Developmental Toxicity and Neurotoxicity Assessment of R-, S-, and RS-Propylene Glycol Enantiomers in Zebrafish (*Danio Rerio*) Larvae

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Research Article

Keywords: Propylene glycol, enantiomers, Danio rerio, developmental toxicity, neurotoxicity.

Posted Date: June 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-579574/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on January 9th, 2022. See the published version at https://doi.org/10.1007/s11356-021-17538-8.

Abstract

Propylene glycol (PG) is widely used in the foods, pharmaceuticals, oil industry, animal feed, cosmetics and other industries. Because of the existence of a chiral carbon center, PG forms R (*Rectus*)- and S (*Sinister*)-enantiomers. Currently, the toxicity study of its R-, S-enantiomers is still very scarce. In this study, we have assessed the developmental toxicity and neurotoxicity of the R-, S-, and RS-PG enantiomers in zebrafish larvae. We found that exposure to R-, S-, and RS-PG enantiomers did not significantly affect the basic developmental endpoints of embryos or larvae (i.e., embryonic movement, hatching, mortality, malformation, heartbeat, body length), indicating that R-, S-, and RS-PG exposures did not exhibit the basic developmental toxicity in zebrafish larvae. The toxicity of the three enantiomers was lower than that of ethanol, and there was no significant difference between them. However, R-, S-, and RS-PG exposures with high doses could significantly change the eye diameter and the locomotor activity of larval zebrafish, indicating that R-, S-, and RS-PG enantiomers of high doses can potentially exhibit the neurotoxicity and ocular developmental toxicity in zebrafish larvae. Therefore, the potential neurotoxicity and ocular developmental toxicity of R-, S-, and RS-PG enantiomers for infants and toddlers should be considered.

1. Introduction

Propylene glycol (PG) is a colorless, tasteless, water-soluble liquid, which also known as 1,2-propanediol (Center for the Evaluation of Risks to Human Reproduction). It belongs to the group of diols, which is a small aliphatic organic compound. Because of the existence of a chiral carbon center, PG forms R (*Rectus*)- and S (*Sinister*)-enantiomers (Ferreira et al., 2017). The S-enantiomer can be produced by microbes via the fermentation of L-rhamnose or L-fucose while the R-enantiomer is produced from pentoses and hexoses via the methylglyoxal bypass (Ingvadottir et al., 2018). PG can dissolve hydrophobic substances, it is commonly present in poorly water-soluble intravenous medications and activated charcoal preparations (Greene et al., 2020). Because of PG has very low toxicity and is currently considered as a noncarcinogenic or nongenotoxic compound, it is important liquid for industrial and technological applications (Zaripov et al., 2020). As an important and safe intermediate, PG has been used in several industrial areas (Ferreira et al., 2017). It is widely used in the foods, pharmaceuticals, oil industry, animal feed, cosmetics and other industries (Zaripov et al., 2021). In 1999, 1083 million pounds of PG are produced in the United States with apparent consumption of 854 million pounds. About 170 million pounds of PG is consumed in human foods (Center for the Evaluation of Risks to Human Reproduction). Thus, it is generally recognized as a safe food additive (Phillips et al., 2017). Besides, PG has numerous other applications in industry, including as a humectant, as a moisturizer, as a nontoxic antifreeze, and as a carrier in fragrance oils (Gaworski et al., 2010; Binks et al., 2013).

Several studies have examined the toxicity of RS-PG in the context of repeated exposure to these preparations. For instance, embryonic exposure to 0.625% or 1.25% RS-PG not only affect behavioral parameters in the zebrafish (*Danio rerio*) larvae, but cause persisting behavioral effects in adults (Massarsky et al., 2018). Besides, parenteral administration of RS-PG can cause certain behavioral

effects in rodents, including locomotor suppression and anxiolytic effects (Lin et al., 1998; Silva & Elisabetsky, 2001; Harris et al., 2018). However, 0.45 and 2 g/kg RS-PG is intravenously administer for 28 days without exhibiting any discomfort or injury in rat (Pandey et al., 2017). Although the toxicity studies of RS-PG in different animal models have been very common, but about the toxicity study and safety evaluation of its R-, S-enantiomers are still very scarce.

Zebrafish is one of the most widely used model species in previous studies (Ge et al., 2015). Zebrafish has been used not only to assess effects on aquatic biota but also to bridge the gap to other vertebrates (Ma et al., 2019). It has many suitable features, which makes it an ideal vertebrate model for toxicology research (Chen et al., 2020; Sun et al., 2020; Shen et al., 2020a). Due to its unique advantages, such as small size, rapid external development, high fecundity, transparency, easy access and maintenance, make it easy to carry out experiments (Wang et al., 2018; Jia et al., 2020; Wang et al., 2020a). In particular, its high homology with mammalian genes is favorable for assessing developmental toxicity and neurotoxicity of chemical substances (Jia et al., 2020; Shen et al., 2020b). Besides, using zebrafish embryos to address scientific questions is more suitable to meet current legislation (Teng et al., 2019).

In the present study, zebrafish embryos and larvae were employed to assess the developmental toxicity and neurotoxicity of R-, S-, and RS-PG enantiomers. Basic developmental endpoints (i.e., embryonic movement, hatching, mortality, malformation, heartbeat, body length, and diameter of eyes), locomotor activity, and relative transcripts of calcium/sodium ion conduction and γ-amino butyric acid (GABA) receptors involved-genes were assessed in the zebrafish embryos or larvae. These results will play a vital role for the formulation of safety guidelines regarding the potential hazards of PG in early warnings to human health.

2. Materials And Methods

2.1. Chemicals

Racemic RS-PG was purchased from Sigma-Aldrich (St. Louis, MO, USA; > 99% purity). R-PG and S-PG were produced by the recombinant *E.coli-1* and *E.coli-2*, respectively (**S-Methods**). The enantiomeric excess (ee) of isolated PG was determined by chiral chromatography on a 30 m × 0.25 mm × 0.25 μm Alpha DEX™ 120 column. The purity of the isolated PG (R-PG and S-PG) was 98% while the enantiomeric excess was 99.9% (**S-Fig. 1.**). Ethanol (CAS 64-17-5; > 99.7% purity) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals with analytical grade were purchased from commercial sources.

2.2. Zebrafish maintenance and embryonic acute toxicity test

Zebrafish maintenance and embryos collection were performed according to our previous studies (Shen et al., 2020b; Tang et al., 2020). The positive control groups (1 and 5% ethanol, v/v) and test groups of the R-, S-, and RS-PG (0.2, 1, and 5%, v/v, respectively) were prepared in zebrafish culture medium. The blank

control group contained the zebrafish culture medium only. 40 embryos between 0.5-1.0 hours post fertilization (hpf) were cultured in 10 mL exposure solution (three replicates for each treatment) and renewed solutions once daily.

2.3. Embryonic movement, heartbeat, body length, and diameter of eyes assessment

Basic developmental endpoints (embryonic movement, heartbeat, body length, and diameter of eyes) were assessed and analyzed using DanioScope 1.1 (Noldus IT, Wageningen, Netherlands). The embryonic movement ($n \geq 20$ embryos for each treatment) and heartbeat ($n \geq 15$ larvae for each treatment) were recorded videos in a 45 s and 30 s, respectively. The body length and diameter of eyes ($n \geq 18$ larvae for each treatment) were measured from images.

2.4. Locomotor behavioral analysis

The detail locomotor behavioral analysis was implemented according to our previous study (Shen et al., 2020b). Briefly, the larvae treated with R-, S-, and RS-PG for 5 days were selected by simple random sampling and assigned gently into 24-well plates with one larva per well (24 larvae for each treatment). Behavioral movements of larval zebrafish were video-recorded and analyzed using Ethovision XT 11.5 software (Noldus IT, Wageningen, Netherlands).

2.5. Gene expression levels analysis

The total RNA of zebrafish larvae (n = 20 larvae/sample, six biological replicates for each treatment) was extracted using the TRIzol Reagent (TaKaRa, Dalian, China). cDNA synthesis and gene expression levels analysis were performed according to our previous references (Zhou et al., 2019; Shen et al., 2020b). The primer information used for the mRNA expression analysis is listed in **S-Table 1**.

2.6. Statistical analysis

The data were analysed using the Duncan's post hoc test after one-way analysis of variance (ANOVA) and performed using Graphpad Prism 7.0 software. All results were showed as the mean and standard error of the mean (SEM). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

3. Results

3.1. Effects of R-, S-, and RS-PG on the developmental endpoints in zebrafish

Survival rate. Survival rate of zebrafish embryos or larvae after treatment with R-, S-, and RS-PG for 24, 48, 72, 96, and 120 h are shown in **Table 1**. Ethanol was employed as a positive control. Zebrafish embryos after treatment with 5% ethanol for 24 h and 5% R-, S-, and RS-PG for 72 h caused 100% mortality, which indicated that R-, S-, and RS-PG have the less toxic than ethanol. As shown in **Table 1**, zebrafish embryos treated with 5% R-, S-, and RS-PG for 24 h compared with the blank control group decreased the survival rate to 63.33 ± 1.67%, 46.67 ± 3.01%, and 35.83 ± 3.01%, respectively. And zebrafish embryos treated with

5% R-, S-, and RS-PG for 48 h decreased the survival rate to 24.17 ± 2.21%, 24.17 ± 5.07%, and 7.50 ± 1.44%, respectively.

Embryonic movement. The effects of R-, S-, and RS-PG exposure on the embryonic movement are shown in **Fig. 1a**. 5% R-, S-, and RS-PG exposure resulted in significant decreases in the embryonic movement at 24 hpf. However, 1% ethanol exposure significantly increased the embryonic movement compared with the blank control group.

Hatching. The effects of R-, S-, and RS-PG exposure on the hatching rate are shown in **Fig. 1b**. Compared with the blank control group, the hatching rate was not affected at 60 and 72 hpf.

Heartbeat. The effects of R-, S-, and RS-PG exposure on the heartbeat are shown in **Fig. 2a**. The heartbeat was decreased in 1% ethanol, 1% R-PG, and 1% RS-PG treated groups. However, a significant increase was observed in 0.2% S-PG treated group compared with the blank control.

Malformation. The effects of R-, S-, and RS-PG exposure on the spinal curvature rate, pericardial edema rate, and yolk sac edema rate are shown in **Fig. 2b, 2c, and 2d**, respectively. When embryos treated with 1% ethanol for 72 h, the malformation frequency of spinal curvature and pericardial edema were significantly increased. However, embryonic exposure to R-, S-, and RS-PG did not affect the spinal curvature rate and pericardial edema rate in 72 hpf larvae. Besides, the yolk sac edema rate was not affected in R-, S-, and RS-PG and ethanol treated group compared with the blank control.

Body length. The effects of R-, S-, and RS-PG exposure on the body length are shown in **Fig. 3a**. A significant decrease of body length was observed in 1% ethanol treated group. However, a significant increase was observed in 0.2% S-PG treated group.

Diameter of eyes. The effects of R-, S-, and RS-PG exposure on the diameter of eyes X and Y axis are shown in **Fig. 3b and 3c**. Embryonic exposure to R-, S-, and RS-PG for 72 h could significantly increase the diameter of eyes X axis of zebrafish larvae. A significant decrease of the diameter of eyes Y axis was observed in 1% ethanol and 1% RS-PG treated groups. However, a significant increase of the diameter of eyes Y axis was observed in 0.2% R-PG treated group.

3.2. Behavioral effects

Locomotor activity was implemented in 120 hpf larvae (**Fig. 4**). Our results showed that zebrafish larvae exposed with R-, S-, and RS-PG for 120 h displayed incremental total moved distance (**Fig. 4a**) and average movement speed (velocity) (**Fig. 4b**). However, exposure to 1% ethanol did not affect the total moved distance and velocity of larvae. The behavioral trajectory (**Fig. 4c**) of larvae was more chaotic in 1% ethanol, 1% R-, S-, and RS-PG treated groups compared with the blank control group.

3.3. Gene expression analysis

The relative transcripts of genes on calcium/sodium ion conduction. To further understand the effects of R-, S-, and RS-PG on locomoter activity in larvae, the expression levels of genes involved with calcium/sodium ion conduction were analyzed (**Fig. 5a**). The relative transcripts of three genes (*cacna1aa*, *ryr3*, and *slc8a3*) exhibited significant dysregulation compared with the blank control. The expression level of *cacna1aa* was significantly up-regulated in 0.2% and 1% RS-PG treated groups. The expression level of *ryr3* was significantly up-regulated in 0.2% RS-PG, 1% R-, S-, and RS-PG treated groups. However, the expression level of *slc8a3* was significantly down-regulated in 0.2% R- and S-PG and 1% R-PG treated groups.

The relative transcripts of genes on GABA receptors (GABARs). Glutamate decarboxylase (*gad1*) is vitally involved in GABA synthesis (Soghomonian & Martin, 1998). The expression level of *gad1* was not changed after treatment with ethanol or R-, S-, and RS-PG (**S-Fig. 2**). In the central nervous system (CNS) of vertebrates, GABARs play an important part in regulating the vast majority of rapid inhibitory synaptic transmission (Shen et al., 2020b). The relative transcripts of sixteen genes involved in GABARs exhibited significant dysregulation compared with the blank control (**Fig. 5b-e**). The relative transcripts of seven genes (*gabra1*, *gabra2a*, *gabbr1b*, *gabbr2*, *gabrb3*, *gabrb4*, and *gabrg1*) were significantly up-regulated in 0.2% RS-PG, 1% R-, S-, and RS-PG treated groups. The relative transcripts of seven genes (*gabra2b*, *gabra3, gabra4, gabra5, gabrb1*, *gabrb2*, and *gabrg3*) were significantly up-regulated in 0.2% and 1% RS-PG treated groups. In addition, the relative transcripts of nine genes (*gabra1*, *gabra2a*, *gabra3, gabra5, gabbr1b*, *gabbr2*, *gabrb4*, *gabrg1* and *gabrg3*) were significantly up-regulated in 0.2% R-PG treated group. The expression levels of *gabbr1a* and *gabrg2* genes were significantly up-regulated in 0.2% RS-PG treated group. The relative transcripts of seven genes (*gabra1*, *gabra3, gabra5, gabbr1b*, *gabrb2*, *gabrb3* and *gabrb4*) were significantly up-regulated in 1% ethanol treated group. However, the expression levels of *gabra3* and *gabrb1* genes were significantly down-regulated in 0.2% S-PG treated group.

4. Discussion

In the present study, we assessed the developmental toxicity and neurotoxicity of the R-, S-, and RS-PG enantiomers in zebrafish larvae. Basic developmental endpoints of embryos or larvae (i.e., embryonic movement, hatching, mortality, malformation, heartbeat, body length) did not significantly be affected after treatment with R-, S-, and RS-PG enantiomers. The toxicity of the three enantiomers was lower than that of ethanol, and there was no significant difference between them. However, exposure to R-, S-, and RS-PG enantiomers with high doses (0.2% and 1%) significantly changed the diameter of eyes -X and -Y axis and the locomotor activity of larval zebrafish. In addition, the relative transcripts of sixteen genes involved in GABARs and three genes associated with calcium/sodium ion conduction exhibited significant dysregulation compared with the blank control. Altogether, these results indicate that R-, S-, and RS-PG enantiomers of high doses can exhibit the neurotoxicity and ocular developmental toxicity in zebrafish larvae.

The behavior of an organism is directly associated with the feeding, mating, and survival (Wu et al., 2020). Locomotor behaviors have frequently been employed to study the neurodevelopmental effects of

various chemicals (Ding et al., 2020). Usually, animals exposed to hazardous chemicals can change their behavior and these changes may affect their survival, growth, and reproduction (Wang et al., 2018). Previous study has been reported that embryonic exposure to PG can result in behavioral changes in zebrafish larvae (Massarsky et al., 2018). In the present study, we found that embryonic exposure to R-, S-, and RS-PG of high doses could significantly induce the locomotor hyperactivity of zebrafish larvae, demonstrating that R-, S-, and RS-PG of high doses could cause the neurodevelopmental effect in zebrafish larvae. The neurobehavioral abnormalities as a major pathophysiological hallmark is mainly caused by the hypoglutamatergic and hyperGABAergic alterations (Probst et al., 2020). As one of the main inhibitory neurotransmitters in the vertebrate brain, GABA plays a critical role for regulating the circuitry underlying locomotor behavior (Yan et al., 2017). GABA displays rapid inhibitory action through the GABARs (Shen et al., 2020b). We found that the relative transcripts of several genes (*gabra1*, *gabra2a*, *gabbr1b*, *gabbr2*, *gabrb3*, *gabrb4*, and *gabrg1*) involved in GABARs exhibited significant up-regulation in R-, S-, and RS-PG treated larvae. Increased gene expression levels of GABARs could regulate the vast majority of rapid inhibitory synaptic transmission in the CNS (Shen et al., 2020b).

Ryanodine receptors (RyRs) are calcium-dependent calcium release channels embedded in the sarcoplasmic/endoplasmic reticulum (SR/ER), which regulate calcium-dependent signal transduction in neurons or skeletal muscles (Frank et al., 2018; Tanaka et al., 2018). RyRs subunits contain a calcium-binding site that mediates calcium release and triggers intracellular calcium-induced calcium release, which is vital for muscle contraction (Ouyang et al., 2019; Wang et al., 2020b). RyRs have been characterized in many vertebrates including fish, birds, and amphibians (Darbandi & Franck, 2009; Murayama & Kurebayashi, 2011; Wang et al., 2020b). The *ryr3* gene is mainly expressed in brain tissue, and low level in mammalian skeletal muscle (Darbandi & Franck, 2009). Calcium voltage-gated channel subunit alpha1A (*cacna1a*) is mainly expressed in neuronal tissue that plays a crucial part in excitation-contraction coupling via interaction with *ryr3* (Shen et al., 2020b). In the previous study, shen et al. has reported that increased *ryr3* and *cacna1aa* expression could significantly stimulate the neuron-mediated contraction (Shen et al., 2020b). Currently, we found that the relative transcripts of *ryr3* and *cacna1aa* genes were significantly up-regulated in R-, S-, and RS-PG treated larvae, indicating that the intracellular calcium release was significantly increase, and neuron-mediated contraction might be stimulative. Solute carrier family 8 member A3 (*slc8a3*) is mainly expressed in brain and muscle tissue, which contribute to cellular Ca^{2+} homeostasis in excitable cells (Shen et al., 2020b). We found that the relative transcript of *slc8a3* was significantly down-regulated in R-, S-, and RS-PG treated larvae, demonstrating that exposure to R-, S-, and RS-PG of high doses could disrupt the cellular Ca^{2+} homeostasis of neuron and muscles.

Observed teratogenic effects such as small eyes and eye diameter, high ocular distance and large inter-eye distance are characteristic of eye defects (Cadena et al., 2020). These eye defects such as microphthalmia, coloboma, anophthalmia, retinal dystrophies, and congenital cataract can occur in the prenatal and perinatal periods (Kim et al., 2019). Previous studies show that zebrafish exposed to ethanol can cause severe eye defects including microphthalmia and abnormal photoreceptor differentiation (Muralidharan et al., 2015; Muralidharan et al., 2018). The microphthalmia is a small eye normally

defined by corneal diameter or axial length (Huang et al., 2013). Many behaviors are correlative with visual function in vertebrates (Shi et al., 2019). Decreased eye diameter can affect the visual function and lead to a reduced ability to capture prey (Qian et al., 2021). In the present study, we found that embryonic exposure to R-, S-, and RS-PG of high doses could significantly affect the eye diameter of zebrafish larvae, indicating that R-, S-, and RS-PG could impair the visual function of larvae. Besides, RyRs signaling pathway has been directly linked to visual functionality (Ma et al., 2015; Frank et al., 2019). We found that the relative transcript of *ryr3* was significantly up-regulated in R-, S-, and RS-PG treated larvae, demonstrating that exposure to R-, S-, and RS-PG of high doses have the potential to affect the visual sensory system.

From birth to three years old is a crucial window for the promotion of optical growth, health and development (Ojuri et al., 2018). Especially in infants below 16 weeks of age, the enzymes involved in the metabolism of exogenous substances are not as efficient as in adults (Nougadère et al., 2020). The development processes of this period is also more likely disturbed (Nougadère et al., 2020). Infants and toddlers have differing diet patterns than adults, consequently, different intake scenario are required in risk assessment (Stroheker et al., 2019). The variety of foods is ceaselessly growing and changing for infants and toddlers, which can result in specific dietary exposure (Chekri et al., 2019). Processed cereal-based foods and other infant foods should be free from chemical and biological hazards (Ojuri et al., 2018). Currently, PG is generally recognized as a safe food additive, which is oversupplied in Chinese market (Phillips et al., 2017; Tao et al., 2020; Zhao et al., 2020). PG has very low toxicity and is considered to be a non-toxic or non-carcinogenic compound for adults (Zaripov et al., 2020). However, newborns and infants are specially susceptible to the effects of PG (Massarsky et al., 2018). In the present study, our results showed that R-, S-, and RS-PG enantiomers of high doses can exhibit the neurotoxicity and ocular developmental toxicity in zebrafish larvae, indicating that R-, S-, and RS-PG exposures of high doses have the potential neurotoxicity and ocular developmental toxicity for infants and toddlers.

In the present study, we demonstrate that exposure to R-, S-, and RS-PG enantiomers of high doses could significantly change the eye diameter and the locomotor activity of larval zebrafish. Besides, the expression levels of sixteen genes involved in γ-amino butyric acid receptors (GABARs) and three genes associated with calcium/sodium ion conduction exhibited significant dysregulation, indicating that R-, S-, and RS-PG enantiomers of high doses can affect the CNS and visual sensory system. However, the toxicity of the three enantiomers was lower than that of ethanol, and there was no significant difference between them. Taken together, our results indicate that R-, S-, and RS-PG enantiomers of high doses can exhibit the neurotoxicity and ocular developmental toxicity in zebrafish larvae. Therefore, we suggest that the potential neurotoxicity and ocular developmental toxicity of R-, S-, and RS-PG enantiomers for infants and toddlers should be considered.

Declarations

Conflicts of interest statement

The authors declare that they have no conflict of interest.

Funding

This work was supported by the collaborative project propylene glycol enantiomers toxicity assays (XDHT2020543A) from the OAMIC BIOTECH CO., LTD. (Lonemen Road, Haicang Xinyang Industrial Zone, Xiamen, China).

Author contributions

Chao Shen: Conceptualization, Methodology, Investigation, Writing-Original draft preparation, Writing-Reviewing and Editing; **Xijing Zhao:** Methodology, Investigation; **Chengyong He**: Supervision, Validation, Writing-Reviewing and Editing; **Zhenghong Zuo**: Funding acquisition, Project administration, Resources, Visualization, Writing-Reviewing and Editing.

Ethics approval

All experiments using zebrafish were performed according to the animal protocol approved by the guides of Animal Ethics Committee of Xiamen University.

Consent to participate and consent for publication

Not applicable.

Availability of data and materials

The obtained and analyzed data of this study are available from the corresponding author on reasonable request.

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Tables

Table 1 Survival rate of zebrafish embryos or larvae after treatment with R-, S-, and RS-PG for 24, 48, 72, 96, and 120 hours.

	The survival rate (%) of zebrafish embryos or larvae				
Treated groups	24 hpf	48 hpf	72 hpf	96 hpf	120 hpf
Control	74.17±2.21	72.50±1.44	68.33±3.33	68.33±3.33	67.50±3.82
1% ethanol	77.50±5.20	76.67±6.01	73.33±5.47	70.00±3.82	57.50±5.00
5% ethanol	00.00±0.00***	00.00±0.00***	00.00±0.00***	00.00±0.00***	00.00±0.00***
0.2%-(R)-PG	76.67±6.01	75.00±6.29	70.00±8.78	69.17±8.33	68.33±7.95
1%-(R)-PG	80.00±2.50	80.00±2.50	80.00±2.50	80.00±2.50	79.17±2.21
5%-(R)-PG	63.33±1.67*	24.17±2.21***	00.00±0.00***	00.00±0.00***	00.00±0.00***
0.2%-(S)-PG	85.00±5.20	84.17±5.47	84.17±5.47	84.17±5.47	83.33±5.83
1%-(S)-PG	85.00±2.50*	85.00±2.50*	82.50±3.82	82.50±3.82	79.17±3.63
5%-(S)-PG	46.67±3.01**	24.17±5.07***	00.00±0.00***	00.00±0.00***	00.00±0.00***
0.2%-(RS)-PG	90.83±1.67**	89.17±1.67**	86.67±3.01*	85.83±3.33*	81.67±4.17
1%-(RS)-PG	85.00±1.44*	80.83±4.41	70.83±1.67	69.17±2.21	60.00±2.50
5%-(RS)-PG	35.83±3.01***	7.50±1.44***	00.00±0.00***	00.00±0.00***	00.00±0.00***

hpf: hours post fertilization. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figures

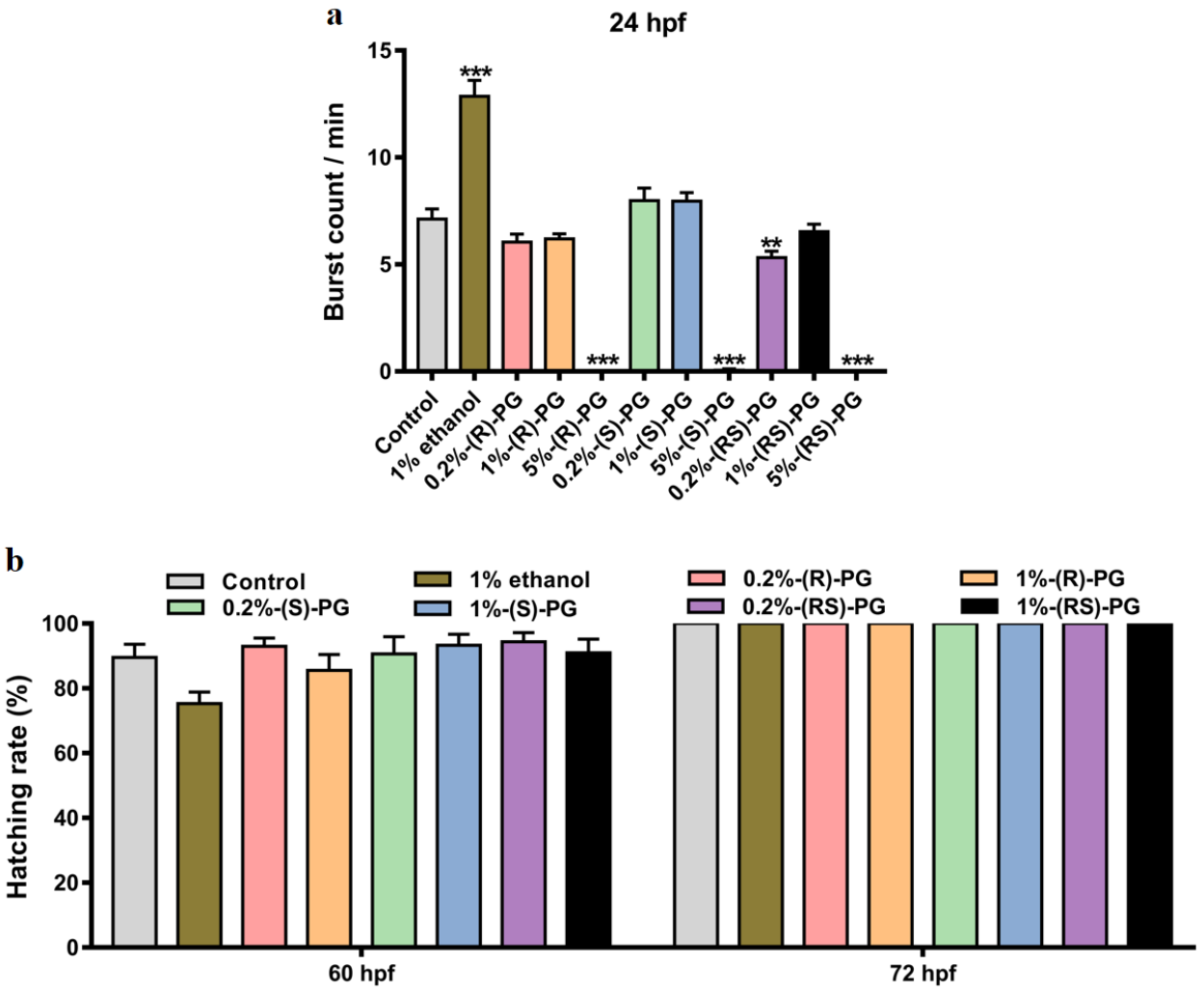

Figure 1

The effects of R-, S-, and RS-PG exposure on the embryonic movement and hatching. (a) Embryonic movement (24 hpf, n ≥ 20 embryos for each treatment), (b) hatching rate (60 and 72 hpf). Data presented as mean ± SE. **P < 0.01, ***P < 0.001.

Figure 2

The effects of R-, S-, and RS-PG exposure on the heartbeat and malformation of larval zebrafish (72 hpf). (a) Heart beats (n ≥ 15 per treatment), (b) spinal curvature rate, (c) pericardial edema rate, (d) yolk sac edema rate. Data presented as mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 3

The effects of R-, S-, and RS-PG exposure on the body length and eye diameter of larval zebrafish (72 hpf). (a) Body length, (b) diameter of eyes-X axis, (c) diameter of eyes-Y axis. Data presented as mean ± SE (n ≥ 18 per treatment). *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 4

Locomotor activity of zebrafish larvae exposed to R-, S-, and RS-PG at 5 dpf. (a) Total moved distance, (b) mean velocity, (c) behavioral trajectory. Data presented as mean ± SE (n = 24 per treatment). **P < 0.01, ***P < 0.001.

Figure 5

R-, S-, and RS-PG exposure induced the mRNA expression of genes involved in calcium/sodium ion conduction and GABA receptors at 5 dpf larvae (n = 6 per treatment). (a) The expression of genes involved in calcium/sodium ion conduction. (b) The expression of genes involved in type A GABA receptor subunit α. (c) The expression of genes involved in type B GABA receptor. (d) The expression of genes involved in type A GABA receptor subunit β. (e) The expression of genes involved in type A GABA receptor subunit γ.

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