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Toxic Effects of Cadmium on Crabs and Shrimps

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1. Introduction

Cadmium (Cd) is one of the most toxic heavy metals for humans; the main source of nonoccupational exposure to Cd includes smoking, air, and food and water contaminated by Cd (Nagata et al., 2005). In addition, herbal medicine is another source of Cd. The World Health Organization (WHO) estimates that 4 billion people or 80 percent of the world population, presently use herbal medicine (Naithani et al., 2010). Several articles have reported of adverse effects of these herbal preparations due to the presence of high level of heavy metals such as Cd, lead, chromium, nickel, etc. (Naithani et al., 2010). Saeed et al. (2010) investigated twenty five herbal products. The results revealed that the concentrations of some heavy metals, including Cd, were far greater than the permissible limits proposed by the International Regulatory Authorities for herbal drugs. Acute or chronic exposure of Cd causes respiratory distress, lung, breast and endometrial cancers, cardiovascular disorders and endocrine dysfunction (Åkesson et al., 2008; Chang et al., 2009; Messner et al., 2009; Nagata et al., 2005; Naithani et al., 2010; Navas-Acien et al., 2004).

In addition, Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources (Ivanina et al., 2008, 2010; Sokolova et al., 2004). It can be accumulated in aquatic animals (e.g. crabs, shrimps, oysters and mussels) after entering through different way such as respiratory tract, digestive tract, surface penetration etc. (Dailianis & Kaloyianni, 2004; Dailianis et al., 2009; Ivanina et al., 2008, 2010; Li et al., 2008b; Sokolova, 2004; Sokolova et al., 2004; Wang L. et al., 2001, 2002a,b, 2008; Wang Q. et al., 2003; Zhao et al., 1995). It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, these animals exposed to Cd might threaten human health.

1.1 Cd accumulation and distribution in crabs and shrimps

Cd in waters can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion (Rainbow & White, 1989; van Hatton et al., 1989). And we can get valuable information for evaluating the level of Cd pollution in waters and sediments by assaying Cd concentration in crabs and shrimps.

1.1.1 The difference of Cd accumulation and distribution in different tissues

Experiments have confirmed that Cd absorption and accumulation by crabs and shrimps had obvious differences among the various body segments. Accumulated Cd was distributed to all organs with the highest proportions of body content being found in the exoskeleton, gills, hepatopancreas, and so on.

The first organ in which Cd accumulates is the exoskeleton. Cd has similar chemical properties to calcium (Ca), the main component of the exoskeleton, such as the same charge number, the similar ion diameter and electronic number. Therefore, the Cd in waters can replace the Ca entering the body via exoskeletons (Jennings & Rainbow, 1979). The gill is a respiratory organ for crabs or shrimps. It plays an important role in the absorption and transport of heavy metals (Silvestre et al., 2004; Silvestre et al., 2005a) and is the target organ of Cd in waters. The hepatopancreas are detoxicating organs in crabs and shrimps which can change the toxic heavy metal into non-toxic compounds and reduce the toxicity of the heavy metal in the body. Thus the Cd concentration is higher in the hepatopancreas.

1.1.2 Factors influencing Cd accumulation and distribution

Due to the different treatment methods, the accumulation and distribution of Cd are different in different organs. When *Carcinus maenas* was exposed to seawater at Cd dose of 10 ppm, the midgut gland contained absorbed 10% of the total Cd, while the exoskeleton contained. When Cd was absorbed from a food source, the midgut gland contained 16.9% of the absorbed Cd whereas the exoskeleton contained only 22.2% (Jennings & Rainbow, 1979). It can be inferred that in bath experiments, the exoskeleton was in direct contact with Cd and accumulated the most Cd; in feeding regimes, the exoskeleton had the lower proportion accumulation. This result was consistent with those in unpolluted areas (Bjerregaard & Depledge, 2002; Davies et al., 1981; Falconer et al., 1986). American lobster, *Homarus americanus* were fed with three kinds of diets containing Cd (based on crab muscle; based on crab muscle adding ascorbic acid; based on casein for protein source). The result showed that Cd accumulated in hepatopancreas was higher in the lobsters fed with the first two diets than in ones fed with casein (Chou et al., 1987). In addition, *Sinopotamon yangtsekiense* had the highest concentration of Cd in the exoskeleton after acute exposure (Silvestre et al, 2005b), while *Eriocheir sinensis* had highest Cd concentration in the gills after chronic exposure for 30 d adding the acute exposure for 3 d (Wang Q. et al., 2003).

The environment can also affect the absorption and accumulation of Cd. An increase in the Cd concentration in the environment will result in increased Cd accumulation. Namely, the accumulation of Cd has obvious dose-dependent relationship (Wang L. et al., 2001; Wang Q. et al., 2003).

Ca in the water environment will prevent the absorption and accumulation of Cd because it can form the competitive relationship with Cd. Therefore, accumulated Cd in the body will be less whenever the Ca concentration in water increases (Wright, 1977).

Beltrame et al. (2010) reported that sex, habitat, and seasonality could influence heavy-metal concentrations in the burrowing crab (*Neohelice granulata*) from a coastal lagoon in Argentina.

The accumulation of Cd in all tissues were markedly higher in postmoult (A1–2 and B1–2) compared to intermoult (C1, C3 and C4) and premoult (D0–3) in male shore crab *C. maenas* (Nørum et al., 2005). This shows that accumulation and distribution of Cd in crabs and shrimps can also be related to the status of the organisms.

1.2 The influence of Cd on the enzyme activity in crabs and shrimps

Small amounts of Cd can be detoxicified into non-toxic substance by metallothionein in the organism (van Hatton et al., 1989). Excessive Cd will damage the body, however, as it will combine with protein molecules having sulphur, hydroxyl and amino group, and restrain some enzyme system activity. In addition, because the affinity of Cd with sulfhydryl groups is stronger than zinc (Zn), it can replace the enzyme-bond Zn and cause the enzyme to lose its function (Müller & Ohnesorge, 1982).

1.2.1 The influence of Cd on antioxidant enzymes system in crabs and shrimps

One of the mechanisms for Cd toxicity to animals is the oxidative damage. On one hand, Cd can cause the body to produce excessive active oxygen. On other hand, it can change the expression and vitality of antioxidant enzymes. Antioxidant enzymes mainly include the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione enzyme turn sulfur (GST), etc. They can effectively scavenge active oxygen in the body and avoid oxidative damage to the body (Wang L. et al., 2007). Numerous studies have been published on the influence of Cd on antioxidant enzymes in terrestrial creatures, while reports about shrimps and crabs are rare. In one study the Cd concentration was 0.025 mg/L and 0.05 mg/L in water, and SOD, CAT and GPX activities in *Charybdis japonica* could be stimulated after 0.5 d, and then reduced during the experimental period (Pan & Zhang, 2006). When crabs (*S. yangtsekiense*) were exposed to the reagent with a dose range of 7.25-116.00 mg/L for 24, 48, 72 and 96 h, the activities of SOD, CAT and GPX increased initially and decreased subsequently (Li et al. 2008; Wang L. et al., 2008; Yan et al., 2007). After Immersing the juvenile crab *E. sinensis* in 2.0 mg/L water, the activities of SOD, CAT and GPX in hepatopancreas were all initially decreased, and then recovered to some degree during the duration of the study (Liu et al., 2003). This showed that low concentration of Cd stimulated antioxidant enzymes activity while high concentration inhibited antioxidant enzymes activity.

1.2.2 The influence of Cd on metabolic enzymes in crabs and shrimps

Glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminease (GOT) are the important aminotransferase in the protein metabolism. Low concentration of Cd stimulated the activity of GPT and GOT in *Scylla serrata* while high Cd concentrations showed apparent inhibition. The results showed the obvious dose-effect relations (Tang et al., 2000). Effects of Cd on GOT and GPT activity are also tissue-specific. GPT and GOT activity decreased significantly in the heart, gills and hepatopancreas after *Macrobrachium rosenbergii* was poisoned by Cd, but increased in the green glands. This may be because green gland is excretory organ with strong detoxicification (Zhao et al., 1995). GPT activity in serum of *E. sinensis* increased with increasing Cd concentration after poisoning. That might be because tissues were damaged and the enzyme released into serum (Lu et al., 1989).

Lactic dehydrogenase (LDH) plays an important role in the carbohydrate metabolism. The crab *Uca pugilator* were immersed in 2.0 mg/L water for 24 h, 48 h, LDH activity reduced in hepatopancreas and that is opposite in the abdominal muscles (Devi et al.,1994).

Alkaline phosphatase is a kind of low-specific phosphomonoesterase which plays an important role in nucleinic acid, protein and lipid metabolic. The influence of Cd on enzymatic activity in *S. serrata* also exhibited dose-effect relationship that was similar to that observed above (Tang et al., 2000).

1.2.3 The influence of Cd on Na^+-K^+-ATPase in crabs and shrimps

Na^+-K^+-ATPase are ubiquitous in organism. It is the most important enzyme during the process of osmotic regulation and ion exchange in crustaceans. It is involved in cellular transmembrane transport of Na^+ and K^+ and sustains the ion gradient and membrane potential inside and outside cells. Cd can be directly combined with ATPase to execute function. In low concentration, the change rule of the enzyme is more complicated. In high concentration, enzyme activity will be loss. When *S. serrata* was exposed to 0.3 μg/L Cd, Na^+-K^+-ATPase activity in hepatopancreas and gills showed temporary activation in 10 d, followed by inhibition at longer exposure times (Daksna, 1988). Crabs *E. sinensis* were submitted to acute (0.5 mg/L for 1, 2 or 3 d), chronic (10 or 50 μg/L for 30 d) or chronic (immediately followed by acute) exposure. After 3 d of acute exposure, the respiratory anterior gill ultrastructure and Na^+/K^+-ATPase activities were significantly impaired. In contrast to acute exposure, chronic exposure did not induce any observable effects. Moreover, crabs submitted to chronic immediately followed by acute exposure showed normal hyper-osmoregulatory capacity with no change in gill Na^+/K^+-ATPase activity. These results demonstrated that a chronic Cd exposure could induce acclimation mechanisms related to osmoregulation in this euryhaline decapod crustacean (Silvestre et al., 2005a).

1.3 The influence of Cd on the ultrastructure of crabs and shrimps

Studies concerning the influence of Cd on the ultrastructure of crabs and shrimps have appeared in the past few years. The published studies have focused on the destruction of membrane systems and morphologic changes of cells. Cd can accelerate cellar lipid peroxidation and cause the accumulation of lipid peroxides. These free radicals and their reaction products, peroxides, can often cause various biological macromolecules, including DNA, to change structures and properties through chemical reactions, such as hydrogen abstraction, oxidation sulfhydryl and carbon chain destruction. Cd can also decompose the unsaturated fatty acid into malondialdehyde (MAD) by peroxiding and cause biological macromolecules to crosslink into abnormal macromolecules which degrade membrane structure and alter the membrane permeability (Shukla et al., 1989).

After the crabs *E. sinensis* were exposed to Cd, many changes appeared in the R-cell in hepatopancreas, such as organells decrease, mitochondria damage, endoplasmic reticulum expansion, and thinning of the cytoplasm matrix (Wang L. et al., 2001). Cd can partly disintegrate the mitochondrial cristae of neurosecretory cells in *E. sinensis* (Li et al., 2008). Whenever injected into the crab *S. yangtsekiense*, Cd resulted in damage to the organells with membrane structure, and the mitochondria was damaged first, which suggested that mitochondria was a sensitive organelle to Cd that could be used to show the amount of damage caused by Cd (Wang L. et al., 2002a,b). Cd could cause the morpha of female ovaries to change markedly in *S. henanese*, such as the increase of fragmentations and adherences. The oval prosenchyma of egg cells became significantly larger. Egg membrane were much thicker. At the same time, the particulate protuterances on the surface of eggs cells decreased. The boundary between egg cells became more and more unclear. These morphological changes may be a form of self-preservation in eggs which can reduce the damage through self-adjustment, whereas with the increase of Cd dosage, the irreconcilable morpha damage would become much larger (Meng, 2006).

1.4 The influence of Cd on ovarian development in crabs and shrimps

1.4.1 The influence of Cd on ovarian development

Studies regarding the effects of Cd on ovarian development in crabs and shrimps have been conducted since the 1990s. The majority of experiments showed that Cd inhibited ovarian growth, reduced hatch rates of the fertilized eggs and led to embryonic deformity.

Reddy et al. (1997) found Cd could inhibit 5-HT-induced ovarian maturation in the red swamp crayfish, *Procambarus clarkia*. Lee et al. (1996) documented that Cd deformed eyespots, reduced hatching success, and inhibited growth of oocytes of *Callinectes sapidus*. Naqvi et al. (1993) reported that *P. clarkia* treated with Cd hatched 48 eggs with a hatching rate of only 17%. In comparison, untreated individuals hatched 203 eggs with a hatching rate of 95%. Some results were not consistent with the above observations. For exemple, red swamp crayfish fed with duckweeds containing Cd for 14 d had significantly bigger ovary index and total fat content than the respective groups fed with unpolluted duckweeds (Devi et al., 1996).

1.4.2 The mechanism for the iInfluence of Cd on ovarian development

There are different views regarding the mechanism of how Cd affects ovary development. Reddy et al. (1997) suggested that the inhibition of Cd on ovarian maturation in P. clarkii was due to the metal inhibiting 5- Hydroxytryptamine (5-HT)-stimulated gonad-stimulating hormone (GSH) release, and preventing the ovaries from responding to this hormone. Rodriguez et al. (2000) studied the effect of Cd on oocyte growth of the fiddler crab *U. pugilator* during the slow vitellogenesis phase of ovarian maturation of this crab. Only when eyestalks were present (intact crabs in vivo experiments or in the incubation media in vitro experiments) , the oocyte growth was inhibited by Cd. So the authors suggested that Cd could act to increase the secretion of the gonad-inhibiting hormone (GIH) from the sinus gland in the eyestalks, and then GIH inhibited the oocytes directly or indirectly. On the contrary, no significant (P >0.05) change of the gonadosomatic index was observed with intact female crab *Chasmagnathus granulata* exposed to 0.5 mg/L Cd, whereas eyestalk-ablated exposed females showed significantly (P<0.05) lower gonadosomatic index values than their respective controls. This indicated that Cd interfered with extra-eyestalk hormones. The experimental results shows a possible interference of Cd with the transduction pathway of methyl farnesoate or 17-hydroxyprogesterone.On the other hand, Cd has an inhibitory effect on GIH secretion from the eyestalk.

2. The reproductive toxicity of the Cd to the Chinese crab *E. sinensi*s

The ovarian growth in the Chinese crab is a process with oogonium multiplication, oocyte enlargement and yolk protein synthesis. It is the basis for the development of follow-up individual and is regulated by their own complex endocrine system. In the condition of internal hormone imbalance or external hormonal stimulation, the process of yolk synthesis will be affected. The gonad-inhibiting hormone (GIH), gonad-stimulating hormone (GSH), methyl ester (MF), progesterone and estradiol in the body can adjust ovarian development together. The existence of heavy metals in water as environment endocrine disruptors will cause certain damage for the shrimps and crabs. In this section, ovarian index (OI), oocyte diameter and yolk protein accumulation, GIH, progesterone and estradiol levels in hemolymph were meassured and ovarian ultrastructural changes were observed after *E. sinensis* was treated with Cd. The influence of Cd on ovarian development and its

mechanism are discussed. The discussion provides information regarding the effects of environmental endocrinal disrupter such as heavy metal on the health of animals and human.

Juvenile female crabs for this experiment were obtained from Baiyangdian Lake, Hebei province, China. In the laboratory, the crabs were maintained for at least 2 weeks prior to the start of an experiment in fresh water, prepared to have a temperature of 25 ^{o}C and were fed uncooked potatoes daily. During the experiment, crabs were distributed into 3 groups of 15 crabs per group. The first group served as the control. Other animals were exposed to Cd concentrations of 0.25 and 0.50 mg/L (Cd added as $CdCl_2 \bullet 2.5H_2O$). The duration of exposure was 12 d. After exposure, the OI, oocyte diameter, yolk protein, GIH, progesterone and estradiol levels in hemolymph were meassured and ovarian ultrastructural changes were observed.

The results showed crabs exposed to 0.50 mg Cd/L had significiantly smaller ovarian index than controls. The difference between crabs exposed to 0.25 mg/L and controls were not significiant. The influence of Cd on OI presented the dose-effect relations.

The influence of Cd on oocyte diameter had the similar regularity.

	OI (%)	oocyte diameter (μm)
controls	0.503±0.162	50.729±2.254
0.25mg/L Cd	0.293±0.149	45.792±1.599
0.50mg/L Cd	0.241±0.026*	40.771±2.097*

* Significant difference to control group ($P < 0.05$)

Table 1. The effect of Cd on ovarian index and oocyte diameter

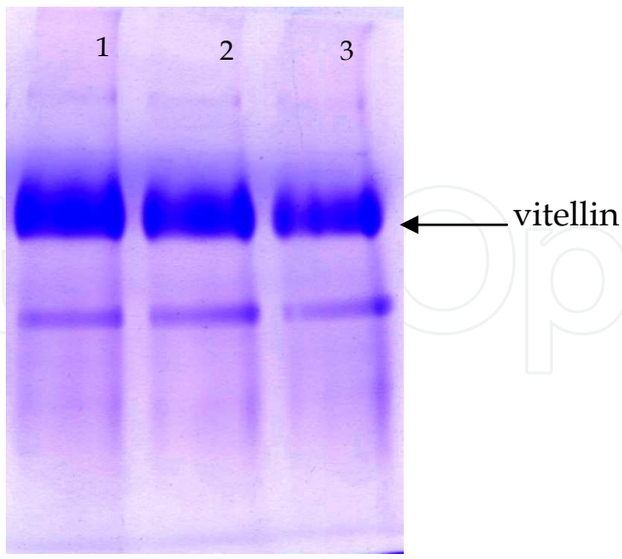

Fig. 1. Native PAGE maps of vitellin

1. map of native PAGE with CBB staining of ovary crude extracts in controls; 2. map of native PAGE with CBB staining of ovary crude extracts exposed to 0.25 mg/L Cd; and 3. map of native PAGE with CBB staining of ovary crude extracts exposed to 0.50 mg/L Cd

Through native PAGE with ovarian coarse extraction fluid of different groups and gray scan with Bandscan 5.0, the control group had the highest vitellin level, the group in 0.25 mg/L Cd had the second highest level, and the group in 0. 50 mg/L Cd had the lowest level. The percentage of ovary total protein charged for livetin had the above regularity. These results documented the accumulation of vitellin and the percentage of ovary total protein charged for livetin decreased with the increase of Cd concentration.

Semi-quantitative analysis of GIH in hemolymph was achieved by enzyme-linked immune sorbent assay (ELISA) method. GIH relative concentration in the crabs exposed to Cd is higher than those in controls. The relative concentration of GIH increased with increasing Cd concentration (see Table 2). These results suggest that Cd might stimulate secretion of GIH.

Progesterone and estradiol levels in hemolymphand measured by radioimmunoassay (RIA) are given in table 2. Compared with control group, groups exposed to Cd had higher progesterone level and lower estradiol level. There were no significant difference between 0.25 mg/L Cd group and control group while there were significant difference between 0.50 mg/L Cd group and control group.

	GIH absorbance	Progesterone level (ng/mL)	Estradiol level (pg/mL)
controls	0.138±0.019	0.91±0.16	180.28±24.01
0.25mg/L Cd	0.168±0.014	1.16±0.17	157.45±24.53
0.50mg/L Cd	0.432±0.021	1.49±0.32*	150.65±26.57*

* Significant difference to control group ($P < 0.05$)

Table 2. GIH absorbance, estradiol and progesterone levels in the hemolymph of each treatment

Observed by transmission electron microscope, normal nuclear appeared round and nuclear matrix was uniformly distributed. The surface of inner nuclear membrane was smooth and perinuclear cisternae was relatively small (Fig.2). In 0.25 mg/L group, outer nuclear membrane appeared folding deformation and swelled slightly. Nuclear material concentrated slightly and the electronic density was not uniform. Perinuclear cisternae became larger (Fig.3). In 0.50 mg/L group, the most notable changes were observed in nuclei. Outer nuclear membrane showed obvious folding deformation and the nuclear material more highly concentrated. The inner nuclear membrane nearly disappeared. Perinuclear cisternae became larger (Fig.4).

In the primary vitellogenesis phase, normal oocyte nuclei exhibit regular roundness. Nuclear membrane looked like moniliform and the moniliform particles distribute uniformly (Fig.5). Most of the vesicles of the endoplamic reticulum in the cytoplasm also showed regular roundness which is attached on by ribosomes (Fig.6). After being exposed to 0.50 mg/L Cd, nuclear membrane were crimpy and distorted, and moniliform particles of nuclear membrane appeared pile and damage (Fig.7). The vesicles of the endoplamic reticulum became swelled and dissolved. Electronic density in vesicles decreased, even vacuolization. Ribosomes on the endoplamic reticulum gradually fell off (Fig.8).

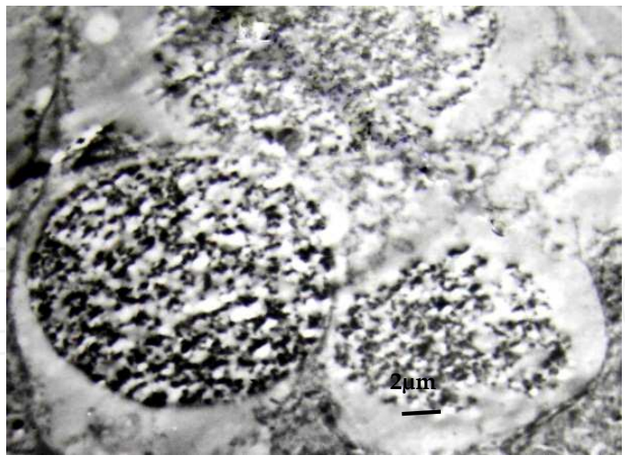

Fig. 2. Normal nuclear of reproductiving

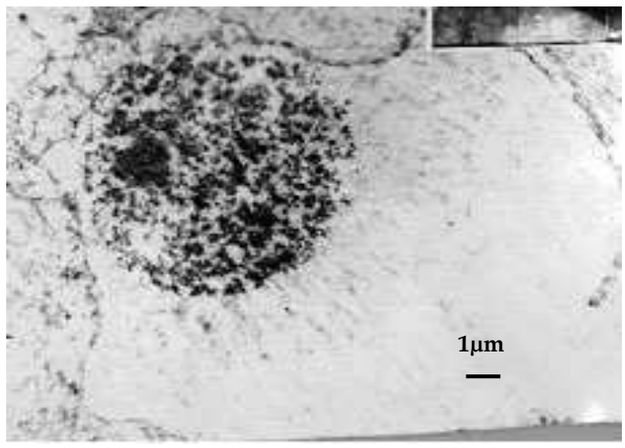

Fig. 3. Nuclear of reproductiving oogonia oogonia exposed to 0.25 mg/L Cd

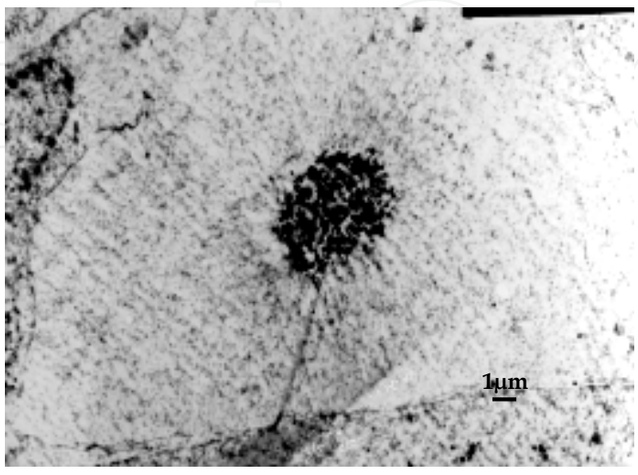

Fig. 4. Nuclear of reproductiving oogonia

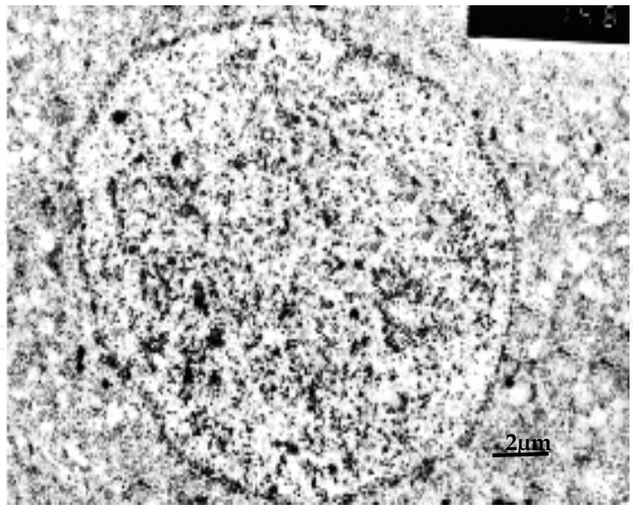

Fig. 5. Normal nuclear of the oocytes in exposed to 0.50 mg/L Cd primary vitellogenesis phase

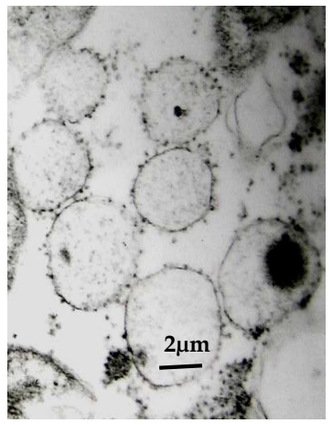

Fig. 6. Normal endoplamic reticulum vesicle

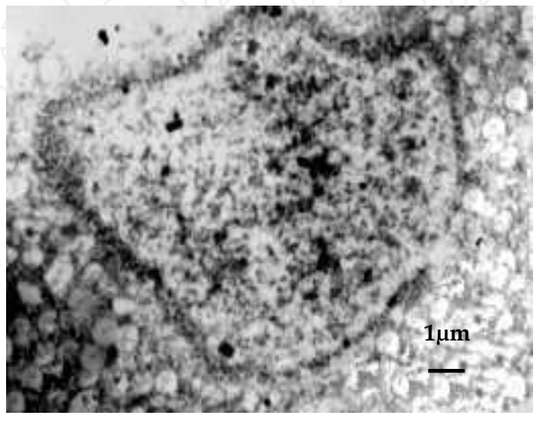

Fig. 7. Nuclear of the oocytes in primary vitellogenesis phase exposed to 0.50 mg/L Cd

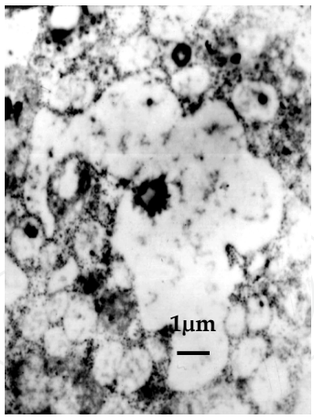

Fig. 8. Endoplamic reticulum vesicles exposed to 0.50 mg/L Cd

3. Effects of Cd on proliferation of spermatogenic cells from *M. nipponense in vitro*

The toxic effects of Cd on male reproductive system is obvious, it can significantly damage the testicles and the testicular parenchyma cells, leading to pathological testicular alterations and morphological abnormalities of spermatozoa, directly affected the reproductive capacity (Luo et al., 1993; Mohan et al., 1992; Saygi et al., 1991).

Culture of spermatogenic cells *in vitro* is important in development. Establishing the model of culture of spermatogenic cells in vitro is helpful for studying the regulate mechanism of spermatogenesis. In addition, the environment factor and presence of a poisonous substance can have grave effect on idioplasm, and thus restrict the development of marine species. Studying the effects of poisonous substances on reproduction and differentiation of spermatogenic cells has theoretical significance on clarifying the mechanism of poisonous substance, and has practical significance on idioplasm profect and health breed aquatics.

Juvenile male *M. nipponense* (20 to 25 mm body length) for the experiments were purchased from Baiyangdian Lake, Hebei Province, China. Spermatogenic cells of *M. nipponense* were isolated and sublimated with the method of trypsinization and differential speed adherence. Cell suspensions were seeded into M199 medium (pH 7.2, supplemented with 10% fetal bovine serum (FBS), 1 g/L glucose, 0.3 g/L glutamine, 0.11 g/L sodium pyruvate, 0.01% 2-mercaptoethanol, 100 IU/mL penicillin, 100 IU/mL streptomycin, 20 μg/mL gentamicin) and kept under 5% CO_2 at 26°C for 12 h before being incubated with various concentrations of Cd (5, 50, 500, 1 000 ng/mL). Equal volumes of culture medium containing no Cd were added to the control groups. Subsequently, MTT [3-(4, 5-dimethylthia- zole-2-yl)-2,5-diphenyl-tetrazoliumbromide] assay (Mosmann, 1983) was used to evaluate the proliferation of spermatogenic cells after 0 h, 24 h, 48 h, 72 h, 96 h exposure.

MTT assay is widespread method to assess cell viability. In living cells, MTT is deoxidized by mitochondrial dehydrogenases to a blue formazan product. The results can be read on a multi-well scanning spectrophotometer (ELISA reader) and the absorption of dissolved formazan correlates with the number of alive cells (Mosmann, 1983). Cytotoxic compounds (e.g. heavy metals) are able to damage and destroy cells, and thus decrease the reduction of MTT to formazan, the absorbance value therefore will decline.

A concentration-response curve for Cd obtained with the MTT assay is shown in Fig. 9. Before 24 h, the absorbance curve of each group showed no regularity; downward trend of the curve was not obvious. 24 h later, the absorbance of groups exposed to Cd at dose of 50 ng/mL, 500 ng/mL, 1 000 ng/mL, but not 5 ng/mL, were significantly lower than those of the controls ($P < 0.01$). The cell proliferation rate was found to decrease with increasing Cd concentration, and after 24 h exposure the absorbance of each concentration was significantly different from the absorbance at the start of the experiment ($P < 0.01$). In brief, rate of cell proliferation showed negative correlation with dose and exposure time at 50 ng/mL, 500 ng/mL, 1 000 ng/mL after 24 h.

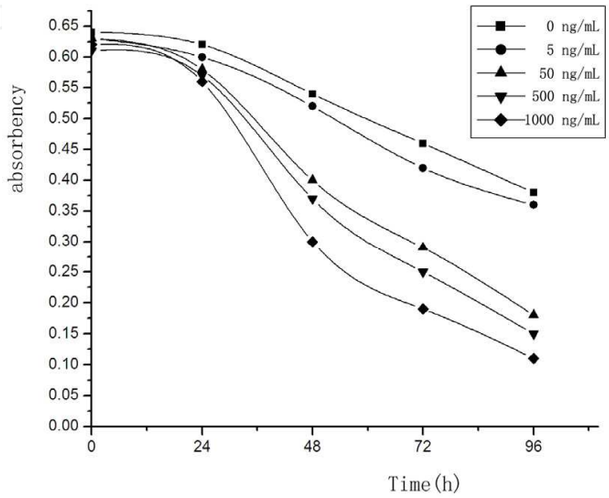

Fig. 9. Absorbency of spermatogenic cells disposed with Cd

There is growing evidence that suggests the mechanism of cytotoxicity of Cd may be mitochondrial dysfunction (Sokolova, 2004; Sokolova et al., 2004; Ivanina et al., 2010). In terrestrial plants and mammals, Cd is known as a powerful modulator of mitochondrial function, inhibiting electron transport chain, increasing generation of reactive oxygen species (Sokolova, 2004, as cited in Miccadei & Floridi, 1993 and Wallace & Starkov, 2000), and stimulating proton leak through the inner mitochondrial membrane (Sokolova, 2004, as cited in Belyaeva et al., 2001). In marine mollusks, such as oysters, Cd also affects mitochondrial function (Sokolova, 2004; Sokolova et al., 2004; Ivanina et al., 2010). These data strongly suggest that mitochondria are key intracellular targets for Cd (Sokolova, 2004). Heavy metals, such as Cd, are known to induce apoptosis and necrosis in invertebrates and vertebrates and result in increased cellular mortality (Benoff et al., 2004; Sokolova et al., 2004, as cited in Li et al., 2000 and Sung et al., 2003). In vertebrates, undergoing Cd stress, cells activate the classical intrinsic death pathway, in which mitochondria have a central role (Sokolova et al., 2004, as cited in Shih et al., 2004 and Hüttenbrenner et al., 2003). Cd exposure induces apoptosis in oyster immune cells and does so through a mitochondria/caspase-independent pathway (Sokolova et al., 2004). These results suggest that the mechanism of apoptosis induced by Cd exposure is very complex.

In our study, the results of MTT assay showed that Cd restrained the proliferation of isolated spermatogenic cells from *M. nipponense*. According to other investigations, it is due to cells apoptosis or necrosis induced by Cd exposure. The cause is unclear and further research will be needed.

4. Conclusion

As noted above, Cd exhibits biochemical and physiological toxicity for crabs and shrimps, affecting on activity of antioxidant enzymes, affecting metabolic enzymes, affecting Na+-K+-ATPase, etc. In some cases, Cd had a stimulating action at low concentration and inhibiting activity at high concentration.

Cd showed noticeable effects on the reproduction of crabs and shrimps. 1. Female crabs exposed to 0.50 mg/L Cd showed significantly ($P < 0.05$) lower the gonadal somatic index, oocyte diameter values and the ovary vitellin than controls. These proved certain concentration of Cd inhibited ovary development in *E. sinensis*. 2. Cd stimulated the secretion of GIH, increased progesterone level and decreased estradiol level in haemolymp. 3. The vesicles of the endoplamic reticulum became swelled and dissolved; ribosomes on the endoplamic reticulum gradually fell off by Cd toxicity. 4. Cd restrained the proliferation of isolated spermatogenic cells from *M. nipponense* at dose of 50, 500, 1 000 ng/mL after 24 h exposure.

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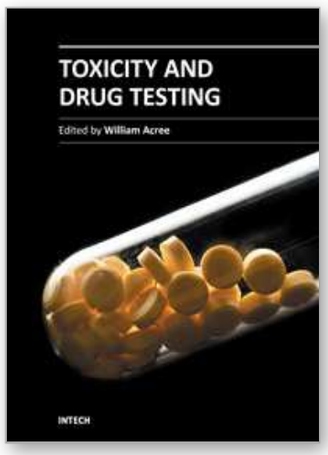

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Modern drug design and testing involves experimental in vivo and in vitro measurement of the drug candidate's ADMET (adsorption, distribution, metabolism, elimination and toxicity) properties in the early stages of drug discovery. Only a small percentage of the proposed drug candidates receive government approval and reach the market place. Unfavorable pharmacokinetic properties, poor bioavailability and efficacy, low solubility, adverse side effects and toxicity concerns account for many of the drug failures encountered in the pharmaceutical industry. Authors from several countries have contributed chapters detailing regulatory policies, pharmaceutical concerns and clinical practices in their respective countries with the expectation that the open exchange of scientific results and ideas presented in this book will lead to improved pharmaceutical products.

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