The Protective Effect of Astaxanthin on Fetal Alcohol Spectrum Disorder in Mice

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Fetal alcohol syndrome (FAS), is diagnosed based on (i) prenatal and/or postnatal growth retardation; (ii) craniofacial abnormalities, including microcephaly, short palpebral fissures, and a deficient philtrum; (iii) central nervous system dysfunction. (1973 Jones and Smith)
Background

The Face in Fetal Alcohol Syndrome

Discriminating Features

- short palpebral fissures
- flat midface
- indistinct philtrum
- thin upper lip

Associated Features

- epicanthal folds
- low nasal bridge
- minor ear anomalies
- short nose
- micrognathia

In the Young Child
FASD (fetal alcohol spectrum disorders, FASD) is an umbrella term that describes the range of effects that can occur in an individual whose mother drank alcohol during pregnancy. These effects can be physical, mental, or behavioral, with possible lifelong implications. The rate of FASD is 8-10 per 1000 live births. FASD constitute a major public health problem.
FASD -- Foetal Alcohol Spectrum Disorders

Diagram:
- Peroxidation of lipids, nucleic acids, and proteins
- Reactive oxygen species (ROS)
- Direct:
  - CH₃·OH (Hydroxyethyl)
  - •OH (Hydroxyl)
- Indirect:
  - C₂H₅·OH (Ethanol)
  - Glutathione peroxidase activity
  - Mitochondrial Damage
  - Glutathione pool
Alcohol

$\text{ROS} \uparrow$

$\text{Cytokines} \uparrow$

$\text{FASD}$
Animal Models
Mice
Embryos of Mouse

The measurements of Embryonic Morphology

HL
HW
CRL (crown rump length)
Effect of different concentration of alcohol on mouse embryos

<table>
<thead>
<tr>
<th>EtOH (ml/g)</th>
<th>0</th>
<th>0.005</th>
<th>0.01</th>
<th>0.015</th>
<th>0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
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<tr>
<td>Lateral</td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
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</tbody>
</table>
**Effect of different concentration of alcohol on mouse embryos**

<table>
<thead>
<tr>
<th>Concentration (25%, ml/g)</th>
<th>Embryos</th>
<th>HL (mm)</th>
<th>HW (mm)</th>
<th>CRL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>2.40 ± 0.04</td>
<td>1.54 ± 0.02</td>
<td>5.00 ± 0.06</td>
</tr>
<tr>
<td>0.005</td>
<td>23</td>
<td>2.23 ± 0.05*</td>
<td>1.44 ± 0.02*</td>
<td>4.71 ± 0.09*</td>
</tr>
<tr>
<td>0.01</td>
<td>22</td>
<td>2.23 ± 0.05</td>
<td>1.44 ± 0.02*</td>
<td>4.69 ± 0.09*</td>
</tr>
<tr>
<td>0.015</td>
<td>20</td>
<td>2.17 ± 0.04*</td>
<td>1.34 ± 0.03*</td>
<td>4.53 ± 0.07*</td>
</tr>
<tr>
<td>0.02</td>
<td>19</td>
<td>1.86 ± 0.05*</td>
<td>1.16 ± 0.02*</td>
<td>4.12 ± 0.08*</td>
</tr>
</tbody>
</table>
Expression of TLRs in FASD

[Graph showing expression levels of TLRs in different groups with error bars].

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Expression of TLRs in FASD

Ethanol induced the expression of TLR4 signaling.
Expression of Cytokines in FASD

Ethanol induces production of TNF-α (A), and IL-1β (B) in embryos.
Intervention of AST on the TLRs system

Ethanol induced the expression of TLR4 signaling, that can be blocked by AST pretreatment.
AST inhibited ethanol-induced production of TNF-α(A), and IL-1β(B) in embryos.
Mechanisms of Oxidative Stress Related FASD and Prevention of AST

AST rescued up-regulation of MDA, H2O2 and down-regulation of GSH-PX induced by alcohol.
Mechanisms of Oxidative Stress Related FASD and Prevention of AST

AST rescued Otx1 and Sox2 mRNA down-regulation induced by alcohol.
Mechanisms of Oxidative Stress Related FASD and Prevention of AST

AST rescued Otx1 and Sox2 protein down-regulation induced by alcohol.

**Graph A**

<table>
<thead>
<tr>
<th>AST (mg/kg.d)</th>
<th>0</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>100</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH (0.02ml/g)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Graph B**

Relative protein level (%) vs EtOH (0.02ml/g) and AST (mg/kg.d)

- Otx1
- Sox2

*Significant difference
AST prevented ethanol-induced developmental retardation in mouse embryos
Alcohol ➔ ROS ➔ TLRs ➔ AST ➔ Cytokines ➔ FASD

- Otx1, Sox2 ➔
Conclusions

1. Morphological observations showed protective effects of AST treatment against ethanol-induced growth retardation in mouse embryos.
2. AST can rescue the Otx1 and Sox2 low expression on mRNA and protein level and GSH-PX overexpression caused by alcohol.
3. Oxidative stress and toll-like receptor signaling associated inflammatory reaction were involved in this process.
4. Our data confirmed the protective effect of astaxanthin (AST) on fetal alcohol spectrum disorder.
Acknowledgments

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Dr. Yi Li
Thanks!