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Evaluation of the toxicity of fluorine in Antarctic krill on soft tissues of Wistar rats

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Abstract Antarctic krill are a potential food source for humans and animals, but krill are known to contain high levels of fluorine (F). In this study, we investigated the toxicity of F in Antarctic krill using Wistar rats. There were three experimental groups: The control group were fed a basal diet, the krill treatment group were fed the same basal diet mixed with krill powder (150 mg·kg^{-1} F), and the sodium fluoride (NaF) treatment group were fed the basal diet with added NaF (150 mg·kg^{-1} F). General toxicity indicators including body weight and food intake were measured during the experiment. After three months the rats were dissected and tissue samples were collected from the liver, kidney, spleen, brain, and testis. Morphological changes in the cells of these tissues were assessed using HE staining. There were no significant differences in the body weight, the food intake, or the viscera coefficients among the three groups. In both treatment groups some pathological changes were observed in all soft tissue samples except the testis, although there were fewer and less severe pathological changes in the krill treatment group than in the NaF treatment group. The results showed that the toxicity of F in Antarctic krill was lower than for an equivalent amount of F in NaF, but it was still toxic to rats consuming large quantities of krill. The findings of this study highlight the need for further investigation into potential F toxicity if krill is to be used for human consumption.

Keywords **Antarctic krill, fluorine, animal experiment, HE staining, pathological change**

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1 Introduction

The high nutritional value of krill and its potential as a food source for animals and humans[1-4], as well as the increasing size of the fishery[5], have resulted in many recent studies focusing on Antarctic krill[6-8]. Every year, Norway, Japan, and Russia harvest Antarctic krill from the Antarctic Ocean to produce food or health care products[9]. However, Antarctic krill has not been widely used as human food because of its high fluorine (F) content. Zhang et al.[10] found F concentrations of 1 102–1 432, 3 828–4 278, and 178–285 mg·kg^{-1} dry weight (DW) in whole krill, shell, and muscle, respectively. Although F is one of the essential trace elements for humans and animals, and small amounts of F can help prevent dental caries and strengthen bones, excessive amounts have adverse effects on soft tissues including the liver, kidney, spleen, testis and brain[11-15].

Many recent studies have focused on F in Antarctic krill, but the issue of F toxicity has been controversial. Xie et al.[16] found that there was no skeletal fluorosis in the bones of penguins although F concentrations were as high as 10 000 mg·kg^{-1}. Yin et al.[17] proposed that the F in Antarctic krill may exist as a less toxic species. However, to date there have been no direct toxicological experiments to evaluate F toxicity in Antarctic krill. In the present study, the toxicity of

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F in Antarctic krill was investigated using laboratory rats, and the results provided scientific evidence for the need for further investigation of the Antarctic krill resource.

2 Materials and methods

2.1 Materials

An ion analyzer (PXSJ-226), fluoride ion selective electrodes, and reference electrodes were all obtained from the Ray Magnetic Instrument Factory in Shanghai. Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) provided all chemicals of analytical grade or better. All chemical reagents were prepared using ultra-pure water produced using a Millipore Milli-Q purification system.

2.2 Experimental animals and treatment

For this experiment, 30 newly weaned Wistar rats were obtained from the Shanghai Laboratory Animal Center Co. Ltd (Shanghai, China). The animals were randomly divided into three groups with ten rats in each group. The average body weight of the rats in each group was approximately 66.4 g. Rats in the control group were fed with a basal diet obtained from the Shuangshi Laboratory Animal Feed Science Co. Ltd. (Suzhou, China), and the F concentration in the feed was 30.3±1.0 mg·kg^{-1} DW. Rats in the NaF treatment group and the krill treatment group were fed with feeds prepared by mixing NaF or Antarctic krill powder with the basal feed, respectively. The final concentration of F in both diets was about 150 mg·kg^{-1}, one tenth of the LD50 for F. During the experiment, samples of the three feeds were selected randomly, and the F concentration in the feeds was monitored according to the GB/T 13083-2002 standard method[18]. The rats were kept for three months at a temperature of 23±2℃, in well-ventilated and hygienic conditions, with a 12 h dark/light cycle. They had ad libitum access to food and distilled water. The weight of the food eaten was recorded every day, and the rats were weighed every week during the experiment.

2.3 Sample collection and diagnosis

After three months, the rats were euthanized using ether, and dissected. Tissue samples were collected from the liver, kidney, spleen, testis, and brain, and viscera coefficients (the ratio of the weight of the organ to the weight of the rat) were calculated. Three rats were randomly selected from each group and their fresh organs were fixed in 10% formalin solution. After dehydration, the tissue samples were rinsed, embedded in paraffin, sectioned, mounted on glass slides, deparaffinized, and stained using hematoxylin and eosin. The slides were then examined under an optical microscope.

3 Results

3.1 Concentration of F in feeds

The basal feed, freeze-dried Antarctic krill powder, and the two mixed feeds were homogenized to determine the F concentrations. The concentrations of F in the basal feed and in freeze-dried Antarctic krill powder were 30.3±1.0 mg·kg^{-1} DW and 2 415.8±3.9 mg·kg^{-1} DW, respectively. The feeds mixed with Antarctic krill powder and NaF had F concentrations of 144.3±3.2 mg·kg^{-1} DW and 146.9±8.0 mg·kg^{-1} DW, respectively, which met the target F concentration for the study of 150 mg·kg^{-1} DW. The accuracy of the determination of F concentrations was monitored using standard sample recovery, and the average standard sample recovery rate was 90%–110%.

3.2 Food intake, weights, and viscera coefficients

During the experimental period, there were no apparent abnormal symptoms in the control group or the treatment groups. Statistical analysis using one-way ANOVA in SPSS (version 19.0) revealed that there were no significant differences (α=0.05) in food intake, body weight, or viscera coefficients among the rats in the three groups (Figure 1).

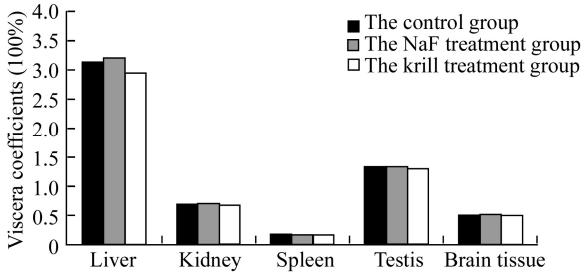

Figure 1 Viscera coefficients for organs from Wistar rats in the two treatment groups and the control group.

3.3 Histological analysis

3.3.1 Liver

The histological sections of liver from rats in the control group and two treatment groups are shown in Figure 2. The sections from rats in the control group appeared normal and healthy (Figure 2 A1, A2, A3). However, the liver sections from the Antarctic krill treatment group showed some vacuolization in the cytoplasm, hepatic sinus expansion, and loss of integrity of the epithelium lining the central veins (Figure 2 B1, B2, B3). The same abnormalities were observed in the NaF treatment group, but to a greater extent than in the krill treatment group (Figure 2 C1, C2, C3), and some of the liver cells in the NaF treatment group showed signs of necrosis.

3.3.2 Kidney

In the control group, the histoarchitecture of the kidney sections was normal (Figure 3 A1, A2, A3). A small degree of vacuolar degeneration in kidney cells and some loss of integrity in the epithelial lining of the renal tubules were observed in the krill treatment group (Figure 3 B1, B2, B3). Similar but more advanced changes were seen in the slides from the NaF treatment group (Figure 3 C1, C2, C3).

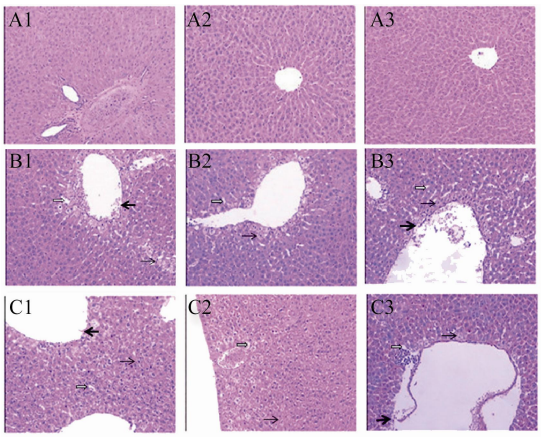

Figure 2 Pathological changes in liver sections viewed using an optical microscope (100×magnification). Control group A1, A2, A3; Krill treatment group B1, B2, B3; NaF treatment group C1, C2, C3. The thin arrows indicate vacuolar degeneration, the thick arrows indicate disruption of the epithelium lining, and the hollow arrows indicate extensive vacuolization in the cytoplasm.

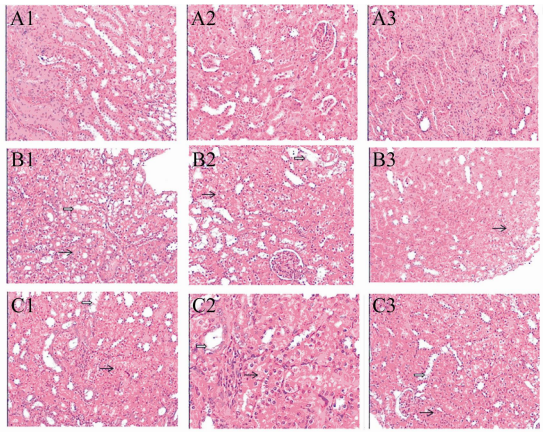

Figure 3 Pathological changes in kidney sections viewed using an optical microscope (100×magnification). Control group A1, A2, A3; Krill treatment group B1, B2, B3; NaF treatment group C1, C2, C3. The thin arrows indicate vacuolar degeneration, and the hollow arrows indicate disintegration of the renal tubular epithelium.

3.3.3 Spleen

The histopathological sections of spleen from the control group and the two treatment groups are shown in Figure 4. Compared with the control group, lymphocyte nodules increased and white pulp decreased in the two treatment groups. The increase in lymphocyte nodules and the decrease in white pulp were more pronounced in the NaF treatment group than in the krill treatment group.

3.3.4 Brain

Compared with the control group (Figure 5 A1, A2, A3), the number of neurocytes decreased and the number of spongiocytes increased in brain sections from the two treatment groups (Figure 5 B1, B2, B3 and C1, C2, C3). The sections from the NaF treatment group revealed large areas of vacuolar degeneration and there was a greater reduction in neurocyte numbers compared with the krill treatment group.

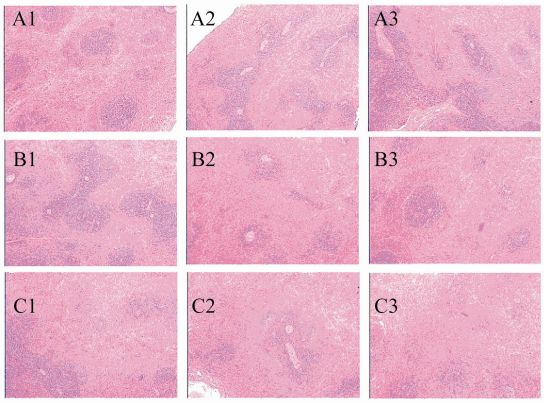

Figure 4 Pathological changes in spleen sections viewed using an optical microscope (100×magnification). Control group A1, A2, A3; Krill treatment group B1, B2, B3; NaF treatment group C1, C2, C3.

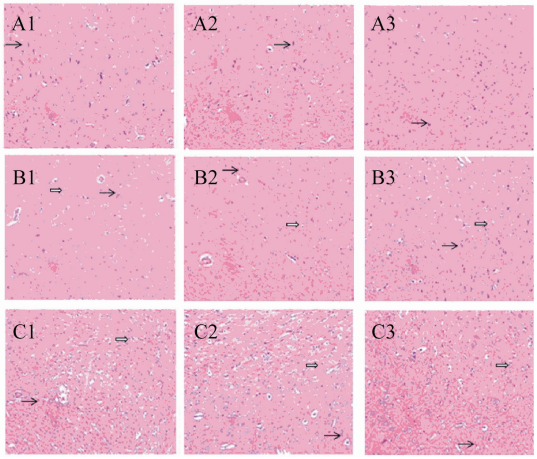

Figure 5 Pathological changes in brain sections viewed using an optical microscope (100×magnification). Control group A1, A2, A3; Krill treatment group B1, B2, B3; NaF treatment group C1, C2, C3. The thin arrows indicate neurocytes, and the hollow arrows indicate vacuolar degeneration.

3.3.5 Testis

There were no significant histopathological changes seen in the sections of testis from the three groups. The number and development of sperm cells showed no obvious pathological changes.

4 Discussion

The consumption of contaminated water is one cause of chronic F exposure in humans and animals. However, high levels of F in food provide another potentially important cause of F toxicity. In this study, Wistar rats were fed with

food mixed with Antarctic krill powder to assess the toxicity of F in Antarctic krill. The aim of the study was to provide scientific reference for exploring Antarctic krill as a potential food source for humans.

The body weight of the rats and the viscera coefficients were considered as general indices for toxicity. Shanthakumari et al.[19] found that the body weight of rats gradually decreased as the dose of F increased. However, Wei et al.[20] reported that the viscera coefficients for testis were unchanged after six months of treatment with 50 $mg \cdot L^{-1}$ of F. Sun et al.[21] found that body weight and viscera coefficients for kidney did not change significantly even after six months of treatment with F at 200 $mg \cdot kg^{-1}$. The present study showed that body weight and viscera coefficients did not differ significantly between the control group and two groups treated with 150 $mg \cdot kg^{-1}$ of F for three months, suggesting that Antarctic krill containing less than 150 $mg \cdot kg^{-1}$ of F was not toxic to Wistar rats.

Liver, kidney, and spleen are organs commonly affected by fluorosis. The liver and kidney are important for metabolism and excretion, and the spleen is the largest lymphoid organ. Many studies have shown that overexposure to F can cause morphological and functional changes in the liver, including vacuolar degeneration, disruption of the endothelial integrity of the central veins, expansion of liver sinuses[11], and local necrosis[22]. In the kidney F toxicity can cause expansion of renal tubules[12], vacuolar degeneration, glomerular atrophy and necrosis, and disruption of the integrity of the epithelium in renal tubules[11]. Fluorine toxicity caused a reduction in white pulp and an increase in red pulp in the spleens of laboratory mice[13]. In the present study, liver, kidney, and spleen sections from rats in the treatment groups showed obvious histological changes. In liver sections there was evidence of vacuolization in the cytoplasm, expansion of hepatic sinuses, and loss of integrity of the epithelium lining the central veins. Kidney sections showed vacuolar degeneration and loss of integrity in the epithelial lining of the renal tubules. A reduction in white pulp and an increase in red pulp were seen in spleen sections from the treatment groups. However, at the same dose of F, the pathological changes were less severe in the Antarctic krill treatment group than in the NaF treatment group, suggesting that F in Antarctic krill was less toxic than the equivalent amount in of F in NaF.

Fluorine can pass through the blood-brain barrier and the blood-testis barrier, and excessive F can be harmful to the nervous and reproductive systems[14-15]. According to epidemiological studies[23-24], the intelligence of children living in areas with high levels of naturally occurring F was lower than that of children in areas with low levels of F, suggesting that high F levels can also be harmful to the neonatal development of the nervous system. Zhang et al.[25] found that an increased intake of F significantly reduced the learning ability of mice in a Y-Maze Spontaneous Alternation Test. In the present study, sections of brain from the two treatment groups displayed obvious histopathological changes, including a reduction in the numbers of neurocytes, an increase in the numbers of spongiocytes, and vacuolar degeneration, indicating that F from Antarctic krill could pass through the blood-brain barrier and cause damage to brain. However, the absence of histological changes in testis sections suggests that the F in Antarctic krill could not cross the blood-testis barrier. Further studies are needed to confirm this observation.

The mechanism of injury to animal tissues caused by F is not clear, but most researchers believe the damage is caused by free radicals. Over-exposure to F can upset the balance between oxidation and the antioxidant system[26]. Fluorosis is associated with an increased level of methane dicarboxylic aldehyde (MDA) and increased lipid peroxidation (LPO), while levels of glutathione peroxidase (GSH-Px), super oxygen dehydrogenases (SOD), and other antioxidant enzymes decrease[26]. However, the findings of the present study indicated that equivalent levels of F in Antarctic krill were less toxic than F in NaF. This could be related to the high levels of selenium (Se, 2.48–4.15 $mg \cdot kg^{-1}$) and zinc (Zn, 153.9±5.7 $mg \cdot kg^{-1}$) in Antarctic krill[27]. Selenium has been shown to increase the activity of GSH-Px, SOD, and other antioxidant enzymes, which could eliminate peroxide and hydroxy free radicals, decreasing the toxicity of F[28]. Zinc is an important component of metalloenzyme, and it can promote the expression of metallothionein (MT). Zinc can also protect the integrity of the cell membrane, and increase antioxidant activity[29]. In the gastrointestinal system and in blood, Zn can combine with free F, and reduce its toxicity[30].

In 2000, the annual catch quota for Antarctic krill was increased to 4 million tons by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). Currently, most of the Antarctic krill catch is directly processed to produce feed for aquaculture and aquariums, and only 5.29% is deshelled[31]. In Japan, 43% of the Antarctic krill catch is deshelled for use as food or processed to extract oil[32]. The results of the present study show that the toxicity of F in Antarctic krill should not be ignored, although F in krill is less toxic than equivalent levels in NaF. In 2012, the recommended daily intake of F for adults was less than 4 $mg \cdot d^{-1}$. Therefore, when krill is used as a food for humans, the intake should be controlled to minimize the risk of fluorosis.

5 Conclusions

Wistar rats were fed a basal diet or a diet containing F at a concentration of 150 $mg \cdot kg^{-1}$, either contained in Antarctic krill powder or as NaF. After three months the body weight of the rats, viscera coefficients, and histopathological features in sections of testis in the treatment groups showed no significant differences compared with the control group. However, significant histopathological changes were observed in liver, kidney, spleen, and brain sections. Although the F in Antarctic krill was less toxic than an equivalent amount of F in NaF, the toxicity of F must be taken into

account if Antarctic krill is to be used as a food source for humans.

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