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RESEARCH ARTICLE

Flavor compound geraniol induces inhibited nutrient utilization and developmental toxicity on embryonic–larval zebrafish

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Abstract

Flavors are widely utilized in the food and oral pharmaceutical industry, particularly in products for children, to enhance palatability and promote ingestion willingness. The complex compositions of flavors potentially induce severer toxicity especially in children. In this study, zebrafish embryos are applied for toxicity screening of flavor and its compounds by immersing in flavor solutions followed by the assessment of morphology of zebrafish larvae. Geraniol is identified as the prominent toxic compound and is considered highly toxic to zebrafish embryo. In further toxicology study, geraniol demonstrates the concentration-dependent developmental toxicity as the obvious reduction of body and eye lengths, as well as the increased prevalence of tail deformities, pericardial edema, and spine deformation. Zebrafish larvae treated with geraniol exhibit reduction in liver area and exocrine pancreas length, increase in yolk sac area, as well as elevation of triglycerides and total cholesterol, which indicate the inhibited nutrient utilization. Transcriptome analysis reveals that under geraniol treatment, 248 differentially expressed genes (DEGs) are downregulated, whereas 23 DEGs are upregulated, and 110 DEGs are related to metabolic process. Biological processes of lipid metabolism, carbohydrate metabolism, protein hydrolysis, and transmembrane transport, including their involved functional genes, are all downregulated. These findings reveal the developmental toxicity of geraniol by affecting the nutrient utilization-related organs development and biological processes. This study

establishes an efficient screening model for identifying toxic flavor compounds during developing stages, thereby elucidating the potential safety risks of geraniol exposure in zebrafish and providing a comprehensive understanding of its potential toxicity mechanism.

KEYWORDS
developmental toxicity, flavor, geraniol, nutrient utilization, zebrafish

1 | INTRODUCTION

Flavors are widely used in food industry to modify the food flavor, as well as in oral medications to optimize the palatability and facilitate the willingness for medicine ingestion (Guedes et al., 2021; Mamusa et al., 2021). Both natural and artificial flavors contain diverse classes of flavor compounds (Kfoury et al., 2024). For instance, berry fruit flavors consist of multiple ionones, damascones, irones, hexenol, ethyl butyrates, and maltols (Reshna et al., 2022); citrus flavors usually contain pinene, myrcene, linalool, and decanal (Fan et al., 2015). The presence of these complex organic compounds in flavors raises the potential safety concerns. In particular, many pharmaceutical excipients and food additives exhibited greater toxicity in children than in adults due to children's immature organ systems and developing barriers (Becker et al., 2010). It has been reported that glycerin, which is considered to be relatively nontoxic in adults, presents the neurotoxic effects in children (Peiré, 2019), and meanwhile, the accumulation of sorbitol could contribute to diabetic complications and even pose a risk of inducing liver damage (Rowe et al., 2009; Yochana et al., 2012). Therefore, the toxicity of flavor compounds in individuals during developmental stages should be given more consideration. In China, the use of flavor compounds adheres to the regulations stipulated in Chinese National Standard GB2760 (http://www.nhc.gov.cn/zwgk/cyb). The safety data of flavors also primarily originate from the sources such as Flavor Extract Manufacturers Association (https://www.femaflavor.org/) and Joint FAO/WHO Expert Committee on Food Additive (https://www.who.int/groups/joint-fao-who-expert-committee-on-food-additives-(jecfa)) worldwide. However, these existing standards and databases are still lack of safety studies specifically for children in developing stages.

According to a market survey conducted by Guangdong Institute for Drug Control, a kind of commercial flavor, ranked among top 10 most frequently used in food and medicines, is consist of a combination of 15 distinct flavor compounds. Based on the available safety data and standard, this commercial flavor is deemed suitable for adding to food products and oral pharmaceuticals. Notably, the components of this commercial flavor include geraniol. Geraniol, an acyclic monoterpene alcohol, is extensively utilized due to its rose-like odor and the fruity citrus taste (Burdock, 2016). In natural flavors, geraniol can be found in multiple essential oils such as monarda fistulosa, palmarosa, citronella, rose, and ninde (Chen & Viljoen, 2010). Within artificial flavors, geraniol also plays an important role in various formulations, contributing significantly to the overall flavoring system (Chen & Viljoen, 2010). In market, 76% of deodorants, 41% detergents, and 33% cosmetics contain geraniol (Maczka et al., 2020). In addition, geraniol is reported to have antimicrobial, antioxidant, anticancer, and anti-inflammatory activities (Chen & Viljoen, 2022; Lira et al., 2020). Despite being a widespread flavor compounds, limited attention has been given to the toxicity of geraniol in previous study. Geraniol may induce the positive patch test reaction, and the sensitization to geraniol would be enhanced as a result of autoxidation and metabolism (Hagvall et al., 2012). Inhalation of geraniol could lead to an elevation in serum alanine transaminase activity and lipid peroxidation, subsequently inducing hepatic tissue injury (Andrade et al., 2014). In addition, it has been documented that geraniol induces chromosomal damage and genotoxic effects on cells (Mamur, 2022; Singulani et al., 2018). Although geraniol has been reported to have embryotoxicity on zebrafish embryos (Singulani et al., 2018), the further developmental toxicity and its mechanism have not been fully investigated.

Zebrafish is an excellent model to study the toxicity during developing stages (Shi et al., 2011), whose main organs are formed within the first 72 h post fertilization (hpf) and keep developing at larval stage (McCollum et al., 2011). The development of zebrafish is mostly comparable to that of mammals, including similar tissues and organs (McCollum et al., 2011), sharing up to 70% genetic homology and over 80% of identical disease-related proteins with humans (Howe et al., 2013). Additionally, zebrafish are capable of facilitating high-throughput screenings in a short period (Mikami et al., 2020). Furthermore, the toxicity on metabolism organs, such as hepatotoxicity, could be determined on the embryonic–larval zebrafish, and the impacts on organs morphology can be visualized on transgenic strains of zebrafish (Zhang et al., 2020).

In this study, the toxicity of the commercial flavor listed in the top 10 commonly used in market according to the survey by Guangdong Institute for Drug Control is evaluated using embryonic–larval zebrafish model. The major toxic compound is screened from the 15 flavor compounds that are contained in the commercial flavor. Geraniol is found to be toxic, and the mechanism of caused toxicity is explored. Developmental toxicity of geraniol is investigated from zebrafish morphology, larval yolk absorption to metabolism-related organs levels. Moreover, the impact on biological processes such as lipid metabolism, protein hydrolysis, and carbohydrate metabolism, as well as their key genes, is explored at transcriptomic level to deeply explain how geraniol affects zebrafish development. This study provides a platform for screening

the toxicity of flavors during developmental stages and reveals the developmental toxicity of key compound, which is beneficial for guiding their proportion in children's products and reducing potential safety risks.

2 | MATERIALS AND METHODS

2.1 | Materials

The commercial flavor and its compounds were collected and provided by Guangdong Institute for Drug Control. 1-phenyl-2-thiourea (PTU) and tricaine were obtained from Sigma Aldrich.

2.2 | Zebrafish husbandry and embryo culture

Zebrafish (*Danio rerio*) were supplied and housed according to the standard protocols from the Zebrafish Model Organism Database (http://zfin.org). A/B wild type and *Tg(fabp10a:dsRed;ela3l:EGFP)* transgenic zebrafish were employed in this study. Transgenic line *Tg(fabp10a:dsRed;ela3l:EGFP)* was obtained from Wei Ge's Lab at University of Macau. Adult zebrafish were raised in an aquaculture system with a light (14 h) and dark (10 h) cycle, being fed at least twice a day with newly hatched shrimp. Embryos were obtained via natural mating and cultured in E3 medium (13.7 mM NaCl, 540 μM KCl, 25 μM $NaHPO_4$, 44 μM KH_2PO_4, 300 μM $CaCl_2$, 100 μM $MgSO_4$, 420 μM $NaHCO_3$, pH 7.4) at 28.5°C. During the initial 48 hpf period, methylene blue was introduced into the E3 medium. For the transgenic strains, PTU was added into medium from 24 hpf. The E3 medium for all embryos was refreshed daily. All the animal experimental protocols were approved by Animal Research Ethics Committee in University of Macau.

2.3 | Flavor and flavor compounds exposure

The flavor and its compounds (Table S1), including geraniol, were first dissolved in equal volume of 1,2-propanediol, respectively, according to their concentration ratio in the commercial flavor, followed by dilution in E3 culture medium to prepare the flavor and its compounds solutions. E3 medium containing the same concentration of 1,2-propanediol as in the flavor and its compounds solution served as the solvent control (vehicle group), whereas pure E3 medium was used as the blank control (blank group). Zebrafish embryos were cultured in these solutions at a temperature of 28.5°C in dark environment from 6 hpf.

2.4 | Mortality and hatching assessment

Mortality and hatching assessments were conducted on the basis of the Organization for Economic Cooperation and Development guidelines and subsequently modified (OECD, 2013). Fertilized eggs in cleavage stage were collected and distributed into 6-well cell culture plates (five eggs/well). Initially, embryos in E3 medium were incubated with flavor and flavor compounds, including geraniol solutions from 6 hpf. Culturing medium was renewed every 24 h throughout 96 hpf. The hatching rate and mortality rate were assessed at 12, 24, 48, 72, and 96 hpf. The mortality rate was calculated as the accumulative number of dead embryos compared with total number of the treated group. The hatching rate was expressed as the accumulative number of embryos that had hatched compared with total number of the treated group.

2.5 | Morphology assessment

After exposure to geraniol from 6 hpf, zebrafish larvae from each group were anesthetizeed with 0.02% tricaine and positioned with the sagittal plane facing upwards, then observed and photographed using stereomicroscope (Nikon, SMZ800N) at 96 hpf. The body length, eye length, and yolk sac area of larvae were measured through ImageJ software. The tail deformity (TD), pericardial edema (PE), and spine deformation (SD) rate was calculated as the number of larvae with TD, PE, or SD compared with total number of hatched larvae.

2.6 | Hepatopancreatic developmental toxicity assessment

Transgenic strain zebrafish *Tg(fabp10a:dsRed;ela3l:EGFP)* was established to investigate the development and toxicity on liver and pancreas. The transgenic zebrafish was incubated in geraniol solutions from 6 hpf until 96 hpf and anesthetizeed with 0.02% tricaine at 96 hpf. To observe the phenotype of liver and pancreas, the zebrafish larvae of each group were imaged using a fluorescence microscope (Leica, Dmi8). The area and fluorescence intensity of liver and pancreas were determined by ImageJ software.

2.7 | Total cholesterol (TC) and triglyceride (TG) measurement

After exposure to geraniol, zebrafish larvae in each group were collected at 96 hpf and homogenized for determination. Each group contained about 120 zebrafish larvae. The levels of total cholesterol (TC) and triglyceride (TG) in larval zebrafish were quantified using specific commercial assay kits (Jiancheng).

2.8 | Transcriptomic analysis of zebrafish larvae

Zebrafish was treated with 1,2-propanediol or geraniol from 6 to 96 hpf, and the geraniol-treated group exhibits the phenomenon of developmental toxicity. Zebrafishes from vehicle and geraniol groups were sent to BGI Genomics for RNA extraction and RNA sequencing

(RNA-seq), each group contained 3 biological replicated samples, and each sample consisted of 25–30 larval zebrafish.

The raw data were filtered with SOAPnuke (v1.6.5) by removing reads containing adapters. Clean reads that unknown base ("N" base) ratio was more than 1%, and low-quality base ratio was more than 40% were obtained and store in FASTQ format. Subsequently, the clean data were, respectively, mapped to the reference genome by HISAT (v2.2.1). After these two steps of quality control, the genes were annotated using Kyoto Encyclopedia of Genes and Genomes (KEGG) and GO databases, and the differentially expressed genes (DEGs) analysis was performed using DEseq2/DEGSeq. The KEGG pathway, gene ontology (GO) terms enrichment and category analysis, and Gene Set Enrichment Analysis (GSEA) were conducted on Dr. Tom in BGI platform (https://biosys.bgi.com/#/report/login).

2.9 | Statistical analysis

All data were analyzed by the GraphPad Prism 9 software (GraphPad Software Inc.). All difference significance was analyzed using one-way analysis of variances (ANOVA) followed by multiple comparisons (compare each group with every other group) using Tukey's test or two-way ANOVA followed by multiple comparisons (within each time point, compare each group with every other group) using Tukey's test. A p value of less than .05 was considered statistically significant; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$.

3 | RESULTS

3.1 | Toxic compounds screening from flavor on zebrafish embryos and larvae

According to a marketing survey conducted by the Guangdong Institute for Drug Control, a commercial flavor is the commonly used flavor with the large market in food, beverage, and pharmaceutics in China. The commercial flavor is composed of 15 flavor compounds, including geraniol, dissolved in 1,2-propanediol (Table S1).

Initially, the commercial flavor was diluted in E3 medium to 0.15, 0.3, 0.6, and 0.8 μL/mL to assess its toxicity on embryonic–larval zebrafish through the detection of the mortality rate, hatching rate, and body length of zebrafish embryos and larvae after treating with the flavor. Interestingly, the acute and developmental toxicity was observed when incubating zebrafish with flavor. At 96 hpf, compared to normal treatment zebrafish, the mortality rate in the groups treated with 0.6 and 0.8 μL/mL flavor was significantly increased from 5.56% ± 1.57% to 15.56% ± 4.16% ($p = .0009$) and 42.23% ± 3.14% ($p < .0001$), respectively, whereas the hatching rates in these two groups obviously decreased from 94.44% ± 1.57% to 73.33% ± 5.44% ($p = .0008$) and 50.00% ± 2.72% ($p < .0001$), respectively (Figure 1a,b). Meanwhile, compared to the average body length of 3.926 ± 0.083 mm for normal zebrafish, the treatment with 0.6 and 0.8 μL/mL flavor resulted in noticeable reduction in body length to 3.233 ± 0.161 mm ($p < .0001$) and 3.005 ± 0.182 mm ($p < .0001$), respectively (Figure 1c). These changes all exhibited a concentration-dependent trend in the dilution gradient.

Following the study of toxic effects on embryonic–larval zebrafish, the contribution of each component to toxicity in this flavor was assessed. According to the results obtained, the dilution concentration of 0.6 μL/mL was selected for subsequent toxic compound screening. First, the 15 flavor compounds in the commercial flavor were divided into 5 groups (Table S1) based on their chemical functional groups for toxicological screening. The flavor compounds in each group were dissolved in 1,2-propanediol at the consistent concentration within the commercial flavor to prepare subgroup samples, and then the subgroup samples were diluted in E3 medium to 0.6 μL/mL. At 96 hpf, the mortality and hatching rates of the blank group were 11.67% ± 2.36% and 88.33% ± 2.36%, and the body length was 3.726 ± 0.112 mm. Under treatment with Group 2, compared to the blank group, the mortality and hatching rates significantly changed to 20.00% ± 4.08% ($p = .0111$) and 71.67% ± 4.71% ($p = .0015$), and the body length decreased to 2.650 ± 0.264 mm ($p < .0001$), whereas the other four groups exhibited no statistically significant alterations (Figure 1d–f), suggesting the only Group 2 compound exists potential toxic effect.

Next, the toxicity evaluation of each compound in group 2 (Table S1) was individually conducted. The four flavor compounds in Group 2 were dissolved in 1,2-propandiol following their concentrations in the commercial flavor to prepare single-compound samples, whereafter the single-compound samples were diluted to 0.6 μL/mL in E3 medium. It was found that geraniol significantly affected the mortality, hatching rate, and body length of zebrafish larvae compared to the blank group at 96 hpf. The mortality rate increased from 15.00% ± 2.36% to 25.00% ± 4.08% ($p = .0465$), whereas the hatching rate exhibited the notable decline from 83.33% ± 2.36% to 68.33% ± 2.36% ($p = .0015$). Additionally, a significant reduction in body length was observed, with measurements decreasing from 3.566 ± 0.102 to 2.735 ± 0.135 mm ($p < .0001$). (Figure 1g–i). However, other three compounds displayed almost no changes, thereby indicating the great contribution of geraniol to the flavor toxicity. The content of geraniol in all the commercial flavor and subgroup/single-compound samples was 0.1%, and the final concentration of geraniol in the 0.6 μL/mL diluent of all the commercial flavor and subgroup/single-compound samples was calculated to be 0.6 μg/mL.

3.2 | Developmental toxicity of geraniol on zebrafish embryos and larvae

Based on the preliminary study, geraniol was screened out as the major compound in flavor caused toxicity. To evaluate the toxicity level of geraniol, the LC_{50} curve was constructed by assessing 96 hpf zebrafish mortality with concentrations of 0.4, 0.7, 1.0, 1.2, and 1.4 μg/mL. The 96 h-LC_{50} value of geraniol was 0.9209 μg/mL, with a 95% confidence interval of between 0.8877 and 0.9534 μg/mL (Figure 2a). Subsequently, the mortality and hatching rates of zebrafish embryos and larvae that treated with 0.2, 0.5, and 0.8 μg/mL geraniol as well as

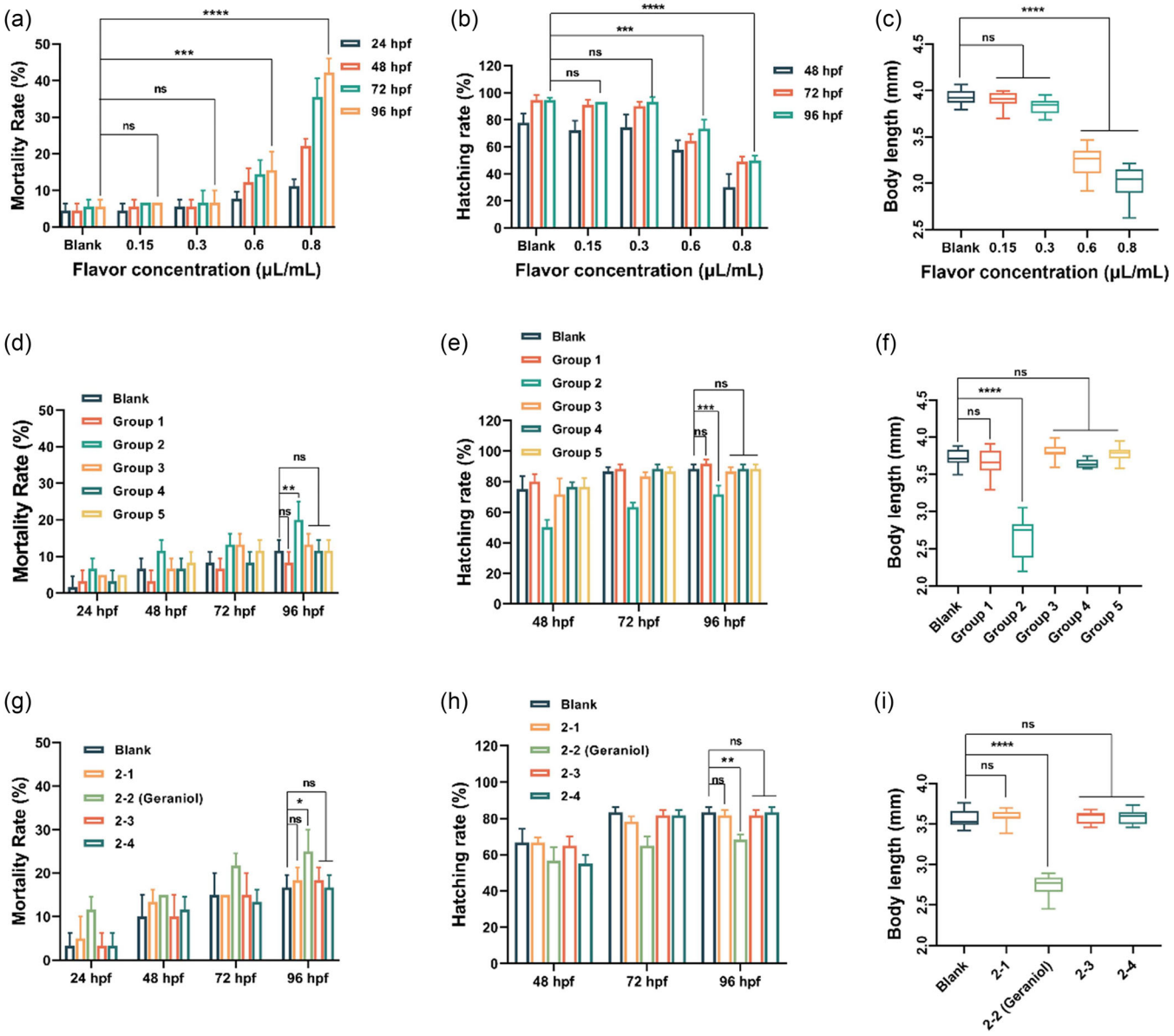

FIGURE 1 Toxic compounds screening from a commercial flavor. After exposure to flavor from 6 h post fertilization (hpf), the mortality (a) and hatching rates (b) of embryonic–larval zebrafish at 24, 48, 72, and 96 hpf; and body length (c) of zebrafish larvae at 96 hpf. After exposure to five mixtures of compounds contained in the flavor from 6 hpf, the mortality rate (d) and hatching rate (e) of embryonic–larval zebrafish at 24, 48, 72, and 96 hpf; and body length (f) of zebrafish larvae at 96 hpf. After exposure to compounds contained in Mixture 2 from 6 hpf, the mortality rate (g) and hatching rate (h) of embryonic–larval zebrafish at 24, 48, 72, and 96 hpf; and body length (i) of zebrafish larvae at 96 hpf. In mortality and hatching rate, data are presented as mean ± SD ($n = 20$ or 30 embryos in each group replicated thrice). In body length, data are presented as boxes and whiskers; boxes represent 25th and 75th percentiles, whiskers represent min to max, and solid lines represent the medians ($n = 10$ larvae in each group). One-way ANOVA or two-way ANOVA followed by Tukey test were conducted to evaluate the statistical differences between the groups. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; and $^{****}p < .0001$.

1,2-propanediol were assessed. At 96 hpf, the mortality rate and hatching rate of zebrafish larvae treated with 0.8 μg/mL geraniol greatly changed compared to the blank group resulting in the increase of mortality rate from 13.33% ± 4.71% to 40.00% ± 4.08%, and the decrease of hatching rate from 86.67% ± 4.71% to 61.67% ± 2.36% (Figure 2b,c). However, the treatment of 0.2 and 0.5 μg/mL geraniol and 1,2-propanediol did not result in any significant alterations in acute toxicity. It revealed that geraniol at the concentration of 0.8 μg/mL exhibited both acute and developmental toxicity on developing zebrafish.

Thereafter, whether geraniol induced further developmental toxic effects on zebrafish larvae at concentrations of 0.2 and 0.5 μg/mL was detected by observing the body length, eye length, TD, PE, and SD. After incubating with 0.2 and 0.5 μg/mL of geraniol, the body and eye lengths of zebrafish larvae were getting shorter at 96 hpf, whereas the TD, PE, and SD were also observed with 0.2 and

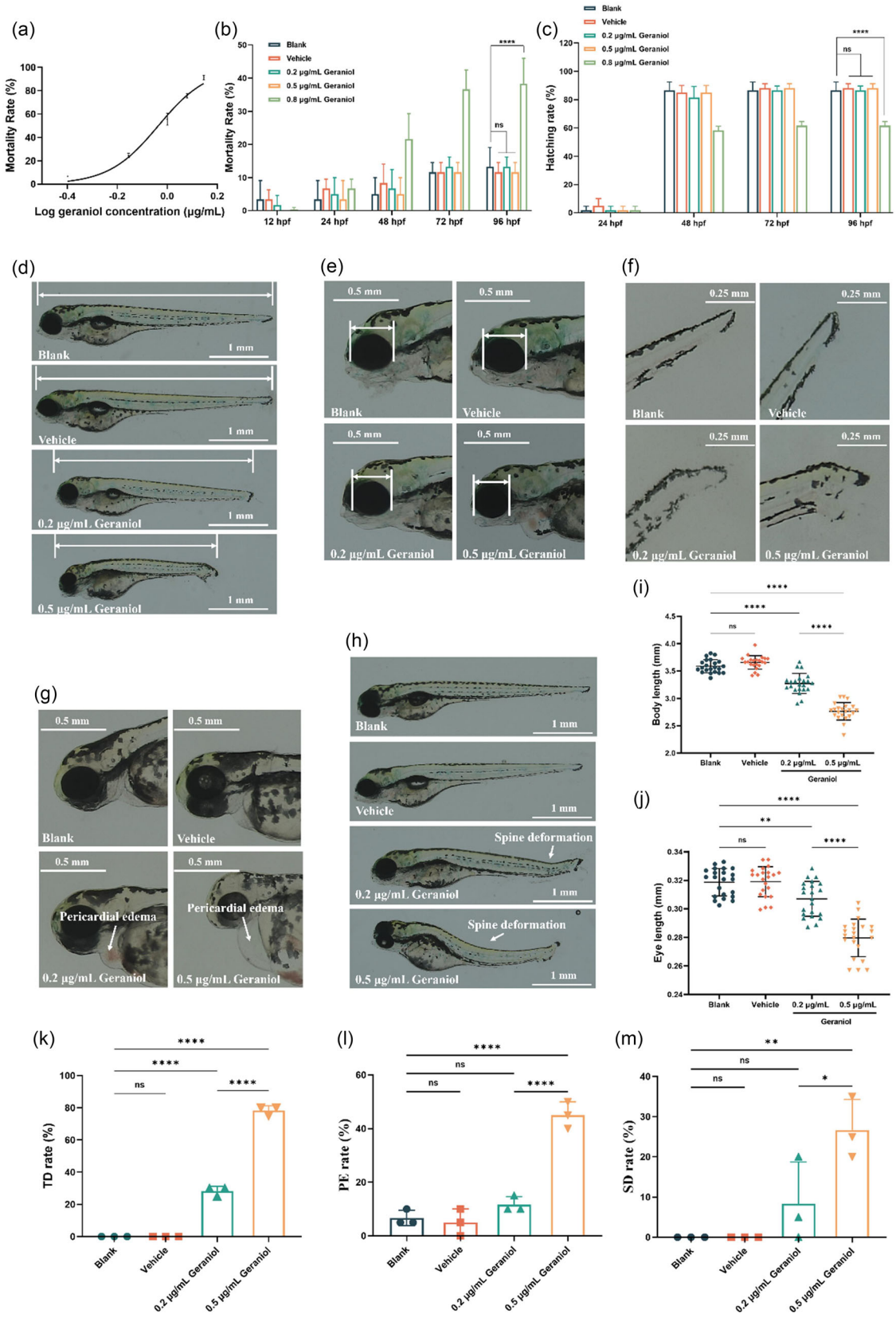

FIGURE 2 Developmental toxicity of geraniol on zebrafish larvae at different concentrations: (a) 96 h post fertilization (hpf)-LC_{50} curves of geraniol on embryonic–larval zebrafish. Mortality (b) and hatching rates (c) of zebrafish embryos and larvae at 12, 24, 48, 72, and 96 hpf. Zebrafish $n = 30$ in each group replicated thrice. Representative images of body length reduction (d), eye length reduction (e), tail deformity (f), pericardial edema (g), and spine deformation (h) of zebrafish larvae at 96 hpf. Body (i) and eye lengths (j) of zebrafish larvae at 96 hpf. Zebrafish larvae $n = 22$ in each group. Tail deformity (TD) (k), pericardial edema (PE) (l), and spine deformation (SD) rates (m) statistics of zebrafish larvae at 96 hpf. Zebrafish larvae $n = 20$ in each group, replicated thrice. One-way ANOVA or two-way ANOVA followed by Tukey test were conducted to evaluate the statistical differences between the groups. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; and $^{****}p < .0001$.

0.5 μg/mL geraniol treatment (Figure 2d–h). As Figure 2i,j displays, the body length in blank group and 0.2 and 0.5 μg/mL geraniol treatment group were measured as 3.587 ± 0.118, 3.274 ± 0.179, and 2.765 ± 0.157 mm, respectively. Similarly, the eye length was determined to be 0.319 ± 0.009, 0.307 ± 0.012, and 0.280 ± 0.013 mm for these three groups. The incidence of TD and SD in the blank group was negligible (0.00% ± 0.00%), whereas the PE rate was 6.67% ± 2.36%. However, the TD rate significantly increased to 28.33% ± 22.36% and 78.33% ± 2.36%, respectively, following the treatment with geraniol at concentrations of 0.2 and 0.5 μg/mL. Although there were no significant changes after treated with 0.2 μg/mL geraniol, the PE and SD rates significantly increased to 45.00% ± 4.08% and 26.67% ± 6.24% in 0.5 μg/mL geraniol group. (Figure 2k–m). Moreover, the reduction of body and eye lengths, as well as the increase of TD, PE, and SD rate, exhibited a concentration-dependent manner. So, geraniol could induce developmental inhibition on zebrafish larvae at the concentrations of 0.2 and 0.5 μg/mL. Additionally, geraniol caused more serve developmental toxicity but no acute toxic effects at 0.5 μg/mL, then the deep study of developmental toxicity mechanism was conducted at this concentration.

3.3 | Effect of geraniol on yolk utilization and related organs

In view of the observed toxicity of geraniol to the development of zebrafish larvae, the impact of geraniol on development and growth of zebrafish was investigated in terms of yolk sac utilization and major organs development associated with nutrient absorption. First, the area of zebrafish larvae yolk sac was measured to evaluate the absorption of nutrients storing in the yolk sac. The yolk retention was observed in zebrafish larvae after expose to geraniol (Figure 3a). The yolk sac area of geraniol group exhibited an obvious increase to 0.184 ± 0.026 mm^2 compared to 0.133 ± 0.020 mm^2 in the blank group (Figure 3b), illustrating the nutrients utilization of zebrafish larvae was inhibited by geraniol. So, it could be contemplated that the developmental toxicity of geraniol was related to nutrients utilization during the developing stage of zebrafish larvae. To validate the impact of geraniol on major organs associated with nutrients metabolism, the liver and exocrine pancreas were investigated on the zebrafish line *Tg(fabp10a:dsRed;ela3l:EGFP)* (Figure 3c) after geraniol treatment (Li et al., 2023). Geraniol exposure resulted in significant decrease on the areas of liver and the length of exocrine pancreas (Figure 3d,e), as the liver area was 0.024 ± 0.005, 0.025 ± 0.004, and 0.011 ± 0.003 mm^2 (Figure 3f), and the exocrine pancreas length was 0.412 ± 0.031, 0.389 ± 0.027, and 0.191 ± 0.070 mm for the blank, vehicle, and geraniol groups (Figure 3g). Moreover, the mean fluorescence intensity of liver and exocrine pancreas was both significantly reduced after exposure to geraniol (Figure 3h,i), revealing the downregulation of expressed *fabp10a* and *ela3l* in liver and exocrine pancreas that were represented by dsRed and EGFP in transgenic zebrafish. These results supported that liver and exocrine pancreas were affected by geraniol, leading to the trend of developmental delay.

To further validate the utilization of major nutrients in the yolk sac, the TGs and TC were determined in zebrafish. As depicted in Figure 3j,k, no significant differences were observed in TGs and TC between the blank and the vehicle groups. However, following geraniol treatment, there was a substantial increase in TC and TG levels, indicating reduced utilization and enhanced retention of nutrients.

3.4 | Transcription alterations of zebrafish larvae induced by geraniol

To elucidate the impact of geraniol on nutrition utilization process and associated functions at transcriptomic level, RNA-seq was performed to analyze global transcription changes of zebrafish larvae treated with 1,2-propanediol and geraniol at 96 hpf (Figure 4). An average of 44.41 Mb and 6.52 Gb of clean reads and clean bases for each sample were acquired with the average valid ratio of 96.49% (Table S2). A cumulative total of 25,660 genes were identified. More than 86.97% of clean reads were totally mapped to reference genome, and more than 74.33% of clean reads were uniquely mapped to reference genome (Table S3), indicating the satisfied quantity and quality of clean data for further analysis. A total of 271 genes between vehicle and geraniol groups were identified as DEGs, including 23 of upregulated and 248 of downregulated (Q value < .05 and $|\log_2(\text{fold change})| \geq 1.2$) (Figure 4a,b).

KEGG pathway enrichment analysis was performed on all DEGs to explore the main impacts of geraniol on functions of zebrafish larvae. Nine pathways were significantly enriched in Figure S1. The KEGG pathways "pancreatic secretion," "bile secretion," "protein digestion and absorption," "fat digestion and absorption," "retinal metabolism," "starch and sucrose metabolism," and "steroid hormone biosynthesis" listed in top 10 identified pathways with most enriched DEGs and Q value < .05, indicating the major impacts on metabolic functions and mainly related to liver and exocrine pancreas. It was consistent with the previously observed insufficiency in hepatic and pancreatic development after treatment with geraniol. Subsequently, the total 271 DEGs were classified, 110 DEGs were involved in metabolic process according to GO annotation (Figure S2), 41 DEGs were involved in digestive system, and 40 DEGs were involved in metabolism according to KEGG pathway annotation (Figure S3). The gene enrichment and classification analysis provided evidence that the processes of metabolism and the digestive system were affected by geraniol.

To further explored the impacts of geraniol on metabolic processes, the DEGs classified as metabolism and digestive system categories were conducted GO enrichment analysis. The top eight significantly enriched GO biological process terms were all subordinate to category of metabolic process or localization. Within the metabolic processes category, the top three enriched terms were "proteolysis," "carbohydrate metabolic process," and "lipid metabolic process." Similarly, within the category of localization, the top three enriched terms were "dipeptide transmembrane transport," "oligopeptide transport," and "transmembrane transport" (Figure 4c). The DEGs enriched in "dipeptide transmembrane transport" and "oligopeptide transport" were all

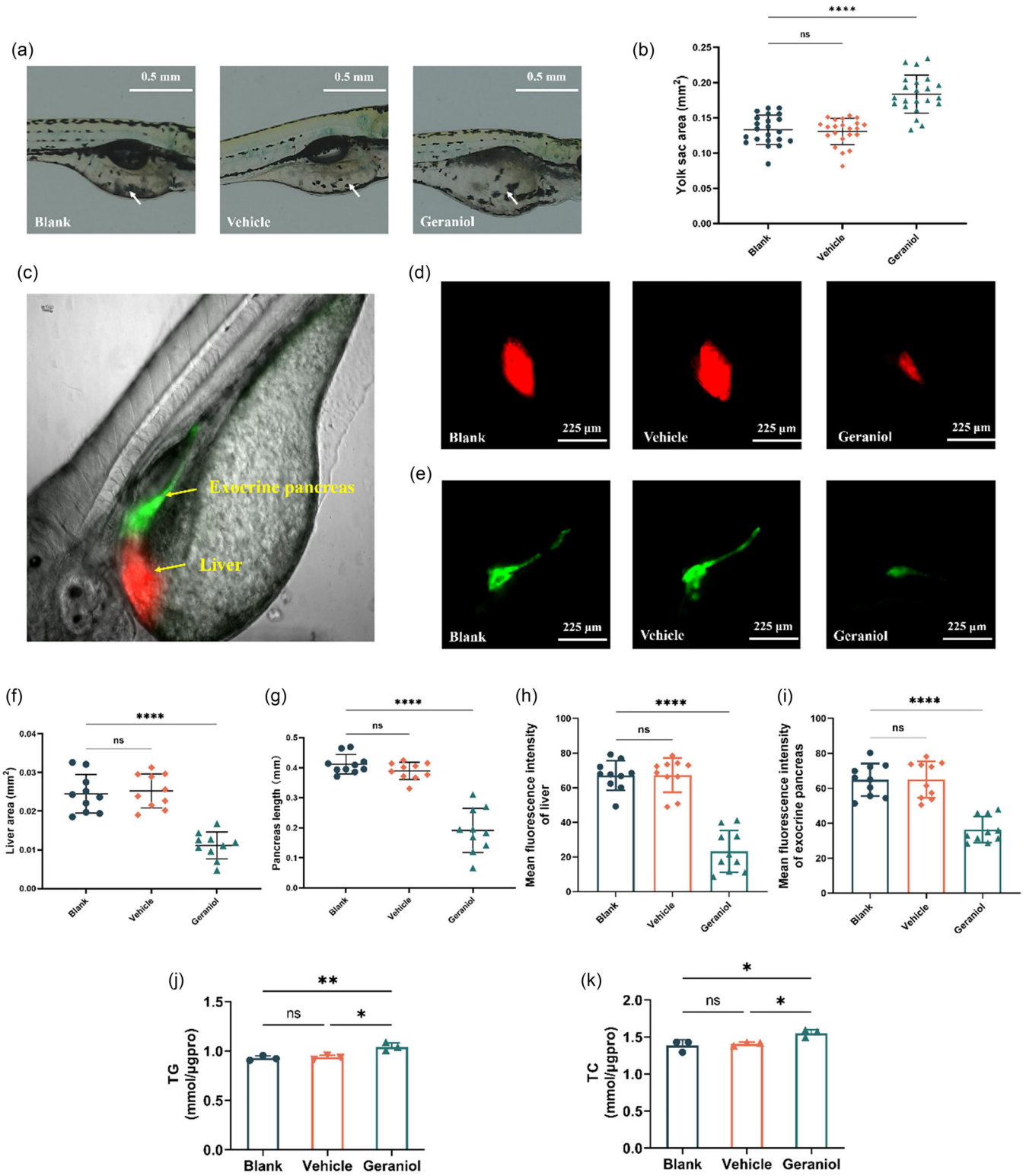

FIGURE 3 The impacts of geraniol on yolk, liver, and exocrine pancreas in zebrafish larvae: (a) Representative images of yolk retention in zebrafish larvae exposed to geraniol. (b) Yolk sac area of zebrafish larvae at 96 h post fertilization (hpf). Zebrafish larvae $n = 22$ in each group. (c) Representative image of zebrafish line *Tg(fabp10a:dsRed;ela3l:EGFP)*. Representative images of developmentally toxic effect on liver (d) and exocrine pancreas (e) of zebrafish larvae exposed to geraniol at 96 hpf. (f) Liver area of zebrafish larvae at 96 hpf. (g) Exocrine pancreas length of zebrafish larvae at 96 hpf. Mean fluorescence intensity of liver (h) and exocrine pancreas (i) at 96 hpf. Zebrafish larvae $n = 10$ in each group. The content of triglycerides (TG) (j) and total cholesterol (TC) (k) in zebrafish at 96 hpf. One-way ANOVA or two-way ANOVA followed by Tukey test were conducted to evaluate the statistical differences between the groups. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; and $^{****}p < .0001$.

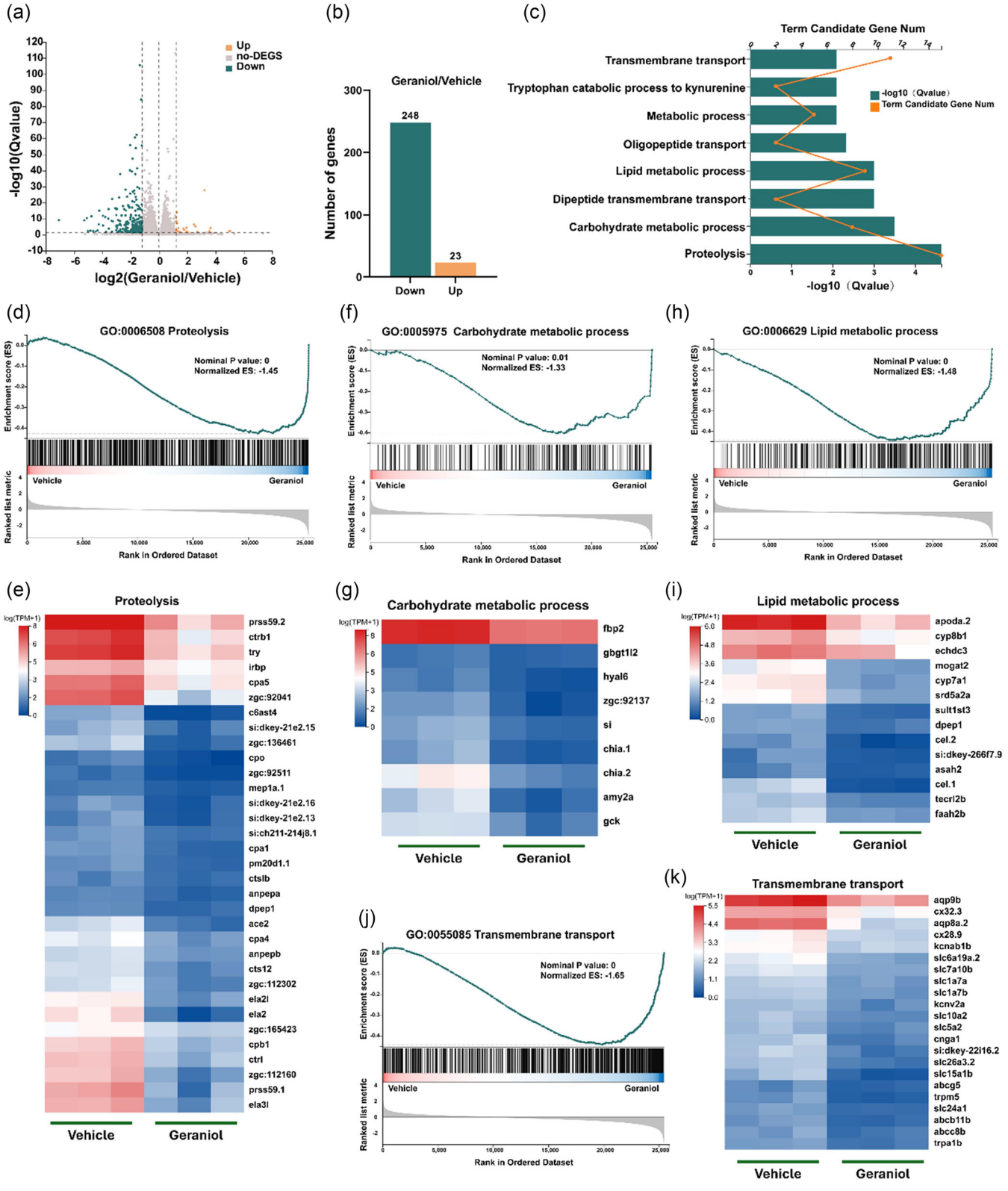

FIGURE 4 Transcription expression changes and enrichment analysis of zebrafish larvae between vehicle and geraniol groups. (a) Volcano plot of DEGs between vehicle and geraniol groups. (b) Amount of up/downregulated DEGs. (c) GO biological process enrichment analysis on DEGs classified into metabolism and digestive system categories. (d) GSEA enrichment analysis on term "proteolysis." (e) Key genes regulation in term "proteolysis." (f) GSEA enrichment analysis on term "carbohydrate metabolic process." (g) Key genes regulation in term "carbohydrate metabolic process." (h) GSEA enrichment analysis on term "lipid metabolic process." (i) Key genes regulation in term "lipid metabolic process." (j) GSEA enrichment analysis on term "transmembrane transport." (k) Key genes regulation in term "transmembrane transport."

involved in "transmembrane transport" as well, indicating that "transmembrane transport" contained the affected functions of "dipeptide transmembrane transport" and "oligopeptide transport."

Subsequently, GSEA was performed on all expressed genes in vehicle and geraniol groups to fully understand the gene expression pattern of biological process terms "proteolysis," "carbohydrate metabolic process," "lipid metabolic process," and "transmembrane transport." The candidate key genes in significantly enriched terms were identified from leading edge subset by applying the standard of $|\log_2(\text{geraniol/vehicle})| > 1$, Q value < .05, and TPM value with the higher expression levels between vehicle and geraniol groups > 1. It revealed a significant enrichment in proteolysis with a nominal p value of 0, a Normalized Enrichment Score (ES) of −1.45, and a False Discovery Rate (FDR) Q value below 0.2 (Figure 4d). Within this term, key genes, such as *try*, *cpa1*, *cpa4*, *cpa5*, *mep1a.1*, *ela2*, *ela2l*, *ela3l*, and *ctrl*, exhibited a notable decreased expression (Figure 4e). Similarly, Figure 4f depicts the significant enrichment of the carbohydrate metabolic process, in which nominal p value was .01, a Normalized ES was −1.33, and an FDR Q value was under .25. Functional genes in this process, including *fbp2*, *chia.1*, *chia.2*, *amy2a*, and glucokinase (*gck*), were also found to be downregulated (Figure 4g). In the context of lipid metabolism, there was an obvious enrichment, as evidenced by a nominal p value of 0, a Normalized ES of −1.48, and an FDR Q value less than .25 (Figure 4h). The key genes involved *cyp7a1*, *cyp8b1*, *cel.1*, *cel.2*, and *apoda.2* showed a considerable reduction in expression levels (Figure 4g). Meanwhile, a significant enrichment in transmembrane transport was observed with a nominal p value of 0, a Normalized ES of −1.65, and an FDR Q value below .25 (Figure 4h), in which substantial downregulation was observed in functional genes, including *slc1a7a*, *slc1a7b*, *slc5a2*, *slc6a19a.2*, *slc7a10b*, *slc10a2*, *slc15a1b*, *slc24a1*, *slc26a3.2*, *abcg5*, *abcb11b*, *abcc8b*, *aqp8a2*, and *aqp9b* (Figure 4g). The downregulation of these four terms and their key genes illustrated that the processes of lipid, protein, and carbohydrate metabolism of zebrafish were disturbed with the treatment of geraniol.

4 | DISCUSSION

Flavors were the widespread used and complex compounds that should be given more attention on their safety risk. In the current study, by investigating mortality rate, hatching rate, and body length of embryonic–larval zebrafish in the first 96 hpf, it was rapidly screened out geraniol as toxic compound from a commercial flavor formulation. In consist with the previous study, it had been investigated the embryotoxicity of geraniol on zebrafish, which demonstrated the feasibility of applying zebrafish embryo as the potential platform to rapidly and accurately screen toxic compounds from flavors (Singulani et al., 2018). In previous studies, multiple cell lines were commonly used to detect the cytotoxicity of flavors, including *Foeniculum vulgare* and *Citrus aurantiifolia* (Fagodia et al., 2017; Sharopov et al., 2017). Toxicity of benzyl alcohol, phenethyl alcohol, and cinnamaldehyde was determined on *Caenorhabditis elegans* (Lu et al., 2021). Compared to these models, zebrafish can not only achieve high-throughput toxicity screening but also be a more advanced animal and visualized platform to directly observe in vivo toxicity and further explore the potential toxicology mechanism due to its transparent body and highly similar tissues, organs, and genes to humans. In this study, geraniol was found to be the major toxicity compound, and its impacts on the development of metabolic organs, the related biological process, and functional genes were deeply studied.

In this study, the 96 hpf-LC_{50} value of geraniol was determined to be 0.9209 μg/mL, with a 95% confidence interval of between 0.8877 and 0.9534 μg/mL. Based on the toxicity grading standards of China (GB/T 31270.12-2014, Ministry of Agriculture Pesticide Inspection Center), geraniol is considered highly toxic to zebrafish embryos. According to the information obtained from the flavor manufacturer by the Guangdong Institute for Drug Control, the content of the commercial flavor is found to be 0.5–1.5 μL/mL in food industry, whereas 5–20 μL/mL in medicine industry, with the final concentration of geraniol in products is 0.5–1.5 μg/mL and 5–20 μg/mL. In this study, all the detected concentrations are 25–100 times lower than the actual industrial used maximum concentration. In other words, geraniol exhibited toxic effects on zebrafish at concentrations that are 25–100 times lower than those in food and medication industry, indicating greater toxicity at concentrations in real-world conditions. The larval zebrafish showed concentration-dependent decrease in eye and body lengths after exposure to geraniol, as well as the enriched KEGG pathway "phototransduction" served as evidence of abnormalities in the visual system. In properly developing zebrafish, the retinal layers were clear, and the size of the eyes was normal. Microphthalmia was a defect on eye development, manifested as a reduction in eye length that could be caused by toxic chemicals (Chen et al., 2022; Qian et al., 2021). Developmental delay could be displayed by the phenomenon of the reduced eye size and visual dysfunction, meanwhile the shorten body length (Cairns et al., 2021; Kashyap et al., 2007). The nutritional absorption was proved to have correlation with the development of larval zebrafish, including body length at early stage (Schwartz et al., 2021), and the decrease of body length and eye size were widely observed in the zebrafish larvae with yolk retention as previously reported. (Al-Jamal et al., 2020; Yu et al., 2022) Additionally, it has been reported that the decrease in nutrient supply could affect the TD (Chahardehi et al., 2020). Accordingly, TD was also viewed on zebrafish larvae exposed to geraniol in the current study. Consequently, it was hypothesized that the developmental delay and tail malformation observed in larval zebrafish following exposure to geraniol were caused by decrease in nutrient utilization.

The yolk sac served as a nutritional reservoir for the zebrafish embryos and larvae at early stages (Sant & Timme-Laragy, 2018). Until 5 dpf, zebrafish primarily relied on the yolk as their exclusive energy source without feeding behavior and sustainable provided nutrients throughout all developmental processes (Halbach et al., 2020; Quinlivan & Farber, 2017). It has been regarded the increased or decreased of remaining yolk as the symbol of delayed or accelerated nutrients utilization during the period of 0–120 hpf (Jiang et al., 2020; Sant et al., 2017). In the present study, the yolk sac area of larval zebrafish was

getting larger after exposure to geraniol, which revealed the decrease in nutrients absorption. Meanwhile, the primary constituents of nutrients within the yolk sac are lipids, including TGs and cholesterol (Sant & Timme-Laragy, 2018). Quantitative analysis conducted on zebrafish revealed that geraniol induced an elevation in residual TG and TC levels, aligning with delayed absorption from the yolk sac and confirming reduced nutrient utilization. As reported, the size of the yolk sac was also suggested as an endpoint for assessing liver function in zebrafish (Hill, 2011; Jiang et al., 2020; Sant et al., 2017). Since the predominant metabolism of nutrients was conducted in liver, hepatotoxicity would disturb the resorption of yolk (Jiang et al., 2020). Based on these, it was inferred that liver could be affected by geraniol and then induced the decrease of yolk utilization and developmental toxic effects.

In zebrafish line *Tg(fabp10a:dsRed;ela3l:EGFP)*, it was observed that the size and fluorescence intensity of liver were reduced. The size change of liver could reflect hepatotoxicity, including the inhibition of early liver development as the result of liver shrinkage and reduction in specific expressed fluorescence intensity (Feng et al., 2023; Manjunatha et al., 2021). The result of RNA-seq excavated the downregulated key gene *mep1a*.1, which played a very important role in cell differentiation and proliferation by activating the growth factors [49]. It was proved that downregulation of *mep1a* could induce developmental hepatotoxicity and delayed nutrient absorption in the previous study [32]. Meanwhile, the size and fluorescence intensity of pancreas were observed to be decreased after geraniol treatment, indicating the development of pancreas was also interrupted. Since liver and pancreas were both differentiated from the foregut endoderm in zebrafish (Dong et al., 2007), geraniol may affect the differentiation and normal development of the endoderm into the liver and pancreas. Additionally, the decrease of fluorescence illustrated that expression of *ela3l* and *fabp10a* was reduced in exocrine pancreas and liver. Fatty acid–binding proteins (*Fabps*) have been reported to be involved in fatty acid and lipid metabolism, regulating fatty acid transport (Bayir et al., 2015). Elastase (*ela*) was reported to be associated with protein digestion and absorption (Li et al., 2023). The downregulation of *ela3l* and *fabp10a* suggested that the metabolism process related to nutrients utilization was disturbed in larval zebrafish exposed to geraniol.

Furthermore, the biological processes, including proteolysis, carbohydrate metabolism, lipid metabolism, and transmembrane transport in larval zebrafish, were all downregulated after treated by geraniol. Key downregulated genes in proteolysis terms encoded multiple enzymes involved in the proteins and peptide hydrolysis, suggesting the utilization of protein was inhibited. In carbohydrate metabolic process, *chia.1*, *chia.2*, *amy2a*, and *amy2al2*, reported to be involved in hydrolysis of chitin and breakdown of polysaccharides and oligosaccharides, were all downregulated (Lanes et al., 2021; Roy-Carson et al., 2017). Except that fructose-1,6-bisphosphatase (*Fbp2*), reported as a rate limiting enzyme in gluconeogenesis (Dhanasiri et al., 2013), and *gck*, as a glycolysis-related gene (Lu et al., 2023), were both downregulated after geraniol treatment, suggesting the glucose homeostasis was affected (Jiang et al., 2020). These changes on functional genes expression revealed that the process of utilizing carbohydrates for energy was disturbed. In lipid metabolic process, the transcription of cytochrome P450s (*cyp7a1*, *cyp8b1*) related to bile acid biosynthesis was decreased, resulting in the impacts of cholesterol metabolism (Mu et al., 2015; Qi et al., 2015; Tomaszewski et al., 2008). The decreased transcription of carboxylic ester lipase (*cel.1 and cel.2*) leads to the reduction of cholesterol resorption and delayed development of larval zebrafish (Camarota et al., 2011; Qiu et al., 2020). So the process of lipid metabolism, especially cholesterol metabolism, was inhibited by geraniol. In transmembrane transport, the transcription of solute carriers (*slc*) and adenosine triphosphate-binding cassettes (*abc*) was decreased. *Slc5a2* has been reported as a glucose transport; *slc6a19a*, *slc1a7a*, *slc1a7b*, *slc7a10b*, and *slc15a1b* were observed to be involved in amino acid and peptide transport; and *slc10a2* could perform bile acids transport closely associated with lipid metabolism (Gesemann et al., 2010; Jersin et al., 2021; Orozco et al., 2018; Sander et al., 2019; Vacca et al., 2019; Zhu et al., 2024). *Abcb11b* was response for transporting cholate conjugates from hepatocytes to the bile (Ren et al., 2015). All phenomena indicated the process and function of protein, lipid, and carbohydrate utilization were inhibited by geraniol.

The genes implicated in zebrafish by geraniol have also demonstrated analogous functions in previous studies on mammal. Taking lipid metabolism as an exemplar, *gck* has been reported to be involved in aberrant lipid accumulation and lipolysis in the mice liver (Zhu et al., 2023). Mice with lipid metabolism disorders exhibited diminished levels of *cyp7a1*, and augmenting *cyp7a1* expedited the conversion of TC into bile acids (Ge et al., 2023). Suppression of *cyp8b1* expression in mouse liver led to impaired bile acid synthesis during infection (Lane et al., 2023). Reduced expression of *slc10a2* in mice was associated with decreased reabsorption of luminal bile acids (Pasquereau-Kotula et al., 2018). *Abcb11*-deficient mice manifest intrahepatic cholestasis and liver fibrosis, whereas even *abcb11* expression is significantly downregulated in human liver cancer tumors (Wang et al., 2021). Therefore, the developmental toxicity induced by geraniol via its impact on organs and biological processes associated with nutrient utilization may also manifest in developing mammals and even children.

Although zebrafish serves as an exceptional platform for rapid toxicity screening and toxicology research, further validation of its toxicity in mammalian models is imperative due to interspecies disparities. Additionally, given that zebrafish are coincubated with compound solutions rather than subjected to oral or systemic administration, it is crucial to conduct additional investigations on the conversion of toxic doses across different dosing modes and animal species.

5 | CONCLUSION

The potential toxicity of flavors and flavor compounds during the developmental stage requires greater attention. In this study, the embryonic–larval zebrafish was used to screen the toxicity of flavor compounds. Geraniol, determined to be highly toxic to zebrafish embryos, was identified as the major toxicity caused compound to induce developmental toxic effects in zebrafish larvae, including reduced body length, decreased eye length, increased TD, PE, and SD. Further investigation revealed that zebrafish larvae exposed to

geraniol had delayed yolk resorption, increased level of residual TGs and TC, as well as reduced liver and exocrine pancreas sizes and fluorescence intensity. Furthermore, four biological processes related to metabolism and functional genes involved in these processes were significantly downregulated. The developmental toxicity study of geraniol on zebrafish embryo from morphology, organ, metabolism process, and gene expression level integrally revealed how geraniol affects the development of zebrafish. Therefore, it is crucial to consider and investigate the potential risks of using geraniol in food and children's products in the future.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

All procedures involving animals were approved by the Animal Research Ethics Committee of the University of Macau, Macau SAR, China on 30th October, 2023. (Ethics approval ID: UMARE-038-2023, valid until 29th October, 2026).

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REFERENCES

Al-Jamal, O., Al-Jighefee, H., Younes, N., Abdin, R., Al-Asmakh, M. A., Radwan, A. B., Sliem, M. H., Majdalawieh, A. F., Pintus, G., Yassine, H. M., Abdullah, A. M., Da'as, S. I., & Nasrallah, G. K. (2020). Organ-specific toxicity evaluation of stearamidopropyl dimethylamine (SAPDMA) surfactant using zebrafish embryos. *Science of the Total Environment*, *741*, 140450. https://doi.org/10.1016/j.scitotenv.2020.140450

Andrade, B. F., Braga, C. P., Dos Santos, K. C., Barbosa, L. N., Rall, V. L., Sforcin, J. M., Fernandes, A. A., & Fernandes Júnior, A. (2014). Effect of inhaling cymbopogon martinii essential oil and geraniol on serum biochemistry parameters and oxidative stress in rats. *Biochemistry Research International*, *2014*, 493183. https://doi.org/10.1155/2014/493183

Bayir, M., Bayir, A., & Wright, J. M. (2015). Divergent spatial regulation of duplicated fatty acid-binding protein (*fabp*) genes in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *14*, 26–32. https://doi.org/10.1016/j.cbd.2015.02.002

Becker, M., Edwards, S., & Massey, R. I. (2010). Toxic chemicals in toys and children's products: Limitations of current responses and recommendations for government and industry. *Environmental Science & Technology*, *44*(21), 7986–7991. https://doi.org/10.1021/es1009407

Burdock, G. A. (2016). *Fenaroli's handbook of flavor ingredients*. CRC Press.

Cairns, G., Burté, F., Price, R., O'Connor, E., Toms, M., Mishra, R., Moosajee, M., Pyle, A., Sayer, J. A., & Yu-Wai-Man, P. (2021). A mutant *wfs1* zebrafish model of Wolfram syndrome manifesting visual dysfunction and developmental delay. *Scientific Reports*, *11*(1), 20491. https://doi.org/10.1038/s41598-021-99781-0

Camarota, L. M., Woollett, L. A., & Howles, P. N. (2011). Reverse cholesterol transport is elevated in carboxyl ester lipase-knockout mice. *The FASEB Journal*, *25*(4), 1370–1377. https://doi.org/10.1096/fj.10-169680

Chahardehi, M. A., Arsad, H., & Lim, V. (2020). Zebrafish as a successful animal model for screening toxicity of medicinal plants. *Plants*, *9*(10), 1345. https://doi.org/10.3390/plants9101345

Chen, W., & Viljoen, A. M. (2010). Geraniol—A review of a commercially important fragrance material. *South African Journal of Botany*, *76*(4), 643–651. https://doi.org/10.1016/j.sajb.2010.05.008

Chen, W. Y., & Viljoen, A. M. (2022). Geraniol—A review update. *South African Journal of Botany*, *150*, 1205–1219. https://doi.org/10.1016/j.sajb.2022.09.012

Chen, X., Qiu, T., Xiao, P., & Li, W. (2022). Retinal toxicity of isoflucypram to zebrafish (*Danio rerio*). *Aquatic Toxicology*, *243*, 106073. https://doi.org/10.1016/j.aquatox.2021.106073

Dhanasiri, A. K., Fernandes, J. M., & Kiron, V. (2013). Liver transcriptome changes in zebrafish during acclimation to transport-associated stress. *PLoS ONE*, *8*(6), e65028. https://doi.org/10.1371/journal.pone.0065028

Dong, P. D., Munson, C. A., Norton, W., Crosnier, C., Pan, X., Gong, Z., Neumann, C. J., & Stainier, D. Y. (2007). Fgf10 regulates hepatopancreatic ductal system patterning and differentiation. *Nature Genetics*, *39*(3), 397–402. https://doi.org/10.1038/ng1961

Fagodia, S. K., Singh, H. P., Batish, D. R., & Kohli, R. K. (2017). Phytotoxicity and cytotoxicity of essential oil and its major constituents: Limonene and citral. *Industrial Crops and Products*, *108*, 708–715. https://doi.org/10.1016/j.indcrop.2017.07.005

Fan, H., Wu, Q., Simon, J. E., Lou, S. N., & Ho, C. T. (2015). Authenticity analysis of citrus essential oils by HPLC-UV-MS on oxygenated heterocyclic components. *Journal of Food and Drug Analysis*, *23*(1), 30–39. https://doi.org/10.1016/j.jfda.2014.05.008

Feng, C., Bai, H., Chang, X., Wu, Z., Dong, W., Ma, Q., & Yang, J. (2023). Aflatoxin B1-induced early developmental hepatotoxicity in larvae zebrafish. *Chemosphere*, *340*, 139940. https://doi.org/10.1016/j.chemosphere.2023.139940

Ge, H., Ye, X., Chen, Q., Ye, J., & Chen, J. (2023). *Ligusticum chuanxiong* prevents high-fat-diet-induced lipid metabolism disorder in mice by modulating the genes in the cholesterol pathway. *Food Frontiers*, *4*(4), 1958–1972. https://doi.org/10.1002/fft2.249

Gesemann, M., Lesslauer, A., Maurer, C. M., Schönthaler, H. B., & Neuhauss, S. C. F. (2010). Phylogenetic analysis of the vertebrate excitatory/neutral amino acid transporter (SLC1/EAAT) family reveals lineage specific subfamilies. *BMC Evolutionary Biology*, *10*(1), 117. https://doi.org/10.1186/1471-2148-10-117

Guedes, M. D. V., Marques, M. S., Guedes, P. C., Contri, R. V., & Kulkamp Guerreiro, I. C. (2021). The use of electronic tongue and sensory panel on taste evaluation of pediatric medicines: A systematic review. *Pharmaceutical Development and Technology*, *26*(2), 119–137. https://doi.org/10.1080/10837450.2020.1860088

Hagvall, L., Karlberg, A. T., & Christensson, J. B. (2012). Contact allergy to air-exposed geraniol: Clinical observations and report of 14 cases. *Contact Dermatitis*, *67*(1), 20–27. https://doi.org/10.1111/j.1600-0536.2012.02079.x

Halbach, K., Ulrich, N., Goss, K. U., Seiwert, B., Wagner, S., Scholz, S., Luckenbach, T., Bauer, C., Schweiger, N., & Reemtsma, T. (2020). Yolk sac of zebrafish embryos as backpack for chemicals? *Environmental Science & Technology*, *54*(16), 10159–10169. https://doi.org/10.1021/acs.est.0c02068

Hill, A. (2011). Hepatotoxicity testing in larval zebrafish. In: P. McGrath (Ed.), *Zebrafish: Methods for assesing drug safety and toxicity* (pp. 89–102). America: Wiley.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J. C., Koch, R., Rauch, G. J., White, S., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503. https://doi.org/10.1038/nature12111

Jersin, R. Å., Tallapragada, D. S. P., Madsen, A., Skartveit, L., Fjære, E., McCann, A., Lawrence-Archer, L., Willems, A., Bjune, J. I., Bjune, M. S., Våge, V., Nielsen, H. J., Thorsen, H. L., Nedrebø, B. G., Busch, C., Steen, V. M., Blüher, M., Jacobson, P., Svensson, P. A., … Dankel, S. N. (2021). Role of the neutral amino acid transporter SLC7A10 in adipocyte lipid storage, obesity, and insulin resistance. *Diabetes*, *70*(3), 680–695. https://doi.org/10.2337/db20-0096

Jiang, J., Chen, L., Wu, S., Lv, L., Liu, X., Wang, Q., & Zhao, X. (2020). Effects of difenoconazole on hepatotoxicity, lipid metabolism and gut microbiota in zebrafish (*Danio rerio*). *Environmental Pollution*, *265*(Pt A), 114844. https://doi.org/10.1016/j.envpol.2020.114844

Kashyap, B., Frederickson, L. C., & Stenkamp, D. L. (2007). Mechanisms for persistent microphthalmia following ethanol exposure during retinal neurogenesis in zebrafish embryos. *Visual Neuroscience*, *24*(3), 409–421. https://doi.org/10.1017/S0952523807070423

Kfoury, M., Legrand, F. X., Ruellan, S., & Fourmentin, S. (2024). Low melting mixtures: Neoteric green solvents for flavor formulation. *Journal of Molecular Liquids*, *394*, 123696. https://doi.org/10.1016/j.molliq.2023.123696

Lane, J. M., Brosschot, T. P., Gatti, D. M., Gauthier, C. M., Lawrence, K. M., Pluzhnikova, V., & Reynolds, L. A. (2023). Chronic small intestinal helminth infection perturbs bile acid homeostasis and disrupts bile acid signaling in the murine small intestine. *Frontiers in Parasitology*, *2*, 1214136. https://doi.org/10.3389/fpara.2023.1214136

Lanes, C. F. C., Pedron, F. A., Bergamin, G. T., Bitencourt, A. L., Dorneles, B. E. R., Villanova, J. C. V., Dias, K. C., Riolo, K., Oliva, S., Savastano, D., & Giannetto, A. (2021). Black soldier fly (*Hermetia illucens*) larvae and prepupae defatted meals in diets for zebrafish (*Danio rerio*). *Animals*, *11*(3), 720. https://doi.org/10.3390/ani11030720

Li, A., Yan, C., Qiu, J., Ji, Y., Fu, Y., & Yan, W. (2023). Adverse effects of plastic leachate and its component 2,4-DTBP on the early development of zebrafish embryos. *Science of the Total Environment*, *904*, 167246. https://doi.org/10.1016/j.scitotenv.2023.167246

Lira, M. H. P., Andrade Júnior, F. P., Moraes, G. F. Q., Macena, G. S., Pereira, F. O., & Lima, I. O. (2020). Antimicrobial activity of geraniol: An integrative review. *Journal of Essential Oil Research*, *32*(3), 187–197. https://doi.org/10.1080/10412905.2020.1745697

Lu, K., Wu, J., Zhang, Y., Zhuang, W., & Liang, X. F. (2023). Role of phosphoenolpyruvate carboxykinase 1 (*pck1*) in mediating nutrient metabolism in zebrafish. *Functional & Integrative Genomics*, *23*(1), 67. https://doi.org/10.1007/s10142-023-00993-6

Lu, S., Liu, S. S., Huang, P., Wang, Z. J., & Wang, Y. (2021). Study on the combined toxicities and quantitative characterization of toxicity sensitivities of three flavor chemicals and their mixtures to *Caenorhabditis elegans*. *ACS Omega*, *6*(51), 35745–35756. https://doi.org/10.1021/acsomega.1c05688

Maczka, W., Winska, K., & Grabarczyk, M. (2020). One hundred faces of geraniol. *Molecules (Basel, Switzerland)*, *25*(14), 3303. https://doi.org/10.3390/molecules25143303

Mamur, S. (2022). Geraniol, a natural monoterpene, identifications of cytotoxic and genotoxic effects in vitro. *Journal of Essential Oil Research*, *34*(1), 54–64. https://doi.org/10.1080/10412905.2021.1974581

Mamusa, M., Resta, C., Sofroniou, C., & Baglioni, P. (2021). Encapsulation of volatile compounds in liquid media: Fragrances, flavors, and essential oils in commercial formulations. *Advances in Colloid and Interface Science*, *298*, 102544. https://doi.org/10.1016/j.cis.2021.102544

Manjunatha, B., Sreevidya, B., & Lee, S. J. (2021). Developmental toxicity triggered by benzyl alcohol in the early stage of zebrafish embryos: Cardiovascular defects with inhibited liver formation and degenerated neurogenesis. *Science of the Total Environment*, *752*, 141631. https://doi.org/10.1016/j.scitotenv.2020.141631

McCollum, C. W., Ducharme, N. A., Bondesson, M., & Gustafsson, J. A. (2011). Developmental toxicity screening in zebrafish. *Birth Defects Research Part C: Embryo Today: Reviews*, *93*(2), 67–114. https://doi.org/10.1002/bdrc.20210

Mikami, I., Takahashi, Y., Koiwa, J., Okamura, M., & Tanaka, T. (2020). Zebrafish yolk sac microinjection of thalidomide for assessment of developmental toxicology. *Congenital Anomalies*, *60*(2), 71–72. https://doi.org/10.1111/cga.12335

Mu, X., Wang, K., Chai, T., Zhu, L., Yang, Y., Zhang, J., Pang, S., Wang, C., & Li, X. (2015). Sex specific response in cholesterol level in zebrafish (*Danio rerio*) after long-term exposure of difenoconazole. *Environmental Pollution*, *197*, 278–286. https://doi.org/10.1016/j.envpol.2014.11.019

OECD. (2013). Test No. 236: Fish embryo acute toxicity (FET) test. In: *OECD guielines for the testing of chemicals* (section 2). Paris: OECD Publishing.

Orozco, Z. G. A., Soma, S., Kaneko, T., & Watanabe, S. (2018). Spatial mRNA expression and response to fasting and refeeding of neutral amino acid transporters *slc6a18* and *slc6a19a* in the intestinal epithelium of *Mozambique tilapia*. *Frontiers in Physiology*, *9*, 212. https://doi.org/10.3389/fphys.2018.00212

Pasquereau-Kotula, E., Martins, M., Aymeric, L., & Dramsi, S. (2018). Significance of *Streptococcus gallolyticus subsp. gallolyticus* association with colorectal cancer. *Frontiers in Microbiology*, *9*, 614. https://doi.org/10.3389/fmicb.2018.00614

Peiré García, M. A. (2019). *Farmacología Pediátrica*. (1st ed.). Argentina: Ediciones Journal.

Qi, Y., Jiang, C., Cheng, J., Krausz, K. W., Li, T., Ferrell, J. M., Gonzalez, F. J., & Chiang, J. Y. (2015). Bile acid signaling in lipid metabolism: Metabolomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids*, *1851*(1), 19–29. https://doi.org/10.1016/j.bbalip.2014.04.008

Qian, L., Qi, S., Wang, Z., Magnuson, J. T., Volz, D. C., Schlenk, D., Jiang, J., & Wang, C. (2021). Environmentally relevant concentrations of boscalid exposure affects the neurobehavioral response of zebrafish by disrupting visual and nervous systems. *Journal of Hazardous Materials*, *404*(Pt A), 124083. https://doi.org/10.1016/j.jhazmat.2020.124083

Qiu, Y., Sun, S., Yu, X., Zhou, J., Cai, W., & Qian, L. (2020). Carboxyl ester lipase is highly conserved in utilizing maternal supplied lipids during early development of zebrafish and human. *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids*, *1865*(6), 158663. https://doi.org/10.1016/j.bbalip.2020.158663

Quinlivan, V. H., & Farber, S. A. (2017). Lipid uptake, metabolism, and transport in the larval zebrafish. *Frontiers in Endocrinology*, *8*, 319. https://doi.org/10.3389/fendo.2017.00319

Ren, J., Chung-Davidson, Y. W., Yeh, C. Y., Scott, C., Brown, T., & Li, W. (2015). Genome-wide analysis of the ATP-binding cassette (ABC) transporter gene family in sea lamprey and Japanese lamprey. *BMC Genomics [Electronic Resource]*, *16*(1), 436. https://doi.org/10.1186/s12864-015-1677-z

Reshna, K. R., Gopi, S., & Balakrishnan, P. (2022). Introduction to flavor and fragrance in food processing. In: P. Balakrishnan & S. Gopi (Eds.), *Flavors and fragrances in food processing: Preparation and characterization methods* (pp. 1–19). America: American Chemical Society.

Rowe, R. C., Sheskey, P., & Quinn, M. (2009). *Handbook of pharmaceutical excipients*. Libros Digitales-Pharmaceutical Press.

Roy-Carson, S., Natukunda, K., Chou, H. C., Pal, N., Farris, C., Schneider, S. Q., & Kuhlman, J. A. (2017). Defining the transcriptomic landscape of the developing enteric nervous system and its cellular environment. *BMC Genomics [Electronic Resource]*, *18*(1), 290. https://doi.org/10.1186/s12864-017-3653-2

Sander, V., Salleh, L., Naylor, R. W., Schierding, W., Sontam, D., O'Sullivan, J. M., & Davidson, A. J. (2019). Transcriptional profiling of the zebrafish proximal tubule. *American Journal of Physiology Renal Physiology*, *317*(2), F478–F488. https://doi.org/10.1152/ajprenal.00174.2019

Sant, K. E., Jacobs, H. M., Borofski, K. A., Chen, P., Park, Y., & Timme-Laragy, A. R. (2017). Pancreas development and nutrient uptake and utilization are disrupted by embryonic exposures to the environmental toxicant perfluorooctanesulfonic acid in the zebrafish. *The FASEB Journal*, *31*(S1), 792.8–792.8. https://doi.org/10.1096/fasebj.31.1_supplement.792.8

Sant, K. E., & Timme-Laragy, A. R. (2018). Zebrafish as a model for toxicological perturbation of yolk and nutrition in the early embryo. *Current Environmental Health Reports*, *5*(1), 125–133. https://doi.org/10.1007/s40572-018-0183-2

Schwartz, A. V., Sant, K. E., Navarrete, J., & George, U. Z. (2021). Mathematical modeling of the interaction between yolk utilization and fish growth in zebrafish, *Danio rerio*. *Development (Cambridge, England)*, *148*(9), dev193508. https://doi.org/10.1242/dev.193508

Sharopov, F., Valiev, A., Satyal, P., Gulmurodov, I., Yusufi, S., Setzer, W. N., & Wink, M. (2017). Cytotoxicity of the essential oil of fennel (*Foeniculum vulgare*) from Tajikistan. *Foods*, *6*(9), 73. https://doi.org/10.3390/foods6090073

Shi, X., Gu, A., Ji, G., Li, Y., Di, J., Jin, J., Hu, F., Long, Y., Xia, Y., Lu, C., Song, L., Wang, S., & Wang, X. (2011). Developmental toxicity of cypermethrin in embryo-larval stages of zebrafish. *Chemosphere*, *85*(6), 1010–1016. https://doi.org/10.1016/j.chemosphere.2011.07.024

Singulani, J. L., Pedroso, R. S., Ribeiro, A. B., Nicolella, H. D., Freitas, K. S., Damasceno, J. L., Vieira, T. M., Crotti, A. E., Tavares, D. C., Martins, C. H., Mendes-Giannini, M. J., & Pires, R. H. (2018). Geraniol and linalool anticandidal activity, genotoxic potential and embryotoxic effect on zebrafish. *Future Microbiology*, *13*(15), 1637–1646. https://doi.org/10.2217/fmb-2018-0200

Tomaszewski, P., Kubiak-Tomaszewska, G., & Pachecka, J. (2008). Cytochrome P450 polymorphism ń molecular, metabolic, and pharmacogenetic aspects II. participation of CYP isoenzymes in the metabolizm of endogenous substances and drugs. *Acta Poloniae Pharmaceutica Drug Research*, *65*(3), 307–318.

Vacca, F., Barca, A., Gomes, A. S., Mazzei, A., Piccinni, B., Cinquetti, R., Del Vecchio, G., Romano, A., Rønnestad, I., Bossi, E., & Verri, T. (2019). The peptide transporter 1a of the zebrafish *Danio rerio*, an emerging model in nutrigenomics and nutrition research: Molecular characterization, functional properties, and expression analysis. *Genes & Nutrition*, *14*(1), 33. https://doi.org/10.1186/s12263-019-0657-3

Wang, L., Luo, Q., Zeng, S., Lou, Y., Li, X., Hu, M., Lu, L., & Liu, Z. (2021). Disordered farnesoid X receptor signaling is associated with liver carcinogenesis in *Abcb11*-deficient mice. *The Journal of Pathology*, *255*(4), 412–424. https://doi.org/10.1002/path.5780

Yochana, S., Yu, M. K., Alvi, M., Varenya, S., & Chatterjee, P. (2012). Pharmaceutical excipients and pediatric formulations. *Chimica Oggi-Chemistry Today*, *30*(5), 56–61.

Yu, H., Zhang, J., Chen, Y., Chen, J., Qiu, Y., Zhao, Y., Li, H., Xia, S., Chen, S., & Zhu, J. (2022). The adverse effects of fluxapyroxad on the neurodevelopment of zebrafish embryos. *Chemosphere*, *307*(Pt 1), 135751. https://doi.org/10.1016/j.chemosphere.2022.135751

Zhang, M. Q., Chen, B., Zhang, J. P., Chen, N., Liu, C. Z., & Hu, C. Q. (2020). Liver toxicity of macrolide antibiotics in zebrafish. *Toxicology*, *441*, 152501. https://doi.org/10.1016/j.tox.2020.152501

Zhu, X., Hao, R., Lv, X., Zhou, X., Li, D., & Zhang, C. (2023). Nuciferine ameliorates high-fat diet-induced disorders of glucose and lipid metabolism in obese mice based on the gut-liver axis. *Food Frontiers*, *5*(1), 188–201. https://doi.org/10.1002/fft2.292

Zhu, Y., Zhang, Q., Pan, J., Li, T., Wang, H., Liu, J., Qian, L., Zhu, T., Pang, Y., Li, Q., & Chi, Y. (2024). Evolutionary analysis of SLC10 family members and insights into function and expression regulation of lamprey NTCP. *Fish Physiology and Biochemistry*, *50*, 1109–1122. https://doi.org/10.1007/s10695-024-01324-7

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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