Iranian Journal of Pharmaceutical Sciences
2022: 18 (1): 1-7
www.ijps.ir

Original Article

Therapeutic Potential of Astaxanthin Against Cadmium-Induced Hepatic Failure: An Experimental Study

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Abstract

Cadmium is a toxic metal that can lead to liver failure in humans and animals. Astaxanthin (ASX) is one of the well-known xanthophyll carotenoids in food that has remarkable antioxidant properties. Hence, this study investigated the effect of ASX against cadmium-induced hepatic failure. Twenty-four mice were divided into four groups of six each and treated intraperitoneally as follows: group 1 (sham) received normal saline and olive oil; group 2 received 10 mg/kg/day of ASX; groups 3 and 4 were treated with cadmium (1 mg/kg/day) and ASX (10 mg/kg/day) + cadmium (1 mg/kg/day), respectively. After 14 consecutive days, mice were sacrificed and blood and liver samples were isolated for histopathological and biochemical experiments. Our findings showed a significant increase in serum alanine aminotransferase level, as a hepatic marker, following cadmium administration ($p < 0.05$). In this regard, cadmium led to hepatic leukocyte infiltration, dilated sinusoids, and increased hepatic metallothionein level ($p < 0.01$). Following the administration of ASX, a significant improvement was found in the metallothionein level and hepatic enzymes alongside histopathologic alterations. The present study revealed that the administration of ASX could prevent cadmium-induced hepatic failure, which may be related to the antioxidant properties of this carotenoid.

*Keywords***:** Cadmium; Astaxanthin; Liver; Metallothionein; Mice; Enzyme.

1. Introduction

Cadmium (Cd) is one of the environmental pollutants which can damage vital organs such as the brain, lungs, kidney, liver [1, 2]. Human exposure to Cd generally results from industrial processes, smoking and food contamination [3]. Among the vital organs, the liver plays a critical role in metabolism and body detoxification. Cd can cause lipid peroxidation, DNA damage, destroy membrane proteins and subsequently hepatocyte necrosis by creating free radicals and depletion of intracellular antioxidant stores [4]. In addition, Cd induces the hepatic inflammation process following the release of inflammatory mediators from kupffer cells and macrophages [5, 6].

Metallothionein (MT) is a rich protein of cysteine with low-molecular-weight, which

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Cite this article as: Mir Mohammadrezaei F., Ghasempour Ganji Z., Nili-Ahmadabadi A., Hajizadeh Moghaddam A., *Therapeutic Potential of Astaxanthin Against Cadmium-Induced Hepatic Failure: An Experimental Study*, 2022, 18 (1): 1-7.

participates in toxic metals detoxification, such as Cd. MT acts as an antioxidant and can protect organs such as the liver and kidney against oxidative damages induced by various toxic metals [7, 8].

Astaxanthin (ASX) is a xanthophyll carotenoid in foods such as salmon, trout, shrimp, lobster, and fish eggs (Figure 1) [9, 10]. The previous studies showed that ASX, as a strong antioxidant and free radical scavenger, protects the liver from oxidative damages and decreases the expression of lipogenic and lipid-uptake genes in mice [10-12]. However, the therapeutic effect of ASX on Cd-hepatic failure is poorly understood.

Based on existing evidence, this study was designed to evaluate the therapeutic effects of ASX on Cd-induced hepatotoxicity.

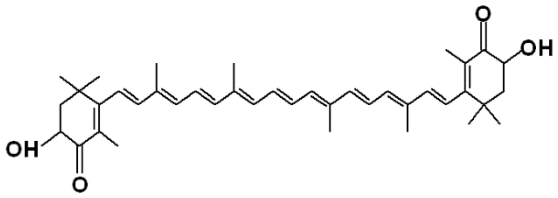

Figure 1. The chemical structures of Astaxanthin.

Materials and Methods

2.1. Materials

ASX was provided from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and cadmium chloride monohydrate ($CdCl_2 \cdot H_2O$) was purchased from Merck (Darmstadt, Germany).

2.2. Experimental Design

Twenty-four adults' male mice, 5–6 weeks old and 20-24 g were purchased from the Iranian Pasteur Institute. Mice were kept in conventional conditions at a temperature of 24 ± 2°C, 12 h light/dark cycle, the humidity of 45-55% and free access to water and standard laboratory diet. For adaptation, mice were maintained in standard condition for a week. After seven days, the animals were divided into four groups of six each and treated intraperitoneally as following: group 1 (sham group) received normal saline and olive oil; group 2 received 10 mg/kg/day of ASX; group 3 was treated with Cd (1 mg/kg/day) and group 4 (therapeutic group) received ASX (10 mg/kg/day) + Cd (1 mg/kg/day). After 14 consecutive days, mice were sacrificed, and blood and liver samples were collected. A part of hepatic tissue was separated and immediately stored in liquid nitrogen at -80°C, and the other part was fixed in a 10% formaldehyde solution for the histopathological experiment. The blood samples were collected from the heart and centrifuged for 15 min at 5000 rpm to acquire clear serum then kept at -20 °C for biochemical experiments.

All ethical aspects were approved by Mazandaran University Ethics Committee with the code number of IR.UMZ.REC.1397.107.

2.3. Liver Function Test (LFT)

The serum hepatic enzymes including alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in blood serum samples were assayed by commercial kits (Bionic, Iran).

2.4. Measurement of Metallothionein in Liver Tissue

Briefly, 100 mg of hepatic tissue and 1 ml of phosphate-buffered saline (PBS, pH=7.4) were homogenized and centrifuged (at 3000 RPM/ 4°C) for 15 min and its supernatant was

removed. The level of MT protein in liver homogenate supernatant was measured using quantitative sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) kit (Shanghai crystal Day Biotech Co., LTD, China), based on the direct sandwich procedure. The samples were evaluated by a microplate reader set at the wavelength of 450 nm against the standard curve. Finally, MT contents were reported as µg/mg tissue.

2.5. Histopathologic Examination

Liver tissues were collected from mice and samples were fixed in 10% formaldehyde for 24 h at room temperature. Then, the specimens were embedded in paraffin and sections were prepared at a thickness of 5 µm by a microtome. Finally, they were stained with hematoxylin and eosin (H&E) dye and were observed using a light microscopy (Olympus, BX41, Japan) [13].

2.6. Statistical Analysis

All data were expressed as Mean±SD at least triplicate and were analyzed by Prism software. One way ANOVA and Tukey post-test were used to compare groups at a significant level of $p < 0.05$.

2. Results and Discussion

Cadmium-induced hepatotoxicity is one of the most important pharmacological models used to evaluate the therapeutic potential of medicines. This study has shown that Cd at a dose of 1 mg/kg induced hepatic injury in male mice, and ASX prevented its hepatotoxic effects.

As shown in Table 1, LFT findings showed an increase in liver enzymes following Cd administration, which was a significant increase for ALT serum level ($p < 0.05$). ASX administration could improve ALT serum level ($p < 0.05$) and ALP ($p < 0.05$), in mice exposed to Cd. The ALT serum enzyme is a specific marker to diagnose early acute hepatic damage [13, 14]. In agreement with our findings, several studies have described Cd-hepatic failure in different animal models. For instance, the studies showed that administration of Cd, for eight weeks, led to enhance the levels of ALT and AST enzymes in mice [15]. In this regard, increased hepatic aminotransferases (ALT and AST enzymes) and subsequently Cd-induced hepatic failure were confirmed by Fahim [16]. The elevated serum hepatic enzymes activities, especially the ALT enzyme, could be due to cellular leakage and loss of functional integrity of the hepatic cell membrane, which is the outcome of free radical-mediated oxidative stress in the hepatic tissue [14, 17].

Table 1. Effect of ASX on hepatic serum enzymes in Cd-exposed mice.

Values are expressed as means ± SD, n = 6 for each group. $^{*}P < 0.05$ *vs* sham group; $^{\#}P < 0.05$ *vs* Cd group. ASX, Astaxanthin; Cd, Cadmium chloride monohydrate ($CdCl_2 \cdot H_2O$).

Group	ALT(U/L)	AST(U/L)	LDH(U/L)	ALP(U/L)
Sham	80.33±15.01	160.5±53.03	1745±623.66	296.5±6.36
Cdcl2 (1mg/kg/day)	135±8.48 *	262.5±82.73	2131±428.50	317±69.34
ASX (10 mg/kg/day)	91.66±29.14	253±38.31	2146±53.74	233.5±21.92
Cdcl2+ ASX (1mg/kg/day +10 mg/kg/day)	67±12.49#	221±52.32	1543.66±389.30	242.43±46.54#

ASX was able to prevent the increase in serum hepatic enzymes (ALT and AST) and some of the Cd-induced pathological changes. Previously, the protective effects of ASX have been reported on liver failure induced by some hepatotoxic agents such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin [18], carbon tetrachloride [19]. This therapeutic effect may be attributed to the free radical scavenging activity of ASX, and our findings in this research are in agreement with earlier reports. ASX was able to accumulate in the liver and reduce oxidative stress and the production of ROS induced by high glucose [20] and improved liver function and inflammation [11]. As shown in Figure 2, hepatic pathological alterations such as inflammation, sinusoidal dilatation, disruption of hepatocytes and leukocyte infiltration around the central vein were observed in the Cd group. ASX could prevent the alterations especially leukocyte infiltration, inflammation and sinusoidal dilatation in hepatic tissue.

It was previously showed that ASX could decrease liver fibrosis induced by CCl4 [21]. In this regard, the previous studies have confirmed that ASX is several folds more active as an antioxidant than α-tocopherol and β-carotene. It seems that the presence of thirteen double bonds in ASX has caused its antioxidant properties to increase significantly compared to other similar carotenoids [22]. In addition, polar hydroxyl groups are existent in the 3 and 3' positions of ASX allowing it to sit near the lipid/water interface of the cell membrane bilayers where free radicals attack occur mostly [19]. These structural features may be one of the reasons for the therapeutic effects of ASX in preventing Cd-induced hepatic oxidative damage.

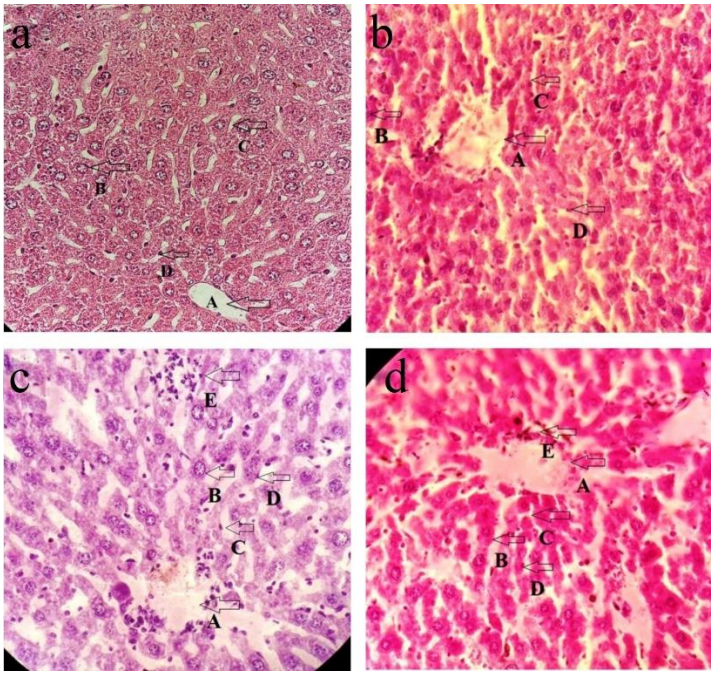

Figure 2. Effects of ASX on hepatic tissue of Cd-exposed mice. (a), Sham group; (b), ASX group (10 mg/kg/day); (c), Cd group (1 mg/kg/day); (d), Cd (1 mg/kg/day) + ASX (10 mg/kg/day). A, central vein; B, hepatocyte; C, sinusoid; D, Kupffer cells; E, Lymphocytic infiltration. ASX, Astaxanthin; Cd, Cadmium chloride monohydrate ($CdCl_2 \cdot H_2O$). (× 40)

As shown in Figure 3, the administration of Cd could significantly increase MT hepatic level compared to the sham group ($p < 0.01$). In contrast, ASX can prevent the inductive effects of Cd on MT ($p < 0.05$). MT is a cysteine-rich protein, which is a part of the defense system against Cd toxicity. It seems that the inhibition effects of ASX on MT protein expression indirectly could be related to its chelating effects. Previously, Hernández-Marin et al. showed that ASX reacts with metal ions such as Cd that the existence of oxygen atoms on ASX is vital for the formation of these complexes [23].

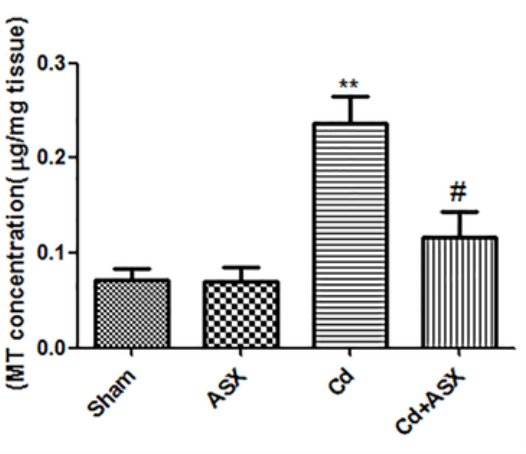

Figure 3. Effect of ASX on hepatic metallothionein in Cd-exposed mice. Values are expressed as means ± SD, n = 6 for each group. $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$ *vs* Cd group. ASX, Astaxanthin; Cd, Cadmium chloride monohydrate ($CdCl_2 \cdot H_2O$); metallothionein (MT).

4.Conclusion

The present study proved that ASX is an effective supplement against Cd-induced hepatic failure. This therapeutic potential may be associated with the structural properties and chelating effects of ASX on Cd, which are recommended to be investigated in future studies.

Acknowledgement

The authors thank from the research deputy of University of Mazandaran for partial support of funding this work.

Conflict of interest

None

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