ORIGINAL RESEARCH

Effects of Embryonic Exposure to Ethanol on Zebrafish Survival, Growth Pattern, Locomotor Activity and Retinal Development

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ABSTRACT

Alcohol intake can cause a wide range of visual system abnormalities. In this study, we characterized how ethanol affects the growth, external morphology and locomotion of zebrafish, particularly with regard to retinal development. Zebrafish embryos were divided into 5 groups and put into hatching liquid for 6 hours. The embryos from 4 groups were treated with varying concentrations of ethanol (0.5%, 1.5%, 2.5% and 3% by volume) from 6 to 24 hours post-fertilization. The toxic effects of ethanol on embryonic development were assessed by mortality, hatching rate and morphologic deformity. The effects of ethanol on locomotive activity were assessed by autonomous motion detection and swimming behavior analysis. The effects of ethanol on retinal morphology were assessed by histologic, immunohistochemical and electron microscopy analyses. Ethanol treatment increased the mortality and induced growth retardation in zebrafish larvae. The locomotive activities of zebrafish embryos/larvae were impeded by exposure to higher (1.5% and 2.5%) concentrations of ethanol. Embryos exposed to higher levels of ethanol at the early developmental stage had a reduction in eye size. The ethanol treatment disrupts the architecture of the retina and reduces retinal size. Embryos exposed to 2.5% concentration of ethanol had morphologic abnormalities of the photoreceptors. Ethanol exposure also inhibited retinal cell differentiation and proliferation, but did not affect apical epithelial polarity.

These findings suggest that ethanol affects the growth and external morphology of zebrafish, and higher levels of ethanol exposure can cause defects of locomotor activity and photoreceptor development. (*Altern Ther Health Med.* 2021;27(5):120-128).

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INTRODUCTION

As has been demonstrated experimentally and clinically, alcohol is a common teratogen. Drinking alcohol during pregnancy can have a variety of adverse effects on fetal development. The severity of the damage caused by alcohol exposure depends on many factors, such as the amount and timing of alcohol exposure.[1] Fetal alcohol syndrome (FAS) is the most serious condition caused by fetal alcohol exposure in the uterus.[2,3]

Alcohol can enter the placenta, hindering the growth and weight of the fetus. Alcohol itself and its metabolites, in a variety of ways, cause damage to all systems of the body, in which the nervous system is one of the target organs. The intake of a large amount of alcohol may eventually cause damage to many parts of the nervous system.[4] Ethanol-induced defects include cardiac, central nervous system, craniofacial, skeletal, learning, motor, sensory, ocular and other defects.[5-9]

The mechanism of nervous system damage caused by ethanol has not yet been fully elucidated, but it is believed that oxidative stress and lipid peroxidation play an important role.[10,11] Studies of the changes in the retina and optic nerve caused by ethanol usually assess the level of oxidative stress and lipid peroxidation. They also measure the visual system function using electroretinograms (ERGs) and explore whether antioxidants have any protective effect.[12] However, there are only a few studies focused on the accompanying and subsequent pathologic changes in the retina and optic nerve.

Zebrafish have become an important animal model for studying alcohol developmental toxicity. The effect of ethanol on zebrafish embryos is similar to that in other species. In

addition, zebrafish share similar cellular and physiologic characteristics with other higher vertebrates.[13] Development is simple to observe in zebrafish, and ethanol can easily pass through the egg membrane. Together, these characteristics facilitate studies of the effect of ethanol on embryo development.[13]

To the best of our knowledge, the effects of ethanol on retinal cell specification, differentiation and proliferation remain unclear. Therefore, we designed a series of experiments to expose zebrafish embryos to different concentrations of ethanol from 6 hours post-fertilization (hpf) to 24 hpf, at which point the eyecups become well-formed.[14] Moreover, we evaluated how embryonic exposure to ethanol influences zebrafish growth, external morphology, locomotion and retinal development.

MATERIALS AND METHODS

All animal experiments in this study were approved by the Animal Care and Use Committee of Zhejiang University, China. The use of animals was in accordance with the Association for Research in Vision and Ophalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and the tenets of the Declaration of Helsinki.

Animal Source and Breeding

The AB wild-type zebrafish were provided by the Translational Medicine Institute of Zhejiang University. Zebrafish were all placed in the same tank. The male to female ratio was 2:1, and the light/dark cycle ratio was 14:10. The fertilized eggs were collected and washed according to Schulte's and Nagel's methods,[15] and the normal divided fertilized eggs were selected for toxicity testing using a stereoscopic zoom microscope (Leica Microsystems, Buffalo Grove, Illinois, USA). Embryos were raised in hatching liquid at 28.5° C for 6 hours.

Animal Grouping and Ethanol Treatment

Before ethanol treatment, the embryos were divided into 5 groups and put into beakers sealed with parafilm. The embryos from 4 groups were treated with varying concentrations (0.5%, 1.5%, 2.5% and 3% by volume) of ethanol (Sigma, St Louis, Missouri, USA) starting at 6 to 24 hpf. After completion of ethanol treatment, the embryos were put into the hatching liquid for further development until 120 hpf.

Toxicity Test and Morphologic Deformity Assessment

The mortality and hatching rate of larvae at 120 hpf were assessed in a total of more than 100 embryos for each concentration. The malformations (eg, pericardial edema, abdominal edema, rounded forebrain and irregular jaw) of larvae were recorded for each group. Larvae were observed and photographed using a stereoscopic zoom microscope at 120 hpf, and the body length and eye diameter of larvae were measured and analyzed with ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). The body length of the zebrafish larvae is defined as the distance from the center of the retina to the end of the tail, and the eye diameter is defined as the distance along the anterior-posterior (AP) axis.

Locomotor Activity Analysis

From each group, 15 embryos at 24 hpf were used for autonomous motion detection. Embryos were placed in a Petri dish, and the frequency of alternating spontaneous movements of the tail from side to side within 60 seconds were counted using a stereoscopic zoom microscope. At 120 hpf 15 larvae from each group were used for swimming behavior analysis.

Histology

From each group, at 120 hpf 15 larvae were selected and fixed with 4% paraformaldehyde overnight at 4° C. The fixing solution was changed once. After fixation, the embryos were transferred to 70% alcohol, subjected to agarose/paraffin double embedding and 3-μm sectioning, and then stained with hematoxylin and eosin (HE) using the standard protocols.[16,17]

Immunohistochemistry

From each group, 15 embryos/larvae at 36 hpf or 120 hpf were fixed with 4% paraformaldehyde solution, refrigerated at -80° C for 1 hour, and then cut into 8-μm continuous frozen sections. Immunohistochemistry analysis was performed using standard protocols.[18] The primary antibody solution contained the blocking solution and either phosphorylated-Histone H3 (pH3) (Sigma, St Louis, Missouri, USA; 1:200 dilution), zpr1 (ZIRC, Wuhan, China; 1:200 dilution), monoclonal mouse anti-ZO-1 (Invitrogen, Carlsbad, California, USA; 1:200 dilution), zn8 (ZIRC, Wuhan, China; 1:200 dilution), HuC/D (Invitrogen; 1:200 dilution), Crb2a (gift from Jian Zou; 1:200 dilution), rabbit anti-rhodopsin (gift from Jian Zou; 1:200 dilution), or rabbit anti-red opsin (gift from Jian Zou; 1:200 dilution).

Quantification of Mitotic Cells

At 120 hpf, the number of pH3-positive retinal cells in 5 sections from 15 wild-type and 2.5% ethanol-treated zebrafish retinas each were counted. The retinal regions counted were contoured and measured with MetaMorph® v6.1 software (Molecular Devices, Carlsbad, California, USA) and the average number of pH3-positive cells per 3600 μm^2 of retinal area was calculated in each group.

Transmission Electron Microscopy

From each group, 15 larvae at 120 hpf were fixed with 2.5% glutaraldehyde after phosphate buffer saline washing and refrigerated at 4° C overnight. Larvae were then fixed with 1% osmium tetroxide for 2 hours. Larvae were dehydrated via acetone series, embedded directionally, and ultrathin sections (60- to 70-nm) were made. The ultrastructural changes of photoreceptor cells were observed using Hitachi H600 TEM (Hitachi, Tokyo, Japan).

Statistical Analysis

All experiments were repeated at least 3 times independently. All data were analyzed with OriginLab® 6.0 statistical software. All results were presented as mean ± standard deviation (SD). For comparison between 2 groups, 2 independent sample *t* tests were used groups, and multiple groups of data were compared using one-way ANOVA. *P* values < .05 were considered statistically significant.

RESULTS

Survival and Hatching Ability of Zebrafish Larvae Exposed to Ethanol

Larvae exposed to low ethanol concentrations (0.5%) showed no deaths at 120 hpf. However, zebrafish larvae exposed to higher concentrations of ethanol (1.5% and 2.5%) had mortality rates of 17% and 37%, respectively. Mortality increased with increased ethanol concentrations. In the 3% ethanol-treated group, mortality reached 100%. The hatching success rates of zebrafish larvae at 120 hpf decreased with increasing concentrations of ethanol (from 0.5% to 2.5%, the hatching rates were 100%, 95.6%, and 82.3%.).

Locomotor Activity of Zebrafish Embryos and Larvae Exposed to Ethanol

The results of measuring autonomous motion in zebrafish embryos at 24 hpf are shown in Figure 1. Embryos exposed to 1.5% and 2.5% ethanol displayed a significant decrease in spontaneous tail movement at 24 hpf compared with the unexposed control group.

At 120 hpf, no erratic movements in the control group were observed. Larvae exposed to 0.5% ethanol were active and swam normally around the Petri dish. However, larvae treated with 1.5% and 2.5% ethanol were mostly motionless and swam very little.

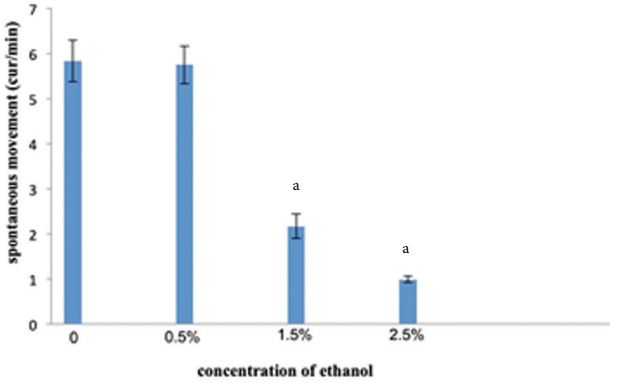
Morphologic Features of Zebrafish Larvae Exposed to Ethanol

External morphologic changes in zebrafish larvae after ethanol treatment were assessed at 120 hpf (Figure 2). Larvae exposed to 0.5% ethanol appeared morphologically normal and showed no malformation (Figure 2B). At 1.5% or 2.5% concentrations of ethanol, larvae were smaller than larvae in the control group and exhibited a high incidence (60.1% and 96.6% of larvae, respectively) of external morphologic malformations (Figures 2C and 2D). The defects increased in severity and frequency as the concentration of ethanol increased.

Eye and Body Size of Zebrafish Larvae Exposed to Ethanol

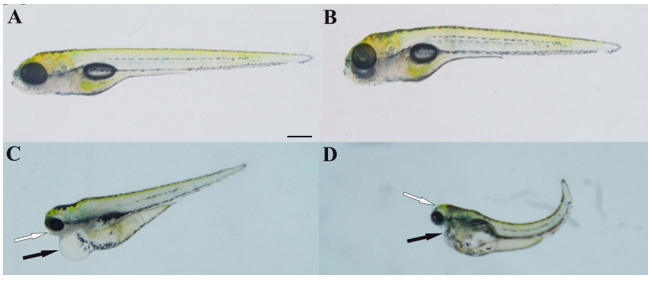
Figures 2C and 2D show a dramatic reduction in eye and body size in larvae treated with 1.5% and 2.5% ethanol. At 120 hpf, the eye diameter and body length of the 1.5% ethanol-treated zebrafish were approximately 70% and 78% of the measurements in the control siblings (Figures 2A and 2C). The difference between eye and body size of ethanol-treated and control larvae increased with exposure to higher ethanol concentrations. The eye diameter and body length of the 2.5% ethanol-treated zebrafish were approximately 45% and 49% of those in the control group (Figures 2A and 2D).

Figure 1. Spontaneous movements per minute in zebrafish embryos at 24 hpf after exposure to different concentrations of ethanol (0% [control], 0.5%, 1.5%, and 2.5% by volume) from 6 hpf through 24 hpf. Compared with the control embryos, the spontaneous movement of embryos wassignificantly decreased as the concentration of ethanol increased (n = 100, 20 embryos in 5 replicates each).



[a]*P* < .05

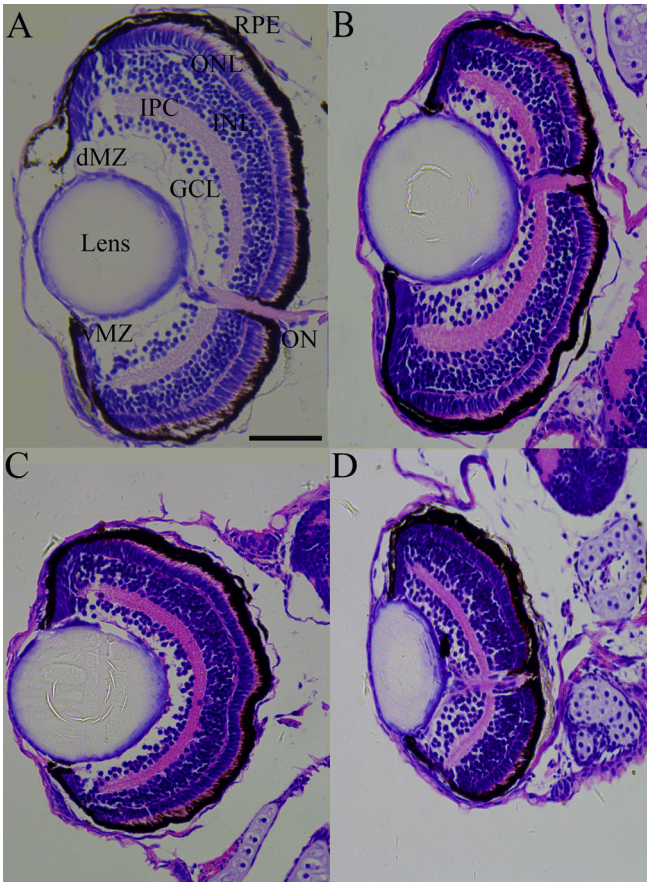
Figure 2. Ethanol treatment of zebrafish embryos causes reduction in eye size. A-D. Zebrafish embryos were raised in fish water (A) or fish water supplemented with 0.5% ethanol (B), 1.5% ethanol (C), or 2.5% ethanol by volume from 6 hpf through 24 hpf. (A, B) Treatment with low concentrations of ethanol (0.5%) resulted in no observable morphologic differences compared with untreated controls at 120 hpf (C, D). Increasing the concentration of ethanol resulted in smaller eye and body size, swollen hearts (black arrow, C), swollen guts (black arrow, D), irregular jaw (white arrow, C) and rounded forebrain (white arrow, D). Scale bar: (A-D) 20 μm.



Retinal Structure of Zebrafish Larvae Exposed to Ethanol

At 120 hpf, the retinal laminae could be easily identified in the larvae in the control group (Figure 3A). All retinas from the ethanol-treated larvae maintained proper lamination and had normally differentiated lenses (Figures 3B-D), but

Figure 3. Ethanol treatment disrupts the architecture of the retina and reduces retinal size. **A-D**. Zebrafish embryos were raised in fish water (A) or fish water supplemented with 0.5% ethanol (B), 1.5% ethanol (C), or 2.5% ethanol (D) by volume from 6 hpf through 24 hpf. Increasing the concentration of ethanol resulted in smaller retinas, but lamination and cellular components were similar at 120 hpf. Scale bar: (A-D) 50 μm.



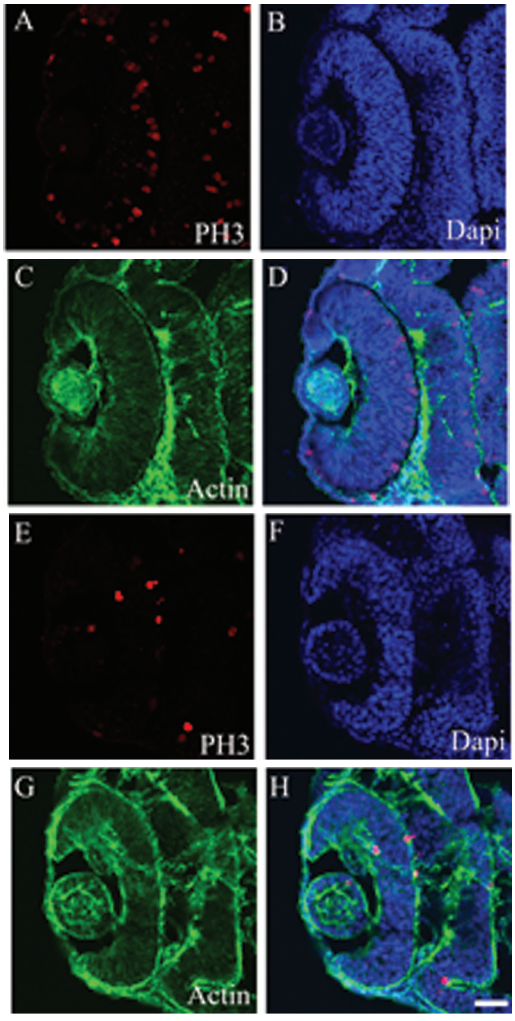
Abbreviations: Lens, the lens; ON, optic nerve; RPE, retinal pigment epithelia; ONL, outer nuclear layer; INL, inner nuclear layer; OPL, inner plexiform layer; dMZ, dorsal marginal zone; vMZ, ventral marginal zone; GCL, ganglion cell layer.

were noticeably smaller than those of the untreated controls. The size of the retina negatively correlated with the concentration of ethanol to which the larvae were exposed. Larvae treated with higher concentrations of ethanol (1.5% and 2.5%) had smaller ciliary marginal zones (CMZs) (Figures 3C and 3D).

Retinal Cell Proliferation of Zebrafish Embryos Exposed to Ethanol

At 36 hpf, the level of active cell proliferation was analyzed in both control and ethanol-treated zebrafish embryos. The retinas were stained with anti-pH3 antibody, which specifically stains cell nuclei in cell cycle M-phase (Figures 4A-H). The average number of positive cells per

Figure 4. Ethanol treatment disrupts cell proliferation in retina. A-H. M-phase nuclei (visualized by anti-PH3 [red]) are observed in both normal (A-D) and ethanol-treated retinas (E-H) at 36 hpf. All nuclei are stained with Dapi (blue). The actin distribution as revealed by phalloidin staining highlights the plexiform layers. D is the merged image of A, B and C. H is the merged image of E, F and G. I. A significant difference was found between the relative numbers of pH3-positive cells in ethanol-treated retinas and control retinas at 36 hpf ($P < .05$). The error bars denote the standard error of the mean. Scale bar: (A-H) 20 μm.



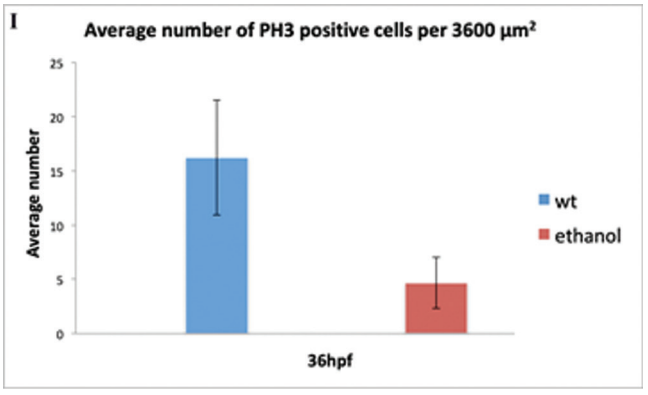
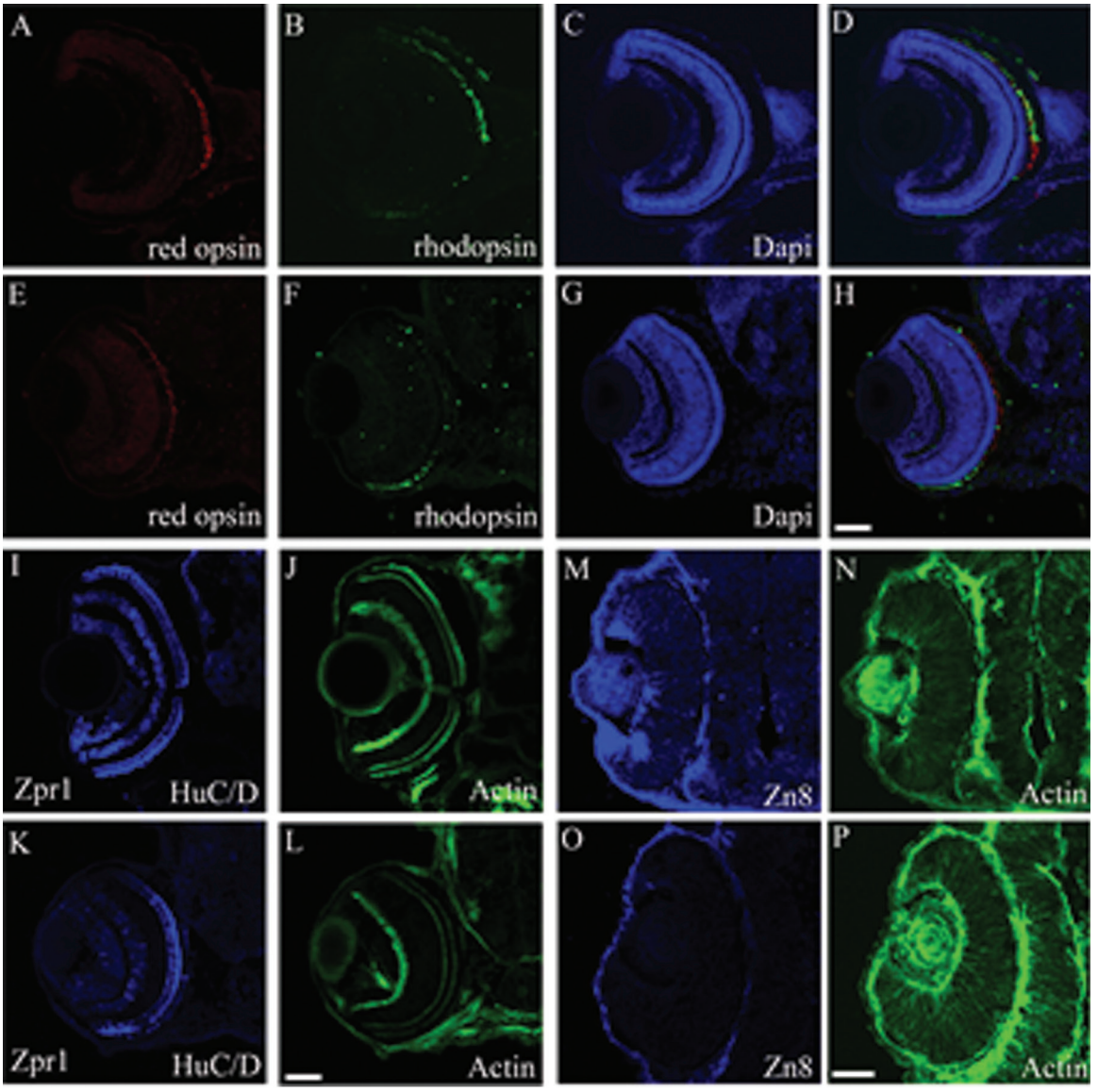


Figure 5. Retinal cell differentiation is delayed by ethanol treatment. A-H. At 120 hpf, red opsin (red) and rhodopsin (green) are observed in both ethanol-treated (E-H) and normal retinas (A-D). All nuclei are stained with Dapi (blue, G and K). D and H are merged images. I-L. At 120 hpf, green/red double cones (G/R, blue) and amacrine cells (blue) are observed in both ethanol-treated (K and L) and control retinas (I and J). Zn8 (blue) expressing ganglion cells are specified in both normal and ethanol-treated retinas. The actin distribution as revealed by phalloidin staining highlights the plexiform layers. M-P. The ganglion cells stained with zn8 antibody (blue) are present in control retinas (M) but not in the ethanol-treated retinas (O) at 36 hpf. Scale bar: (A-P) 20 μm.



3600 μm^2 of retinal section was calculated, and a significant decrease was found in the relative number of pH3-positive cells in 2.5% ethanol-treated retinas compared with the retinas of control group embryos (Figure 4I; $P < .05$).

Retinal Cell Specification of Zebrafish Embryos and Larvae Exposed to Ethanol

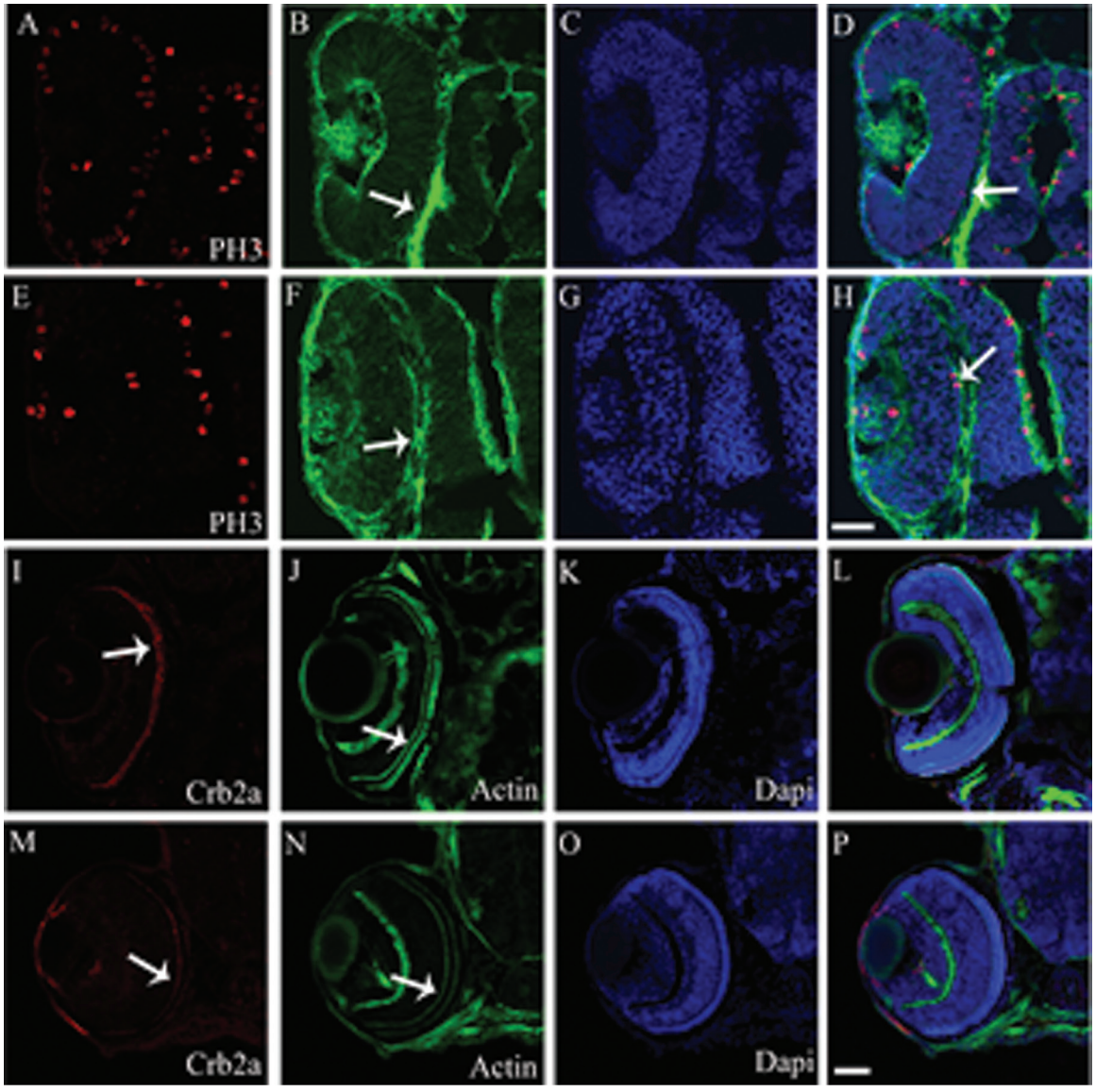
At 120 hpf, all of the retinal cell-specific markers appeared in both untreated and 2.5% ethanol-treated zebrafish (Figures 5A-L). Rhodopsin and red opsin were expressed in the outer segments of photoreceptors in the 2.5% ethanol-treated zebrafish. Photoreceptors in retinas treated with 2.5% ethanol did not have the typical elongated shape (Figures 5K and 5L).

At 36 hpf, a considerable number of inner nuclear (INL) cells were observed in the 2.5% ethanol-treated embryos. However, the ganglion cells were not detected at this stage (Figures 5O-5P).

Retinal Epithelial Polarity of Zebrafish Embryos and Larvae Exposed to Ethanol

As shown in Figure 6**,** the distribution patterns of the apical polarity markers Crb2a, adherens junction-associating actin bundles and M-phase nuclei in the control and ethanol-treated retinas were compared.

Figure 6. Ethanol treatment does not affect apical epithelial polarity. A-H. At 36 hpf, the M-phase nuclei (visualized with anti-phosphorylated-Histone H3 antibody (PH3, red)) localized to the apical regions of ethanol-treated retinas (E-H) and normal retinas (A-D). Apical localization of adherens junctions in the retina (arrows) of the 36 hpf normal and ethanol-treated embryos is visualized by the staining patterns of adherens junction-associated actin bundles (green). Cell nuclei were labeled with Dapi (blue). I-L. Apical localization of adherens junctions in the retina (arrows) of the 120 hpf normal embryo is visualized by the staining patterns of Crb2a (red) and adherens junction-associated actin bundles (green). M-P. Crb2a (red) and adherens junction-associated actin bundles (green) localize properly to the apical surface of the retina (arrows) in ethanol-treated larvae at 120 hpf. Cell nuclei were labeled with Dapi (blue). Scale bar: (A-P) 20 μm.



There was no between-group difference in the localization of all these apical markers in the retinas at 36 hpf and 120 hpf.

Structure of Photoreceptors of Zebrafish Larvae Exposed to Ethanol

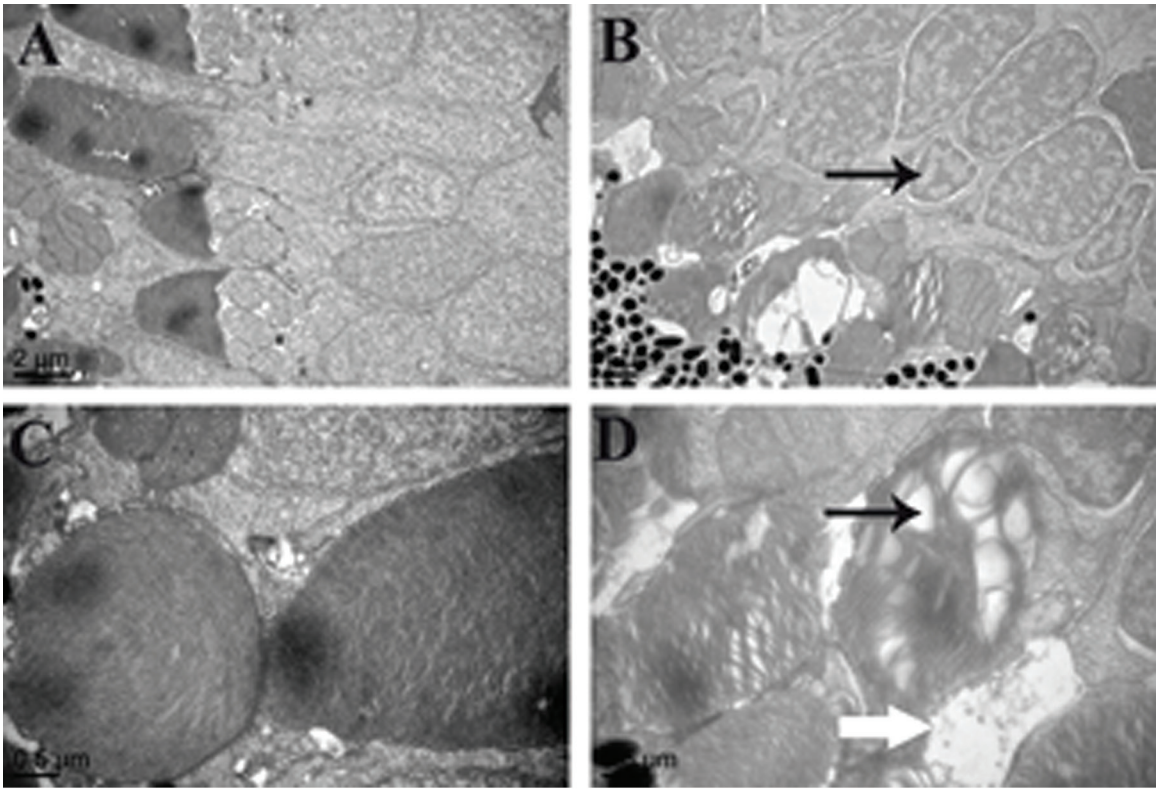
As shown in Figure 7, the retinal photoreceptor cells at 120 hpf were arranged in regular and orderly lamellae, and the membrane disc was clearly demarcated (Figures 7A and 7C). The photoreceptor cells were arranged irregularly and the normal lamellar structure was lost in the 2.5% ethanol-treated larvae (Figure 7B). The membrane discs of the photoreceptor outer segments were most significantly affected by exposure to ethanol. The gap between the discs increased; vacuole-like changes were observed and the discs were dissolved, fused and indistinguishable locally (Figure 7D). In the 2.5% ethanol-treated retina, the nuclei of photoreceptor cells lacked elongated morphology (Figure 7B).

DISCUSSION

Effects of Ethanol on Zebrafish Survival and growth Pattern

Zebrafish are highly sensitive to environmental pollutants.[19] The results of this study demonstrate that ethanol

Figure 7. Ethanol treatment affects the development of photoreceptors. A-D. Embryos were untreated (A, C) or treated with 2.5% ethanol (B, D) from 6 hpf through 24 hpf. Photoreceptors were arranged in a disorderly fashion and the normal lamellar structure was lost in 2.5% ethanol-treated embryos (black arrow, B). Severely disrupted outer segments (OS) showing tubulation and vesiculation (black arrow, D) of disc structures were observed in ethanol-treated embryos. Many vacuoles and holes (white arrow, D) were found between the outer segments. In ethanol-treated retinas, the nuclei of photoreceptors cells lacked elongated morphology (black arrow, B).



exposure increases mortality and induces growth retardation in zebrafish embryos. The results also show that the toxicity of ethanol becomes more discernible at higher concentrations. In addition to an apparent reduction in eye and body size, the zebrafish embryonic deformities induced by ethanol were mainly manifested as curved body axis, paracardiac edema, abdominal edema, rounded forebrain and irregular jaw.

Hatching rate is an important index of toxicity in embryonic development.[20] Our results showed that ethanol inhibits zebrafish embryonic hatching and delays incubation time. Zebrafish hatching stripping requires a close combination of 2 processes: the softening of the egg film through embryonic release of the hatching enzymes and the rupture of the egg membrane leading to hatching through embryonic autonomous movement.[21,22] The results of the zebrafish embryonic behavior test showed that autonomous movement dramatically decreased after ethanol exposure; slowing of autonomic movement can explain why ethanol inhibits embryonic hatching. Slowing down autonomic movement, leading to a delay in egg membrane rupture and consequently, the hatching process. We speculate that incubation time delay is the leading cause of morphologic deformities. The mechanism of ethanol leading to embryonic developmental malformation needs to be further studied.

Locomotor Activity Defects in Ethanol-Exposed Zebrafish

Ethanol interferes with the development of glial cells, which may lead to changes in cell migration, neuron survival and differentiation. The influence of ethanol on the neurotransmitter system and its receptor is an important factor affecting the development of the central nervous system.[23] The early damage caused by ethanol is irreversible and has a great influence on the first 3 months of pregnancy in humans. The zebrafish nervous system has many similarities to the mammalian nervous system. Neurologic disorders and mental retardation are often associated with compound exposure toxicity. Necrosis and apoptosis of neurons in the brain can be used as an index for detecting the neurotoxicity of compounds, and motor neuron apoptosis is associated with motor defects.

Previous studies have investigated the swimming behavior of zebrafish after various toxin exposures, such as chronic sublethal dietary selenomethionine, domoic acid, perfluorooctane sulfonate, alcohol and sodium hypochlorite.[24,25] Our results showed a dramatic decrease in spontaneous tail movement at 24 hpf and movement speed at 120 hpf compared with the control group after ethanol exposure at higher levels (1.5% and 2.5%). These findings suggest that ethanol has latent neurotoxicity.

Effects of Ethanol on Zebrafish Retinal Development

Alcohol intake can cause a wide range of abnormalities in the visual system. Research shows that the retina may be another susceptible area of oxidative stress induced by alcoholism.[12] The threshold level of alcohol tolerance in the retina is low because the outer disc membrane of rod cells contains a lot of long-chain unsaturated fatty acids, which makes them sensitive to oxidative damage. A study of monkeys with long-term ethanol intake showed a significant decrease in brain and retinal fatty acids, mainly DHA, which is abundant in the disc membrane of rod cells.[26] Therefore, although the nutritional deficiency caused by ethanol intake will affect the function of the visual system, the toxic effect of ethanol should not be ignored.

In order to investigate the effects of ethanol on visual physiology, the effects of ethanol before birth in ERG of rats were investigated. The results showed that the adaptability of ethanol-treated rats to light and darkness was poor, and the content of rhodopsin in the retina was decreased.[27] In a similar fashion, fetal alcohol exposure induced anatomical changes within the retinal ganglion cell layer that are reflected in the increased photosensitivity of the cone photoreceptors.[28]

In order to investigate the effects of exposure to ethanol on retinal development, the zebrafish model was used in this study. In zebrafish, the first differentiated retinal ganglion cells appear at approximately 28 hpf.[14,29] However, in our study, the retinal ganglion cells did not differentiate at 36 hpf, suggesting that ethanol exposure at the early stage of embryonic development leads to delayed cell differentiation. After embryonic development to 120 hpf, all retinal cells differentiate. However, the eye becomes smaller with increased ethanol concentration.

The retinal patterning defect in zebrafish treated with ethanol might be explained by suppressive retinal cell proliferation during the early stage of embryonic development, which could affect both eyes and retina size and result in inconsistent migration and/or differentiation signals to the newly emerged retinal cells. This is consistent with the finding that the germinal zone at the ciliary margin of the ethanol-treated retina appears abnormal. While ethanol exposure causes retinal patterning defects, it does not disrupt retinal epithelial polarity. Retinoic acid or folic acid co-supplementation with ethanol is known to rescue Wnt signaling and retinal differentiation.[30]

At present, the mechanism of nervous system damage caused by chronic alcoholism is not completely clear, but the main factors are related to the production of oxygen-free radicals and the imbalance of oxides and antioxidants caused by lipid peroxidation.[31] In the visual system, the retina is rich in membrane structures containing polyunsaturated lipids, which makes it very sensitive to oxidative stress and lipid peroxidation. Research shows that oxidative stress and lipid peroxidation play an important role in some degenerative diseases of the eye (such as diabetic retinopathy, uveitis and others). They also play a role in the retina damage caused by chronic alcoholism.[32,33] Long-term drinking can lead to the accumulation of lipid peroxidation products such as malondialdehyde (MDA), and a decrease in antioxidants such as glutathione (GSH) and its related enzymes, accompanied by a significant increase of anti-apoptotic protein Bcl-2. These changes can be reduced by the use of antioxidants.[12,34,35]

Photoreceptors begin to differentiate in zebrafish at approximately 43 hpf, shortly after they become postmitotic.[5,29] Morphologic differentiation of these cells progresses quickly thereafter, with the outer segments first becoming visible by 60 hpf.[29] Our results showed that embryonic exposure to ethanol is harmful for maintaining the proper structure of the photoreceptors during early development. Ethanol can damage many system organs of the human body, among which the nervous system is one of the important target organs systems.[36] Large intake of ethanol has a wide and strong inhibitory effect on the central nervous system, which can lead to diffuse damage to the nervous system including dehydration, degeneration, necrosis and loss of nerve cells, atrophy of nerve cell bodies and decrease of dendrites, leading to cell atrophy.[37] Photoreceptors are special nerve cells in the retina that send signals to the brain when stimulated by light. In mammals, photoreceptors do not regenerate on their own and do not divide once they mature.[38] Therefore, the toxic effects of ethanol on photoreceptors will directly lead to damage of visual function.

In future studies, we will further verify the effect of ethanol on visual function.

CONCLUSION

In summary, our study revealed that embryonic exposure to ethanol at early stages of development affects the growth and external morphology of zebrafish, which might subsequently impede the locomotive activity of larvae. Furthermore, our study demonstrated that zebrafish exposure to ethanol affects retinal cell proliferation and differentiation in the early stages of development, thus causing retinal patterning defects. Oxidative stress and lipid peroxidation may play an important role in the damage of photoreceptors caused by ethanol toxicity.

CONFLICT OF INTEREST

None.

ACKNOWLEDGMENTS

The authors thank Jian Zou for helpful suggestions and technical support, and Mingjie Zheng for embryos preparation and literature searching. The work was supported by the National Natural Science Foundation (81970836), the International Cooperation Project of Science and Technology Department of Jilin Province (20190701044GH), the Training Program for Outstanding Young Teachers of Jilin University (419080520313), and the Natural Science Foundation Project of Science and Technology Department of Jilin Province (20200201360JC).

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