Research investigating antioxidation of astaxanthin extracted from *Haematoccus pluvialis* in mice

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OBJECTIVE: To evaluate the antioxidative effect of astaxanthin extracted from *Haematoccus pluvialis* in mice.
METHODS: Fifty mice were randomly divided into five groups (high-dose [astaxanthin 4.0 mg/kg]; medium dose [astaxanthin 2.0 mg/kg]; low dose [astaxanthin 1.0 mg/kg]; model control; and blank control). The experimental substance was administered every day. Mice in the model group and the control group received an equal volume of vegetable oil for 30 days. After 30 days, blood antioxidant enzyme activity was measured in tail blood. Except for the blank control group, the other groups were exposed to 8 Gy ^{60}Coγ irradiation. On the fourth day after irradiation, all animals were euthanized and liver tissue was tested for malondialdehyde, superoxide dismutase and glutathione peroxidase activity levels.
RESULTS: Superoxide dismutase levels in all three groups that received astaxanthin were significantly higher than in the model control group, and malondialdehyde levels in all three groups that received astaxanthin were significantly lower than in the control group. Glutathione peroxidase enzyme activity in the high-dose group was significantly higher in the model control group.
CONCLUSION: According to the evaluation standards in *Inspection and Assessment Standard for Health Food*, 2003 edition, the present study demonstrated that astaxanthin extracted from *Haematoccus pluvialis* has antioxidative effects in a mouse model.

Key Words: *Antioxidation; Astaxanthin;* Haematococcus pluvialis; *Mice*

Haematococcus pluvialis is a major source of natural astaxanthin (1). Production of astaxanthin can be stimulated by dry, high temperatures, ultraviolet light, nutritional deficiency and other conditions. It produces strong antioxidants, which can remove oxides and restore balance (2). Modern research demonstrates that astaxanthin has a strong antioxidant effect, along with other multiple effects in the body (3). Therefore, using *Haematococcus pluvialis* to produce astaxanthin to develop astaxanthin-containing drugs, cosmetics and health food has been a hot scientific research topic. We extracted natural astaxanthin from *Haematococcus pluvialis* using supercritical CO_2 extraction technology, and evaluated its antioxidant effect.

METHODS

Test substance

Astaxanthin oil (Yunnan Green A Bioligical Project Co Ltd, China) was diluted with vegetable oil.

Experimental animals

Fifty healthy female Kunming mice, weighing between 18 g and 22 g, were provided by the Experimental Animal Center of Zhongshan University (China). The mice were quarantined for one week before the experiment. The mice were fed in a barrier level animal room, which was mainted at a temperature between 20°C and 23°C, and a relative humidity between 55% and 65% for the duration of the experiment. Mice were provided with food and water ad libitum.

Equiptment and reagents

For the present study, the following equiptment was used: 722 spectrophotometer; water bath maintained at a constant temperature; micro-adjustable pipette; high- and low-speed centrifuges; vortex mixers; a DY89-1 electric glass homogenizer; and malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) assay kits (Nanjing Jiancheng Bioengineering Institute, China). Chloroform, ethanol, acetic acid and other chemical reagents were analytical grade.

Dose group

Fifty mice were randomly divided into five groups (high-dose [astaxanthin 4.0 mg/kg], medium dose [astaxanthin 2.0 mg/kg], low-dose [astaxanthin 1.0 mg/kg], model control and blank control). The amount of astaxanthin administered to the groups receiving astaxanthin, repectively, were equivalent to 20×, 10× and 5× the human recommended amount.

Experimental methods

The three groups receiving aztaxanthin were administered treatment according to the corresponding dose for 30 days. The model control group and blank control group were given equal volumes of vegetable oil for 30 days. After 30 days, blood samples taken from the tail were tested for antioxidant enzyme activity. In addition to the blank control group, each group was exposed to 8 Gy ^{60}Coγ ray exposure once. Four days after irradiation, all animals were euthanized to test for MDA content, SOD activity and GSH-Px in liver tissue according to the corresponding kit instructions.

Statistical analysis

ANOVA was performed using PEMS 3.0 (Chinese Medical Encyclopedia Medical Statistics, China).

RESULTS

Effect of astaxanthin on body weight of mice

There were no adverse effects on body weight of the mice ($P>0.05$) (Table 1).

SOD activity in mice before irradiation

There was no significant difference among any of the groups in SOD activity before irradiation ($P>0.05$) (Table 2).

Effect of astaxanthin MDA, SOD and GSH-Px activity in liver tissue

The activity of MDA in the model group was significantly higher than that of the control group ($P<0.05$), and MDA content of the three

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TABLE 1
Effect of astaxanthin on the body weight of mice

		Weight, g		
Group	n	Starting	Mid-experiment	Final
Blank control	10	20.5±2.0	33.7±3.1	42.3±3.9
Model	10	20.2±2.3	33.2±3.2	43.1±4.0
Low dose	10	20.3±2.3	33.5±3.8	44.1±3.3
Medium dose	10	20.3±1.7	34.1±4.4	44.9±3.7
High dose	10	20.6±1.9	33.8±2.9	43.5±3.1

Data presented as mean ± SD unless otherwise indicated

TABLE 2
Superoxide dismutase (SOD) activity in mice before irradiation

Group	n	SOD activity, NU/mL
Blank control	10	3143.6±198.6
Model	10	3054.2±267.6
Low-dose	10	3038.6±242.0
Medium dose	10	3198.9±250.5
High-dose	10	3228.7±232.5

Data presented as mean ± SD unless otherwise indicated

TABLE 3
Effect of astaxanthin on malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities in liver tissue

Group	n	MDA activity, nmol/g liver wet weight	SOD activity, nmol/g liver wet weight	GSH-Px, activity units/g liver wet weight
Blank control	10	110.2±46.4	33579.5±885.8	4088.0±703.2
Model	10	190.6±73.0*	27853.8±1566.4*	1950.0±1028.5*
Low dose	10	130.6±40.0†	31999.0±969.8†	2048.0±730.4
Medium dose	10	123.3±38.1†	31983.5±921.7†	2708.0±1461.7
High dose	10	104.4±42.3†	32595.6±984.2†	3524.0±810.9†

*Data presented as mean ± SD unless otherwise indicated. *Significant difference compared with the control group (P<0.01); †Significant difference compared with the control group (P<0.05)*

groups that received antaxanthin were significantly lower than in the control group (P<0.05). SOD activity in the model group was significantly lower than in the control group (P<0.05), and SOD activity of the three groups that received astaxanthin were significantly higher than in the control group (P<0.05). The GSH-Px activity in the model group was significantly lower than in the control group (P<0.05), and the GSH-Px activity in the high-dose group was significantly higher than in the model control group (P<0.05) (Table 3).

DISCUSSION

Astaxanthin (3,3′-hydroxy-β, β′-carotene-4, 4′-dione) is a type of short-chain antioxidant that has strong antioxidant properties. It can provide an electron or a radical to react with another radical, which can then absorb excess energy, making the radical transform to a nonactive or more stable compound, thereby interrupting the radical chain reaction process. The main activity of its antioxidant mechanism has several aspects (4-6):

1. Quenching singlet oxygen and scavenging oxygen-free radicals: astaxanthin has a strong ability to quench singlet oxygen capacity. Its molecular structure, which contains both hydroxyl and keto groups, may also promote keto-hydroxyl hydrogen transfer to peroxide radicals;
2. Stabilizing membrane structure and reducing membrane fluidity: astaxanthin contains polar ends, which act as bridge-like molecules across the cell membrane, which can increase its stability and mechanical strength, and can reduce membrane permeability, limiting penetration of intracellular oxidants and protecting cells from oxidative damage;
3. Increasing antioxidant enzyme activity: astaxanthin can act as an antioxidation system by activating the cells to decrease MDA production, reducing its concentration in the body, and increase SOD and GSH-Px activity, protecting cells from free radical-induced oxidative damage;
4. Reducing the oxidative damage to mitochondria: after hydrogen peroxide damage, the contents of MDA and NO increased, while the activities of GSH, SOD, ATP enzyme decreased in mitochondria. Astaxanthin can reverse these changes.
5. Inhibition of lipid peroxidation: astaxanthin inhibits unsaturated fatty-acid methyl ester peroxide, phosphatidylcholine protection from oxidation, delay time phosphatidylcholine single large bubble (liposome) peroxidation.

Astaxanthin extracted from *Haematoccus pluvialis* using supercritical CO_2 extraction technology, compared with synthetic astaxanthin, has more stable and secure advantages. The results of the present study demonstrate that SOD activity in the three groups that received astaxanthin were significantly higher than in the model control group. MDA activity in the three groups that received astaxanthin was significantly lower than in the model group. Finally, GSH-Px activity in the high-dose group was significantly higher than the model control group. According to the *Inspection and Assessment Standard for Health Food* (2003 edition), the results produced by astaxanthin extracted from *Haematoccus pluvialis* suggest that it produced antioxidant effects.

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