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RESEARCH ARTICLE

Effects of cadmium on liver function in turtle *Mauremys reevesii*

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Abstract

This research was designed to investigate the effects of cadmium (Cd) on liver function in turtle *Mauremys reevesii*. Turtles were divided into 4 groups at random. The turtles were injected intraperitoneally with Cd at 0, 7.5, 15, 30 mg kg^{-1} Cd chloride separately. Liver index was calculated. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the content of TP in liver were examined with biochemical methods. The results indicated that the liver index of turtles changed obviously only at higher dose and longer time. The activities of ALT and AST in liver increased with prolongation of exposure time in a dose-dependent manner. TP content in liver was lower than that in the control. In summary, Cd had an obvious toxic effect on liver tissues of freshwater turtle *Mauremys reevesii*, and it was dose dependent with the extension of exposure time. But the results also showed that the turtle had strong tolerance to Cd.

Keywords Turtle *Mauremys reevesii* · Cadmium · Liver

Introduction

Cadmium (Cd) is an important pollutant in water environment (Mehinto et al. 2014; Novelli et al. 2000; Theron et al. 2012). Cd can enter an animal's body by breathing, drinking, feeding and even through the skin (Willers et al. 2005; Zhang and Wang 2007). Because Cd has a prolonged biologic half-life (15–20 years), it can accumulate in the body of the upper animals through the food web (Fowler 2009; Joseph. 2009; Rose et al. 2015).

Cd has poisoning effects on the organisms (Engström et al. 2012; Enli et al. 2010). The toxic mechanism of Cd is not known completely, and some explanations have been presented (Varoni et al. 2010; Yamano et al. 1998). Studies have demonstrated that Cd toxicity is primarily affiliated to accumulation of metals in the liver, leading to toxicity of liver cell (Gong et al. 2014; Van et al. 2007). Some reports demonstrate that Cd changes liver enzymes (Brucka-Jastrzebska and Protawicki 2005).

The most important sign of hepatocyte injury caused by Cd is the release of certain enzymes from liver, for example aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Honda et al. 2010). The release of AST and ALT is the indicator commonly used for assessing liver injury (Hayes 1989). Marked variations of the activities of AST and ALT indicate the damage of liver tissue (Cinar et al. 2011).

Cd is able to be enriched to the upper animals through the food web (Simon et al. 2000). High levels of Cd have been detected in the body of some sea turtles (Cortés-Gómez et al. 2017; Macêdo et al. 2015; Ross et al. 2016; Storelli et al. 2008). However, there is very little toxicological information of Cd in freshwater turtles (Dayna et al. 2016; Huo et al. 2017b, 2018, Huo et al. 2020a, b).

In this research, the effects of Cd on the turtle liver index, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the content of total protein (TP) in liver of the turtles *Mauremys reevesii* were investigated.

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Aiguo Dong and Hui He are first co-authorship, and contributed equally to this work.

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Materials and methods

Animals and treatment

Turtles *Mauremys reevesii* were divided into 2 groups, which included control group and experiment groups. The turtles of the control group were administered 0.85% saline solution by intraperitoneal injection. The turtles of the experiment groups were administered 7.5, 15, 30 mg/kg Cd respectively by intraperitoneal injection. All the turtles were treated once. The feeding conditions of the turtles used in the experiment were the same as those used when they adapted to the environment.

Sample collection

Five turtles were sacrificed 1 week (1w), 2 weeks (2w) and 3 weeks (3w) after Cd was administered. The liver samples were cut promptly and weighed. Nine times 0.85% saline solution was added to liver tissue by weight (g): volume (ml), homogenized, centrifuged at 4 °C at 2500 r/min for 10 min. The supernate of the liver tissue was reserved at − 80 °C for detecting liver function.

Calculation of the liver index

$$\text{Liver index (\%)} = (\text{wet liver weight} / \text{body weight}) \times 100\%$$

Biochemical tests

The activities of AST, ALT and the contents of TP in supernate of the liver tissue were determined using the kit according to the method of the manufacturer.

Statistical analysis

All experimental data were statistically analyzed using the SPSS 20.0 software package. The test data represented the mean of five animals per group, and the test results were expressed as the mean ± standard deviation (SD). The probability value less than 0.05 was statistically different. If the probability value is less than 0.01, the difference is statistically significant.

Results

Correlation of ALT activities and Cd exposure in liver tissues

ALT activities of liver tissue in the 15 mg/kg and 30 mg/kg groups were significantly higher than that of the control and 7.5 mg/kg groups in 1 week. The activities of ALT in liver tissue in the 7.5 mg/kg, 15 mg/kg and 30 mg/kg groups were significantly higher than that of the control group, and the activities of ALT in liver tissue in the 15 mg/kg and 30 mg/kg groups were significantly higher than that of the 7.5 mg/kg group in 2 week. ALT activities of liver tissue in the 7.5 mg/kg, 15 mg/kg and 30 mg/kg groups were significantly higher than that of the control group, and the activity of ALT in liver tissue in the 30 mg/kg group was significantly higher than that of the 7.5 mg/kg and 15 mg/kg group in 3 week (Fig. 1).

Correlation of AST activities and Cd exposure in liver tissues

AST activities of liver tissue in the 15 mg/kg and 30 mg/kg groups were significantly higher than that of the control group, and the activity of AST in liver tissue in the 30 mg/kg group was significantly higher than that of the 7.5 mg/kg and 15 mg/kg group in 1 week. AST activities of liver tissue in the 7.5 mg/kg, 15 mg/kg and 30 mg/kg groups were significantly higher than that of the control group, and the activities of AST in liver tissue in the 15 mg/kg and 30 mg/kg groups were significantly higher than that of the 7.5 mg/kg group in 2 weeks and 3 weeks (Fig. 2).

Correlation of the concentrations of TP and Cd exposure in liver tissues

The content of TP of liver tissue in the 15 mg/kg and 30 mg/kg groups was significantly lower than that of the control

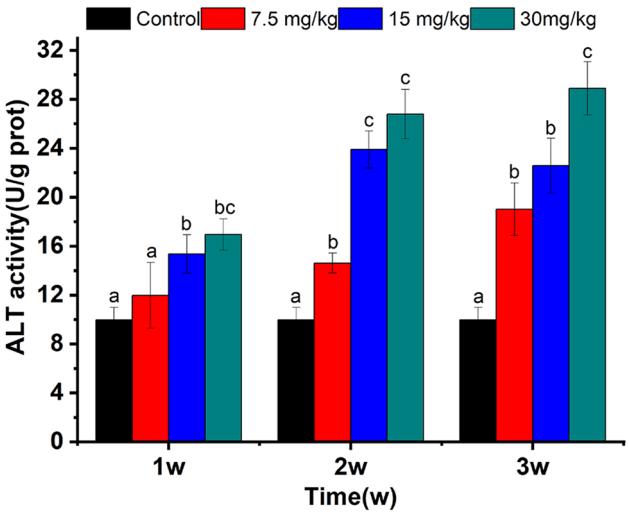

Fig. 1 The activities of ALT in liver of turtles exposed to Cd. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$)

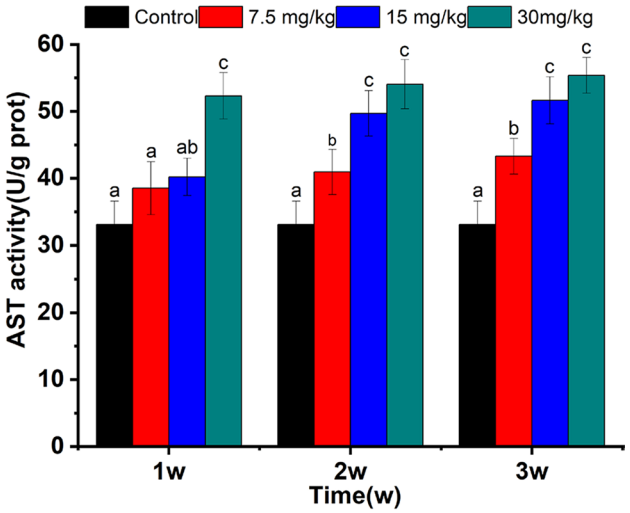

Fig. 2 The activities of AST in liver of turtles exposed to Cd. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$)

group and the 7.5 mg/kg group in 1 week. TP content of liver tissue in the 7.5 mg/kg, 15 mg/kg and 30 mg/kg groups was significantly lower than that of the control group in 2 weeks. The content of TP of liver tissue in the 30 mg/kg group was significantly lower than that of the control group, the 7.5 mg/kg group and 15 mg/kg group in 3 weeks (Fig. 3).

Correlation of Cd treatments and the liver index

The liver index of liver in the 30 mg/kg group was significantly higher than that of the control group, the 7.5 mg/kg group and 15 mg/kg group in 1, 2 and 3 weeks (Fig. 4).

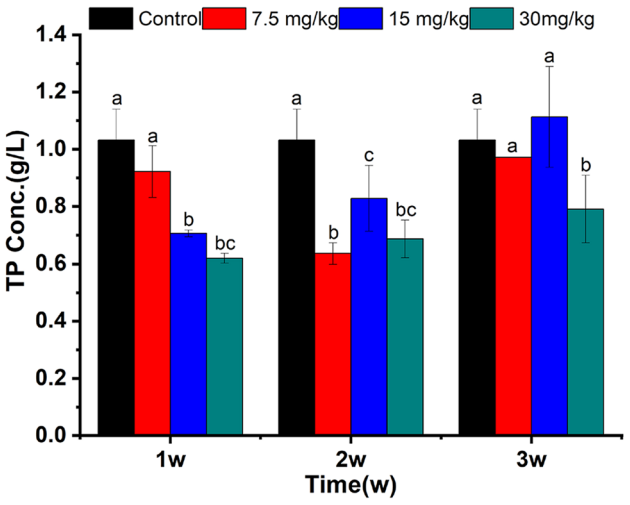

Fig. 3 The content of TP of liver in turtles exposed to Cd. Comparison between groups at the same exposure time: the difference of different letters was statistically significant (P < 0.05)

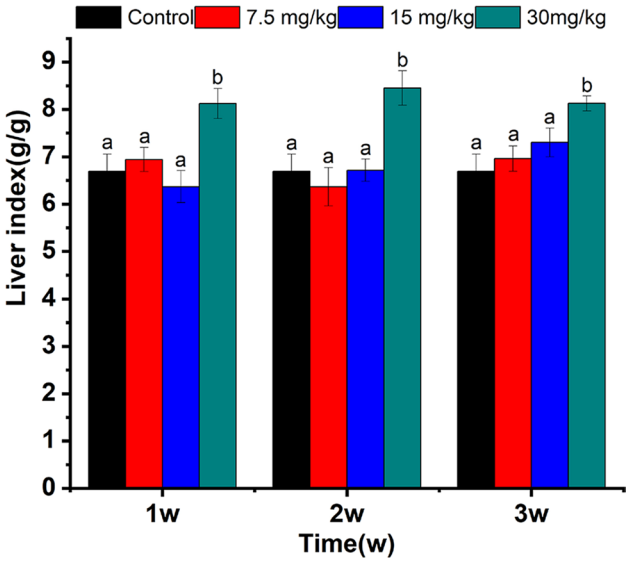

Fig. 4 The liver index of liver in turtles exposed to Cd. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$)

Discussion

The general population is exposed to Cd either voluntarily or involuntarily (Meeker et al. 2008). The most important sign of hepatocyte injury caused by Cd is the release of certain enzymes from liver, for example AST and ALT (Honda et al. 2010). The release of AST and ALT is the indicator commonly used for assessing liver injury (Hayes 1989). Marked variations of the activities of AST and ALT indicate the damage of liver tissue (Cinar et al. 2011). Changes of ALT and AST activities can be regarded as a signal of liver damage (Miandare et al. 2016). Increased activities of ALT and AST are the result of the discharge of ALT and AST from the liver cytoplasm (Navarro et al. 1993). Under some circumstances, organelles are damaged, and then the cytoplasm is released to the cell membrane (Jaswal and Shukla 2015). When the cell membrane is damaged, these enzymes are released from the cell, which means that the increasing of the activities of AST and ALT can be biological indicators for evaluating susceptibility to liver injury (Jaswal and Shukla 2015; Miandare et al. 2016). Although there are more studies on the activities of ALT and AST in blood, there are fewer studies on ALT and AST activities in liver. Wang et al. (2015) report that ALT and AST activities in the livers of the mice exposed to Cd increase. The level of ALT and AST in the livers of rats exposure to Cd has ascended (An et al. 2018). These were also confirmed by our research, where Cd caused a significant increase of the activities of ALT and AST, which was caused by liver cell injury. However, there is one report that ALT and AST activities in the livers

of the fish Tilapia nilotica exposed to Cd are decreased (Hui et al. 2000).

The TP content of the liver tissues of turtles indicated a trend of decreasing first and then increasing with the extension of the exposure time, which may be that 7.5, 15 and 30 mg/kg of Cd in the initial stage of the exposure induced lipid peroxidation injury in the liver tissues of turtles, affecting the liver function and inhibiting the synthesis of protein. With the prolongation of administered time, the increase of TP content may be due to the synthesis of a large amount of metallothionein induced by Cd on the one hand and the partial functional recovery of liver tissues after the stress of Cd stress on the other hand.

There are some reports on the effect of Cd on liver index. Dong et al. (2010) report that liver index in mice exposed to Cd is increased. The liver index in rats exposed to Cd is increased (An et al. 2018). Consistent with these studies, our results indicated that the liver index of turtles increased obviously only at higher dose and longer time. However, there have been some reports to the contrary. Huang et al. (2018) report that liver index in rats exposed to Cd is decreased. The results in vivo showed that Cd had the effects of reducing the liver index of rats (Du et al. 2008).

The biochemical signals of liver injury are confirmed by histopathological proof (Huo et al. 2017a). These observed results indicated that the structure and function of the liver changed significantly under the action of Cd.

Conclusions

The activities of ALT and AST in liver increased with exposure time in a dose-dependent manner. TP content in liver was lower than that in the control. The liver index of turtles changed obviously only at higher dose and longer time. In summary, Cd had an obvious toxic effect on liver cells of freshwater turtle *Mauremys reevesii*, and it is dose dependent with the extension of exposure time. But this research also showed that turtle *Mauremys reevesii* had strong tolerance to Cd.

Author contribution AD designed the study, performed the research, analyzed data and wrote the paper. HH analyzed data and wrote the paper. TZ wrote the paper. XJ was one contributor in writing the manuscript. YM was one contributor in writing the manuscript. XW was one contributor in writing the manuscript. HD was one contributor in writing the manuscript. WL was one contributor in writing the manuscript. KF designed the study. JH designed the study, performed the research, analyzed data and wrote the paper. All authors read and approved the final manuscript.

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Data availability Not applicable.

Declarations

Ethics approval and consent to participate This study was approved by Shanxi University of Chinese Medicine (permit number: 2022DW037).

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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