



The acute toxicity of cadmium on turtle *Mauremys reevesii*

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Abstract This research was designed to investigate the acute toxic effect of cadmium chloride on freshwater turtle *Mauremys reevesii*. *Mauremys reevesii*s were exposed to a wide range of cadmium chloride by intraperitoneal injection for 7 days and the survival numbers of the animals were noted to determine the dose of cadmium chloride for 0% mortality rate (Dn) and the dose of cadmium chloride for 100% mortality rate (Dm). Karber's method was used to test the LD₅₀ of cadmium chloride in *Mauremys reevesii*. The results showed that cadmium has acute toxic effect on freshwater turtle *Mauremys reevesii*. Dm and Dn were 500 and 20 mg·kg⁻¹ separately. The LD₅₀ value was 89.8 mg·kg⁻¹ for cadmium chloride, with the 95% confidence limit of 85.2–98.5 mg·kg⁻¹. The results indicated that cadmium had acute toxicity on turtle *Mauremys reevesii*.

Keywords Turtle *Mauremys reevesii* · Cadmium · Acute toxicology · LD₅₀

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Introduction

Cadmium (Cd) is a worldwide pollutant in aquatic systems (Helen et al. 2011; Järup et al. 1998). Cadmium enters animals from breathing, food, water (Zelikoff et al. 1995).

Due to the lack of elimination mechanisms in animals, cadmium can accumulate in the body and has a biological half-life of 15–20 years (Joseph. 2009). So, the toxic effects of cadmium are accumulated through the food web (Fowler. 2009; Järup et al. 1998; Rose et al. 2015; Waalkes 2003). It induces morphological deformities, physiological dysfunctions and biochemical alterations (Dieter et al. 2014; Lei et al. 2011).

Cadmium is able to accumulate in the water environment and be transported through the food chain (Simon et al. 2000). High levels of cadmium are found in some marine turtles (Cortés-Gómez et al. 2014, 2017, 2021; Ehsanpour et al. 2014; Ley-Quinóñez et al. 2013; Macêdo et al. 2015; Maffucci et al. 2005; Rie et al. 2001; Ross et al. 2016; Storelli et al. 2008). However, toxicological information on cadmium contamination in freshwater turtles is limited (Dayna et al. 2016; Dong et al. 2021a, b, 2023a, b; Huo et al. 2017a, b, 2018, 2020a, b; Noppadon et al. 2008; Yu et al. 2011; Yu et al. 2013).

Turtles have been used for traditional medicine, nutriment and pets in some countries in the world for many years, (Fordhama et al. 2007; Xu et al. 2014). From Japan to southern China, the turtle *Mauremys reevesii* is usually found in some ponds, rivers and

lakes in East Asia (Hoshi and Nakao 2008). Turtle *Mauremys reevesii* is one of the most valuable aquaculture species in China (Du et al. 2007).

This research was designed to investigate the acute toxic effect of cadmium on turtle *Mauremys reevesii* and determined the LD₅₀ of cadmium on the turtle *Mauremys reevesii*.

Materials and methods

Materials

Cadmium chloride (CdCl₂, batch number: 20150504) was purchased from Tianjin guangfu technology development co., LTD. Sterilized saline solution (batch number: 31215073109) was purchased from Shijiazhuang Pharmaceutical Group Company.

105 *Mauremys reevesii* with 130 ± 10 g in weight was purchased from Taiyuan birds and fish market.

Methods

Dn and Dm determined

The dose of cadmium chloride of 0% mortality rate on turtle *Mauremys reevesii* (Dn) and the dose of cadmium chloride of 100% mortality rate on turtle *Mauremys reevesii* (Dm) for subsequent experiment of LD₅₀ were determined.

Twenty five *Mauremys reevesii* were randomly divided into five groups (Ethical and collected permit numbers (SXZYDXLL022)). After fasting for one day, they were injected intraperitoneally with different concentration (500, 100, 20, 4, and 0 mg·kg⁻¹) of sterilized CdCl₂ solution. After 7 days of observation with three times a day, the numbers of dead *Mauremys reevesii* from each group were noted. Then, the minimum dose that could cause the death of all turtle in the experimental group was considered as lethal dose Dm. The maximum dose that did not induce any death of the turtle in the experimental group was Dn.

LD₅₀ determined

Group interval (*i*, the different dose logarithm between the two groups) was calculated from the formula

$$i = (\log D_m - \log D_n) / (n - 1)$$

where D_m = 100% mortality rate, D_n = 0% mortality rate, *n* = the number of treatment groups.

The dosage ratio (*k*) was calculated from the formula

$$k = 10^{-i}$$

where *i* = group interval.

The Karber method (Klassen. 1991) was used to investigate LD₅₀ of CdCl₂ on turtle *Mauremys reevesii*. Regardless of gender, 80 *Mauremys reevesii* were used in the study. They were divided into eight groups, which included one control group and seven treatment groups (Table 1). After fasting for one day, the control group was injected intraperitoneally with 1 ml sterilized saline solution and the seven treatment groups were injected intraperitoneally with sterilized CdCl₂ solution: 500, 290, 168, 97, 56, 32, and 19 mg·kg⁻¹. Throughout the seven-day test cycle, the turtles were carefully observed for changes in behavior, signs of poisoning or death, and the incubation period for death. All the dead turtles *Mauremys reevesii* were promptly get out with plastic tweezers to avert potential metamorphic of the quality of the water. The turtles were feed with fodder during the assay. After 7 days of observation, the numbers of dead turtles *Mauremys reevesii* from each group were noted.

The mortality percentages of turtles *Mauremys reevesii* in each concentration of CdCl₂ after 7 days of exposure were counted. The values and the confidence limits (CLs, 95%) of LD₅₀ were counted.

Table 1 Cumulative mortality of turtles *Mauremys reevesii* in acute poisoning of CdCl₂

Dose (mg·kg ⁻¹ bw)	Mortality (%)
Control	0
19	0
32	20
56	40
97	50
168	70
290	90
500	100

The LD_{50} was calculated from the following formula:

$$LD_{50} = \log^{-1} \left[X_m - i \left(\sum p - 0.5 \right) \right]$$

where X_m = the logarithm of the Maximum dose, i = group interval, $\sum p$ = the sum of mortality of treatment groups.

The standard error ($S_{X_{50}}$) was calculated from the formula:

$$S_{X_{50}} = i \sqrt{\sum pq/n}$$

where i = group interval, p = the mortality rate of each treatment group, q = the survival rate of each treatment group, n = the number of the turtles in each group.

The 95% CLs for LD_{50} were estimated using the formula:

$$LD_{50}(95\% \text{ CL}) = \log^{-1} (LD_{50} \pm 1.96 \times S_{X_{50}})$$

Results

The acute signs effect of cadmium on freshwater turtle *Mauremys reevesii*

The turtles *Mauremys reevesii* injected with higher dose cadmium had some symptoms such as vomit, diarrhea, asynergy, even death. But the turtles *Mauremys reevesii* injected with lower dose cadmium had lighter symptoms such as anepithymia.

The D_m and D_n of cadmium on the turtle *Mauremys reevesii*

The minimum dose that could cause the death of all turtles in the experimental group (the dose of cadmium chloride of 100% mortality rate on turtle *Mauremys reevesii*) was considered as lethal dose D_m . The maximum dose that did not induce any death of the turtle in the experimental group (the dose of cadmium chloride of 0% mortality rate on turtle *Mauremys reevesii*) was D_n . D_m and D_n were 500 and 20 $\text{mg} \cdot \text{kg}^{-1}$ separately.

The LD_{50} of cadmium on the turtle *Mauremys reevesii*

Mortality of studied turtles *Mauremys reevesii* for cadmium doses 500, 290, 168, 97, 56, 32, and 19 $\text{mg} \cdot \text{kg}^{-1}$ were investigated within the time of treated on 1, 2, 3, 4, 5, 6, and 7 d (Table 1, Fig. 1).

Although the test period was 7 days, the main time of turtle death was 24–72 h after the administration of cadmium. In the turtles *Mauremys reevesii* injected with CdCl_2 , the number of dead individuals increased significantly within 24–72 h as the concentration increased. There was a statistically significant difference in the number of deaths within 24–72 h in each group. All the turtles *Mauremys reevesii* died 100% within 24 h at a concentration of 500 $\text{mg} \cdot \text{kg}^{-1}$ for all the *Mauremys reevesii*, and no mortality at 19 $\text{mg} \cdot \text{kg}^{-1}$ within the exposure times. Dead turtles for 500, 290, 168, 97, 56, 32, and 19 $\text{mg} \cdot \text{kg}^{-1}$ group were 10, 9, 7, 5, 4, 2, 0.

The calculated LD_{50} , the standard error ($S_{X_{50}}$) and 95% confidence limits of CdCl_2 in turtles *Mauremys reevesii* were 89.8 $\text{mg} \cdot \text{kg}^{-1}$, 0.0718, 85.20–98.50 $\text{mg} \cdot \text{kg}^{-1}$ (Table 2).

Discussion

D_m was 500 $\text{mg} \cdot \text{kg}^{-1}$ and D_n was 20 $\text{mg} \cdot \text{kg}^{-1}$. The difference was 25 times, which indicated that the

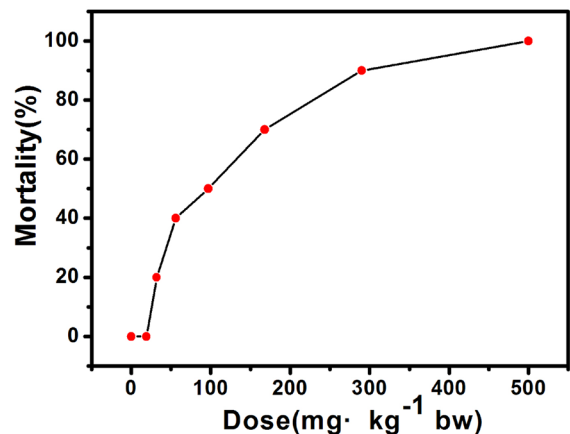


Fig. 1 Cumulative mortality of turtles *Mauremys reevesii* in acute poisoning of CdCl_2

Table 2 The calculated parameters

parameter	data
Group interval (<i>i</i>)	0.233
The dosage ratio (<i>k</i>)	0.58
The LD ₅₀	89.8 mg·kg ⁻¹
The standard error (S _{X50})	0.0718
95% confidence limits	85.20–98.50 mg·kg ⁻¹

tolerance range of turtles *Mauremys reevesii* to cadmium is very large.

From Table 1, it could be seen that the mortality of the turtles increased with the increasing of the dose of cadmium. The mortality of the turtles increased from 20 to 40% (the mortality rate was increased by onefold) with a 0.75-fold increasing of the dose of cadmium. The mortality of the turtles increased from 40 to 50% (the mortality rate was increased by 0.25-fold) with a 0.73-fold increasing of the dose of cadmium. The mortality of the turtles increased from 50 to 70% (the mortality rate was increased by 0.40-fold) with a 0.73-fold increasing of the dose of cadmium. The mortality of the turtles increased from 70 to 90% (the mortality rate was increased by 0.28-fold) with a 0.73-fold increasing of the dose of cadmium. The mortality of the turtles increased from 90 to 100% (the mortality rate was increased by 0.11-fold) with a 0.72-fold increasing of the dose of cadmium. This indicated that the mortality of the turtles changed greatly when the magnitude of the increasing of the dose changed very little. The mortality of the turtles increased most significantly when the dose of cadmium increased from 32 mg·kg⁻¹ bw to 56 mg·kg⁻¹ bw. In addition, although the amplitude of dose increased little, the increase of the mortality of the turtles became smaller with the increase of the dose of cadmium.

Cadmium had evident acute toxicity on turtle *Mauremys reevesii*, which was similar with its poisonousness to other animals (Fowler 2009; Lei et al. 2011). Cadmium have an acute toxicity of 88 mg·kg⁻¹ (oral LD₅₀, rat, Lehman 1951) and 72.4 mg·kg⁻¹ (oral LD₅₀, mice, Li 1997). Cadmium have an acute toxicity of 4.8 mg·kg⁻¹ (subcutaneous injection LD₅₀, mice, Feng 1981). But cadmium have an acute toxicity of 18.37 mg·kg⁻¹ (intraperitoneal LD₅₀, rat, Lu 2010) and 4.14 mg·kg⁻¹ (intraperitoneal LD₅₀, mice, Li 2003) (Table 3). These results showed that the acute toxicity of cadmium on different animal in diverse administration was various. The value of LD₅₀ per g of body weight was 0.106 for mice (calculated as 20g body weight), 0.092 for rats (calculated as 200g body weight) and 0.691 for turtle *Mauremys reevesii* (calculated as 130g body weight), indicating that the tolerance of turtle *Mauremys reevesii* to cadmium was significantly higher than that of mice and rats (Table 3). From Table 3 and Fig. 2, we could see that turtle *Mauremys reevesii* had more tolerance to cadmium than other animals such as mice and rats.

Macroscopically and microscopic changes of cadmium on turtle *Mauremys reevesii* can be seen in the major organs such as liver (Huo et al. 2017a, 2017b). In the 7.5 mg/kg cadmium treatment group, the livers have lost luster and become light. The surface of the liver has hemorrhagic spots. The liver is slight congestion. However, the cells of the liver are arranged closely and the outline of liver cells and nucleus are clear. Hepatic cords and hepatic sinusoids are clearly visible. The nucleus of cell is round with complete nuclear membrane and a homogeneous pattern of chromatin disperse throughout the nucleus. The cytoplasm is rich in lipid drops. Most mitochondria exhibit volume expansion and uneven reduction of the metrical density. Apparent distension, twisting and fracture of rough endoplasmic

Table 3 LD₅₀ of different animals

Animals	Administration route	LD ₅₀ (mg·kg ⁻¹)	95% confidence limits (mg·kg ⁻¹)
Mice	Intraperitoneal injection	2.12	1.71–2.67
Rat	Intraperitoneal injection	18.37	16.56–20.38
Turtle	Intraperitoneal injection	89.8	85.20–98.50
Mice	Subcutaneous injection	4.8	3.47–4.79
Mice	Oral	72.4	48.30–108.70
Rat	Oral	88	

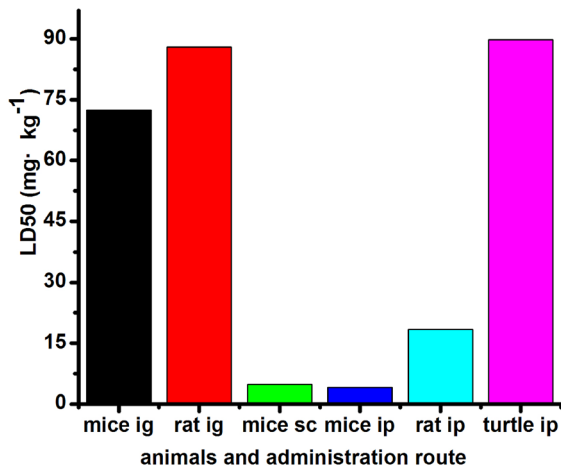


Fig. 2 LD₅₀ of different animals

reticulum (RER) are observed. Incomplete microvilli and kupffer cell are also observed (Huo et al. 2017a). In the 15 mg/kg cadmium stress group, liver surface has lesion plaques. Livers are swollen and margins are obtuse. Liver tissue become serious congestion and light dye in partial region. Some cells are swollen and show degeneration as well as necrosis. The outline of liver cells and nucleus are not clear. The nuclear envelope is dispersed or outright missing accompanied by chromatin condensation and marginalization. Chromatin is condensed into large clumps under the periphery of the nucleus. The cytoplasm are rich in lipid drops. Most mitochondria exhibit volume expansion, swollen matrix, membrane disintegration. Distension, twisting and fracture of RER are also observed. Liver cells show deformation and microvilli irregularity. The number of organelles decrease and fat accumulate (Huo et al. 2017a). In the 30 mg/kg cadmium stress group, livers are also swollen and margins are obtuse. The color of the livers turn to yellowish. Hydropic degeneration occurs, and the boundaries of the liver cells are fuzzy or disappeared. Chromatin distributes along the nuclear membrane side. Arrangements of hepatic cords and hepatic sinusoids are not neat. Chromatin is condensed into large clumps under the periphery of the nucleus and the structure of the nuclear envelope is dispersed or outright missing accompanied by chromatin condensation and marginalization. The cytoplasm is rich in lipid drops. Many damages of mitochondrial

appear. Most mitochondria exhibit volume expansion, swollen matrix, membrane disintegration, and uneven reduction of the metrical density. Mitochondrial disassembly is observed. In a subset of mitochondria with ruptured membranes, some of the cristae disappear and double-membrane-bounded vesicles are noted. Apparent distension, twisting and fracture of RER are observed, and a large number of ribosomes are detached from the surface of the RER. Liver cells show deformation and microvilli deletion. Autolysosomes and large granular lymphocyte significantly increase in liver cells (Huo et al. 2017a).

Conclusions

Our results showed that cadmium caused acute toxicity on turtle *Mauremys reevesii*. The value of LD₅₀ was 89.8 mg·kg⁻¹ for CdCl₂ on turtle *Mauremys reevesii*, with the 95% confidence limit of 85.2–98.5 mg·kg⁻¹. So the mechanisms of cadmium on turtle *Mauremys reevesii* should be studied later.

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Authors contribution Aiguo Dong and Junfeng Huo designed the study, performed the research, analyzed data, and wrote the paper. Huidong Dong, Tianmiao Zhang, Xuejie Jing and Hui He was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This study was approved by Shanxi University of Chinese Medicine (permit number: SXZYDXLL022).

Consent for publish Not applicable.

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