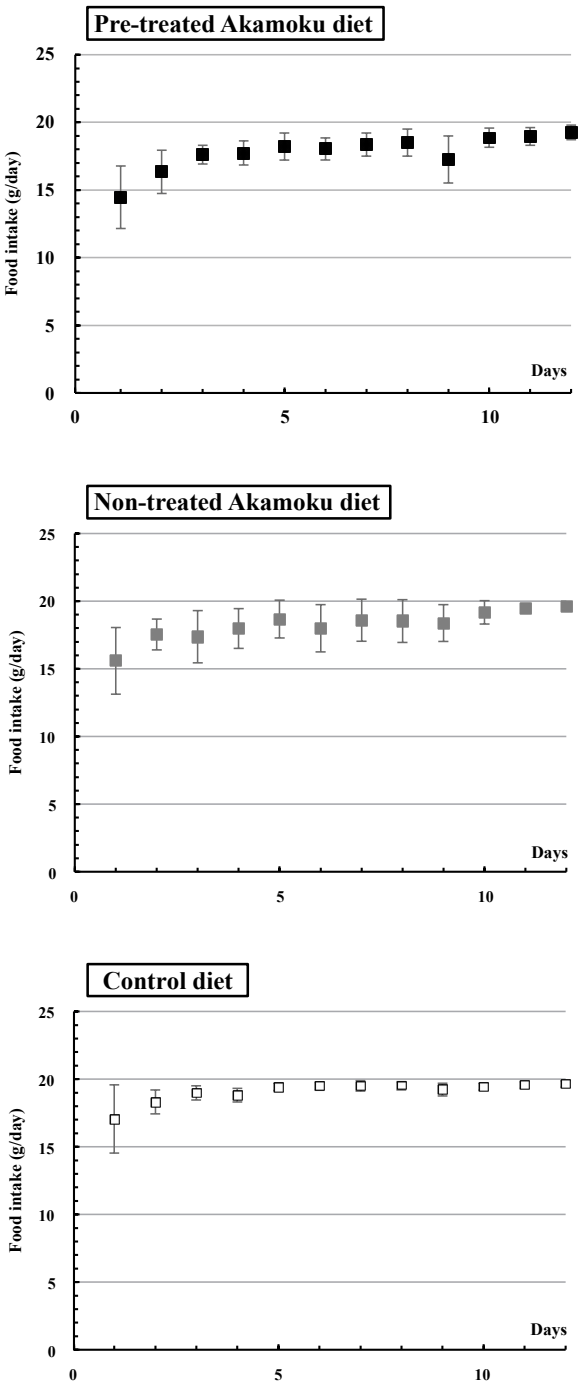

Fig. 2 Daily food intake by the rats of the respective groups. After the pre-feeding for one week, rats of the three groups were fed the respective diets indivisually for two weeks. Bars express the standard deviation.

Table 2. Organ weights per 100 g body weight

Diet \ Organ	Liver	Kidney	Spleen	Heart	Lung	Adrenal gland	Thymus	Testis	Muscle	Femur
Pre-treated Akamoku	3.068±0.120	0.882±0.059	0.228±0.028	0.391±0.051	0.460±0.018*	0.0165±0.0055	0.310±0.047	1.030±0.082	1.544±0.074	0.757±0.090
Non-treated Akamoku	3.093±0.138	0.849±0.067	0.245±0.030	0.356±0.033	0.441±0.030	0.0228±0.0098	0.309±0.027	0.960±0.067	1.517±0.154	0.707±0.103
Control	3.091±0.196	0.873±0.051	0.223±0.032	0.363±0.020	0.420±0.028*	0.0178±0.0027	0.347±0.061	0.941±0.090	1.485±0.056	0.738±0.059

Note: Organ weights (g) were expressed as average ± standard deviation (7 samples). *Significantly different ($p < 0.05$)

This may suggest that, except for the lung, the arsenic level in the organs did not affect the general metabolism of the organs during the experimental period.

3. Arsenic contents in diets

The daily arsenic intake from the diets was as shown in Table 3.

4. Arsenic excretion

Arsenic from the diets was, in part, excreted daily via the feces and urine (Table 4). The arsenic concentrations excreted via the feces was 19 μg As/g weight for the non-treated Akamoku diet group and 10 μg As/g weight for the pre-treated Akamoku diet group. For the control diet group, less than 0.1 μg As/g dry weight of feces was excreted daily. In the feces, un-absorbed arsenic in food will be also included. Arsenic concentrations excreted via the urine averaged 3.5 μg As/ml for the non-treated Akamoku diet group and 1.8 μg As/ml for the pre-treated Akamoku diet group. For the control diet group, less than 0.05 μg As/ml of urine was excreted (Table 4). The ratios of the excreted arsenic in feces and urine from the pre-treated and non-treated Akamoku diets were shown in Table 5. Arsenic from pre-treated Akamoku was more easily excreted.

Table 3. Arsenic contents in algae and diets (ppm)

Diet	Algae	Diet
Pre-treated Akamoku	59	2.6
Non-treated Akamoku	160	7.5
Control	0	0

Table 4. Arsenic contents in feces and urine (ppm)

Diet	Feces	Urine
Pre-treated Akamoku	10.30±0.67	1.75±0.53
Non-treated Akamoku	19.14±3.56	3.54±0.90
Control	< 0.1	< 0.05

Note: Arsenic contents were expressed as average ± standard deviation (7 samples).

Table 5. Ratio of arsenic present in the diets and that excreted via feces and urine.

Diets	Feces	Urine
0.347	0.538	0.494

Note: Average values were used.
Diets: Ratio of the arsenic concentration in the pre-treated Akamoku diet to that in the non-treated Akamoku diet.
Excretion: Ratio of arsenic excreted from the pre-treated Akamoku diet group to that from the non-treated Akamoku diet group.

5. Arsenic accumulation in the respective organs (Table 6)

Arsenic concentrations accumulated in the spleen averaged 70 ppm on the dry weight basis for the non-treated Akamoku group and 28 ppm for the pre-treated Akamoku diet group. For the control diet group, arsenic accumulation was less than 2 ppm. In the lung, arsenic concentrations averaged 44 ppm, and in the liver and kidney, they were 11~13 ppm for the non-treated Akamoku diet group. For the pre-treated Akamoku diet group, arsenic accumulations were 13 ppm in the lung and 5~6 ppm in the liver and kidney.

Arsenic concentrations accumulated in the heart averaged 15 ppm and in the femur, 7 ppm for the non-treated Akamoku diet group. For the pre-treated Akamoku diet group, arsenic accumulations in the heart and femur, were 5 ~ 3 ppm, respectively, and for the control diet group, they were less than 2 ppm and less than 0.5 ppm, respectively.

Table 6. Arsenic content in organs (ppm as As)

Diet \ Organ	Liver	Kidney	Spleen	Heart	Lung	Adrenal gland	Thymus	Testis	Muscle	Femur
Pre-treated Akamoku	4.73±1.09	6.07±1.77	27.71±5.26	4.91±0.65	12.99±2.42	Trace**	< 2	1.40±0.23***	0.90±0.25	2.56±0.41
Non-treated Akamoku	10.80±1.04	13.00±2.33	69.57±8.53	14.57±1.84	44.14±6.62	Trace*	2.84±1.06***	2.60±0.54	3.29±0.71	7.37±1.59
Control	< 0.5	< 1	< 2	< 2	< 1	Trace**	< 2	< 1	< 0.5	< 0.5

Note: Arsenic content (ppm) were expressed as average ± standard deviation (7 samples). *: mostly, 5~10. **: less than 5. ***: or less.

Table 7. Ratios of arsenic accumulated in the respective organs, between the pre-treated and non-treated Akamoku diet groups.

Organs	Liver	Spleen	Kidney	Adrenal gland	Heart	Thymus	Testis	Lung	Muscle	Femor
Ratio	0.438	0.398	0.467	−	0.337	−	< 0.538	0.294	0.274	0.347
Remarks	***		***				***	*	*	

Note: Average values of the respective organs were used. *** The ratio is greater than that of the diet. * The ratio is less than that of the diet.

6. Effects of Akamoku pre-treatment on arsenic accumulation

The differences of arsenic accumulation in the organs of the Akamoku subjected to the pre-treatment suggested its presence in the plant in different chemical or physical states or forms. This may have an effect on the degree of accumulation in the respective organs (Table 7). Arsenic from the pre-treated Akamoku accumulated more in the liver, kidney and testis (Table 7 ***) in contrast to the lung and muscle (Table 7 *). The ratio of arsenic accumulation in the spleen, heart and femur was similar to the ratio in the diet (Table 5, 7). Some factors making these organ specificity in arsenic accumulation are interested.

The arsenic behavior in the rat body seems to be not simple, as that found in the results obtained by Hijiki feeding experiments[8,9]. Some components in Hijiki were suggested to promote the arsenic metabolism in rats[10]. The arsenic retained in the pre-treated Akamoku, showing an organ specificity (Table 7), may behave by different mechanism from the Hijiki-diet rats.

Conclusion

We found that pre-treatment even by simply passing the Akamoku through hot saline water for 1 minute reduced the arsenic content by 70%, resulting in less accumulation in the rat organs. Further work is needed to determine the mechanism of the different behavior of arsenic accumulation between non-treated and pre-treated Akamoku.

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