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In Vivo Developmental Toxicity and Teratogenic Evaluation of *Cucumis melo* L. var. Gama Melon Parfum Extract on Wader Pari Fish (*Rasbora lateristriata*) Embryos

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Abstract: Cucumis melo L. var. Gama Melon Parfum (GMP) is a superior hybrid cultivar developed by the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, characterized by its distinctive sweet aroma and bitter taste. Previous in vitro studies have demonstrated the anticancer potential of GMP extract through the inhibition of cell proliferation and the induction of apoptosis; however, its in vivo toxicity and developmental safety profile remain largely unexplored. This study aimed to evaluate the developmental toxicity and teratogenic potential of GMP extract using Rasbora lateristriata embryos as a biological model. This Indonesian native species offers several advantages, including embryo transparency, high environmental sensitivity, and rapid development, with hatching occurring within 24 hours. The study was conducted in accordance with the Fish Embryo Toxicity (FET) guidelines (OECD Test No. 236, 2025). A preliminary range-finding test indicated 100% mortality at 1000 ppm, followed by a definitive test using concentrations of 50, 100, 250, and 500 ppm in 0.1% DMSO. Early-stage embryos (2–4 hpf) were exposed up to 96 hpf, and endpoints including hatching rate, survival rate, heart rate, and morphological abnormalities were assessed. The results revealed a concentration-dependent response. At 50–250 ppm, no significant effects were observed on survival, hatching, or heart rate; however, notable teratogenic effects were identified, including spinal deformities, ocular abnormalities (microphthalmia and anophthalmia), hemorrhage, and impaired swim bladder development. In contrast, exposure to 500 ppm resulted in acute lethal toxicity, as evidenced by complete mortality, absence of hatching, and loss of cardiac activity. In conclusion, GMP extract exhibits developmental toxicity characterized by a transition from sublethal (teratogenic) to lethal effects with increasing concentration. These findings underscore the importance of dose optimization and comprehensive in vivo evaluation in the development of GMP extract as a potential biomedical agent.

Keywords: *Cucumis melo* var. GMP, *Rasbora lateristriata*, Embryogenesis, Teratogenicity, Morphological malformations.

Introduction

The global rise in non-communicable chronic diseases, including diabetes mellitus, oxidative stress–related disorders, and cancer, has driven intensive exploration of plant-derived bioactive compounds as complementary and preventive therapeutic alternatives (Acimovic et al., 2026). Several anticancer agents currently used in clinical practice are derived from plants or represent synthetic derivatives of plant secondary metabolites, such as paclitaxel, vincristine, vinblastine, and camptothecin (Vieira et al., 2026). Although natural compounds are often

assumed to possess lower toxicity profiles than synthetic counterparts, this assumption is not consistently supported by scientific evidence. Toxicological studies have demonstrated that the LD_{50} values of natural bioactive compounds vary substantially depending on their chemical structure, test organisms, and routes of exposure (Hasan et al., 2025). Therefore, both natural and synthetic compounds require comprehensive toxicological evaluation, including reproductive and developmental toxicity assessments, prior to being considered safe for use (Vilas-Boas et al., 2021).

Embryogenesis represents a highly vulnerable stage of development to xenobiotic exposure. During this phase, rapid cellular proliferation increases susceptibility to disruptions in cell growth and the regulation of intracellular signaling pathways. Natural anticancer compounds generally exert their effects through inhibition of cell cycle progression and induction of apoptosis in target cells (Kang et al., 2025). However, these mechanisms may also produce unintended effects on normal developmental processes. Interference with cellular proliferation during embryogenesis can disrupt molecular signaling pathways that regulate body pattern formation, ultimately leading to developmental abnormalities (Lee et al., 2024). This highlights a biological paradox in which cytotoxic activity that is beneficial in the context of cancer therapy may be detrimental during embryonic development.

One promising source of bioactive compounds is *Cucumis melo* L. var. Gama Melon Parfum (GMP), a cultivar developed through hybridization between the female parent ♀NO3 and the male parent ♂MR5 by the Laboratory of Genetics and Plant Breeding (Maryanto et al., 2015). GMP extract has been reported to exhibit cytotoxic activity against MCF-7 and T47D breast cancer cell lines in vitro (Salamah & Widiyanto, 2022; Salamah et al., 2025). As a member of the Cucurbitaceae family, GMP contains cucurbitacins, a group of triterpenoid compounds responsible for its characteristic bitterness and widely recognized for their anticancer properties. The degree of fruit bitterness is positively correlated with cucurbitacin content (Hua et al., 2019; Fan et al., 2024). Interestingly, despite its strong sweet fragrance resembling perfume, GMP exhibits a distinctly bitter taste, suggesting a relatively high cucurbitacin content compared to other melon varieties. This unique characteristic positions GMP as a potential source of bioactive compounds with potent cytotoxic activity.

Despite its reported in vitro cytotoxic activity, the developmental safety profile of GMP extract has not yet been systematically evaluated. Considering the mechanisms of action of cucurbitacins—including modulation of the cell cycle, induction of apoptosis, and regulation of proliferation-related signaling pathways (Kim et al., 2020; Alafnan et al., 2022) —as well as the chemical complexity of crude extracts, the assessment of developmental toxicity is critical to identify potential teratogenic or embryotoxic effects. The absence of such data represents a significant knowledge gap that must be addressed to ensure the safe application of GMP in biomedical contexts.

Evaluation of teratogenic and embryotoxic potential requires an in vivo model that is highly sensitive to xenobiotic exposure during early developmental stages. Fish embryos have been widely utilized in developmental toxicity studies due to their rapid development, external fertilization, and optical transparency, which enable real-time observation of morphological changes and continuous monitoring (Nawaji et al., 2024). Among freshwater fish species, *Rasbora lateristriata*, an Indonesian native species belonging to the Cyprinidae family—closely related to *Danio rerio*—has emerged as a promising model (Kusuma et al., 2016; Tan & Armbruster, 2018). Compared to commonly used models such as zebrafish and medaka, *R. lateristriata* exhibits a faster embryonic development rate, with hatching occurring within approximately 24 hours, whereas zebrafish and medaka require around 48 hours post-fertilization (hpf) and up to 9 days, respectively (Retnoaji et al., 2023; Lailiati et al., 2022). These characteristics make *R. lateristriata* an efficient and relevant model for assessing toxic effects during early development.

Based on this background, the present study aims to evaluate the developmental toxicity and teratogenic potential of Gama Melon Parfum extract in vivo using *Rasbora lateristriata* embryos. The assessment includes analysis of hatching rate, survival rate, heart rate and morphological abnormalities. The findings of this study are expected to provide fundamental insights into the developmental safety profile of GMP extract and support its potential application in biomedical research.

Method

Experimental Design

This study employed an experimental design consisting of six treatment groups: four groups exposed to Gama Melon Parfum (GMP) extract at concentrations of 50, 100, 250, and 500 ppm, one negative control group (egg water medium), and one solvent control group (0.1% DMSO). Fish embryos were selected at a uniform

developmental stage, specifically the pre-gastrulation stage (2–4 hours post-fertilization; hpf), and were randomly distributed into each treatment group. Each group consisted of 20 embryos, resulting in a total of 120 embryos used in this study. Exposure to the extract was conducted for 96 hours under a static system with renewal of the test medium every 24 hours. Each embryo was maintained individually in separate wells of a 24-well plate to ensure independence of observations. Observations were performed every 24 hours to assess developmental parameters, including hatching rate, heart rate, and survival rate. Morphological abnormalities were evaluated at the end of the exposure period (96 hpf).

Materials

The materials used in this study included ethanolic extract of Gama Melon Parfum fruit, reverse osmosis (RO) water, salt stock solution, and 0.1% DMSO. The egg water medium for embryo maintenance was prepared by mixing 1 L of RO water with 1.5 mL of 3.5% (b/v) salt solution and two drops of 300 ppm methylene blue. The equipment used included 24-well plates, beakers, measuring cylinders, Erlenmeyer flasks, glass bottles, aquaria ($30 \times 20 \times 15$ cm^3), and an Olympus CX43 microscope.

Preparation of GMP Extract

Fresh Gama Melon Parfum (GMP) fruits were cleaned, sliced into thin sections, and oven-dried at 45–50°C. The dried material (simplisia) was air-dried and ground into a fine powder. Extraction was performed using the maceration method with 70% ethanol for 48 hours at a sample-to-solvent ratio of 1:10. The extract was filtered, and the solvent was evaporated to obtain a concentrated crude extract. The crude extract was subsequently re-dissolved in 0.1% DMSO to obtain working concentrations of 50, 100, 250, and 500 ppm.

Fish Embryo Preparation

Broodstock fish were selected based on reproductive readiness, which was assessed by gentle abdominal massage. Spawning pairs were arranged in a ratio of two males to one female. Spawning was conducted in the afternoon in aquaria containing ijuk (palm fiber) as a substrate to simulate natural conditions (Boyhaki, 2016). Egg collection was performed the following morning. Fertilized eggs were collected, cleaned, and screened to ensure uniform developmental stages (pre-gastrulation stage, 2–4 hpf). Each treatment group consisted of 20 embryos, resulting in a total of 120 embryos. The embryos were maintained in egg water prior to exposure to GMP extract.

Fish Embryo Acute Toxicity Test

Embryo exposure was conducted using a 24-well plate following the OECD Test Guideline No. 236 (2025). At 2–4 hpf, embryos were rinsed and examined under a stereo microscope (Olympus), and only fertilized embryos were selected for further experimentation. A total of 20 embryos were allocated to each treatment group. The treatments included GMP extract at concentrations of 50, 100, 250, and 500 ppm, a negative control (egg water), and a solvent control (0.1% DMSO). Each well contained 2 mL of the respective test solution. Observations and media renewal were performed every 24 hours for 96 hours (4 days). The evaluated parameters included hatching rate, heart rate, and survival rate. Daily developmental progress was documented to assess potential teratogenic effects on larval morphology.

Developmental Endpoints

Embryo mortality and developmental abnormalities were documented using an Olympus CX43 microscope at 3.2× magnification with a light intensity setting of 6. The observed parameters included hatching rate (24–48 hpf), survival rate (24–120 hpf), heart rate (measured daily), and morphological abnormalities (96 hpf). Observed abnormalities included spinal deformities, eye abnormalities (microphthalmia and anophthalmia), hemorrhage, and swim bladder defects. Edema was recorded descriptively due to its low incidence and was not included in quantitative analysis. Normal morphological development was assessed based on established developmental criteria (Retnoaji et al., 2023).

Heart Rate Assessment

Hatching rate was calculated as the percentage of embryos that hatched within the first 24 hours relative to the total number of embryos. Embryos that hatched after 24 hpf were categorized as delayed hatching. Embryo mortality was also recorded, with dead embryos identified by coagulation of fertilized eggs (Raharjeng & Retnoaji, 2021).

$$Heart\ Rate\ (\%) = \frac{Number\ of\ hatched\ embryos}{Total\ number\ of\ embryos} x\ 100$$

Heart Rate Measurement

Larvae were placed in a Petri dish in a lateral position and observed under a stereo microscope (Olympus). Heart rate was measured by counting the number of heart beats per minute for each larva. Measurements were performed daily for four consecutive days.

Survival Rate Assessment

Survival rate was calculated as the percentage of surviving embryos relative to the initial number of embryos over a 4-day observation period. This parameter reflects the proportion of embryos that remained viable following exposure (Huyen et al., 2024; Zhu et al., 2021).

$$Survival\ Rate\ (\%) = \frac{Number\ of\ surviving\ embryos}{Initial\ number\ of\ embryos} x\ 100$$

Larval Developmental Assessment

Larval development was assessed both quantitatively and qualitatively to evaluate the effects of exposure on morphology. The incidence of morphological abnormalities was calculated using the following formula:

$$Malformation\ Incidence(\%) = \frac{Number\ of\ abnormal\ larvae}{Initial\ number\ of\ larvae} x\ 100$$

Morphological abnormalities were classified based on predefined criteria and compared across treatment groups.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 25. Normality testing indicated that the data were not normally distributed; therefore, non-parametric statistical methods were applied. Differences among treatment groups for hatching rate and survival rate were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney U test for post hoc comparisons when significant differences were detected. Data were presented as median values with interquartile range (IQR). Morphological abnormalities were analyzed descriptively based on incidence percentages in each treatment group.

Results and Discussion

Developmental Toxicity

Effect of GMP Extract on Embryonic Development in R. lateristriata

The evaluation of the effects of Gama Melon Parfum extract on the embryonic development of *R. lateristriata* was conducted by assessing several parameters, including hatching rate, hatching delay, survival rate, and embryonic heart rate across different treatment concentrations. The results of the observations on hatching rate and hatching delay are presented in Table 1.

Table 1. Hatching rate and delayed hatching of *R. lateristriata* embryos following exposure to gama melon parfum extract

Group	Hatching (%)	Delay (%)
Normal Control	80 (75-82.5)	00 (00-15)
Negatif Control	70 (67.5-85)	22 (11.11-25)
50 ppm	85 (67.5-90)	5 (2.78-25)
100 ppm	80 (70-87.5)	0 (00-18.42)
250 ppm	85 (70-85)	10 (5.26-27.76)
500 ppm	00 (00-15)	00 (00-00)

*Differences among groups were analyzed using the Kruskal–Wallis test. Data are presented as median (interquartile range, IQR).

Based on the obtained results, the hatching rate of embryos following exposure to the extract ranged from 0% to 85%. Exposure at concentrations of 50 ppm and 250 ppm resulted in relatively high hatching rates (85%), while the 100 ppm concentration showed a value comparable to the normal control (80%). In contrast, no hatching was observed at the highest concentration (500 ppm). This finding indicates that high concentration (500 ppm) exerts a toxic effect, as evidenced by the high level of embryonic mortality in this treatment group. Meanwhile, the percentage of hatching delay varied among treatment groups. The highest delay was observed in the negative control (22%), followed by the 250 ppm concentration (10%). The use of 0.1% DMSO as a solvent is suspected to contribute to the occurrence of delayed hatching, particularly at 24 hours post-fertilization, resulting in some embryos hatching at 48 hpf. At 100 ppm, embryos tended not to exhibit hatching delay, whereas at 500 ppm, embryos died before showing any signs of delayed hatching.

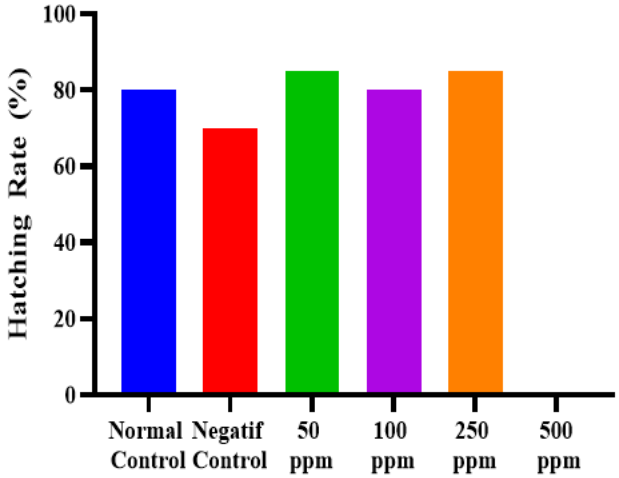

Figure 1. Hatching rate (%) graphic of *R. lateristriata* embryos following exposure to gama melon parfum extract

Based on Figure 1, the embryonic hatching rate was relatively high at low to moderate concentrations, whereas no hatching was observed at the highest concentration (500 ppm). Overall, the graph illustrates that low to intermediate concentrations did not exert toxic effects on embryonic hatching, while high concentration clearly impaired embryonic development. Consistent with these findings, the survival rate of embryos exhibited a similar pattern, in which increasing extract concentration was associated with decreased embryonic viability. The results of the survival rate observations are presented in Table 2.

Table 2. Survival rate of *R. lateristriata* embryos following exposure to gama melon parfum extract

Group	Survival Rate (%)			
	24 hpf	48 hpf	72 hpf	96 hpf
Normal Control	85 (82,5-92,5)	75 (70-87.5)	75 (70-87.5)	75 (70-85)
Negatif Control	95 (90-97.5)	85 (80-90)	80 (77.5-97.5)	75 (72.5-85)
50 ppm	95 (92.5-95)	90 (77.5-87.5)	85 (85-90)	85 (85-87.5)
100 ppm	95 (87.5-97.5)	95 (80-97.5)	95 (75-95)	90 (72.5-92.5)
250 ppm	95 (90-95)	95 (77.5-95)	90 (75-92.5)	85 (67.5-90)
500 ppm	0 (0-47.5)	0 (0-45)	0 (0-12.5)	0 0 (0-12.5)

* Differences among groups were analyzed using the Kruskal–Wallis test. Data are presented as median (interquartile range, IQR).

The survival rate in the normal control group remained relatively stable, ranging from 75–85% throughout the observation period, whereas the negative control exhibited a higher initial value (95% at 24 hpf) followed by a gradual decline to 75% at 96 hpf (Figure 2). Exposure to Gama Melon Parfum extract at low to moderate concentrations (50–250 ppm) resulted in consistently high survival rates (approximately 85–95%) with minimal fluctuations, indicating that these concentrations remain within the biological tolerance limits of the embryos. In contrast, at the highest concentration (500 ppm), the survival rate decreased drastically to 0% as early as 24 hpf and remained at this level until the end of the observation period, indicating acute lethal effects. The trend shown in Figure 2 confirms that increasing extract concentration is associated with a decline in survival rate, and the differences among treatment groups were statistically significant ($p < 0.05$).

The stability of survival rates at low to moderate concentrations suggests the presence of adaptive responses or detoxification mechanisms in embryos against the compounds present in the extract. Conversely, the high mortality observed at 500 ppm indicates that the accumulation of bioactive compounds exceeded the embryos' tolerance capacity, thereby disrupting embryogenesis, particularly during the early developmental stages that are highly sensitive to toxic exposure.

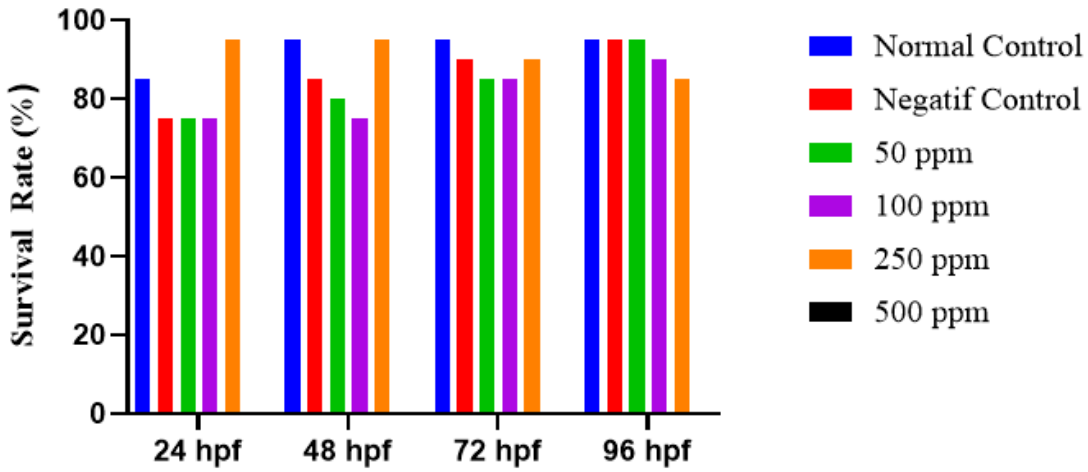

Figure 2. Survival rate (%) graphic of *R. lateristriata* embryos following exposure to gama melon parfum extract

Embryonic heart rate is an important sublethal parameter that has been widely used as an indicator of toxicity. The heart rate of *R. lateristriata* embryos following exposure to Gama Melon Parfum extract at various concentrations is presented in Table 3. In general, heart rate values in the normal control group remained relatively stable throughout the observation period, ranging from 186–198 bpm at 24–72 hpf and slightly decreasing to 192 bpm at 96 hpf.

Table 3. Heart rate of *R. lateristriata* embryos following exposure to gama melon parfum extract

Group	Heart Rate (%)			
	24 hpf	48 hpf	72 hpf	96 hpf
Normal Control	186 (180-195)	195 (183-204)	198 (180-204)	192 (180-204)
Negatif Control	162 (00-183)	186 (171-192)	186 (171-201)	186 (84-198)
50 ppm	183 (174-198)	201 (183-219)	198 (180-207)	192 (162-207)
100 ppm	189 (177-195)	204 (192-216)	207 (192-222)	210 (195-234)
250 ppm	186 (168-198)	192 (186-204)	192 (186-198)	204 (189-222)
500 ppm	186 (180-192)	186 (174-204)	00 (00-72)	00 (00-72)

* Differences among groups were analyzed using the Kruskal–Wallis test. Data are presented as median (interquartile range, IQR).

In the treatment groups, concentrations of 50–250 ppm exhibited heart rate values that generally remained within the normal range and were comparable to the control. At 50 ppm, the heart rate gradually increased from 183 bpm (24 hpf) to 192 bpm (96 hpf). A similar pattern was observed at 100 ppm and 250 ppm, where heart rate increased over the observation period, reaching peak values at 96 hpf of 210 bpm and 204 bpm, respectively. Exposure to Gama Melon Parfum extract at low to moderate concentrations (50–250 ppm) did not result in significant changes in embryonic heart rate compared to the control group. This indicates that within this concentration range, embryos were still able to maintain normal cardiac physiological function.In contrast, at the highest concentration (500

ppm), the absence of detectable heart rate during later developmental stages (72–96 hpf) indicates a lethal toxic effect. The loss of cardiac activity at this stage suggests a cessation of cardiovascular function, ultimately leading to embryonic death. This finding is consistent with other observed parameters, such as reduced hatching and survival rates at the same concentration, which collectively indicate a high level of toxicity. Cardiac dysfunction during early developmental stages can lead to arrhythmia and impaired blood circulation, which in turn may result in growth retardation due to insufficient nutrient supply (Shaikh et al., 2019).

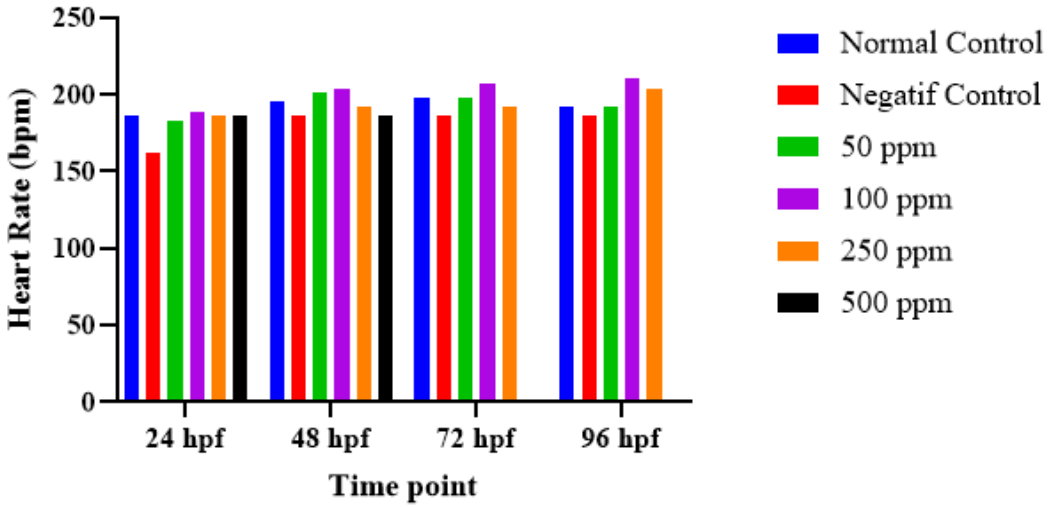

Figure 3. Heart rate graphic of *R. lateristriata* embryos following exposure to gama melon parfum extract

Teratogenic Evaluation

Morphological Abnormalities in R. lateristriata Larvae Following GMP Exposure

Based on the obtained results, exposure to Gama Melon Parfum (GMP) extract exhibited teratogenic effects on the development of R. lateristriata embryos, as indicated by the occurrence of various morphological abnormalities in several organs. The incidence of morphological abnormalities in R. lateristriata embryos at 96 hpf following exposure to GMP extract is presented in **Table 4.** In general, variations in both the types and percentages of abnormalities were observed across almost all treatment groups, with distinct patterns among different concentrations. The non-linear pattern of incidence in relation to concentration suggests that the embryonic response to exposure is complex and may be influenced by tolerance thresholds as well as the differential sensitivity of specific organs to the active compounds.

Table 4. Incidence of morphological abnormalities in *R. lateristriata* embryos at 96 hpf

Group	Malformation Incidence (%)			
	Spinal	Eye	Hemorrhage	Swim Bladder
Normal Control	4,26	0	0	8,51
Negatif Control	2,08	0	2,08	20.83
50 ppm	9,26	5,77	9,62	25,00
100 ppm	6,25	4,17	4,17	33,33
250 ppm	10,87	4,35	6,52	19,57
500 ppm	0,00	0,00	0,00	80,00

Spinal deformities were observed in all groups, with the highest incidence at 250 ppm (10.87%), followed by 50 ppm (9.26%) and 100 ppm (6.25%). In contrast, the normal and negative control groups exhibited lower incidences, at 4.26% and 2.08%, respectively. This impairment is likely associated with disruptions in somite development and osteogenesis during embryogenesis. Previous studies have reported that exposure to toxic compounds in zebrafish embryos can induce spinal deformities through mechanisms involving cellular stress and apoptosis, which interfere with tissue development (Li et al., 2022).

Eye abnormalities (Figure 6 EF) were detected only in the treatment groups, specifically at concentrations of 50 ppm (5.77%), 100 ppm (4.17%), and 250 ppm (4.35%), while no abnormalities were observed in the control groups. Although no major morphological abnormalities were generally observed, this study identified limited occurrences of microphthalmia (reduced eye size) and anophthalmia (absence of eye formation). The low

frequency of these abnormalities suggests that they do not represent a dominant response to the extract exposure, but rather sporadic effects under certain conditions.

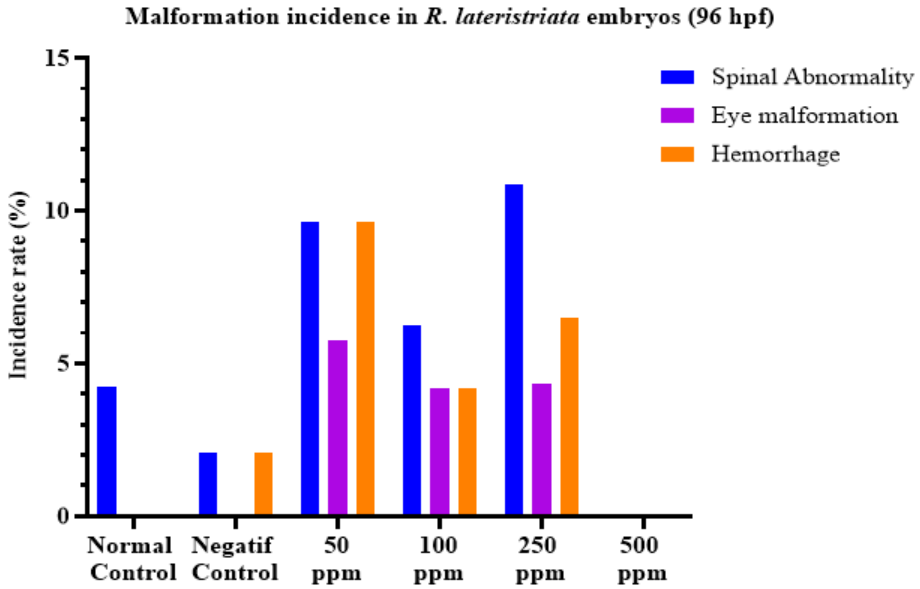

Figure 4. Incidence of spinal abnormalities, eye malformations, and hemorrhage in *R. lateristriata* larvae at 96 hpf

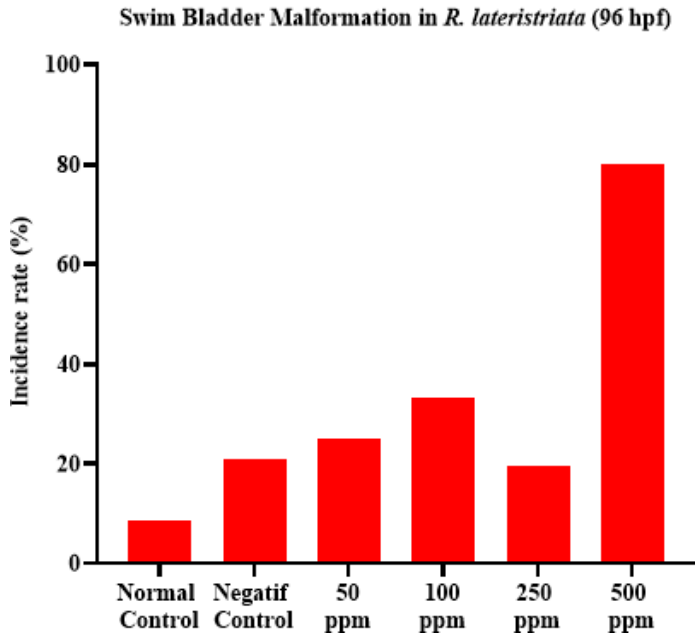

Figure 5. Incidence of swim bladder malformations in *R. lateristriata* larvae at 96 hpf following exposure to gama melon parfum extract

The incidence of hemorrhage (Figre 6G) increased at 50 ppm (9.62%), decreased at 100 ppm (4.17%), and increased again at 250 ppm (6.52%) (Figure 4). No hemorrhage was observed in the normal control group, whereas the negative control exhibited a low incidence (2.08%). The occurrence of hemorrhage suggests potential disruption of the embryonic vascular system. This condition may be attributed to compromised vascular integrity or impaired cell adhesion, both of which are essential for maintaining tissue structure. Similar findings have been reported in studies involving exposure to other toxic compounds, which induce edema and hemorrhage through physiological disturbances and cellular apoptosis (Ge et al., 2023)

Swim bladder abnormalities (Figure 6 CD) exhibited a variable pattern (Figure 5), with the highest incidence observed at 100 ppm (33.33%), followed by 50 ppm (25.00%) and the negative control (20.83%). The lowest incidence was found in the normal control group (8.51%). These findings are supported by previous studies indicating that swim bladder development is highly dependent on a properly functioning circulatory system,

particularly cardiac function. Cardiac impairment may hinder swim bladder growth due to inadequate oxygen and nutrient distribution (Ge et al., 2023).

At the highest concentration (500 ppm), no abnormalities were observed in spinal, ocular, or hemorrhagic parameters. However, this was attributed to the high level of embryonic mortality at this concentration. All surviving larvae at 500 ppm exhibited swim bladder abnormalities, with an incidence of 80.00%. This finding indicates that at high concentrations, the toxic effect tends to be lethal, and even the surviving individuals experience developmental impairment. This phenomenon suggests a shift from sublethal (teratogenic) effects to lethal effects with increasing exposure concentration. Although no significant ocular morphological abnormalities were generally observed, this study identified limited occurrences of microphthalmia (reduced eye size) and anophthalmia (absence of eye formation) (Figure 6 EF). The low frequency of these abnormalities suggests that they do not represent a dominant response to extract exposure, but rather may occur as sporadic effects under certain conditions. These abnormalities are likely associated with disruptions in embryonic developmental processes, particularly during the stages of neural differentiation and tissue organization. This is consistent with previous reports indicating that exposure to certain compounds, such as cucurbitacin B, can affect nervous system development, including neuronal differentiation and axonal pathway formation, without causing widespread morphological defects in embryos (Huang, 2023). Disruption of axonal organization in the cranial region may consequently affect the development of sensory organs, including the eyes. Therefore, although the incidence of eye abnormalities in this study was relatively low, these findings still indicate a potential effect on neurodevelopmental processes and organogenesis, which under certain conditions may influence the formation of ocular structures.

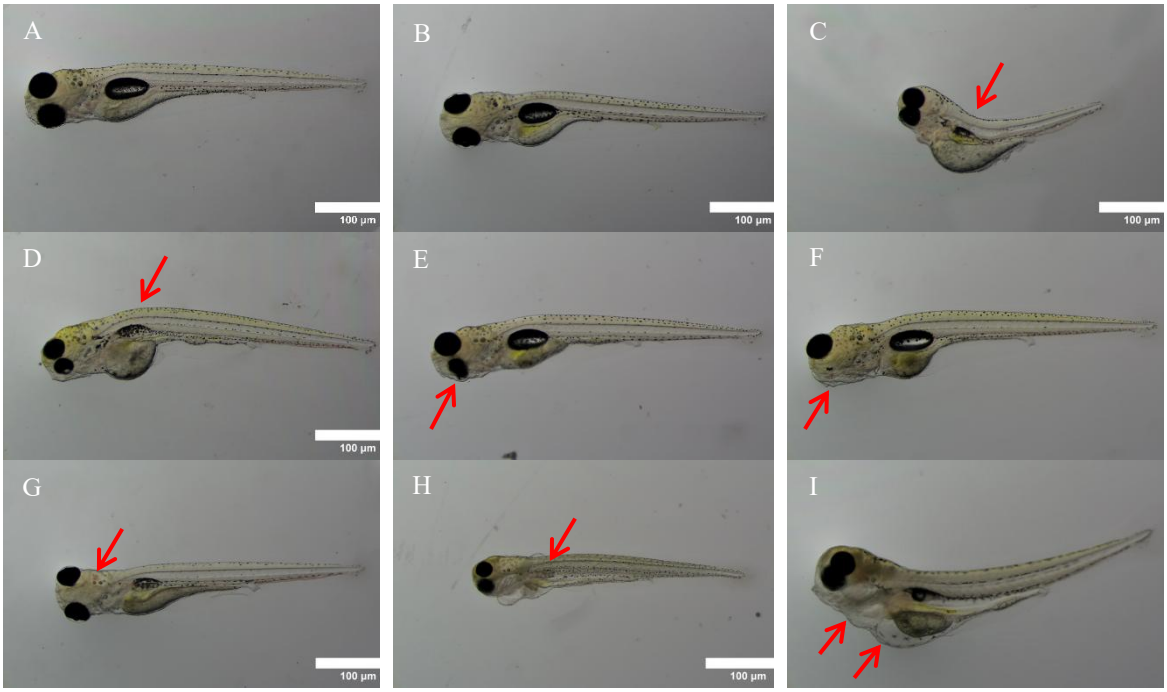

Figure 6. Representative of morphological abnormalities in *R. lateristriata* larvae at 96 hpf following exposure to gama melon parfum extract. mag 3,2X. **(A-B)** control *R. lateristriata* at 96 hpf. A: normal control. B: Negatif control. **(C-D)** Spinal abnormality. C: Lordosis. D: Kyphosis. **(E-F)** Eye malformation. E: microphthalmia. F: Anophthalmia. G: Hemorrage. H: Swim bladder malformation. I : Pericardial and yolk edema.

Conclusion

Gama Melon Parfum extract exhibited concentration-dependent effects on the embryonic development of *R. lateristriata*. At low to moderate concentrations (50–250 ppm), no significant alterations were observed in hatching rate, survival rate, or heart rate; however, teratogenic manifestations were detected, including spinal deformities, eye abnormalities (microphthalmia and anophthalmia), hemorrhage, and impaired swim bladder development. In contrast, exposure to the highest concentration (500 ppm) resulted in acute lethal toxicity, evidenced by complete inhibition of hatching, total mortality, and absence of cardiac activity. Collectively, these findings indicate that GMP extract induces developmental toxicity, characterized by a concentration-dependent

shift from sublethal (teratogenic) effects to overt lethality, and provide a foundational basis for assessing its developmental safety profile in biomedical applications.

Recommendations

Further studies are recommended to investigate the effects of Gama Melon Parfum extract on later developmental stages, including larval and adult fish, as well as to evaluate the safety of the extract in other model organisms, such as rodents.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

* This study was approved by the Animal Ethics Committee of Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval No. 00064/XII/UNI/LPPT/EC/2025, issued on 18 Dec 2025)

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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