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# Inhibition of inflammation by astaxanthin alleviates cognition deficits in diabetic mice

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## HIGHLIGHTS

- Cognition deficits and inflammation were ameliorated by astaxanthin in diabetic mice.
- Astaxanthin protected neurons against hyperglycemia injury.
- Astaxanthin inhibited NF-κB nuclear translocation reducing neuronal inflammation/death.

## ARTICLE INFO

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## ABSTRACT

Neurons in the hippocampal and cortical functional regions are more susceptible to damage induced by hyperglycemia, which can result in severe spatial learning and memory impairment. Neuroprotection ameliorates cognitive impairment induced by hyperglycemia in diabetic encephalopathy (DE). Astaxanthin has been widely studied in diabetes mellitus and diabetic complications due to its hypoglycemic, antioxidant and anti-apoptotic effects. However, whether astaxanthin can alleviate cognition deficits induced by DE and its precise mechanisms remain undetermined. In this study, DE was induced by streptozotocin (STZ, 150 mg/kg) in ICR mice. We observed the effect of astaxanthin on cognition and investigated its potential mechanisms in DE mice. Results showed that astaxanthin treatment significantly decreased the latency and enhanced the distance and time spent in the target quadrant in the Morris water maze test. Furthermore, neuronal survival was significantly increased in the hippocampal CA3 region and the frontal cortex following treatment with astaxanthin. Meanwhile, immunoblotting was used to observe the nuclear translocation of nuclear factor-kappaB (NF-κB) p65 and the expression of tumor necrosis factor-α (TNF-α) in the hippocampus and frontal cortex. The results indicated that astaxanthin could inhibit NF-κB nuclear translocation and downregulate TNF-α expression in the hippocampus and frontal cortex. Overall, the present study implied that astaxanthin could improve cognition by protecting neurons against inflammation injury potentially through inhibiting the nuclear translocation of NF-κB and down-regulating TNF-α.



## 1. Introduction

Diabetic encephalopathy (DE), which is characterized by cognitive deficits, involves dysfunction of the nervous system caused by hyperglycemia and neuronal injury [1,2]. DE is one of the most common diabetic complications in both type 1 diabetes mellitus and type 2 diabetes mellitus [3–5]. The present study shows that the hippocampus and frontal cortex are the important pathological centers in diabetes-induced behavior deficit development and progression [6,7]. It has been reported that the nuclear translocation of nuclear factor-κB (NF-κB) aggravates neurocyte injury in the brain [8]. Furthermore, NF-κB is associated with increased expression of tumor necrosis factor-α (TNF-α) in the brain. Therefore, diabetes associated cognitive deficits could possibly be actively regulated by intervening the NF-κB signaling pathway [9,10].

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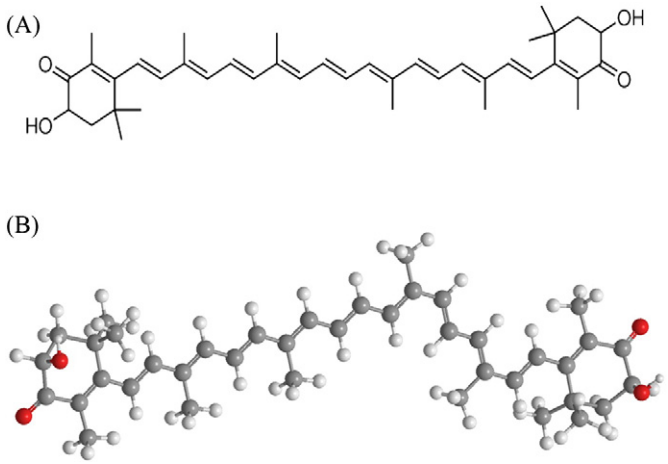


**Fig. 1.** The chemical structure of astaxanthin. (A) Molecular structural formula. (B) Ball-and-stick model.

It is not advisable to alleviate DE by injecting insulin in the clinic because applying insulin can result in the side-effect of hypoglycemia [11,12]. An increasing number of studies show that some new therapies for DE prevention and treatment in experimental DE can not only ameliorate DE cognitive deficits but also decrease blood glucose. In addition, the alternative medicines are suitable for long-term using because their adverse effects are minimal [13–16]. Astaxanthin (Fig. 1) is a ketocarotenoid red pigment which is found in red yeast, microalgae and marine animals [17]. Astaxanthin possesses pharmacological functions such as anti-oxidative and anti-inflammatory, anti-apoptotic, anti-cancer, and immunomodulating effects [18–22]. Recent reports have indicated that astaxanthin can protect against the harmful effects attributed to high glucose exposure and it has been used to reduce diabetic complications in animal models [23,24]. Moreover, due to its ability to traverse the blood–brain-barrier, astaxanthin has neuroprotective properties and anti-aging potential and can improve cognition [25,26].

However, whether chronic administration of astaxanthin could alleviate behavior abnormalities in an experimental model of DE and its possible mechanisms are still unknown. Based on previous studies, we hypothesized that astaxanthin could ameliorate cognitive deficits in DE mice by decreasing blood glucose and inhibiting neuronal injury. Our study sought to determine whether treatment with astaxanthin could improve cognitive function and protect hippocampal and frontal cortical neuron morphological characteristics from damage induced by hyperglycemia in DE mice. Furthermore, we aimed to determine the influence of astaxanthin on the nuclear translocation of NF-κB and its relative protein TNF-α in the hippocampus and frontal cortex and whether the astaxanthin induced attenuation of cognitive deficits in DE mice can be attributed to inhibition of the NF-κB signaling pathway.

## 2. Materials and methods

### 2.1. Materials

Astaxanthin (sc-391006, purity: 99%) was purchased from Santa Cruz Biotechnology. Streptozotocin (STZ, s0130-500 MG) was obtained from Sigma. A glucometer and glucose test strips were purchased from Johnson & Johnson Ltd. β-Actin and NF-κB were purchased from Santa Cruz Biotechnology. TNF-α antibodies were obtained from Abcam Biotechnology. The secondary antibodies and other chemicals employed in our experiments were purchased from Sigma unless otherwise indicated.

### 2.2. Animals

Adult male ICR mice weighing 18–20 g were obtained from the Shanghai Experimental Animal Center (Chinese Academy of Sciences). Mice were maintained in standard housing conditions (room temperature: 25 ± 1 °C and humidity: 55–65%) on a 12 h light/12 h dark cycle and had ad libitum access to food and water. All experimental procedures were conducted according to the Provision and General Recommendation of the Chinese Laboratory Association.

### 2.3. Assessment of diabetes and drug administration

After mice were fasted for 12 h, a single dose of STZ (150 mg/kg) dissolved in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally into the mice to induce diabetes [27]. Age-matched control mice received an equal volume of citrate buffer and were designated as the control group (Con, n = 12); animals receiving only astaxanthin were designated as the N + AST group (n = 12). The diabetes model was verified 72 h after STZ injection; blood samples were collected through the tail vein and glucose levels were measured by a glucometer. Animals with a fasting blood glucose level of more than or equal to 16.7 mmol/L

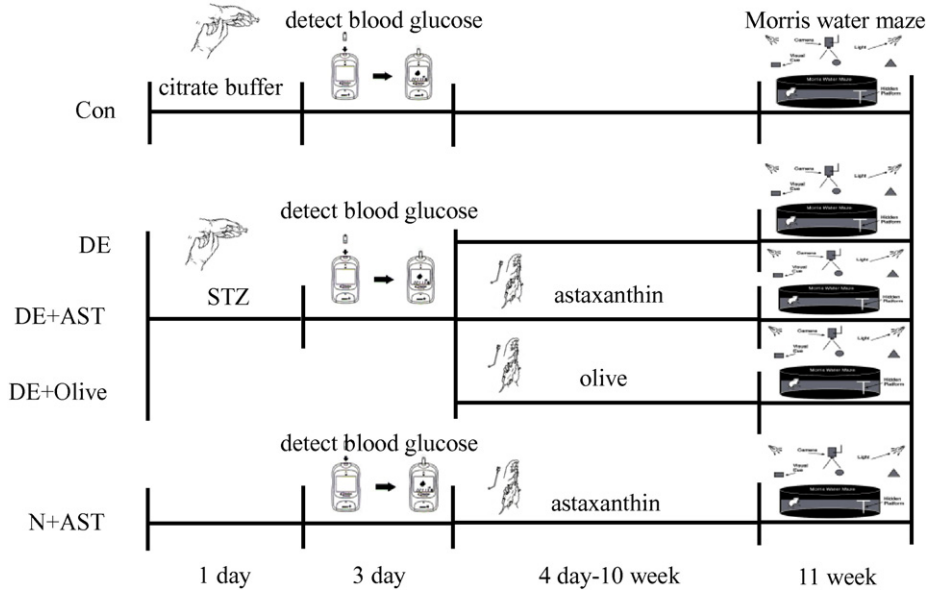


**Fig. 2.** Schematic overview of the present experimental procedure.