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Protective Effect of Astaxanthin on Prenatal Bacterial Lipopolysaccharide Exposed Behavioral Deficits in Adult Mice

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Abstract

Background: Prenatal maternal lipopolysaccharide (LPS) exposure causes behavioral deficits in adulthood. LPS-exposure cause oxidative damage and cytokines production. In contrast, astaxanthin (Ast) is a carotenoid antioxidant that shows protective effects through its antioxidant capacity.
Objectives: This study investigates the effect of prenatal treatment with astaxanthin on the behavioral deficit (including sexual, depressive, and anxiety-like behavior) caused by prenatal maternal LPS in adult male offspring.
Methods: Pregnant mice were randomly divided into 4 groups: (1) control, (2) LPS: injecting with LPS (20 μg/kg, sc.) on gestation day 11, (3) Ast: receiving astaxanthin (4 mg/kg for 3 days, i.p.) on 11 - 13th gestation day, (4) LPS+Ast: injecting with LPS (20 μg/kg, sc.) on gestation day 11 and receiving astaxanthin (4 mg/kg for 3 days, i.p.) on 11 - 13th gestation day. Then in each group, 23 day old male offspring (3 and 12 male children from each mother and group, respectively) were separated from mothers and then the sexual, depressive and anxiety-like behaviors were examined in adult male mice.
Results: Findings showed that prenatal LPS-exposed mice had more anxiety and spent less time in open arms of the elevated plus-maze test ($P < 0.05$). In addition, it decreased sexual behaviors, the amount of which was significant in the number of sniffing, following behaviors ($P < 0.01$). Also, there was no significant difference between different groups in the forced swimming test ($P < 0.05$). On the other hand, prenatal treatment with astaxanthin significantly elevated the percentage of open arm time and open arm entry, without altering in locomotor activity ($P < 0.05$). Also, it significantly increased sexual behavior in Ast and LPS+Ast groups ($P < 0.01$).
Conclusions: The obtained results suggest that prenatal maternal exposure to LPS impaired several aspects of male sexual behavior and resulted in behavioral deficits in adulthood, while astaxanthin has an antianxiety effect and improves the deficits of sexual behavior presumably via its antioxidant property.

Keywords: Lipopolysaccharide, Astaxanthin, Sexual Behavior, Depressive-Like Behavior, Anxiety-Like Behavior

1. Background

Lipopolysaccharide (LPS) is a bacterial cell wall toxic component in gram-negative strains and is widely used to create bacterial infection models (1). Infection perpetually exposes humans to low levels of LPS (2). Recent results of various studies have demonstrated that exposure to LPS at prenatal period could cause structural damage and dysfunction for cerebral cortex and hippocampal neurons, therewith inducing autism, schizophrenia, anxiety and cerebral palsy at adulthood (3, 4). However, there is little information about the effects of maternal exposure to LPS during pregnancy on reproductive performance in offspring.

During pregnancy, LPS has been widely used to investigate the behavioral and histological abnormalities in the adult offspring in rodents (5).

Maternal exposure to LPS, before the birth of child, enhances the pro-inflammatory cytokines. These cytokines can cross the placental barrier and intervene with the early stages of brain development.

Development-related abnormality of offspring is revealed at the behavioral level and during the adult stage of life. It has been shown that prenatal exposure to LPS increases the level of lipid peroxidation, nitric oxide and also

deplete the glutathione level in the placenta, embryo and maternal liver (6).

On the other hand, astaxanthin (Ast) is a carotenoid antioxidant that its antioxidant capacity is 100 - 500 times greater than α-tocopherol (7). Kuhn et al. in 1938 discovered Ast in lobsters. Nowadays researches on Ast are on the rise, because of the persistent request for natural Ast in the progression of the human health world (8). The main and richest source of Ast for human utilization is microalgae Haematococcus pluvialis. It has the highest potential to provide Ast among proposed sources (9). Astaxanthin is able to cross the blood-brain barrier, and its neuroprotective properties are through the restoration of antioxidant enzymes. In addition, treatment with astaxanthin improves depressive-like behavior by diminishing IL-β and IL-6 levels in the frontal cortex (10).

Today, it is well identified that the environment of gestation can have enduring effects on an individuals' life and health span and also have long-lasting effects on the future generation through potential epigenetic changes. Moreover, it's known that the influences of additional developments such as toxins, nutrition and stress can affect reproductive development (11).

2. Objectives

In the present study, we assume that astaxanthin, as an antioxidant, may improve the impaired brain function by scavenging oxidative stress in the brain. Therefore, this research investigates the protective effect of astaxanthin against negative effects of prenatal bacterial lipopolysaccharide exposure on depressive and anxiety-like behaviors and also sexual behaviors in adult male offspring in NMRI mice.

3. Methods

3.1. Animals

This experimental study with a randomized block design was done in the Physiology Laboratory of the Science and Research branch of Islamic Azad University, Tehran, Iran, in 2018. For this study, adult female NMRI mice weighing 30 g were purchased from the Pasteur Institute of Iran. Animals were housed in a room with controlled conditions including temperature range of 22 $\pm$ 1°C, humidity 40 - 45%, and luminosity with 12 hours light and 12 hours dark. Mice had free access to water and food. Feeding, maintenance protocols and animal experiments were carried out according to the ethics committee of the Science and Research branch of Islamic Azad University (ethical code: IR.IAU.SRB.REC.1398.081). The study was performed after a week of environmental adaptation.

3.2. Experimental Design

Female mice after mating and ensuring pregnancy (by checking the vaginal plaque one day after mating) were randomly divided into 4 groups (8 pregnant mice in each group): (1) healthy control group (Control); receiving single dose of PBS as LPS solvent and 5% DMSO at the concentration of 0.2 mM as solvent of astaxanthin. (2) LPS group (LPS): receiving single dose of LPS (Sigma-Aldrich, St Louis, MO) (20 μg/kg, sc.) on gestation day 11. (3) Astaxanthin group (Ast); receiving astaxanthin (Sigma-Aldrich, St Louis, MO) at a dose of 4 mg/kg for 3 days, intraperitoneally (on gestation day 11 - 13). (4) LPS + Astaxanthin group (LPS+Ast): receiving astaxanthin at a dose of 4 mg/kg for 3 days (on gestation day 11 - 13) and single dose of LPS (20 μg/kg) on gestation day 11. Then in each group, 23 days old male offspring (3 and 12 male child from each mother and group, (respectively) were separated from mothers and after the maturity/puberty (at 60 days old), the following tests are performed on them: anxiety, depression and sexual behavior test.

3.3. Behavioral Test

3.3.1. Elevated Plus Maze (EPM)

This test was used to measure the anxiety in mice. The EPM contained two open and two closed arms (35 $\times$ 35 cm) and a center platform (5 $\times$ 5 cm) elevated 50 cm above the floor. The maze was situated in a test room that was completely alike for all mice in terms of sound, temperature, light and objects, and this experiment was carried out in full silence. At the time of the test, the mice were first placed on one of the open arms. Then the time spent in the open arm as well as the closed arm and the number of entered to the open arm as well as the closed arm were recorded using a ceiling camera for 5 minutes. In order to analyze the open arm activity, for each mice, the percentage of open arm entry (OAE) and open arm time (OAT), as well as the rate of motor activity of the mice (locomotor activity), which is equivalent to the number of entries to different arms, were also calculated (12).

3.3.2. Forced Swimming Test

The forced swim test was used to test for depression-like behavior in mice. In this test mice were put into a glass cylinder with 25 cm height and 12 cm diameter that

filled to a 15 cm depth with water at room temperature. After Discontinue the swimming behavior by mice, the time of floating behavior that conventionally called Immobility, where the animal remains almost immobile and with its head above water, was measured with chronometer and used as a parameter to analyze "hopelessness" and thus depression-like behavior. An increase in immobility time equivalent to depression and its reduction was evaluated as the effectiveness of antidepressant treatment. Test duration was 5 minutes (12).

3.3.3. Sexual Behaviors Test

To examine the sexual behavior of male mice, a special protocol was used that was developed by Johansson and his colleagues in 2008. Receptive female mice, prepared by subcutaneously injection of the 50 μg estradiol benzoate and 500 μg progesterone, 48 and 6 hours respectively before starting examination, were used to test male sexual behaviors (sniffing, following, mounting, coupling/ejaculation) in such a way that males were placed in the female's cage; then mentioned items were recorded by a camera for one hour. For each behavior, the delay at the beginning of the behavior and the number of behaviors were measured (13).

3.4. Statistical Analysis

SPSS software was used for analyzing data by using of analysis of variance. One-way ANOVA and Tukey post hoc test were used for comparison between the different groups with the control. It was performed at a significance level of $P < 0.05$. Graph Pad software was used for drawing graphs.

4. Results

4.1. Elevated Plus Maze

LPS group compared to control group had a significant decrease ($P < 0.05$) in the open arm entry (OAE) and percentage of open arm time (OAT). Also Ast and LPS+Ast groups had a significant increase compared to LPS group and no significant difference compared to control group ($P < 0.05$). No significant difference was observed between different groups in locomotor activity (Figure 1)

4.2. Forced Swimming Test

The results of this test showed no significant difference between different groups and the time of immobility was the same in all groups ($P < 0.05$) (Figure 2).

4.3. Sexual Behavior Test

The results of this research suggested prenatal administration of LPS decreased sexual behavior that this decrease was significant in the number of sniffing, following behaviors ($P < 0.01$) in comparison with the control group (Figure 3A - C).

In Ast group compared to control group, the number sniffing and following behavior significantly increased with $P < 0.001$ and $P < 0.01$, respectively. These two behaviors had also increase in LPS+Ast groups ($P < 0.001$ and $P < 0.05$, respectively).

In comparison with the LPS group, sexual behavior in Ast and LPS+Ast groups had increase that this increase was significant in all sexual behavior components except mounting and ejaculation numbers in LPS+Ast group (in sniffing number with $P < 0.001$, following number with $P < 0.001$ and $P < 0.01$ respectively in Ast and LPS+Ast; and with $P < 0.01$ and $P < 0.05$ respectively in mounting and ejaculation number) (Figure 3A - C).

5. Discussion

The result of this study showed that maternal exposure to LPS can lead to anxiety like behaviors in offspring mice. In this study, the EPM test is used to assess anxiety-like behaviors. In this test, anxious animals prefer to stay more in closed arms and spend less time in open arms (14). Our study showed that prenatal LPS-exposure mice had more anxiety and spent less time in open arms. Additionally, prenatal treatment with Ast could increase the percentage of open arm time and open arm entry, without altering in locomotor activity. In this way, it reduced and reversed anxiety-like behaviors, even more than what was seen in the control group.

Our findings are consistent with prior research demonstrating that prenatal exposure to LPS leads to immune activation and evokes behavioral deficits such as reduced locomotor activity, anxiety, depression, and schizophrenia in the adult lives (6, 15-17).

In previous studies, the beneficial effects of postnatal treatment of Ast have been examined. Al-Amin and et al. in a study showed that postnatal treatment with Ast ameliorates impaired behavior, via its antioxidant property, in the prenatal LPS-exposed offspring mice (6). However, the results of our study and others indicate the antianxiety effects of Ast in adult offspring mice following maternal LPS exposure.

Previous studies have shown that LPS reduces locomotor activity (18), while our study did not show this, in addi-

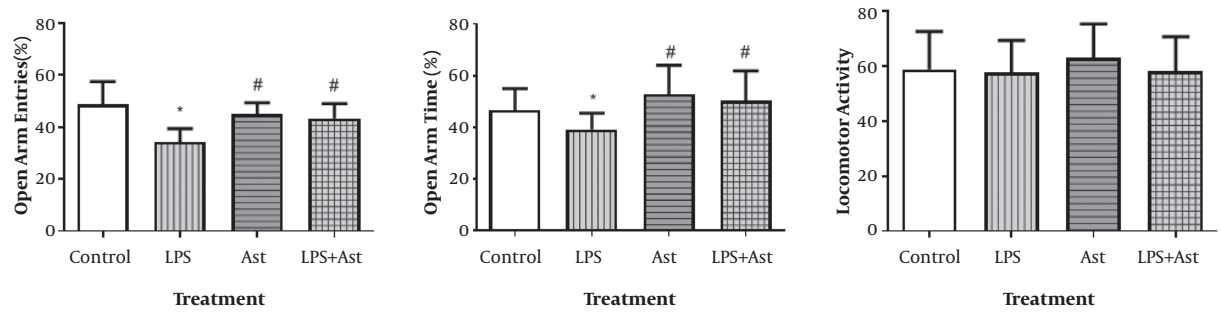

Figure 1. Prenatal effects of LPS and Ast administration on the anxiety-like behavior of male mice observed on EPM (means ± SEM). *P < 0.05 show significant difference with control group; #P < 0.05 show significant difference with LPS group.

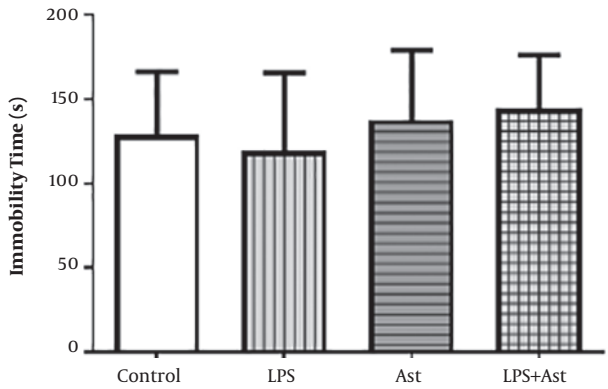

Figure 2. Prenatal effects of LPS and Ast administration on the depressive-like behavior of male mice observed on forced swimming test (means ± SEM).

tion, Ast administration increases locomotor activity and improves these conditions. The reason for this difference is probably due to the use of different methods in assessing the activity of locomotor, since, in previous studies, the open-field test has been used. Or, maybe the time and amount of exposure to LPS and Ast administration have been effective in it.

Force swimming test is one of the most valid and common tests to study the depressive-like behaviors in rodents (14). The result of this test in our study showed that prenatal LPS exposure didn't lead to depressive like behaviors in adult mice and therefore prenatal Ast treatment did not show a significant effect on immobility.

This finding is in contrary with the findings of previous studies suggesting prenatal LPS exposure leads to depressive like behaviors in offspring (18) and Ast treatment ameliorates depressive-like behavior by reducing the level of oxidative markers in the frontal cortex (6).

In another part of the results of the present study, it was found that LPS exposure during neonatal life has a negative effect on sexual behaviors in adulthood. In addition, prenatal treatment with Ast has a preventive effect on it and improves this negative effect and increases these behaviors.

In line with our study and in a study by Wijkstra et al., it has been reported that additional behavioral alterations following prenatal LPS lead to a decrease in sexual behavior. The findings of this study indicated an increase in latency to initiate sexual behavior and decrease in intromission by males (19). Also in other study, it has been revealed that exposure to the bacterial endotoxin in neonatal life disrupts the offspring's sexual behavior in adulthood (1).

5.1. Conclusions

The obtained results suggest that prenatal maternal exposure to LPS impaired several aspects of male sexual behavior and resulted in behavioral deficits in adulthood, while astaxanthin has an antianxiety effect and improves the deficits of sexual behaviors presumably via its antioxidant property.

Footnotes

Authors' Contribution: Mahdi Goudarzvand as second supervisor professor plays an equal role with Nasim Hayati Roodbari as the first supervisor in writing this article.

Conflict of Interests: It was not declared by the authors.

Ethical Approval: IR.IAU.SRB.REC.1398.081.

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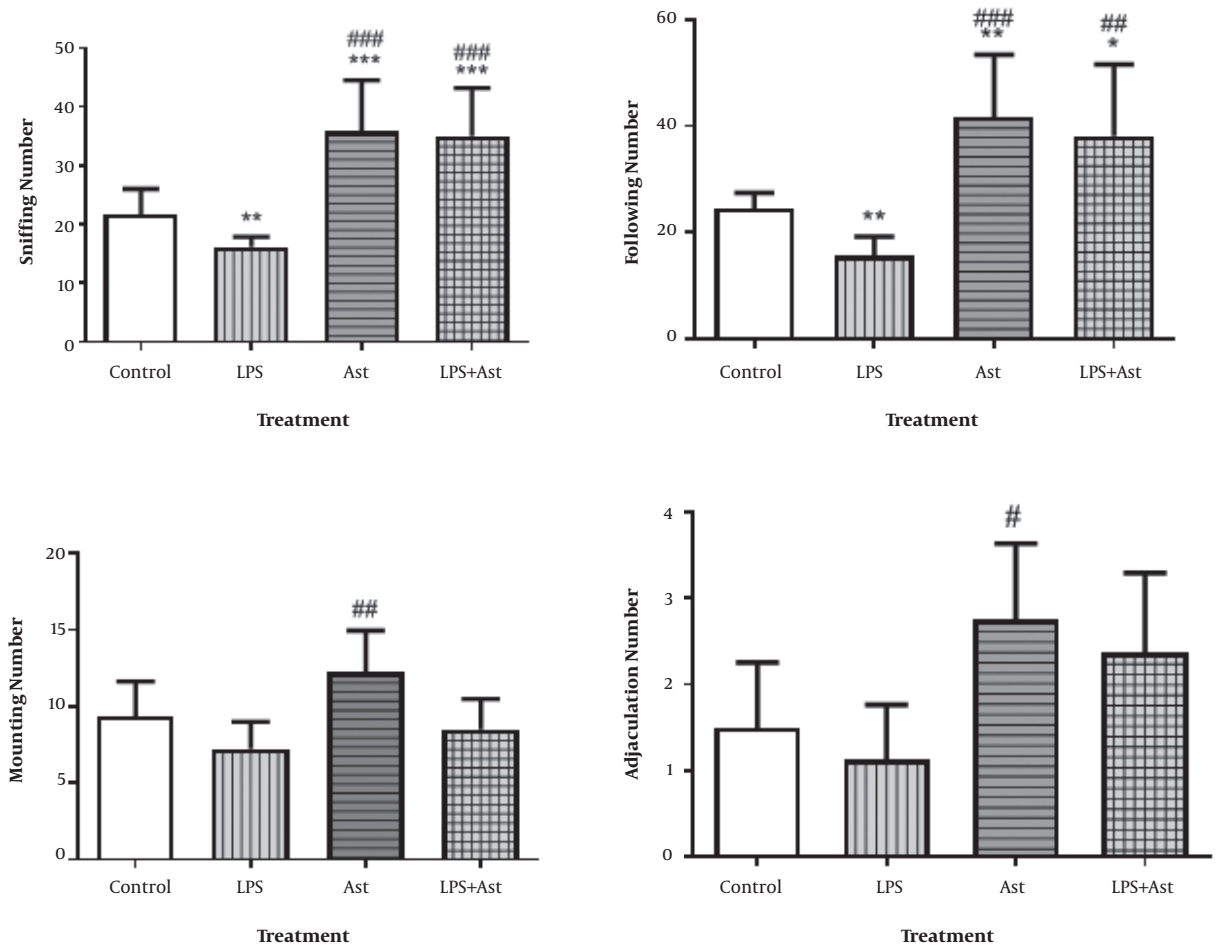

Figure 3. Prenatal effects of LPS and Ast administration on the sexual behavior of male mice observed. On EPM (means ± SEM). *, P < 0.05 show significant difference with control group; **, P < 0.01 show significant difference with control group; ***, P < 0.001 show significant difference with control group. #, P < 0.05 show significant difference with LPS group; ##, P < 0.01 show significant difference with LPS group; ###, P < 0.001 show significant difference with LPS group.

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