ETHANOL-RELATED TERATOGENICITY AND NEUROBEHAVIOURAL IMPAIRMENTS: INFLUENCE OF DIETARY ZINC SUPPLEMENTATION DURING PREGNANCY

A thesis submitted for the degree of Doctor of Philosophy

by

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µl</td>
<td>microlitre</td>
</tr>
<tr>
<td>µmol</td>
<td>micromole</td>
</tr>
<tr>
<td>AE</td>
<td>Acrodermatitis Enteropathica</td>
</tr>
<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>ARBD</td>
<td>Alcohol Related Birth Defects</td>
</tr>
<tr>
<td>ARND</td>
<td>Alcohol Related Neurdevelopmental Disorder</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>BAC</td>
<td>Blood Alcohol Concentration</td>
</tr>
<tr>
<td>Bi</td>
<td>Bismuth</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CRL</td>
<td>Crown Rump Length</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
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<tr>
<td>d</td>
<td>day</td>
</tr>
<tr>
<td>dL</td>
<td>decilitre</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EDC</td>
<td>Ethanol Derived Calories</td>
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<tr>
<td>EP</td>
<td>Escape Platform</td>
</tr>
<tr>
<td>FAS</td>
<td>Fetal Alcohol Syndrome</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>FASD</td>
<td>Fetal Alcohol Spectrum Disorder</td>
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<td>GD</td>
<td>Gestational Day</td>
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<tr>
<td>GLM</td>
<td>General Linear Model</td>
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<tr>
<td>GRE</td>
<td>Glucocorticoid Response Element</td>
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<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>hZTL1</td>
<td>human ZnT-like transporter 1</td>
</tr>
<tr>
<td>IMVS</td>
<td>Institute of Medical and Veterinary Science</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
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<tr>
<td>L</td>
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</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>Mg</td>
<td>Magnesium</td>
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</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>MRE</td>
<td>Metal Response Element</td>
</tr>
<tr>
<td>MT</td>
<td>Metallothionein</td>
</tr>
<tr>
<td>MTF</td>
<td>Metal Transcription Factor</td>
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<td>number</td>
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NaCl  Sodium Chloride
NAD  Nicotinamide Adenine Dinucleotide
NTD  Neural Tube Defect
ORT  Object Recognition Task
PD  Postnatal Day
ppm  parts per million
REML  Restricted Maximal Likelihood
RNA  Ribonucleic Acid
ROS  Reactive Oxygen Species
s  seconds
SEM  Standard Error of Mean
TF IIIA  Transcription Factor III A
TNF-α  Tumor Necrosis Factor alpha
v  volume
VSD  Ventricular Septal Defect
w  weight
Zn  Zinc
ZnSO₄  Zinc Sulphate
Ethanol consumption during pregnancy can result in a wide range of negative outcomes, including pre-and post-natal mortality, growth retardation, physical abnormalities and brain deficits, manifested as behavioral impairments. These outcomes can result from “binge-drinking” (generally defined as >5 standard drinks on a single occasion) or chronic ethanol consumption. Ethanol-induced zinc (Zn) deficiency is one of the mechanisms proposed as a cause of ethanol teratogenicity. We have previously demonstrated in mice that ethanol exposure on gestational day (GD)8 (during organogenesis) can alter Zn homeostasis by inducing the Zn-binding protein metallothionein (MT) in the maternal liver. This causes plasma Zn concentrations to decrease as Zn redistributes into the liver, and consequently decreases the fetal Zn supply and increases the risk of teratogenicity. Subcutaneous Zn treatment with ethanol on GD8 can prevent the deleterious effects of ethanol on the fetus (i.e. physical abnormalities and spatial memory impairments). The main objective of this thesis was to investigate whether a less invasive approach of giving dietary Zn supplementation throughout pregnancy could provide similar protective benefits against a range of adverse outcomes caused by prenatal binge or chronic ethanol exposure.

Binge ethanol exposure in early pregnancy (i.e. where mice are injected with 25% ethanol (0.015 ml/g) intraperitoneally at 0 and 4 hours on GD8) significantly increased the incidence of birth abnormalities measured on GD18. These included craniofacial abnormalities (microphthalmia, anophthalmia) and limb defects. Ethanol
also increased postnatal mortality between birth and postnatal day (PD)60. In a separate study, offspring from dams given ethanol on GD8 were subjected to a physical and behavioural screening protocol (including tests for vision, olfactory, exploratory, anxiety and motor impairments) and subsequently a cohort of phenotypically-normal offspring were randomly selected for testing in a cross-maze escape task (for spatial learning and memory) and an object recognition test (for short-term non-spatial memory). While ethanol did not affect behaviour measured during screening, it resulted in spatial memory and object recognition memory impairments in adult offspring. The most important finding was that dietary Zn supplementation throughout pregnancy significantly increased plasma Zn concentrations at the time of ethanol exposure (avoiding the “typical” ethanol-induced decrease in plasma Zn) and prevented all negative outcomes resulting from early ethanol exposure (birth abnormalities, mortality, spatial and object recognition memory impairments). In the chronic ethanol mouse model (i.e. where mice were fed a liquid diet containing 27% v/v ethanol-derived calories from GD6-18), ethanol did not affect offspring growth between birth and PD21 or spatial memory in adult offspring, thus, the influence of Zn supplementation could not be examined for these parameters. While ethanol decreased offspring weight at PD50 and increased mortality between birth and PD40, they were not prevented by Zn supplementation throughout pregnancy.

The findings from this thesis emphasise that organogenesis is a particularly vulnerable period to ethanol exposure and even a binge of ethanol during this time
can result in dysmorphology, mortality and spatial and object memory impairments in adulthood. In addition, dietary Zn supplementation is protective against the deleterious effects of binge ethanol exposure in early pregnancy.
DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent for this copy of my thesis being made available in the University Library.

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Brooke Lee Summers

Signature………………………………      Date……………..
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**PUBLICATIONS FROM ASSOCIATED RESEARCH**

