Intervention effect of fucoxanthin supplementation on cadmium-induced thyroid injury in mice

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Research

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Abstract

Background: The Intervention effect of fucoxanthin, which is reportedly a powerful antioxidant, on cadmium-induced thyroid damage in mice was evaluated.

Methods: Animals (N = 120) were divided into control group (given pure water, N=20) and CdCl-exposed group (given CdCl orally at a dose of 30 mg/kg body weight (bw)/day for 30 days, N=100). Besides, the CdCl-exposed group were divided into following 5 groups (N=20) to evaluate the intervention effect of fucoxanthin: 1) negative control group (NCG, animals were supplied with pure water); 2) positive control group (PCG, animals were supplied with 50 mg/kg bw/day thyroid tablets); 3) low fucoxanthin concentration group (F1, animals were supplied with 10 mg/kg bw/day fucoxanthin); 4)medium fucoxanthin concentration group (F2, animals were supplied with 25 mg/kg bw/day fucoxanthin); 5) high fucoxanthin concentration groups (F3, animals were supplied with 50 mg/kg bw/day fucoxanthin). A 14-day treatment was conducted for these animals. The levels of T4, T3, MDA, APX and CAT were measured, and the expression levels of Bax, Bcl-2, ERK1, ERK2, MEK1, elf2α, p-elf2α, GRP78 and GRP94 genes were determined using real-time reverse transcriptase-polymerase chain reactions (RT-PCR). In addition, tissue histopathology and ultrastructure were recorded and analyzed.

Results: We found that injection of cadmium chloride (CdCl) decreased blood T4 and T3 levels to 27.10 ng/ml and 837.74 pg/ml, respectively. In addition, CdCl intoxication induced oxidative stress, structural abnormalities and apoptosis in thyroid follicles. Our results showed that treatment of CaCl-exposed mice with 25-50 mg/kg bw/day fucoxanthin appreciably decreased oxidative stress and apoptosis induced by CdCl, and restored the microstructural and ultrastructural organisation of the thyroid gland towards normalcy. Compared with the negative control group, fucoxanthin treatment groups showed significantly upregulated T4 and T3 levels (52.17 ng/ml and 1669.18 ng/ml, respectively; P 0.05), relieved oxidative stress by decreasing Malondialdehyde (MDA) level and increasing Catalase (CAT) and Ascorbate Peroxidase (APX) levels, and increased apoptosis inhibition through inhibiting the ERK1/2 pathway and preventing endoplasmic reticulum stress in thyroid follicular epithelial cells.

Conclusion: Herein, our study provides evidence of the protective effects of fucoxanthin supplementation against thyroid damage, and suggests therapeutic potential of this pigment in cases of Cd intoxication and hypothyroidism.

Introduction

Among heavy metal pollutants, cadmium (Cd) is increasingly present in environment and biological systems (1–3). The Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) recognise Cd as extremely harmful to human health (4). However, due to economic forces, industrialisation and agricultural modernisation are accelerating, and Cd pollution of ecosystems is becoming increasingly serious. Globally, more than 22,000 tonnes of Cd compounds have accumulated in the environment, predominantly in soil. Plants growing in soils with high Cd contents absorb and enrich

the proportion of Cd, and when concentrations in soil reach 11 mg/kg, concentrations in rice can reach 1.19 mg/kg. Gradual accumulation in the food chain significantly amplifies the toxicity of Cd, and ultimately leads to health hazards for animals, plants and humans (5–8). Therefore, strategies for preventing further Cd contamination and controlling existing Cd pollution are crucial for human health and sustainable development.

Cd exerts toxicity by promoting oxidative damage and apoptosis in cells (9). For a long time, studies involved in the toxic mechanisms of Cd focused on oxidative injury in cells, which is considered the main mechanism. Accordingly, Cd-induced oxidative damage in cells and tissues, and consequent DNA damage have been shown in previous studies (10, 11). In addition, apoptosis following Cd poisoning can damage the hypothalamic-pituitary-thyroid (HPT) axis and affect the synthesis and secretion of thyroid hormones. He B *et al*. tested pituitary glands from Cd-poisoned chickens and found reduced antioxidant enzyme activities and increased mRNA expression of the apoptotic genes Fas and caspase-3. With increased Cd exposure time, the apoptotic index of the low-dose group increased more significantly than that of the high-dose group, although the specific mechanism remained to be further studied (12, 13). Zhu W *et al*. found that CdCl affected hormone secretions under conditions of adenohypophysis and induced apoptosis of adenohypophysis cells *in vivo* and *in vitro* (14). Similarly, Poliandri *et al*. also found that Cd could induce apoptosis and produce cytotoxic effects on adenohypophysis cells through the production of antagonistic oxidants (15).

Fucoxanthin is an oxygen-containing derivative of carotene, which belongs to the lutein family of carotenoids (16). Fucoxanthin is widely distributed in algae and invertebrate cells in the natural environment (17). In isodinoflagellates other than euglenophyta and xanthophyta and dinoflagellates, fucoxanthin is taken up as the main carotenoid, especially into algae from algophyta and diatoms, which are rich in fucoxanthin (18). Currently, sources, extraction and purification methods, biosynthetic pathways, physiological activities and metabolic activities of fucoxanthin have been investigated enthusiastically (19). Marine sources of fucoxanthin are extensive and this compound has various pharmacological activities and potential applications. Anti-inflammatory, anti-tumour, anti-obesity, antioxidant, anti-acne, anti-diabetes, anti-malaria and anti-lipid activities of fucoxanthin have been partially confirmed (20). Other potential physiological activities of fucoxanthin are being actively studied.

To further explore the physiological functions of fucoxanthin, expand its range of applications and reduce the harm of Cd pollutants in the body, we investigated the functional effects of fucoxanthin in restoring thyroid structure, promoting thyroid hormone synthesis, increasing antioxidant capacity and inhibiting apoptosis of thyroid follicular epithelial cells in this study, so as to evaluate the protective effects of fucoxanthin on the pathogenesis of CdCl-induced hypothyroidism.

Materials And Method

Materials

Tetraiodothyronine (T4) and triiodothyronine (T3) ELISA kits were purchased from Nanjing Jiancheng Bioengineering Institute. Expression levels of *Bax*, *Bcl-2*, *ERK1*, *ERK2*, *MEK1*, *elf2a*, *p-elf2a*, *GRP78* and *GRP94* genes were determined using transcription-polymerase chain reaction technique (Shanghai bioengineering Limited by Share Ltd). Thyroid tablets were purchased from Biochemical Pharmaceutical Factory (Laiyang city, shandong province, China). Analytical grade CdCl, fucoxanthin and other chemicals were purchased from Solarbio Science & Technology Co., Ltd (Beijing, China).

Experimental Animals

The specific pathogen-free (SPF) adult male Kunming mice (22-26g) were obtained from the institute of drug inspection of Qingdao. The animals were fed with a experimental mice diet (moisture ≤10%, crude protein ≥18%, crude fat ≥4%, crude fiber ≤5%, crude ash ≤8%) and pure water (Jiangshan wahaha hongzhen drinking water co., LTD., Quzhou City, zhejiang province, China). The animals were kept in laboratory animal houses, under a 12h/12h light-dark cycle conditions (lights on at 7: 00, off at 19: 00) with temperature 22°C-25°C and 50% relative humidity. The animals were adapted to laboratory animal houses conditions for 1 week prior to experiments.

All experimental procedures were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Ethical Committee and the protocols were approved by the Committee on the Ethics of Animal Experiments of the Institute of Oceanology, Chinese Academy of Sciences, Shandong, China. All efforts were made to minimise suffering of the animals. The animals were anesthetised using ether before blood sampling. The laboratory animal quality certificate code is scxk20140001.

Experimental Design

Animals (N = 120) were divided into control group (N=20) and CdCl-exposed group (N=100). The blank control group (BCG) were given pure water only, whereas animals of the CdCl-exposed group were given CdCl orally at a dose of 30 mg/kg body weight (bw)/day for 30 days (21). Fucoxanthin was previously administered orally at doses of 1-100 mg/kg bw/day and significant antioxidant effect and health benefits were demonstrated in various organisms (22-25). Herein, fucoxanthin was administered at doses of 10, 25 and 50 mg/kg bw/day. The CdCl-exposed group were divided into following 5 groups (N=20) to evaluate the intervention effect of fucoxanthin supplementation on cadmium-induced thyroid injury in mice: 1) negative control group (NCG, animals were supplied with pure water); 2) positive control group (PCG, animals were supplied with 50 mg/kg bw/day thyroid tablets); 3) low fucoxanthin concentration group (F1, animals were supplied with 10 mg/kg bw/day fucoxanthin); 4)medium fucoxanthin concentration group (F2, animals were supplied with 25 mg/kg bw/day fucoxanthin); 5) high fucoxanthin concentration groups (F3, animals were supplied with 50 mg/kg bw/day fucoxanthin). A 14-day treatment was conducted for these animals.

Animal Sample Collection

After 14-day treatment, these animals were sacrificed and thyroid tissue and blood samples were collected immediately. These samples were kept in 10% neutral-buffered formalin, 2.5% neutral-buffered glutaraldehyde and ultra low temperature refrigerator for further analysis.

Estimation of Malondialdehyde (MDA), Ascorbate Peroxidase (APX), Catalase (CAT), T4 and T3 Levels

Immediately before sacrifice, blood samples were collected from animals under ether anaesthesia. MDA, CAT and APX levels were measured spectrophotometrically. T4 and T3 levels were assayed using enzyme-linked immuno assays. T4, T3, MDA, APX and CAT levels were expressed in ng/ml, pg/ml, ng/ml, μmol/min/g and nmol/min/mL, respectively.

Total mRNA Extraction and Real-Time Quantitative PCR

Total RNA from thyroid tissues was prepared using Trizol reagent. Subsequently, mRNA was reverse-transcribed to cDNA using oligonucleotide dT primers according to the manufacturer's instructions. RT-PCR analysis were then performed using Platinum Taq polymerase and 4S Red Plus nucleic acid stain. Primer sequences were designed using Primer Premier 5.0 software (Table 1).

Microstructures and Ultramicrostructures of Thyroid Tissues

After sacrifice, mice thyroid tissues were removed immediately and fixed in 10% neutral-buffered formalin or 2.5% neutral-buffered glutaraldehyde at 4℃. Tissues that were fixed in formalin were made into paraffin sections, stained with haematoxylin and eosin, and tissue histopathology were recorded and analyzed with a optical microscope(Olympus, BX63). Tissues that were fixed in glutaraldehyde were used to analyze the ultrastructure under an electron microscope (Hitachi, H-9500).

Statistical Analysis

Statistical analysis were performed using SPSS 22.0 software. Data were expressed as means ± standard deviations (SD). Differences between two groups were tested by Student's t-test, while multiple groups were examined by one-way analysis of variance (ANOVA)$P < 0.05$ was considered as a significant difference.

Results

Changes in Blood T4 and T3 Levels

CdCl treatments significantly decreased T4 and T3 levels. Compared with the blank control group, CdCl exposure in the NCG decreased T4 levels by 48.07% ($P < 0.05$) and T3 levels by 45.7% ($P < 0.05$)(Table 2).

As shown in Table 2, treatments with thyroid tablets increased T4 and T3 levels compared with those in the NCG ($P < 0.05$). Compared with the NCG, the treatments with fucoxanthin countered the Cd-induced decreases in T4 and T3 levels ($P < 0.05$), and there were no significant differences between the blank control group and F2 group ($P > 0.05$), indicating that supplementation with fucoxanthin at 25 mg/kg

bw/day completely restored thyroid T4 and T3 levels, with better protection against Cd than that of thyroid tablets.

Microstructures of Thyroid Glands

Optical microscope observation results showed regular round or oval thyroid follicles in the blank control group, and follicular epithelial cells formed simple flat or cubic epithelia (Fig. 1A). Compared with the NCG, heights of thyroid follicular epithelial cells were increased significantly by CdCl treatments (Table 3), and thyroid follicular epithelial cells were enlarged and hyperplastic (Fig. 1B). We also observed decreased average thyroid follicular areas (Table 3) and irregular shapes of thyroid follicles in CdCl treated animals. Treatments with fucoxanthin markedly inhibited CdCl-induced damage in thyroid follicles and restored follicular sizes. Moreover, colloidal materials and epithelial tissue architectures resembled those in the blank control group (Fig. 1D-1F).

Ultrastructures of Thyroid Follicular Epithelial Cells

Compared with thyroid follicular epithelial cells from mice of the blank control group, those in the NCG showed irregularly shaped nuclei (Fig. 2B-a), disappearing mitochondrial crests (Fig. 2B-b), reticular expansion (Fig. 2B-c), thickened and lengthened microvilli (Fig. 2B-d, Table 4), nuclear vacuoles (Fig. 2B-e) and chromatin condensation.

Fucoxanthin and thyroid tablet treatments inhibited CdCl-induced damage to thyroid follicles and restored mitochondrial integrity (Fig. 2C and 2D-b), chromatin and nuclear states (Fig. 2C-a, e and 2D-a, e) and reticular (Fig. 2C-c, 2D-c) and microvilli (Fig. 2C-d, 2D-d) morphologies, which resembled those in cells of mice of blank control group (Table 4). Fucoxanthin treatments and thyroid tablets exerted similar protective effects (Fig. 2C) against CdCl-induced damage to ultrastructures of thyroid follicles.

Effects of Fucoxanthin on antioxidant in Cd-Poisoned Mice

MDA contents in blood samples from NCG mice were significantly increased, compared with those in blank control group, and CAT and APX levels were significantly decreased ($P<0.05$). Fucoxanthin treatments significantly improved redox states compared with those in mice of the NCG. In the high concentration fucoxanthin group, CAT, APX and MDA levels were restored to those observed in blank control group, indicating that fucoxanthin exerted better protective effects against CdCl-induced oxidative stress than thyroid tablets (Fig. 3).

Molecular Mechanisms Underlying the Protective Effects of Fucoxanthin Against Cell Death

Expression levels of Bax, Bcl-2, ERK1, ERK2, MEK1, elf2α, p-elf2α, GRP78 and GRP94 mRNA are shown in Table 5. Compared with the blank control group, relative mRNA levels of MEK1, ERK1, p-elf2α and GRP94 were strongly upregulated in the NCG ($P < 0.05$) and these transcripts were strongly downregulated in the middle and high-dose fucoxanthin treatment groups compared with those in the NCG ($P < 0.05$). Relative mRNA expression levels of GRP78 did not differ significantly among experimental groups ($P > 0.05$). Yet

Bcl-2 expression levels were significantly downregulated in the NCG compared with those in the blank control group and the high-dose fucoxanthin treated group ($P < 0.05$). Finally, there were no significant differences in Bax, Bcl-2, ERK1, MEK1 and GRP94 mRNA expression levels between the high-dose fucoxanthin group and blank control group ($P > 0.05$).

Discussion

Numerous studies have demonstrated the toxic mechanisms of Cd, including interactions between Cd and metallothionein, relationships between Cd and oxidative damage and between Cd and calcium, and abnormal gene expression levels. Among these mechanisms of toxicity, Cd-induced oxidative damage is the most abundantly characterised. Studies showed that Cd exposure could impair organ functions of rats, with abnormal microstructures and ultrastructures and clear morphological alterations to organelles such as mitochondria and lysosomes (26). These observations are consistent with our experiments, which reflect enhanced lipid peroxidation and changes to intracellular antioxidant systems (27).

Fucoxanthin is one of numerous carotenoids, which generally have antioxidant activity. Some studies attribute the antioxidant activity of carotenoids to the presence of oxygen atoms that can accept or donate electrons in redox reactions. Fucoxanthin has six oxygen atoms on its propylene structure, which is highly sensitive to free radicals and has strong antioxidant activity (22). Accordingly, the antioxidant activity of fucoxanthin is mainly reflected by free radical scavenging activity, which is commonly assessed using the radicals 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azobis-2-methyl-propanimidamide, dihydrochlor (AAPH) and 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (18, 23). Although antioxidant activities of fucoxanthin have been confirmed, no specific mechanisms have been identified and further exploration is required.

In present experiments, the protective effects of fucoxanthin against Cd-induced damage to thyroid functions were showed, which may be mediated by the antioxidant properties of fucoxanthin. Short-term Cd exposures through drinking water impose stress on thyroid follicular epithelial structures, as reflected in our morphological and metabolic observations of damage in thyroid tissues originated from Cd-intoxicated mice.

Oxidative stress due to Cd toxicity causes DNA damage in various cell types, leading to abnormal metabolism and structural damage (2). Follicle epithelial cell necrosis, hyperplasia, follicular thickening and deformation and atrophy and cavitation of the entire thyroid were observed after injury caused by CdCl (Fig. 1). We also observed a loss of clarity of the thyroid cell membrane border, with irregular shrinking and wavy nuclei. In contrast, intake of 25–50 mg/kg bw/day fucoxanthin almost completely reversed Cd-induced thyroid microstructural damage.

Previously, Cd-mediated damage was found to be correlated with apoptosis related gene expression (26). In the present CdCl-treated mice, we found increased ERK1, MEK1, p-elf2α and GRP94 expression and decreased Bcl-2 expression, suggesting that Cd may mediate apoptosis of thyroid follicular epithelial cells through activating the ERK1/2 pathway and inducing endoplasmic reticulum stress (28). At medium

and high concentrations, fucoxanthin significantly inhibited ERK1, MEK1, p-elf2α and GRP94 expression, indicating that fucoxanthin can mitigate Cd-induced apoptosis through inhibiting the ERK1/2 pathway and preventing endoplasmic reticulum stress.

Conclusion

In conclusion, our results suggest that fucoxanthin can prevent Cd-induced hypothyroidism. Under conditions that lower T4 and T3 expression levels, fucoxanthin treatments can maintain T4 and T3 levels in the blood by protecting thyroid follicular epithelial cells against oxidative stress and inhibiting CdCl-induced apoptosis. These findings warrant further consideration of the therapeutic potential of fucoxanthin as a choice of treatment for hypothyroidism.

Abbreviations

AAPH
2,2'-azobis-2-methyl-propanimidamide, dihydrochlor
ABTS
2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
APX
Ascorbate Peroxidase
CAT
Catalase
Cd
Cadmium
CdCl
Cadmium Chloride
DPPH
1, 1-diphenyl-2-picrylhydrazyl
FAO
Food and Agriculture Organisation
HPT
Hypothalamic-pituitary-thyroid
MDA
Malondialdehyde
NCG
Negative Control Group
PCG
Positive Control Group
RNA
Ribonucleic Acid

SD
Sprague Dawley
SD
Standard Deviations
T3
Triiodothyronine
T4
Tetraiodothyronine
TBA
Thiobarbituric Acid
WHO
World Health Organisation

Declarations

Ethics approval and consent to participate：All experimental procedures were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Ethical Committee and the protocols were approved by the Committee on the Ethics of Animal Experiments of the Institute of Oceanology, Chinese Academy of Sciences, Shandong, China.

Consent for publication: Not applicable.

Availability of data and material：The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions：HYY and RGX contributed to the conception and design of the study; SL contributed to the acquisition of data; SL and PCL performed the experiments; PCL contributed to the analysis of data; HYY wrote the manuscript; All authors reviewed and approved the final version of the manuscript.

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Tables

Table 1 Housekeeping gene (β-actin) primer sequence

Gene	Primer sequence (5’-3’)
β-actin	Forward: GTGCTATGTTGCTCTAGACTTCG
	Reverse: ATGCCACAGGATTCCATACC
Bax	Forward: AGGATGCGTCCACCAAGAA
	Reverse: CAAAGTAGAAGAGGGCAACCAC
Bcl-2	Forward: TGTGGCCTTCTTTGAGTTCG
	Reverse: TACCCAGCCTCCGTTATCCT
ERK1	Forward: GGCTTTCTGACGGAGTATGTG
	Reverse: GGGGAACCCAAGATACCTAGA
ERK2	Forward: GGTTGTTCCCAAATGCTGAC
	Reverse: GCTCATCACTTGGGTCATAATACT
MEK1	Forward: TGACGCAGAAGCAGAAGGTG
	Reverse: TGAAGACCACTCCACCGTTG
eIf2α	Forward: TAATCAATGTCGCTAACAAGGG
	Reverse: AAGTTGTAGGTTAGGCGTCCC
p-eIf2α	Forward: TTCTACAGAAACCATGCCCAT
	Reverse: TTGATAACTGCCATAGCCTGAT
GRP78	Forward: GCCAACTGTAACAATCAAGGTCT
	Reverse: TCAGGTGTCAGGCGGTTTT
GRP94	Forward: AGGTGTTGTGGATTCCGATGA
	Reverse: AGTTTAGCAAGCCGTGTTCG

Table 2 Changes in T4 and T3 levels after CdCl treatment with fucoxanthin and thyroid tablet supplementation

	Blank control group	NCG	PCG	F1	F2	F3
T4 (ng/ml)	52.17±3.56	27.10±3.28*	53.50±3.57#	40.47±2.45#	52.17±4.96#	52.02±5.60#
T3 (pg/ml)	1542.35±133.19	837.74±181.20*	1143.07±330.19#	1476.59±238.46#	1669.18±82.57#	1006.85±195.77#

Note: compared with blank control group, *P<0.05; compared with NCG, #P<0.05.

表中标记已经修改，按照SCI的惯例在数据的右上角标记角标*，#，以表示和某组比较P<0.05，目前根据作者的分组，NCG统一和空白对照组比较，PCG、F1、F2和F3组统一和NCG相比。

Table 3 Changes in the height of thyroid follicular epithelial cells and average thyroid follicular area after treatment with fucoxanthin and thyroid tablet supplementation

	Blank control group	NCG	PCG	F1	F2	F3
Epithelial cell height (μm)	3.32±0.41	6.02±0.38*	4.19±0.24#	3.49±0.32#	3.22±0.45#	3.53±0.43#
Thyroid follicular area (μm²)	2495.9±263.4	1843.0±230.6*	2262.9±389.2#	2396.3±390.9#	2430.5±298.3#	2351.5±596.2#

Note: compared with blank control group, *P<0.05; compared with NCG, #P<0.05.

Table 4 Average length of microvilli in thyroid follicular epithelial cells

	Blank control group	NCG	PCG	F3
Microvilli length (μm)	0.434±0.102	3.332±0.871*	0.485±0.095#	0.445±0.079#

Note: compared with blank control group, *P<0.05; compared with NCG, #P<0.05.

Table 5 Effect of fucoxanthin on the expression of thyroid function-associated genes in the cadmium thyroid-damaged mouse model

	Bax	Bcl-2	ERK1	ERK2	MEK1	eIf2α	p-eIf2α	GRP78	GRP94
Blank control	26.24 ±0.23	28.86 ±0.50	24.11 ±0.40	24.27 ±0.45	27.47 ±0.37	24.84 ±0.11	26.28 ±0.21	20.37 ±0.11	20.44 ±0.43
NCG	26.61 ±0.29	27.14 ±0.44b*	25.91 ±0.41*	24.51 ±0.40	28.64 ±0.20*	24.73 ±0.17	27.90 ±0.12*	20.71 ±0.33	22.50 ±0.44*
PCG	27.00 ±0.25	27.21 ±0.70	24.70 ±0.29#	24.54 ±0.24	27.51 ±0.48#	24.94 ±0.17	27.00 ±0.35#	20.54 ±0.29	20.66 ±0.34#
F1	26.44 ±0.43	27.55 ±0.29	24.07 ±0.33#	24.75 ±0.39	28.49 ±0.51	24.91 ±0.52	27.75 ±0.22	21.05 ±0.98	20.57 ±0.45#
F2	25.98 ±0.30#	27.85 ±0.35	24.04 ±0.38#	24.31 ±0.19	27.28 ±0.21#	24.53 ±0.10	26.78 ±0.25#	20.41 ±0.51	20.36 ±0.24#
F3	25.64 ±0.13#	28.72 ±0.49#	24.14 ±0.25#	24.97 ±0.28	27.70 ±0.33#	24.27 ±0.39	26.80 ±0.38#	20.56 ±0.43	20.37 ±0.42#

Note: compared with blank control group, *P□0.05; compared with NCG, #P□0.05.

Figures

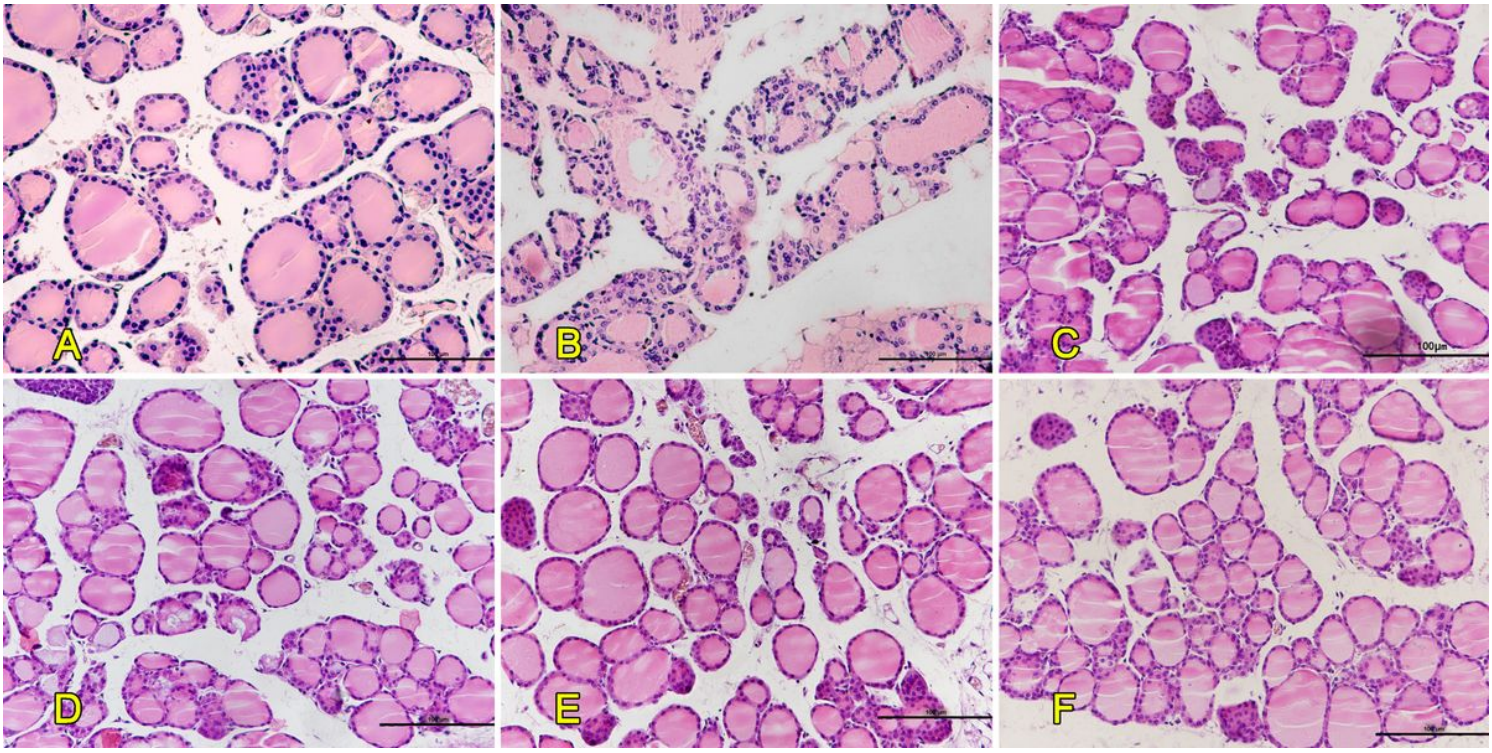

Figure 1

Histological changes after treatment with fucoxanthin and thyroid tablet supplementation. (A) BCG, (B) NCG, (C) PCG, (D) F1, (E) F2 and (F) F3.

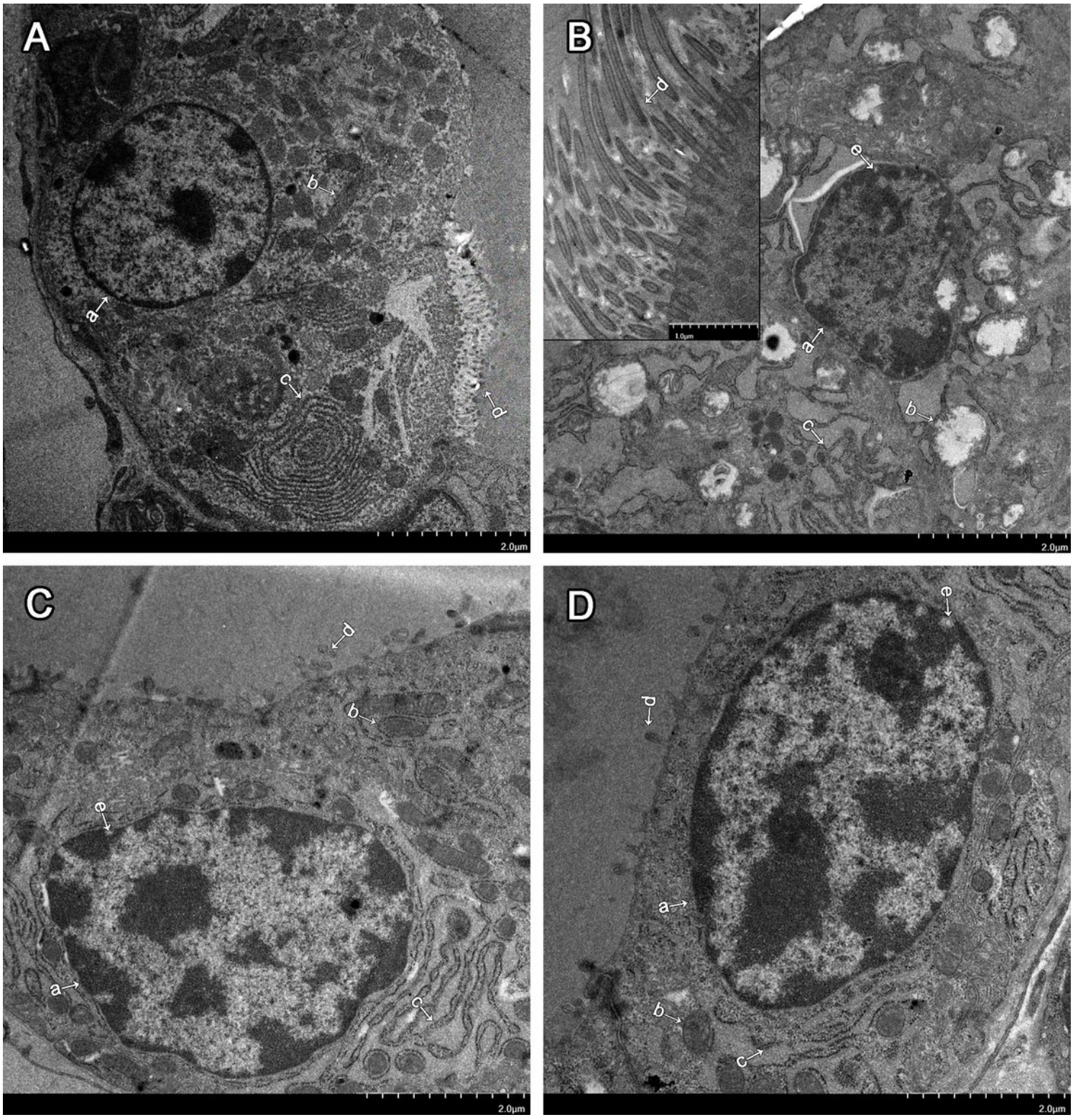

Figure 3

Ultrastructural changes after treatment with fucoxanthin and thyroid tablet supplementation. (A) BCG, (B) NCG, (C) PCG, (D) F3. a: nucleus, b: mitochondrion, c: endoplasmic reticulum, d: microvilli, e: nuclear vacuoles.

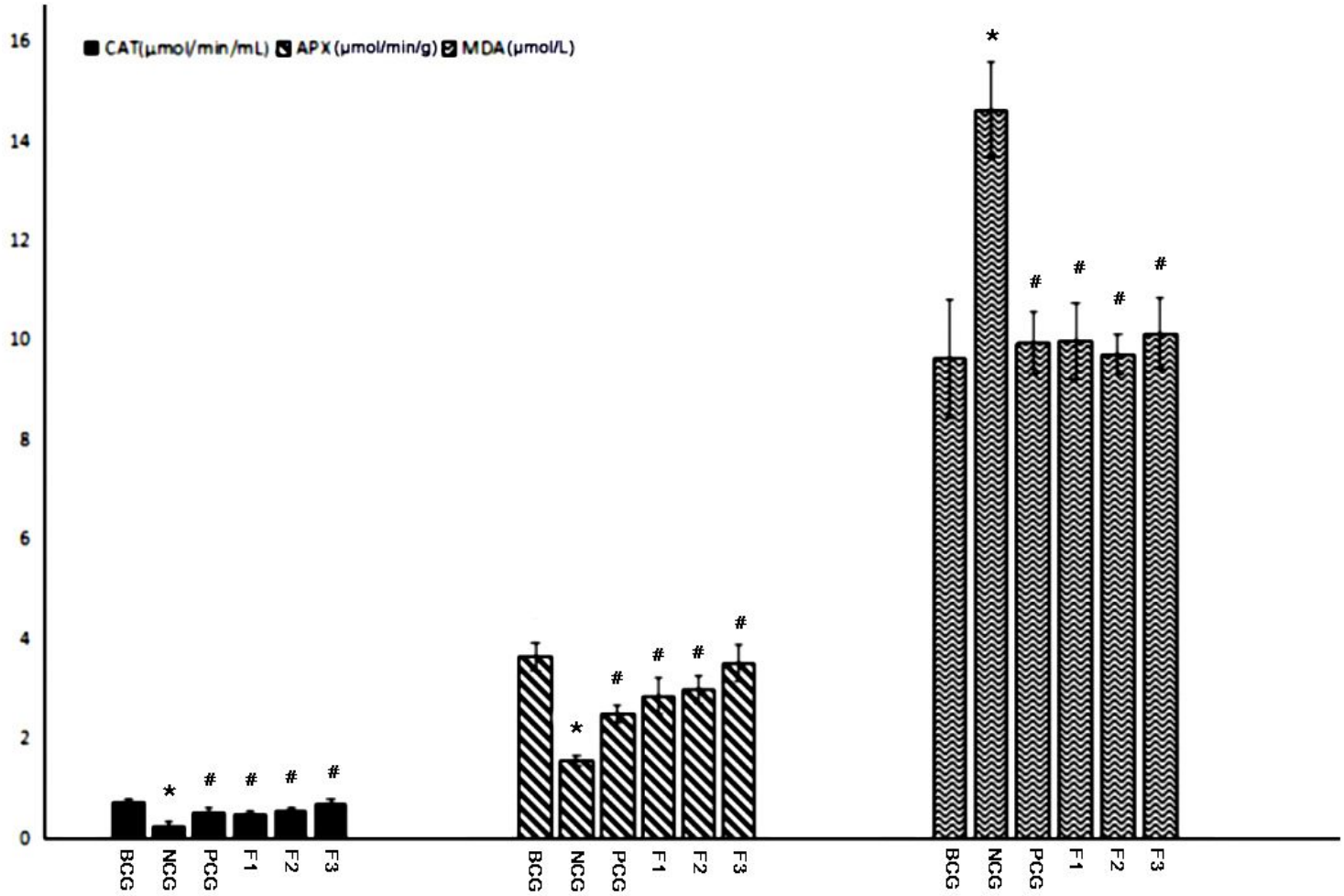

Figure 5

Beneficial effects of fucoxanthin on oxidative stress in cadmium poisoning mouse. The data were expressed as means ± SD. *P□0.05 vs. blank control group; #P□0.05 vs. NCG.