

Influence of Squid Liver Powder on Accumulation of Cadmium in Serum, Kidney and Liver of Mice

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ABSTRACT: In this study, the effect of squid liver powder intake on accumulation of cadmium in mice was investigated. Subjects were divided into 4 groups including the control group (CON), squid liver powder group with lipids not removed (SLP100), and squid liver powder groups with lipids removed (LFSLP50 and LFSLP100). Feed intake and food efficiency ratio of squid liver powder groups was significantly higher than the CON. As a result of investigating cadmium content in hair, serum, liver, and kidney during intake of squid liver powder, all groups showed increase in cadmium accumulation through consistent, long-term intake. Especially, cadmium content in liver and kidney of LFSLP100 was significantly higher than the content of SLP100 and CON. As a result of pathological observation on liver and kidney tissues according to squid liver powder diet, LFSLP100 showed most serious pathological symptoms. In case of kidney tissues, degeneration was significantly more severe in LFSLP100 compared to other groups. Such results suggest that cadmium concentration in human body can be increased by ingestion of whole squid including internal organs and that tissues can be damaged by increased cadmium concentration. More specific and systematic studies are deemed necessary.

Keywords: *Todarodes pacificus*, squid liver, cadmium accumulation, heavy metal, rats

INTRODUCTION

Among harmful heavy metals, cadmium arouses toxicity and carcinogenic effects in the human body through a tendency to accumulate instead of being excreted (1). Studies on cadmium began in earnest during the 1950s after being identified as the cause of itai-itai disease in Japan. Cadmium is known as a heavy metal that causes external bone diseases such as osteomalacia and osteoporosis (2). The mechanism of cadmium-induced tissue damage is based on glass cadmium not bonding with metallothionein. Cadmium is known to affect the sulfhydryl group of cell membrane proteins to change major enzymatic activities in the body, cause structural changes in cell membranes and damage in tissues (3). There are 4 types of metallothioneins that bond to metal ions and proteins in the human body; among them, metallothionein I and II play important roles in maintaining homeostasis of metal ions by bonding with various metals in the body (4). Metallothioneins are known to bond with metals, like copper, zinc, and mercury, to neutralize cadmium toxicity and prevent accumulation of

cadmium in the blood and bones (5,6). Among the minerals, iron and calcium hinder absorption of cadmium by the alimentary tract. Zinc, a cofactor of enzymatic activity, is substituted by cadmium to mimic toxicity when falling below an optimal level in the human body (7). Symptoms of long-term accumulation of cadmium in the body include proteinuria caused by hindered enzymatic activity, aminoaciduria, growth disturbance, hyperglycemia, and hyperlipidemia (8-10), as well as tissue damages from hepatotoxicity, circulatory disease, anemia, damage to the genital gland, bone disorder, and kidney toxicity (11-13). Exposure to low concentrations of cadmium by some Europeans reportedly caused an increase in urinary concentration of cadmium and negatively influenced the kidneys and bones (14,15).

Cadmium can enter the human body by eating crops absorbing high concentrations of cadmium from the soil (0.09 mg/kg), emissions from smelting industries, production of fertilizers, and contaminated sludge. Other methods of cadmium entry into the body include smoking (16) and food intake by non-smokers and other individuals (17,18). Cadmium in seawater (0.2~0.9 µg/L)

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accumulating in marine organisms can ultimately enter the human body through digestion and absorption (19,20). Due to the continued inflow of contaminants from land to the oceans, some high ranked predators among marine organisms show dangerous levels of heavy metal contents (21). Although contents of harmful heavy metals in marine products reported until now were kept under a tolerance limit or at a minor level (22-24), relatively high concentrations of heavy metals are found from special environmental contaminations in specific regions (25,26). In particular, squid is reported as a marine animal with a high concentration of cadmium accumulated within internal organs (27). About 20,000 M/T of squids were caught off the eastern coast of Korea for the past 3 years, and about 70~80% of them are processed into dried products, salt-dried products, and intermediate moisture products nearby the fisheries. Large amounts of wastes, such as generative and internal organs, occur during the processing of squids, and these wastes are either discarded or used as feed or fertilizer. In addition, squid liver takes up 20~30% of total weight. While squid liver is a useful resource that contains high contents of fat, vitamin B, protein, and bioactive substances (28), problems are caused by the high cadmium content. Cadmium cannot easily be excreted out of the human body and therefore remains in the body long after being absorbed. Since the biological half-life of cadmium is 16 years and cadmium concentration increases with increasing age (29), studies must be continuously conducted on accumulation of cadmium in the body from food intake.

In a study on cadmium in squid liver, Tanaka et al. (30) reported that 15~33 ppm of cadmium exists in squid liver. Li et al. (31) raised yellow corvina by adding squid liver in the feed; as a result, the bioconcentration of cadmium was found in the liver and gills. Kim et al. (32) reported that the cadmium concentration in the digestive gland of *Todarodes pacificus* was 21.5~23.1 ppm, extremely higher than the cadmium concentration in the edible parts. Based on such results, either discarding or adding squid liver in feeds can raise dangers to the environment and organisms, respectively. In addition, some regions of Korea include internal organs of squid, such as the liver, as an edible part. Therefore, scientific research on accumulation of heavy metals in the human body from squid liver intake is deemed necessary.

In this study, the liver of squids, as a major fishing resource of the eastern coast of Gangwon Province, was separated and freeze-dried. After classifying subjects into squid liver powder with and without lipids removed, the effect of squid liver powder diet on accumulation of cadmium in serum, liver, and kidneys of rats was investigated.

MATERIALS AND METHODS

Experimental materials

Todarodes pacificus squids (full length of 45~50 cm) used in this study were in the Ommastrephidae family of the Teuthoidea order. Squids were caught in November 2010 off the coast of Jumunjin, Gangneung-si, Gangwon Province, stored in a -20°C cold storage at the National Federation of Fisheries Cooperatives Jumunjin Branch, and were transported to the laboratory. After defrost and removal of foreign substances, the liver was separated for use as specimens to manufacture feed.

Experimental animals

Forty male ICR mice (Orient Bio, Seoul, Korea) 4 weeks old and body weight of 20 ± 3 g were housed in a laboratory environment and allowed to adapt for one week before being used in this study. The mice were divided into 4 groups of 5 mice each according to body weight by using a randomized complete block design. Groups were separated using a plastic cage and raised for 8 weeks. Water and food were supplied *ad libitum* without limitations. The laboratory was kept at a temperature of $23\pm 1^{\circ}\text{C}$ and relative humidity of $60\pm 5\%$ while controlling light so that both photo and dark periods were fixed to 12 hours. Also, all matters related to the animals followed "Management regulation on the use and raising of experimental animals" defined in Regulation no. 116 of Korea Food and Drug Administration.

Experimental diet

In order to verify the degree of cadmium accumulation in the body of mice according to squid liver diet, subjects were divided into 4 groups: 1) control group (CON group) with commercial feed for mice (Diet5053, Orient Bio), 2) squid liver powder group (SLP100 group) with lipids not removed to set 100 times the provisional tolerable weekly intake of codex (PTWI; $7\ \mu\text{g}/\text{kg}$ of body weight) for commercial feed, 3) cadmium, squid liver powder group (LFSLP50 group) with lipids removed to set 50 times the PTWI for commercial feed, 4) cadmium, squid liver powder group (LFSLP100 group) with lipids removed to set 100 times the PTWI for commercial feed and cadmium (Table 1).

Collection of serum and organs

After 8 weeks of raising and 15 hours of fasting, experimental animals were primarily anesthetized through ether inhalation. Serum was collected from the abdominal aorta using a heparinized syringe and stored in a -80°C cryogenic freezer. After blood collection, serum was removed using phosphate buffer solution. The liver and kidneys were removed, washed with physiological saline, and completely dehydrated by a filter paper

Table 1. Diets and grouping for the study

Ingredient ¹⁾	Group ²⁾			
	CON	LFSLP50	LFSLP100	SLP100
Diet5053	1,000.0	952.7	905.2	907.5
LFSLP (cadmium amount)	—	47.3 (0.2 mg)	94.8 (0.4 mg)	—
SLP (cadmium amount)	—	—	—	92.5 (0.4 mg)

¹⁾Diet5053: composed of about 65% carbohydrate, 22% protein, 7% ash, and 6% lipid, LFSLP: lipid free squid liver powder, SLP: squid liver powder.

²⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

(Whatman 3MM, Maidstone, England) before measuring the weight. Some livers and kidneys were used as histopathological specimens. All specimens were quick-frozen with liquid nitrogen and stored in a -80°C freezer until cadmium analysis.

Proximate analysis

Proximate analysis on the feed used in this study was conducted in accordance to the AOAC method (33). Briefly, the moisture content was analyzed using 105°C ambient drying, crude protein content by the micro-Kjeldahl method, crude fat content by the Soxhlet method, and crude ash content by the 550°C ash method. All measurements were expressed as an average of 3 or more measurements.

Food intake, body weight gain, and food efficiency ratio

Food intake was recorded as a value computed by subtracting residual food measured once every 2 days from the amount of food supplied. Body weight gain in mice was measured at a fixed time every week. Food efficiency ratio (FER) was expressed as the ratio of previous body weight gain (g) to previous food intake (g).

Cadmium content analysis

Cadmium content was analyzed using an atomic absorption spectrometer (AAS, Perkin Elmer PK300, Waltham, MA, USA) after decomposing the specimens by wet processing. Specifically, 5 mL of HNO_3 was added to 0.5 g of hair, feces, urine, liver, and kidney tissues and decomposed using a microwave specimen decomposition device (CEM Mars Xpress, Matthews, NC, USA). Decomposed specimens were diluted using distilled water, and cadmium content was quantified with the AAS.

Persistence rate of cadmium

To analyze the persistence rate of cadmium, feces and urine were collected twice in a 24 hour period by transporting animals to a metabolic case 2 days prior to sacrifice. After cadmium content analysis, the following formula was used to find body persistence rate of cadmium.

$$\text{Persistence rate (\%)} = \{(\text{Daily cadmium intake} - \text{cadmium excreted through feces and urine}) / \text{Daily cadmium intake}\} \times 100$$

Histopathological examination

Liver and kidney tissues were observed using an optical microscope (Olympus BX51, Tokyo, Japan) after manufacturing each tissue section and dyeing with hematoxylin-eosin (H&E). Briefly, the specimens were placed in 10% formalin buffer solution for 48 hours at room temperature and washed for 1 hour with tap water. They were then dehydrated step-wise using 70~100% ethanol. Dehydrated tissues were turned transparent through addition of xylene. Tissues were added to pre-heated paraffin for sufficient infiltration, cooled, and made into paraffin blocks before cutting into 4~5 μm sections using a rotary microtome (Thermo HM325, Leicestershire, England). The paraffin was removed and specimens were dehydrated for observation with an optical microscope after hematoxylin-eosin dyeing.

Statistical treatment

For the results of this experiment, means and standard deviations were computed using a statistical analysis program (SPSS package program v18.0, SPSS Inc., Chicago, IL, USA). Significant difference test was conducted by independent *t*-test at significant level of $p < 0.05$ for the difference between mean values of 2 groups. One-way ANOVA was used to analyze mean values among 3 or more groups. Significance of mean values was tested ($p < 0.05$) using Duncan's multiple comparison test.

RESULTS AND DISCUSSION

Proximate composition and cadmium content of feed

Proximate compositions of feed given to the control group and animal feed with squid liver powder are shown in Table 2. Moisture content of groups with squid liver powder (17.21~18.77%) was about 2.5 times higher than that of the control group ($7.56 \pm 0.3\%$), and ash

Table 2. Proximate composition of diets and cadmium concentration in feed (unit : g/100 g)

Group ¹⁾	Moisture	Ash	Crude protein	Crude fat	Carbohydrate ²⁾	Cadmium concentration (mg/kg)
CON	7.56±0.30 ^{a3)}	6.40±0.10 ^d	20.69±0.12 ^a	5.64±0.17 ^b	59.71	<0.01
LFSLP50	18.77±0.18 ^{bc}	5.78±0.05 ^b	21.76±0.42 ^b	5.12±0.13 ^a	48.57	0.54
LFSLP100	17.21±0.17 ^b	5.89±0.06 ^c	23.36±0.22 ^c	5.46±0.22 ^b	48.07	0.97
SLP100	18.46±0.20 ^c	5.53±0.03 ^a	23.59±0.27 ^c	6.38±0.11 ^c	45.59	0.95

¹⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

²⁾Carbohydrate=100−(moisture+ash+crude protein+crude fat).

³⁾Values are mean±SD (n=5), means with different superscripts within a column indicate significant differences (p<0.05).

Table 3. Feed intakes and gain body weights

Period (weeks)	Group ¹⁾	Feed intake (g/day)	Body weight gain (g)	Feed efficiency ratio ²⁾
4	CON	4.95±0.35 ^{b3)}	10.65±3.45 ^{ns4)}	0.08
	LFSLP50	5.12±0.39 ^b	9.53±2.20	0.07
	LFSLP100	5.18±0.46 ^b	10.53±1.34	0.07
	SLP100	5.59±0.64 ^a	9.05±1.15	0.06
8	Control	4.90±0.40 ^c	13.22±1.92 ^b	0.05
	LFSLP50	5.33±0.45 ^b	16.46±3.13 ^a	0.06
	LFSLP100	4.80±0.53 ^b	16.83±3.11 ^a	0.06
	SLP100	5.55±0.64 ^a	12.34±2.36 ^b	0.04

¹⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

²⁾Food efficiency ratio (FER): body weight gain (g/day)/food intake (g/day).

³⁾Values are mean±SD (n=5), means with different superscripts within a column indicate significant differences (p<0.05).

⁴⁾ns: not significant.

content of the control group was significantly higher (p<0.05) than groups with squid liver powder (LFSLP50, LFSLP100, and SLP100 groups). Crude protein content was lowest at 20.69±0.12% for the control group and highest at 23.59±0.27% for the squid liver powder group with lipids not removed (SLP100 group). Fat content was highest at 6.38±0.11% for the SLP100 group, followed by the CON, LFSLP50, and LFSLP100 groups (p<0.05). Cadmium content of animal feed increased with the amount of squid liver powder added, showing highest values in SLP100 and LFSLP100 groups. However, cadmium content was lower than 2 mg/kg, the level permitted in marine products.

Body weight gain and food efficiency ratio

Feed intake, body weight gain, and food efficiency ratio of the experimental animals according to the addition of squid liver powder are shown in Table 3. Feed intake of the control group after 4 weeks of experiment was 4.95±0.35 g/day, lower compared to the squid liver powder groups. The control group was especially low compared to the SLP100 group (5.59±0.64 g/day). After 8 weeks, feed intake of the control group (4.90±0.40 g/day) was significantly lower than feed intake of the squid liver powder groups. The control group was about 12% lower than feed intake (5.55±0.64 g/day) of SLP100 group (p<0.05). While no significant difference in body

weight gain was noted between the control group and squid liver powder groups, body weight gain after 8 weeks of experiment was significantly lower (p<0.05) in the control group (13.22±1.92 g) and SLP100 group (12.34±2.36 g) than the LFSLP groups (16.46~16.83 g). The body weight difference probably correlated with the lowest food efficiency ratio found in the control and SLP100 groups, and highest food efficiency ratio in LFSLP groups. Food efficiency ratio was calculated by dividing body weight gain by feed intake. The ratio was highest at 0.08 for the control group after 4 weeks of experiment, followed by LFSLP (0.07) and SLP100 (0.06) groups. In contrast, food efficiency ratio after 8 weeks was highest in the LFSLP (0.06) group, followed by control (0.05) and SLP100 (0.04) groups. As such, overall food efficiency ratios after 8 weeks were lower than food efficiency ratios after 4 weeks. The food efficiency ratio difference is probably attributed to the increase in feed intake by the experimental animals from growth period (week 4) to maturity period (week 8). However, no correlation was found in feed intake, body weight gain, and food efficiency ratio according to removal of lipids in squid liver and addition of squid liver powder (p<0.05).

Cadmium content in hair, serum, liver, and kidneys

Cadmium content in hair, serum, liver, and kidneys of experimental animals is shown in Table 4. Cadmium

Table 4. The cadmium contents of mouse hair, blood, liver and kidney

Period (weeks)	Group ¹⁾	Cadmium content ($\mu\text{g/g}$)			
		Hair	Blood	Liver	Kidney
4	CON	$0.15 \pm 0.02^{\text{a2)}$	$0.06 \pm 0.00^{\text{ns3)}$	$0.05 \pm 0.00^{\text{c}}$	$0.14 \pm 0.00^{\text{d}}$
	LFSLP50	$0.14 \pm 0.00^{\text{ab}}$	0.06 ± 0.00	$0.21 \pm 0.03^{\text{b}}$	$0.29 \pm 0.02^{\text{c}}$
	LFSLP100	$0.12 \pm 0.02^{\text{c}}$	0.06 ± 0.00	$0.48 \pm 0.07^{\text{a}}$	$0.44 \pm 0.04^{\text{a}}$
	SLP100	$0.13 \pm 0.03^{\text{bc}}$	0.06 ± 0.00	$0.41 \pm 0.49^{\text{a}}$	$0.36 \pm 0.04^{\text{b}}$
8	CON	$1.31 \pm 0.04^{\text{ns}}$	$0.25 \pm 0.07^{\text{c}}$	$0.46 \pm 0.02^{\text{c}}$	$1.52 \pm 0.05^{\text{b}}$
	LFSLP50	1.30 ± 0.04	$0.36 \pm 0.01^{\text{b}}$	$2.04 \pm 0.48^{\text{b}}$	$2.86 \pm 0.28^{\text{a}}$
	LFSLP100	1.31 ± 0.05	$0.40 \pm 0.02^{\text{a}}$	$2.71 \pm 0.42^{\text{a}}$	$3.04 \pm 0.27^{\text{a}}$
	SLP100	1.30 ± 0.04	$0.40 \pm 0.01^{\text{a}}$	$1.83 \pm 0.77^{\text{b}}$	$2.87 \pm 0.50^{\text{a}}$

¹⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

²⁾Values are mean \pm SD (n=5), means with different superscripts within a column indicate significant differences ($p < 0.05$).

³⁾ns: not significant.

content in hair after 4 weeks was significantly higher for the control group ($0.15 \pm 0.02 \mu\text{g/g}$) than squid liver powder groups ($0.12 \sim 0.14 \mu\text{g/g}$), but there was no significant difference ($p > 0.05$) among groups after 8 weeks. Choi et al. (34) reported an increase in cadmium content in hair while increasing the dietary period of cadmium through an experiment on mice. In this study, cadmium content was increased in all groups during the increasing dietary period.

Since cadmium in serum mostly exists in the plasma at the early stage of exposure and moves to corpuscles with passage of time (35,36), cadmium content in serum was investigated using whole blood. Cadmium content in serum after 4 weeks was found to be $0.06 \pm 0.00 \mu\text{g/g}$ in all groups, including the control group, showing no significant difference among groups ($p > 0.05$). However, cadmium content in serum after 8 weeks was significantly increased by addition of squid liver powder. In particular, cadmium content was highest in SLP100 ($0.4 \pm 0.0 \mu\text{g/g}$) and LFSLP100 groups ($0.4 \pm 0.0 \mu\text{g/g}$) and lowest at $0.25 \pm 0.07 \mu\text{g/g}$ in the control group ($p < 0.05$). A notably high content was observed in the LFSLP100 group ($0.4 \pm 0.0 \mu\text{g/g}$) than the LFSLP50 group ($0.36 \pm 0.01 \mu\text{g/g}$) and was probably caused by consistent, long-term intake of feed containing cadmium, despite the fact that cadmium content in the animal feed for the two groups was lower than the level of cadmium permitted for marine products. Lee (37) reported a positive correlation between increased cadmium content in serum from increases in the cadmium intake period and amounts of intake; this result is consistent with the results of this study.

A significant difference in liver cadmium content was clearly shown among the groups, both after 4 and 8 weeks. Cadmium content in liver of the LFSLP100 group after 4 weeks ($0.48 \pm 0.07 \mu\text{g/g}$) was about 900% higher than the control group ($0.05 \pm 0.00 \mu\text{g/g}$), and about

56% higher than the LFSLP50 group ($0.21 \pm 0.03 \mu\text{g/g}$) ($p < 0.05$). No significant difference between the SLP100 ($0.41 \pm 0.49 \mu\text{g/g}$) and LFSLP100 groups was observed. Cadmium content in liver after 8 weeks was highest for the LFSLP100 group at $2.71 \pm 0.42 \mu\text{g/g}$, followed by LFSLP50 ($2.04 \pm 0.48 \mu\text{g/g}$), SLP100 ($1.83 \pm 0.77 \mu\text{g/g}$), and the control ($0.46 \pm 0.02 \mu\text{g/g}$) groups. Interestingly, the SLP100 group with lipids not removed showed significantly lower cadmium content in liver compared to the LFSLP100 group with lipids removed, suggesting that lipids can impede accumulation of cadmium in the body.

A significant difference in cadmium content in kidney was clearly shown among the groups after 4 weeks and 8 weeks. The cadmium content in kidney after 4 weeks was highest for the LFSLP100 group at $0.44 \pm 0.04 \mu\text{g/g}$, followed by SLP100 ($0.36 \pm 0.04 \mu\text{g/g}$) and LFSLP50 ($0.29 \pm 0.02 \mu\text{g/g}$) groups. Similarly, after 8 weeks, cadmium content in kidney was highest for the LFSLP100 group ($3.04 \pm 0.27 \mu\text{g/g}$) and lowest for the control group ($1.52 \pm 0.05 \mu\text{g/g}$), but there was no significant difference in content among the squid liver powder groups ($p > 0.05$).

Cadmium content in liver and kidney slightly differed after 4 weeks in the experiment, but not significantly different; on the contrary, they showed a significant difference after 8 weeks. Once cadmium enters the body, it first accumulates in liver. With an increased intake period, some of the cadmium is transported from the liver to the kidneys, a secondary accumulation site (38-40). Therefore, the reason for higher cadmium content in the kidneys than the liver after 8 weeks is because cadmium accumulated in the liver from the squid liver powder intake is transported to the kidneys for excretion. Coinciding with results by Jarup (19), cadmium content in kidney increases on week 8 with increased amount of cadmium excreted through urine.

Table 5. Cadmium intakes and the persistence rates of cadmium in the body

Period (weeks)	Group ¹⁾	Cadmium intake ($\mu\text{g}/\text{day}$)	Cadmium amount ($\mu\text{g}/\text{day}$)		PRCdB ²⁾ (%)
			Fecal	Urinary	
4	CON	<0.01	<0.01	<0.01	—
	LFSLP50	$0.89\pm 0.07^{\text{c}3)}$	$0.14\pm 0.01^{\text{b}}$	$0.01\pm 0.00^{\text{ns}4)}$	83.2
	LFSLP100	$1.81\pm 0.16^{\text{b}}$	$0.25\pm 0.01^{\text{a}}$	0.01 ± 0.00	85.7
	SLP100	$2.08\pm 0.22^{\text{a}}$	$0.09\pm 0.02^{\text{c}}$	0.01 ± 0.00	95.0
8	CON	<0.01	$0.02\pm 1.16^{\text{b}}$	$0.08\pm 0.00^{\text{c}}$	—
	LFSLP50	$0.92\pm 0.08^{\text{c}}$	$0.33\pm 1.13^{\text{c}}$	$0.12\pm 0.01^{\text{b}}$	51.1
	LFSLP100	$1.68\pm 0.19^{\text{b}}$	$0.50\pm 1.43^{\text{a}}$	$0.13\pm 0.01^{\text{a}}$	62.5
	SLP100	$1.95\pm 0.27^{\text{a}}$	$0.40\pm 2.05^{\text{b}}$	$0.12\pm 0.00^{\text{b}}$	73.3

¹⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

²⁾PRCdB: Persistence rate of cadmium in the body.

³⁾Values are mean \pm SD (n=5), means with different superscripts within a column indicate significant differences (p<0.05).

⁴⁾ns: not significant.

Cadmium intake and persistence rate

Provisional tolerable weekly intake by codex (PTWI) is 7 $\mu\text{g}/\text{kg}$ of body weight. The PTWI computed for the mice used in this experiment was 30.1 ± 0.2 g, which is about $0.21\sim 0.24$ $\mu\text{g}/\text{week}$ or $0.03\sim 0.034$ $\mu\text{g}/\text{day}$. Based on the PTWI, cadmium intake of each group was studied during the experimental period as shown in Table 5. Daily mean cadmium intake of mice after 4 weeks was highest in the SLP100 group (2.08 ± 0.22 $\mu\text{g}/\text{day}$), followed by the LFSLP100 (1.81 ± 0.16 $\mu\text{g}/\text{day}$) and LFSLP50 (0.89 ± 0.07 $\mu\text{g}/\text{day}$) groups (p<0.05). In comparing these results, SLP100 group was found to intake about 66, 61, and 30 times more cadmium than the control, LFSLP100, and LFSLP50 groups, respectively. Daily mean cadmium intake of mice after 8 weeks of experiment was highest in the SLP100 group (1.95 ± 0.27 $\mu\text{g}/\text{day}$), followed by the LFSLP100 (1.68 ± 0.19 $\mu\text{g}/\text{day}$) and LFSLP50 (0.92 ± 0.08 $\mu\text{g}/\text{day}$) group, showing an identical as week 4.

As a result of examining cadmium content in feces (Table 5), content after 4 weeks was highest in the LFSLP100 group (0.25 ± 0.01 $\mu\text{g}/\text{day}$), followed by the LFSLP50 (0.14 ± 0.01 $\mu\text{g}/\text{day}$) and SLP100 (0.09 ± 0.02 $\mu\text{g}/\text{day}$) groups. Cadmium content in feces of the control group was 0.02 ± 1.16 $\mu\text{g}/\text{day}$ after 8 weeks, which is much lower than PTWI. In contrast, cadmium content in feces of squid liver powder groups after 8 weeks was about 2 times the content in the LFSLP group and 4 times that in the SLP100 group after 4 weeks. While cadmium content in urine was low (0.01 ± 0.00 $\mu\text{g}/\text{day}$) with no significant difference in all the groups after 4 weeks of experiment, it was significantly higher (p<0.05) in the squid liver powder groups ($0.12\sim 0.13$ $\mu\text{g}/\text{day}$) than the control group (0.08 ± 0.00 $\mu\text{g}/\text{day}$) after 8 weeks. Furthermore, cadmium content was significantly higher in feces than in urine after 4 weeks. Higher cadmium content was also observed in feces than in urine

after 8 weeks, but the content in urine at 8 weeks was increased by more than 10 times compared to 4 weeks; this rate of increase was higher than the increase of cadmium content in feces. During initial exposure to cadmium, accumulation in the liver occurs first. Cadmium is then transferred from liver to kidney during long-term exposure. Cadmium contents in liver and kidney were higher after 8 weeks of experiment than 4 weeks because cadmium concentration excreted out through urine increased with increasing amount of squid liver powder (38-40). Cadmium is known to be mainly absorbed through the alimentary tract and shows a rapid increase in excretion through urine with kidney dysfunction from long-term exposure (41), which is consistent with the results of this study.

Persistence rate of cadmium in the body (PRCdB) is the percentage of total cadmium intake excreted out of the body through feces and urine. PRCdB after 4 weeks was highest in the SLP100 group (95.0%), followed by the LFSLP100 (85.7%) and LFSLP50 (83.2%) groups. PRCdB after 8 weeks was highest in the SLP100 group (73.3%), followed by the LFSLP100 (62.5%) and LFSLP50 (51.1%) groups. In addition, PRCdB after 8 weeks was lower than the rate after 4 weeks in all groups. PRCdB was high until week 4 due to the high accumulation rate of cadmium in the body. With long-term exposure to cadmium until week 8, excretion of cadmium through the kidneys increased resulting in a lower persistence rate of cadmium after 8 weeks (38,40,41). Therefore, the results on week 4 and 8 showed a positive correlation between persistence rate of cadmium and cadmium intake.

Pathological observation on liver and kidney tissues

Liver is the organ in which the largest amount of cadmium is accumulated. Pathological examination results on liver tissues are shown in Table 6 and Fig. 1. As a result, while hepatolysis, abnormal sinusoid, and liver cell

necrosis did not occur in the control group, significant pathologic abnormalities were shown in liver cells of squid liver powder groups (LFSLP and SLP groups) on

week 4 and 8. Also, mild hepatolysis and abnormal sinusoid were shown from acute toxicity by cadmium in squid liver powder groups on week 4, but the degree of

Table 6. Histological change of mouse liver after feeding of squid liver powder

Group ¹⁾	Weeks	Hepatocyte swelling	Deranged sinusoid	Hepatocyte necrosis	Hepatocyte regeneration
CON	4	— ²⁾	—	—	—
	8	—	—	—	—
LFSLP50	4	+	++	+	+
	8	++	++	+	++
LFSLP100	4	++	++	++	++
	8	+++	+++	++	+++
SLP100	4	+	+	+	+
	8	++	++	+	++

¹⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

²⁾—: negative, +: mild, ++: moderate, +++: severe.

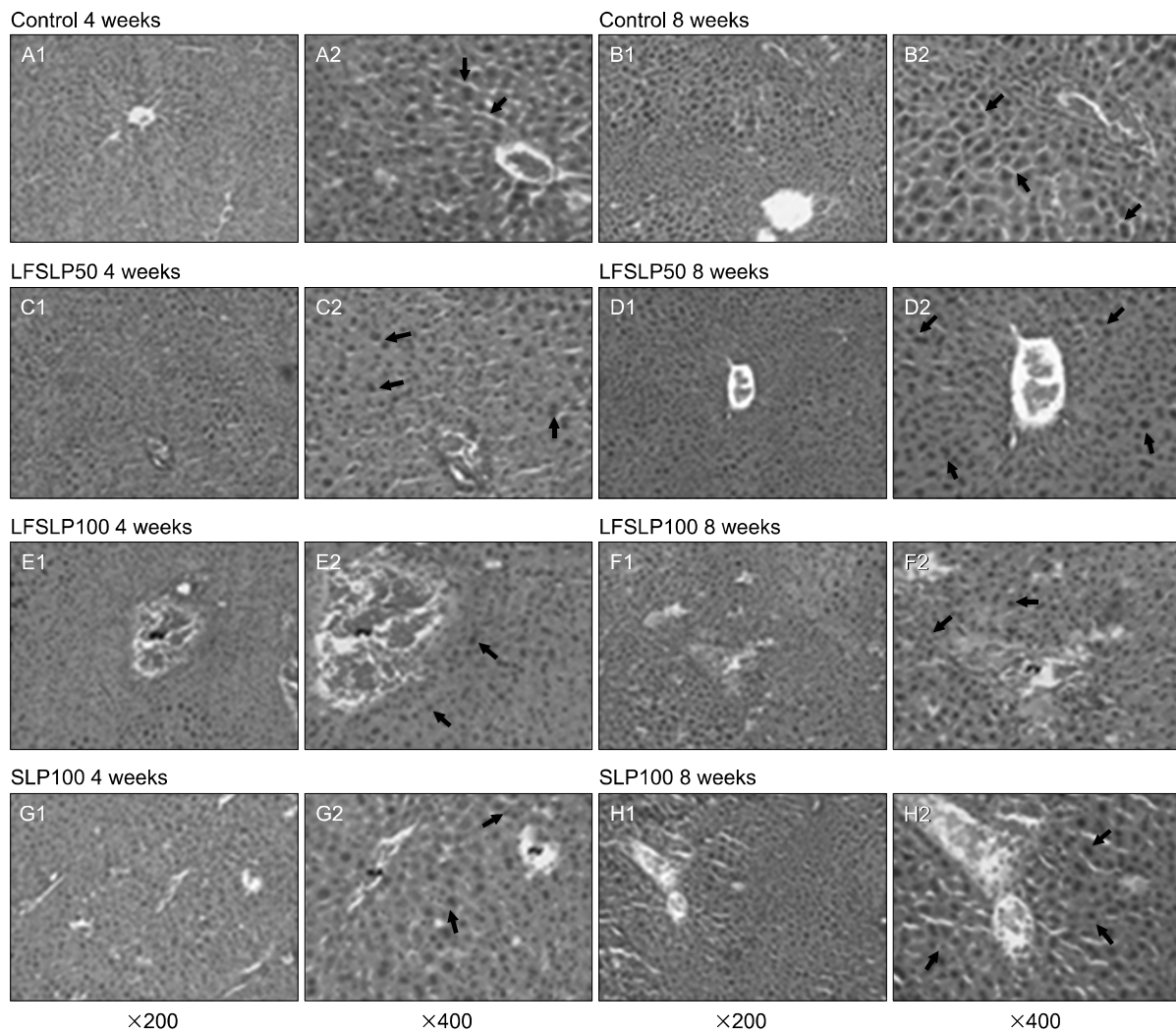


Fig. 1. Photomicroscope of mouse liver after feeding of squid liver addition feed. A, C, E, G: 4 weeks. B, D, F, H: 8 weeks. C: Some hepatocytes were much swelled and their nuclei were also hyper chromatic, and some sinusoidal spaces were distorted. D: Many hepatocytes (arrows, D2) near hepatic artery were sclerosed with hyper chromatic nuclei. E: The portal vein was dilated and filled with red blood cells, and adjacent hepatocytes were degenerative with sclerotic change. F: The hepatic cords (arrows, F2) around portal vein was severely distorted and damaged. G: The hepatocytes between portal veins and hepatic aunties were relatively well presence, but some hepatocytes were degenerative in high magnification veins. H: Many hepatocytes (arrows, H2) became sclerotic and degenerative, and the associated portal vein was dilated and congested with red blood cells.

such abnormalities was weak due to liver cell regeneration and proliferation. In particular, cadmium-induced pathological symptoms of the LFSLP100 group were further degenerated compared to other groups. Pathological symptoms by cadmium were worsened in all groups after 8 weeks compared to week 4, in the following sequence: LFSLP100, LFSLP50, and SLP100 groups. Pathological symptoms of squid liver powder group with lipids removed (LFSLP100 group) were more degenerated than the squid liver powder group with lipids not removed (SLP100 group). When lipids were removed, symptoms were deepened with increasing amounts of squid liver powder. These results suggest that the cadmium content in liver of the SLP100 group was lower than the content of the LFSLP groups, resulting in lower liver cell toxicity by cadmium and weaker pathological symptoms. In addition, pathological symptoms of liver tissues were further degenerated on week 8 compared to week 4 regardless of whether lipids were removed because of liver toxicity and reduction in liver regeneration due to long-term exposure to cadmium. Morita (42) and Kudo et al. (43) medicated high concentrations of cadmium in mice to observe liver cell necrosis, degeneration, cirrhosis, abnormal sinusoid structure, structural changes in Kupffer cells, and discoloration of dead cell nucleus. Such results are similar to the results of this study.

Pathological symptoms of kidney tissues are shown in Table 7 and Fig. 2. At week 4, contraction and stiffening of glomeruli were more severe in the LFSLP100 group (++) compared to the control (-), LFSLP50 (+), and SLP100 (+) groups. The degree of contraction and stiffening was further deepened by week 8, when compared to week 4, and was significantly more severe in the LFSLP100 group than other groups. Epithelial desquamation and necrosis of renal tubules became more severe after 8 weeks than 4 weeks, and the degree was greater in the LFSLP100 group compared to the LFSLP50 and

SLP100 groups. Similar to liver tissues, kidney tissues were damaged more in squid liver powder groups (LFSLP and SLP groups) compared to the control group, regardless of whether lipids were removed. The degree of damage in kidney tissues increased as the food intake period increased. The kidneys filter body wastes and reabsorb nutrients. Since the kidneys have a low ability to regenerate cells unlike the liver, it requires a long time for recovery once damaged. The glomerulus performs the role of filtering out toxic substances and can be weakened and damaged when filtering function is degenerated. Metallothionein (MT) synthesized in liver is combined with cadmium (Cd-MT) and discharged as serum. In serum, cadmium cannot pass through cell membranes of other organs and tissues and selectively penetrates glomeruli (35,44). Cd-MT filtered by the glomerulus membrane is reabsorbed into the renal tubule by pinocytosis and decomposed in the lysosome. When toxic glass cadmium is released, MT is re-synthesized in the renal tubule into a non-toxic form of Cd-MT. However, large amounts of cadmium decomposed from Cd-MT can infringe enzymatic activities of kidney cells. Kidney dysfunction is caused by failure of the renal tubule to generate additional MT (45-47). In this study, contraction and stiffening of glomeruli became severe in squid liver powder groups compared to the control group regardless of whether lipids were removed. Epithelial desquamation and necrosis are probably symptoms of kidney dysfunction caused by cadmium.

Squid is a representative marine product of the eastern coast of Korea. One of the Korean eating habits with squid is to ingest it whole, including the internal organs. Since most of the internal organs in squid are composed of liver, an organ known to have high concentrations of cadmium, our study examined the effects of such an eating habit on the human body. In this study, the effect of squid liver powder intake on accumulation of cadmium in mice was investigated. Such results suggest that cad-

Table 7. Histological change of mouse kidney after feeding of squid liver powder

Group ¹⁾	Weeks	Glomerulus		Convolutated ducts (renal tubule)		
		Constriction	Sclerosis	Ductal dilatation	Epithelial detachment	Epithelial necrosis
CON	4	- ²⁾	-	-	-	-
	8	-	-	-	-	-
LFSLP50	4	+	+	++	+	±
	8	++	++	++	++	+
LFSLP100	4	++	++	++	+	+
	8	+++	+++	++	++	++
SLP100	4	++	+	+	+	+
	8	++	++	++	++	++

¹⁾CON: normal diet, LFSLP50: LFSLP addition to the Diet5053 in the level of 50 times higher than cadmium PTWI, LFSLP100: LFSLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI, SLP100: SLP addition to the Diet5053 in the level of 100 times higher than cadmium PTWI.

²⁾-: negative, ±: rare, +: mild, ++: moderate, +++: severe.

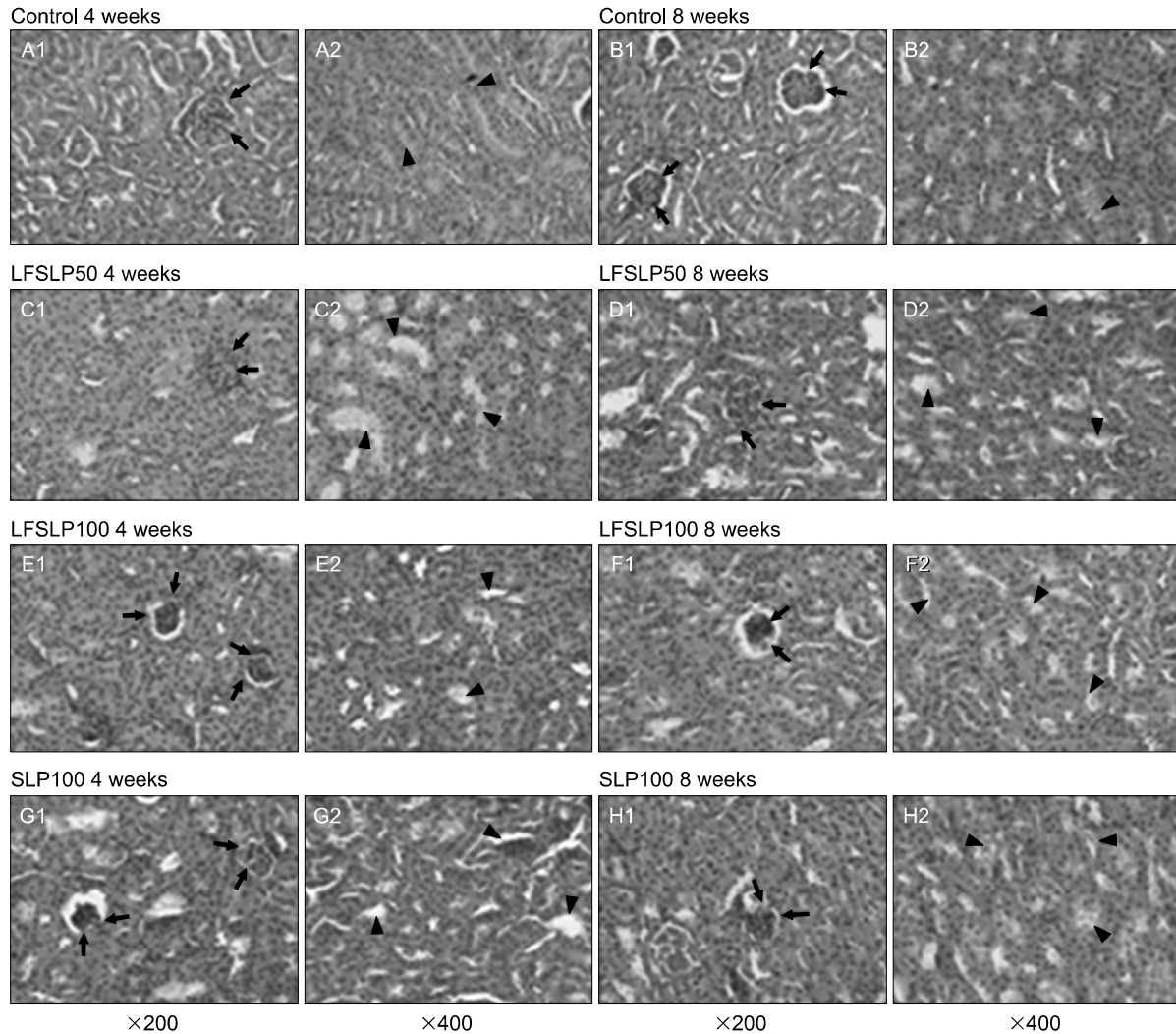


Fig. 2. Photomicroscope of mouse kidney after feeding of squid liver addition feed. A, C, E, G: 4 weeks. B, D, F, H: 8 weeks. B: glomerulus (arrows) and convoluted ducts (arrow heads). The histological structure of glomerulus and convoluted ducts were well preserved. C: The glomerulus was much shrinkaged and the convoluted ducts were greatly dilated. D: There appeared hemorrhage between the glomerulus and ducts were greatly dilated. E: The glomerulus were greatly shrinkage and sclerosed, and the convoluted ducts were remarkably distorted. F: A glomerulus was almost destroyed by hyaline necrosis and the surrounding ductal structure was dilated and degenerative. G: A glomerulus was relatively well preserved, while another glomerulus was almost destroyed. And the convoluted ducts were usually dilated but relatively well preserved. H: A glomerulus was lonely degenerative by hyaline charge, and the convoluted ducts were greatly dilated with the detachment of ductal cell.

mium concentration in the human body can be increased by ingestion of whole squid including internal organs, and that tissues can be damaged by increased cadmium concentration. More specific and systematic studies are deemed necessary.

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REFERENCES

1. IARC. 1993. *Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry*. International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France. Vol 58, p 41.
2. Nogawa K, Suwazono Y. 2011. Itai-Itai disease. In *Encyclopedia of Environmental Health*. Elsevier, Burlington, VT, USA. p 308-314.
3. Chan HM, Cherian MG. 1992. Protective roles of metallothionein and glutathione in hepatotoxicity of cadmium. *Toxicol* 72: 281-290.
4. Kagi JHR. 1993. Biological roles and medical implications. In *Metallothionein III*. Suzuki KY, Imura N, Kimura M, eds. Birkhäuser, Basel, Germany. p 29-55.
5. Brozoska MM, Moniuszko-Jakoniuk J. 2001. Interactions between cadmium and zinc in the organism. *Food Chem Toxicol* 39: 967-980.
6. Oteiza PI, Adonaylo VN, Keen CL. 1999. Cadmium-induced testes oxidative damage in rats can be influenced by dietary zinc intake. *Toxicol* 137: 13-22.
7. Kagi JH, Kojima Y. 1987. Chemistry and biochemistry of metallothionein. *Experientia Suppl* 52: 25-62.

8. Piscator M. 1962. Proteinuria in chronic cadmium poisoning. *Arch Environ Health* 12: 345-359.
9. Goyer RA, Tsuchiya R, Leonard DL, Kahy OH. 1972. Aminoaciduria in Japanese workers in the lead and cadmium industries. *Am J Clin Pathol* 57: 635-642.
10. Ramirez DC, Gimenez MS. 2002. Lipid modification in mouse peritoneal macrophages after chronic cadmium exposure. *Toxicol* 172: 1-12.
11. Anderson HR, Anderson O. 1988. Effect of cadmium on hepatic lipid peroxidation in mice. *Pharmacol Toxicol* 63: 173-177.
12. Basavaraju SR, Jones TD. 1998. Atherosclerotic risks from chemicals: part I. Toxicological observations and mechanisms of atherosclerosis. *Arch Environ Contam Toxicol* 35: 152-164.
13. Liu J, Liu Y, Sultans SH, Klassen CD. 1999. Metallothionein-null mice are highly susceptible to the hematotoxic and immunotoxic effects of chronic CdCl₂ exposure. *Toxicol Appl Pharmacol* 159: 98-108.
14. Buchet JP, Lauwerys R, Roles H, Bernard A, Bruaux P, Claeys F, Ducoffre G, DePlaen P, Staessen J, Amery A, Lijnen P, Thijs L, Rondia D, Sartor F, Saint Remy A, Nick L. 1990. Renal effects of cadmium body burden of the general population. *Lancet* 336: 699-702.
15. Jarup L, Hellstrom L, Alfven T, Carlsson MD, Grubb A, Persson B, Pettersson C, Spang G, Schutz A, Elinder CG. 2000. Low level exposure to cadmium and early kidney damage: the OSCAR study. *Occup Environ Med* 57: 668-672.
16. Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M. 1998. Health effects of cadmium exposure a review of the literature and a risk estimate. *Scand J Work Environ Health* 24: 1-51.
17. WHO. 1992. Environmental Health Criteria 134: Cadmium. Geneva International Program on Chemical Safety.
18. Ando M, Matsui S, Jinno H, Takeda M. 1989. Urinary excretion of cyclic AMP in cadmium intoxicated rats. *J Toxicol Environ Health* 27: 307-315.
19. Jarup L. 2003. Hazards of heavy metal contamination. *Br Med Bull* 68: 167-182.
20. Wang G, Su MY, Chen YH, Lin FF, Luo D, Gao SF. 2006. Transfer characteristics of cadmium and lead from soil to the edible parts of six vegetable species in southeastern China. *Environ Pollut* 144: 127-135.
21. Arnot JA, Mackay D, Bonnell M. 2008. Estimating metabolic biotransformation rates in fish from laboratory data. *Environ Toxicol Chem* 27: 341-351.
22. Jun JY, Xu XM, Jeong IH. 2007. Heavy metal contents of fish collected from the Korean coast of the east sea. *J Korean Fish Soc* 40: 362-366.
23. Sung DW, Lee YW. 1993. A study on the content of heavy metals of marine fish in Korean coastal water. *Korean J Food Hyg* 8: 231-240.
24. Jeoung IG, Ha KS, Choi JD. 2004. Heavy metals in fish and shellfish at the coastal area of Tongyeong, Korea. *J Ins Marine Industry* 17: 39-46.
25. Cho YG, Kim GB. 2007. Bioaccumulation of Pb and Cd in blue mussel (*Mytilus edulis*) and olive flounder (*Paralichthys olivaceus*) exposed to rearing media. *J Korean Soc Marine Environ Eng* 10: 21-28.
26. Bolton JL, Stehr CM, Boyd DT, Burrows DG, Tkalin AV, Lishavskaya TS. 2004. Organic and trace metal contaminants in sediments and English sole tissue from Vancouver Harbour, Canada. *Mar Environ Res* 57: 19-36.
27. Mai K, Li H, Ai Q, Duan Q, Xu W, Zhang C, Zhang L, Tan B, Liufu Z. 2006. Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of Japanese seabass, *Lateolabrax japonicus* (Cuvier 1828). *Aquac Res* 37: 1063-1069.
28. Park YB. 2010. Characteristics of angiotensin converting enzyme inhibitory peptides from salt-fermented squid liver sauce. *J Korean Soc Food Sci Nutr* 39: 1654-1659.
29. Cherian MG, Goyer RA. 1978. Metallothioneins and their role in the metabolism and toxicity of metals. *Life Sci* 23: 1-9.
30. Tanaka T, Hayashi Y, Ishizawa M. 1983. Subcellular distribution and binding of heavy metals in the untreated liver of the squid: comparison with data from the livers of cadmium and silver-exposed rats. *Experientia* 39: 746-748.
31. Li H, Mai K, Qinghui A, Chunxiao Z, Lu Z. 2009. Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of large yellow croaker, *Pseudosciaena crocea* R. *Front Agric China* 3: 78-83.
32. Kim SU, Hwang YO, Park AS, Park YA, Ham HJ, Choi SM, Kim JH. 2011. Contents of heavy metals (Hg, Pb, Cd, Cu) and risk assessment in commercial cephalopods. *J Korean Soc Food Sci Nutr* 40: 606-612.
33. AOAC. 2000. *Official methods of analysis*. International 17th ed. Association of Official Analytical Chemist, Horowitz, ML, USA. p 12-20.
34. Choi SI, Lee JH, Lee SR. 1994. Effect of green tea beverage for the removal of cadmium and lead by animal experiments. *Korean J Food Sci Technol* 26: 745-749.
35. Bremner I. 1987. Cadmium toxicity: Nutritional influence and the role of metallothionein. *World Rev Nutr Diet* 32: 165-197.
36. Dunnick JK. 1995. NTP technical report on toxicity studies of cadmium oxide (CAS No. 1306-19-0) administered by inhalation to F344/N rats and B6C3F1s mice. *NIH Publication* 95: 1-106.
37. Lee IS. 2007. Protective effects of Kamdootang on accumulation of cadmium in rats. *MS Thesis*. Chungnam National University, Daejeon, Korea. p 15-31.
38. Nechay BR, Saunders JP. 1977. Inhibition of renal adenosine triphosphatase by cadmium. *J Pharmacol Exp* 200: 623-629.
39. Klassen CD, Liu J, Choudhuri S. 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. *Annu Rev Pharmacol Toxicol* 39: 267-294.
40. Ellis KJ, Cohn SH, Smith TJ. 1985. Cadmium inhalation exposure estimates: Their significance with respect to kidney and liver cadmium burden. *J Toxicol Environ Health* 15: 173-187.
41. Roh JH, Han CK, Seong KS, Lee NH. 2005. Effects of pork on cadmium detoxification in rats. *Korean J Food Sci Ani Resour* 25: 373-382.
42. Morita S. 1984. Defense mechanisms against cadmium toxicity: I. A biochemical and histological study of the effects of pretreatment with cadmium on the acute oral toxicity of cadmium in mice. *Jpn J Pharmacol* 35: 129-141.
43. Kudo N, Yamashina S, Waku K. 1986. Protection against cadmium toxicity by zinc: Decrease in the Cd-high molecular weight protein fraction in rat liver and kidney on Zn pretreatment. *Toxicol* 40: 267-277.
44. Rhee SJ, Huang PC. 1989. Metallothionein accumulation in CHO cd^r cells in response to lead treatment. *Chem Biol Interact* 72: 347-361.
45. Friedman PA, Gesek FA. 1994. Cadmium uptake by kidney distal convoluted tubule cells. *Toxicol Appl Pharmacol* 128: 257-263.
46. Coleman JE, Vallee BL. 1961. Metallo carboxy peptidases stability constants and enzymetic characteristics. *J Biol Chem* 236: 2244-2249.
47. Parizek J. 1957. The destructive effect of cadmium ion on testicular tissue and its prevention by zinc. *J Endocrinol* 15: 56-63.