

Embryotoxicity and teratogenesis of orthodontic acrylic resin in zebrafish

Amanda Sayuri Cardoso Ohashi

Pontifical Catholic University of Rio Grande do Sul

Christiane Staub Pizzato

Pontifical Catholic University of Rio Grande do Sul

Helena Reis de Souza Schacher (✉ helena.schacher@edu.pucrs.br)

Pontifical Catholic University of Rio Grande do Sul

Monica Ryff Moreira Roca Vianna

Pontifical Catholic University of Rio Grande do Sul

Luciane Macedo de Menezes

Pontifical Catholic University of Rio Grande do Sul

Research Article

Keywords: Animal model, benzoyl peroxide, dibutyl phthalate, formaldehyde, methyl methacrylate, toxicity

Posted Date: April 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1516524/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objectives: To verify *in vivo* the embryotoxicity, teratogenic power and other possible effects of the orthodontic acrylic resin and its components separately using Zebrafish as a model organism. **Materials and Methods:** Embryo and larval stage animals were divided into 5 experimental groups. These were divided into 5 subgroups: three specific doses of each substance tested, one control with the vehicle (0,1% DMSO in water) and one absolute control (water). In the 50 day post fertilization, the animals were submitted to morphological, cardiac, behavioral, and cognitive evaluations. Ten animals were used in triplicate for all experiments. Survival and hatching were analyzed by the Kaplan Meier test and the other measurements were analyzed by one way ANOVA and Tukey post hoc test. **Results:** For all tested substances, statistically significant differences were found between the control and treated groups for heart rate, cognitive responsiveness, and cellular apoptosis, whereas survival, hatching rate and other parameters did not show significant differences. Only the highest dose of the dibutyl phthalate group showed significant difference in survival. **Conclusions:** Chronic exposure to acrylic resin and its components may be associated with decreased cognitive ability and cardiac rhythm and an increase in the level of cellular apoptosis in zebrafish. **Clinical Relevance:** Acrylic resin widely used in dentistry, where not only the patient stays in contact with it for a long period of time, but the dental and laboratory staff also often for a lifetime. Considering this, we emphasize the importance of further investigating the long-term effects of acrylic resin.

Introduction

The biocompatibility of materials has been studied for several years and it represents a matter of high concern in the scientific literature. The use of biocompatible dental materials is indispensable to a biologically safe treatment for patients, considering that they may remain in contact with oral tissues for a long period [1, 2]. In addition, dental professionals and laboratory staff are also exposed to these materials throughout their life [3].

Biological and immunological adverse reactions can be attributed to dental materials and, in general, allergic reactions and hypersensitivity are the most common side effects [1]. Acrylic resin, a widely used material in orthodontics (removable, fixed and retention appliances) and prosthetics (dentures, restorations and temporary crowns), can also be responsible for local reactions and occasionally lead to systemic manifestations such as urticaria, burning sensation and difficulty in swallowing [4]. These reactions are associated with the elution of the toxic components of these resins, which are methyl methacrylate (MMA), formaldehyde, benzoyl peroxide and plasticizers such as dibutyl phthalate [4], among which the main component is MMA (about 96%). All products leached from this resin, and not only the residual monomer, have cytotoxic effects on cell lines, especially the epithelial cell line [5].

The acute oral median lethal dose of MMA in rats has been reported as 8.4-9 g/kg body weight, which indicates very low acute systemic toxicity [6]. MMA is rapidly hydrolyzed by enzymes and subsequently metabolized to toxic substances [7]. MMA vapor caused vertigo in dental professionals [8]. However,

serious problems hasn't been reported by inhaling poly methyl methacrylate (PMMA) ingredients, although MMA may irritate eyes, skin and respiratory system [9]. Acute inhalation of MMA vapor induced a moderate restriction of pulmonary function in dental students, both smokers and non-smokers [10].

There is plenty of evidence concerning about residual monomers release and toxicity of acrylic resin used in dentistry [9], especially with clinical observations and *in vitro* experiments [4, 5, 11–14], but few *in vivo* studies [10, 15]. Clinical observations are highly subject to bias, such as non-commitment of the individual to the treatment or exposure of these individuals to external factors that may interfere with the research, and *in vitro* experiments do not have system integrity to assess the real impact of a substance in a complete organism, so animal models are better suited for these types of studies since they present difficult to simulate responses in cell culture [16]. Traditional mammalian models used to assess toxicity are costly and difficult to work during embryonic development [17]. Zebrafish has characteristics that qualify it as an excellent alternative model for toxicology studies, including being a vertebrate organism, genetic similarity with humans [18], rapid external development and transparency during early development [19], permeability to small molecules [20] and great fecundity [21].

Zebrafish (*Danio rerio*) has been widely used to evaluate cytotoxicity, genotoxicity, nanotoxicity and carcinogenic potential of various substances, such as drugs and nanomaterials [22, 23] and represents an useful model in dental research especially in biocompatibility of dental materials, craniofacial development and toxicological research [24]. In dentistry, there are already studies on fluorosis [25], toxicity of zirconium oxide nanoparticles used in dental implants [26], porcelain-fused-to-metal crowns [16], dental bio ceramics [27], Bisphenol A-glycidyl methacrylate in dental filing composite monomer [28] and Methacrylate [29] using zebrafish models.

The knowledge of the capacity that the materials used in Orthodontics in affecting the biological environment is of great importance to provide the patient with a safe and effective treatment, since these materials often remain for several years in contact with the oral mucosa, in addition to preserving the health of the dental team, who maintains frequent contact with such materials. Considering that, this study aims to verify, *in vivo*, the embryotoxicity, teratogenic power and other possible deleterious effects of the orthodontic acrylic resin and its active components using zebrafish as a model organism.

Material And Methods

Experimental design

Embryonic and larval wild-type AB zebrafish (*Danio rerio*) obtained from the crossing of adult fish with more than seven months of life were used. At 4 hours post-fertilization (hpf), fertilized embryos were observed in a stereomicroscope and those that were developing according to standardization of the developmental stages of zebrafish [30] were selected for the experiments.

The animals were divided into 5 experimental groups. These were divided into 5 subgroups: three specific doses of each substance tested, 1 control with the vehicle (0,1% DMSO in water) and 1 absolute control

(water only), used as a sentinel, but not included in the analyzes because it is not the adequate control for the effects that were measured since the DMSO can have effect by itself (Table 1). The doses were based on LC 50 (Lethal Concentration 50%) of each substance found in the literature and proportion the concentrations of these in the resin monomer.

The selected embryos were sanitized with system water (reverse osmosis water equilibrated with Instant Ocean salt according to the requirements of Westerfield [31] and transferred to 6 well plates where they were exposed to their treatments and kept in a controlled incubator of light / dark cycle (14/10 h) and temperature (27°C + 1°C) until the 5 day post fertilization (dpf).

Table 1
Groups, experimental subgroups and exposure dose.

GROUP	SUBGROUPS	EXPOSURE DOSE
Resin (R)	0	DMSO 0.1%
	R1	0,01 mg/l
	R2	0,1 mg/l
	R3	1 mg/l
Methyl methacrylate (MMA)	0	DMSO 0.1%
	MMA1	0,01 mg/l
	MMA2	0,1 mg/l
	MMA3	1 mg/l
Formaldehyde (F)	0	DMSO 0.1%
	F1	0,12 mg/l
	F2	1,2 mg/l
	F3	12 mg/l
Dibutylphthalate (DB)	0	DMSO 0.1%
	DB1	0,024 mg/l
	DB2	0,24 mg/l
	DB3	2,4 mg/l
Benzoyl peroxide (PB)	0	DMSO 0.1%
	PB1	0,0024 mg/l
	PB2	0,024 mg/l
	PB3	0,24 mg/l

For all groups, proceeded the middle exchange and monitored daily, including survival control, hatching and morphology. In the 5 dpf, the animals were submitted to behavioral and cognitive, morphological, and cardiac evaluations. Ten animals were used in triplicate for all experiments, as established by Nery et al. [32].

This study was approved by the Scientific and Ethical Commission of the School of Health Sciences and the Ethics Committee for the use of Animals of the Pontifical Catholic University of Rio Grande do Sul (CEUA PUCRS, number 7031/15). It followed the directions of the National Council for Control of Animal Experimentation (CONCEA) for the use of fish in research and Brazilian legislation (COBEA No. 11.794 / 08).

Morphological measurements

The teratogenic potential of the elements tested was estimated by morphological defects monitoring in larvae with 5 dpf ($n = 10$ in triplicate) under a stereomicroscope (Nikon, Melville, USA). NIS Elements D software (Nikon Instruments Inc., Melville, USA) and Image J 1.37, both for Windows, was used to determine the body length (Appendix Fig. 1A) and head (Appendix Fig. 1B), distance between the eyes (Appendix Fig. 1B) and ocular height and width (Appendix Fig. 1C). Body length was estimated using the method described by Altenhofen et al. [33]. The other parameters were estimated using the Kramer et al. [28] method.

Appendix Fig. 1. Photographs performed with stereomicroscope (Nikon, Melville, USA) for body, cranial and ocular measurements in zebrafish embryos in μm . Body length, 4x magnification (1A); Length of head and interocular distance, 6x magnification (1B); Height and width ocular, 6x magnification (1C). Dorsal view.

Cardiac system

For cardiac system evaluation, larvae with 5 dpf ($n = 10$ in triplicate) were observed under a stereomicroscope (Nikon, Melville, USA) and had their heart rate counted for 10 seconds. Subsequently these beats were multiplied by 6 to obtain the average heart rate per minute.

Exploratory Behavior

For exploratory behavior and locomotion analysis, larvae with 5 dpf ($n = 10$ in triplicate) and without morphological changes were used individually in 24 well cell culture plates with system water. First the animals were acclimated for 1 minute. After this time exploratory behavior was recorded with a HD digital webcam (Logitech) camera for 5 minutes, according to Nery et al. [32]. The videos were later analyzed with Ethovision software for the following parameters: distance traveled, average speed, immobile and mobile periods, thigmotaxis (exploration of the peripheral versus central area, validated in larvae as an anxiety measure), rotation and "turn angle" during movement.

Aversive behavior

To assess the cognitive ability to escape a visual aversive stimulus, a task adapted from Pelkowski et al. [34] by Nery et al. [32] was performed. The larvae were placed in six well plates (5 larvae per well, n = 10 in triplicate) and exposed to a visual stimulus where a red circle oscillated between the two extremities for 5 minutes. The ability to move to the area without stimulus during the task is indicative of cognitive response to the aversive stimulus. At the end of the session, the percentage of animals in the portion without stimulus was used to assess the cognitive ability of the different groups [32].

Cellular apoptosis

For the quantification of apoptotic cells, the acridine orange technique was used, a dye that fluoridates the degraded nucleic acid [35], thus allowing the observation of apoptotic cell islands throughout the animals' bodies. For this, 24-hour embryos (n = 10 in triplicate) were treated with propylthiouracil (PTU) until the 5° dpf to inhibit the natural pigmentation process of the animals and to facilitate the visualization of fluorescent labeling. In the 5° dpf the animals were immersed in acridine orange solution (2 µg / mL) for 30 min, followed by three washes with system water for 10 minutes [35]. For the photographic record the larvae were fixed in methylcellulose (3%) and observed under stereomicroscope with UV light. The densitometric quantification of each image was performed using Carestream software (Carestream Health), using the ratio of positive and negative pixels.

Statistical analysis

Survival and hatching were analyzed by the Kaplan-Meier test. Once the normality of the data was observed, the other measurements were analyzed by the one-way ANOVA test, followed by the Tukey test when there was a difference between the groups. The significance level considered was $p < 0.05$.

Results

1. Effects of acrylic resin and its components on zebrafish mortality and hatching rate

The survival rate showed a significant difference only for the highest dose in the DB group, where 100% mortality was observed in the 1° dpf. Regarding the hatch rate, there was no delay or acceleration for any of the groups studied. Both were monitored daily and analyzed by the Kaplan-Meier test. All treatments were accompanied by an absolute control group exposed only to water and data from these groups did not differ from the control group of the vehicle.

2. Effects of acrylic resin and its components on behavioral parameters and cognitive abilities.

The individual evaluation of the locomotion and exploratory parameters in the 5° dpf showed no significant difference between the groups tested. Only discrete variations were found, which do not seem to support any larger analysis.

For the evaluation of cognitive abilities was used the aversive behavior test, where the animals submitted to the varied doses of all substances tested showed a lower escape response to the aversive stimulus, dose-dependent, that is, the higher the concentration of the substances, the lower the escape response (Fig. 1), except for the resin group where a non-monotonic dose response curve was observed, in which the lower concentration showed a lower escape response in relation to the intermediate concentration.

Figure 1. Effects of the resin and its components on the cognitive escape response in the 5 dpf. The bars expressed the mean \pm standard error (n = 10, in triplicate). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one-way ANOVA, followed by the Tukey test). The animals exposed to resin and their components separately showed a decrease in the escape response when compared to their respective control. The R and F group showed a significant decrease in the escape response even at its lowest dose.

3. Effects of acrylic resin and its components on the heart rate in zebrafish.

Exposure to resin monomer, dibutyl phthalate and benzoyl peroxide caused a decrease in heart rate at higher concentrations, while exposure to MMA and formaldehyde caused an acceleration in the rate (Fig. 2).

Figure 2. Effects of resin and its components on the heart rate of zebrafish larvae at 5 dpf. The bars expressed the mean \pm standard error (n = 10, in triplicate). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one

way ANOVA, followed by the Tukey test). The heart rate was counted with a stereomicroscope (Nikon, Melville, USA) for 10 seconds and then multiplied by 6 to obtain the average heart rate per minute. All groups tested showed changes in heart rate in relation to their respective controls.

4. Teratogenicity of acrylic resin and its components in zebrafish.

The possible effects of exposure to resin and its components on morphological parameters were also evaluated (Fig. 3). Regarding the resin, there was no significant difference for any of the evaluated parameters. For the other groups only discrete results were found, which do not seem to support any larger analysis.

Figure 3. Morphological effects of acrylic resin and its components in 5^o dpf larvae. A) Body length; B) Head length; C) Interocular distance; D) Eyer diameter and E) Eye depth. The bars expressed the mean \pm standard error (n = 10 animals in triplicate analyzed individually for each subgroup). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one-way ANOVA, followed by the Tukey test).

5. Effects of acrylic resin and its components on cellular apoptosis parameter.

All groups showed a significant increase in the rate of cellular apoptosis at the intermediate and higher concentrations, except for the resin group where only the highest concentration showed a significant difference. The DB group showed a significant increase even at the lowest concentration (Fig. 4).

Figure 4. Apoptosis analysis by means of the acridine orange technique in zebrafish with 5 dpf. The bars expressed the mean \pm standard error (n = 10 animals in triplicate analyzed individually for each subgroup). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one-way ANOVA, followed by the Tukey test). All groups showed significant increase of apoptotic cells relative to their respective controls.

Discussion

In the present study, the effects of different concentrations of the acrylic resin and its components were separately evaluated during the initial development of the zebrafish. This study showed that 120 hours of exposure to the resin and its components can cause significant changes in heart rate, cognitive deficits, and cellular apoptosis. Exposure to treatments did not cause major morphological abnormalities in developing embryos, such as pericardial and yolk sac edema, caudal deformities, and curvature of the spine, among others, which often occurs in embryos exposed to substances known to be toxic [36]. Despite that, there were discrete and isolated alterations in some measurements, such as interocular distance in animals exposed to MMA, formaldehyde, and DB (Fig. 3). Therefore, it is not possible to conclude that such substances have no teratogenic potential, since they may not have been concentrated enough to express such potential.

The acrylic resin group showed no significant difference for survival and hatching parameters. Regarding the morphological and behavioral parameters, only slight changes were observed. No studies have been found in the literature that have evaluated the toxicity of the acrylic resin in animals, but many studies have evaluated allergic contact reactions. In the current study, the acrylic resin caused a significant deficiency in the escape response at all concentrations (Fig. 1), suggesting that it may cause cognitive deficits in zebrafish, in addition to a significant increase in cellular apoptosis levels (Fig. 4). A decrease in heart rate was also observed, suggesting a potential toxic effect on the cardiac system (Fig. 2).

The MMA group, despite being the main component of the acrylic resin (96%), showed a significant increase in heart rate even at its lowest concentration (Fig. 2), while the deficit in the escape response was significant only at the highest concentration, differently from the resin group which even at its lowest concentration showed significant cognitive deficit (Fig. 1). These differences may suggest an important role of the other components on the resin. Regarding the cellular apoptosis parameter, only the lowest concentration showed no significant difference (Fig. 4). *In vitro* [11] and *in vivo* studies [37] have shown toxic effects of MMA. It has also been shown to be toxic in human neuron-enriched primary culture, derived from embryonic brain tissue [38]. Developmental defects were observed in methacrylate exposed embryo, as pericardial edema [29].

Studies in animals have shown the neurotoxic potential of formaldehyde, and that exposure to this compound may induce deficits in spatial learning and memory [39], symptoms of cognitive dysfunction frequently accompanied by anxiety and depressive disorders [40], in addition to intensifying aggressive behaviors in rats [41]. Formaldehyde is also responsible for causing toxicity in the reproductive system of rats in short- and long-term exposure [16] and oxidative stress in cardiac rainbow trout tissue [42]. Studies in humans have shown the neurotoxic potential of this compound and may be an important factor in the induction of Alzheimer's disease, since it may be responsible for loss of memory, cognitive dysfunction, reduction of cholinergic signals and increased acetylcholinesterase activity in humans [43–45]. In this study, formaldehyde showed a significant increase in heart rate (Fig. 2) and cell apoptosis at its highest concentrations (Fig. 4). There was also a decrease in the ability to escape to the aversive stimulus even at its lowest concentration (Fig. 1), as well as in the resin group, which may suggest a great influence of this compound on the toxicity of the acrylic resin for this parameter. These results corroborate the toxicological potential of formaldehyde found in the above-mentioned studies.

In this study, the DB group presented a mortality rate of 100% in its highest concentration, still in the embryonic stage (2^o dpf). Many studies have shown that the resin releases by products into the oral cavity [15, 46], so it is possible that the mortality of 100% of the higher dose embryos in the DB group may be related to resin toxicity observed in clinical and *in vitro* studies. The DB is also responsible for causing reproductive abnormalities, oxidative stress, embryonic toxicity, and neurotoxicity in fish [47–49], rats [50] and even in children [51]. In this study it was observed that DB, even at its lowest concentration, showed a marked level of cellular apoptosis (Fig. 4) and cognitive loss (Fig. 1), which is considered a toxicological parameter.

The PB group showed no significant difference for the survival and hatching parameters. As with the other groups, there were discrete morphological and behavioral changes and significant alterations in cardiac rhythm and cellular apoptosis in their higher concentration, and a decrease in the escape response only at the highest concentration. The literature is still scarce of toxicological studies with this compound in animals, being more explored in research on dermatitis since it is widely used in the treatment of acne.

All groups, at their highest concentrations, showed changes in heart rate (Fig. 2). The Resin, DB and PB groups showed a decrease in heart rate, while the MMA and formaldehyde groups showed increase. The MMA (increased heart rate) and DB (decreased heart rate) groups showed changes even at their lower concentrations. Even though MMA was the main component of the acrylic resin, it still showed a decrease in the rhythm, which suggests that for this parameter dibutyl phthalate exerted a greater influence than the MMA on the resin group.

In addition to assessing mortality and hatch rates, behavioral tests can also be a simple and rapid method to obtain additional information on the toxic effects of different substances on embryonic and nervous system development [52]. In this study, we observed discrete behavioral changes in zebrafish larvae in all groups tested.

Regarding cognitive abilities, all groups showed a decrease in this capacity in the 5 dpf (Fig. 1). Resin and formaldehyde groups showed a significant decrease in the escape response even at their lower concentrations. Formaldehyde showed a dose dependent response. Although MMA is the main component of the resin, only the highest concentration of MMA showed a decrease in the escape response. Formaldehyde was the only component of the resin that showed a significant reduction of this ability at the lowest concentration, which may suggest a great influence of this component on the resin, since it can be considered more toxic than MMA, requiring more attention [53].

Cellular apoptosis is an important process that occurs during the development of vertebrates, but the exacerbated loss of cells by this type of programmed cell death can also have irreversible deleterious consequences throughout life [54]. The results of the present study showed that exposure to acrylic resin and all its components significantly increased apoptosis when compared to its controls, suggesting that all its components cause cell death, some at a higher level than others (Fig. 4). MMA showed a dose dependent relationship, which caused more cell death in its higher doses, in comparison to the lower and the control doses. The DB and PB groups also had a dose dependent relationship. Interestingly the controls of these two groups showed a much higher level of cellular apoptosis than in controls of the other groups, which may suggest that these compounds release toxic vapors that may have interfered in their controls, since all treatments were performed on plaques of 6 wells that were closed after treatment changes. These plates were opened again only the other day for further treatment change.

More *in vivo* research is needed to deepen the knowledge of the toxicological potential that acrylic resin can have since it is a widely used material in dentistry, and where people are exposed for long periods of time either by direct contact, or by inhalation.

Based on the results found, it can be concluded that acrylic resin and all its components present toxicological potential, which can cause cardiac alterations, cognitive losses and increased cellular apoptosis. Although MMA is the main component of acrylic resin, it appeared to have less influence than formaldehyde on cognitive abilities parameters and less influence than dibutyl phthalate on heart rate.

This study showed the toxicological potential of orthodontic acrylic resin on the initial development of zebrafish, reinforcing this animal model as a potential tool for future studies in dentistry.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

1. Bapat RA, Parolia A, Chaubal T, Dharamadhikari S, Abdulla AM, Sakkir N, et al. Recent update on potential cytotoxicity, biocompatibility and preventive measures of biomaterials used in dentistry. *Biomater Sci.* 2021;9(9):3244–83. doi: 10.1039/d1bm00233c.
2. Shahi S, Özcan M, Maleki Dizaj S, Sharifi S, Al-Haj Husain N, Eftekhari A, et al. A review on potential toxicity of dental material and screening their biocompatibility. *Toxicol Mech Methods.* 2019;29(5):368–77. doi: 10.1080/15376516.2019.1566424.
3. Scott A, Egner W, Gawkrödger DJ, Hatton PV, Sherriff M, van Noort R, et al. The national survey of adverse reactions to dental materials in the UK: a preliminary study by the UK Adverse Reactions Reporting Project. *Br Dent J.* 2004;196(8):471–7; discussion 65. doi: 10.1038/sj.bdj.4811176.
4. Gonçalves TS, Morganti MA, Campos LC, Rizzato SM, Menezes LM. Allergy to auto-polymerized acrylic resin in an orthodontic patient. *Am J Orthod Dentofacial Orthop.* 2006;129(3):431–5. doi: 10.1016/j.ajodo.2005.10.017.
5. Siqueira Gonçalves T, Minghelli Schmitt V, Thomas M, Lopes de Souza MA, Macedo de Menezes L. Cytotoxicity of two autopolymerized acrylic resins used in orthodontics. *Angle Orthod.* 2008;78(5):926–30. doi: 10.2319/072407-343.1.
6. Borzelleca JF, Larson PS, Hennigar GR, Huf EG, Crawford EM, Smith RB. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36. doi: 10.1016/0041-008x(64)90019-5.
7. Bereznowski Z. In vivo assessment of methyl methacrylate metabolism and toxicity. *Int J Biochem Cell Biol.* 1995;27(12):1311–6. doi: 10.1016/1357-2725(95)00101-T.
8. Hensten-Pettersen A, Jacobsen N. Perceived side effects of biomaterials in prosthetic dentistry. *J Prosthet Dent.* 1991;65(1):138–44. doi: 10.1016/0022-3913(91)90066-6.
9. Gautam R, Singh RD, Sharma VP, Siddhartha R, Chand P, Kumar R. Biocompatibility of polymethylmethacrylate resins used in dentistry. *J Biomed Mater Res B Appl Biomater.* 2012;100(5):1444–50. doi: 10.1002/jbm.b.32673.
10. Al-Saadi MA, Al-Yasiry A, Al-Jammali Z, Moez A. Effect of acute methyl methacrylate vapor inhalation on smokers' and non-smokers' respiratory function in a sample of male dentistry students. *Dent Med Probl.* 2019;56(1):75–80. doi: 10.17219/dmp/100444.
11. Dahl OE, Garvik LJ, Lyberg T. Toxic effects of methylmethacrylate monomer on leukocytes and endothelial cells in vitro. *Acta Orthop Scand.* 1994;65(2):147–53. doi: 10.3109/17453679408995423.
12. Bural C, Aktaş E, Deniz G, Ünlüçerçi Y, Kızılcın N, Bayraktar G. Effect of post-polymerization heat-treatments on degree of conversion, leaching residual MMA and in vitro cytotoxicity of autopolymerizing acrylic repair resin. *Dent Mater.* 2011;27(11):1135–43. doi: 10.1016/j.dental.2011.08.007.
13. Blasiak J, Synowiec E, Tarnawska J, Czarny P, Poplawski T, Reiter RJ. Dental methacrylates may exert genotoxic effects via the oxidative induction of DNA double strand breaks and the inhibition of their repair. *Mol Biol Rep.* 2012;39(7):7487–96. doi: 10.1007/s11033-012-1582-3.

14. Ahn EJ, Kim SJ, Kim SH, Park KH, Jang WW, Kang YG. Nickel, chromium and methyl methacrylate monomer release from orthopaedic functional appliances. *Eur J Paediatr Dent*. 2021;22(2):144–50. doi: 10.23804/ejpd.2021.22.02.11.
15. Singh RD, Gautam R, Siddhartha R, Singh BP, Chand P, Sharma VP, et al. High performance liquid chromatographic determination of residual monomer released from heat-cured acrylic resin. An in vivo study. *J Prosthodont*. 2013;22(5):358–61. doi: 10.1111/jopr.12004.
16. Zhao L, Si J, Wei Y, Li S, Jiang Y, Zhou R, et al. Toxicity of porcelain-fused-to-metal substrate to zebrafish (*Danio rerio*) embryos and larvae. *Life Sci*. 2018;203:66–71. doi: 10.1016/j.lfs.2018.04.019.
17. Peterson RT, Nass R, Boyd WA, Freedman JH, Dong K, Narahashi T. Use of non-mammalian alternative models for neurotoxicological study. *Neurotoxicology*. 2008;29(3):546–55. doi: 10.1016/j.neuro.2008.04.006.
18. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature*. 2013;496(7446):498–503. doi: 10.1038/nature12111.
19. Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol Sci*. 2014;35(2):63–75. doi: 10.1016/j.tips.2013.12.002.
20. Taylor KL, Grant NJ, Temperley ND, Patton EE. Small molecule screening in zebrafish: an in vivo approach to identifying new chemical tools and drug leads. *Cell Commun Signal*. 2010;8:11. doi: 10.1186/1478-811X-8-11.
21. Legradi J, el Abdellaoui N, van Pomeran M, Legler J. Comparability of behavioural assays using zebrafish larvae to assess neurotoxicity. *Environ Sci Pollut Res Int*. 2015;22(21):16277–89. doi: 10.1007/s11356-014-3805-8.
22. Amatruda JF, Shepard JL, Stern HM, Zon LI. Zebrafish as a cancer model system. *Cancer Cell*. 2002;1(3):229–31. doi: 10.1016/s1535-6108(02)00052-1.
23. Zoupa M, Machera K. Zebrafish as an Alternative Vertebrate Model for Investigating Developmental Toxicity-The Triadimefon Example. *Int J Mol Sci*. 2017;18(4). doi: 10.3390/ijms18040817.
24. Karaman GE, Emekli-Alturfan E, Akyüz S. Zebrafish; an emerging model organism for studying toxicity and biocompatibility of dental materials. *Cell Mol Biol (Noisy-le-grand)*. 2020;66(8):41–6.
25. Zhang Y, Zheng X, Xu R, He H, Duan X. Grading and quantification of dental fluorosis in zebrafish larva. *Arch Oral Biol*. 2016;70:16–23. doi: 10.1016/j.archoralbio.2016.05.021.
26. P K, M P, Samuel Rajendran R, Annadurai G, Rajeshkumar S. Characterization and toxicology evaluation of zirconium oxide nanoparticles on the embryonic development of zebrafish, *Danio rerio*. *Drug Chem Toxicol*. 2019;42(1):104–11. doi: 10.1080/01480545.2018.1523186.
27. Makkar H, Verma SK, Panda PK, Jha E, Das B, Mukherjee K, et al. In Vivo Molecular Toxicity Profile of Dental Bioceramics in Embryonic Zebrafish (*Danio rerio*). *Chem Res Toxicol*. 2018;31(9):914–23. doi: 10.1021/acs.chemrestox.8b00129.

28. Kramer AG, Vuthiganon J, Lassiter CS. Bis-GMA affects craniofacial development in zebrafish embryos (*Danio rerio*). *Environ Toxicol Pharmacol*. 2016;43:159–65. doi: 10.1016/j.etap.2016.02.018.
29. Altayib B, Egilmezer G, Unal I, Ustundag UV, Gozneli R, Emekli-Alturfan E. Effects of Methacrylate Exposure on Developing Zebrafish Embryos. *European Journal of Research in Dentistry*. 2019;3:25–8. doi: 10.35333/ERD.2019.10.
30. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn*. 1995;203(3):253–310. doi: 10.1002/aja.1002030302.
31. Westerfield M. *The zebrafish book. A guide to laboratory use of zebrafish (Danio rerio)*. 4^a ed. Eugene, Oregon.: 2000.
32. Nery LR, Eltz NS, Martins L, Guerim LD, Pereira TC, Bogo MR, et al. Sustained behavioral effects of lithium exposure during early development in zebrafish: involvement of the Wnt- β -catenin signaling pathway. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;55:101–8. doi: 10.1016/j.pnpbp.2014.04.011.
33. Altenhofen S, Nabinger DD, Wiprich MT, Pereira TCB, Bogo MR, Bonan CD. Tebuconazole alters morphological, behavioral and neurochemical parameters in larvae and adult zebrafish (*Danio rerio*). *Chemosphere*. 2017;180:483–90. doi: 10.1016/j.chemosphere.2017.04.029.
34. Pelkowski SD, Kapoor M, Richendrfer HA, Wang X, Colwill RM, Creton R. A novel high-throughput imaging system for automated analyses of avoidance behavior in zebrafish larvae. *Behav Brain Res*. 2011;223(1):135–44. doi: 10.1016/j.bbr.2011.04.033.
35. Tucker B, Lardelli M. A rapid apoptosis assay measuring relative acridine orange fluorescence in zebrafish embryos. *Zebrafish*. 2007;4(2):113–6. doi: 10.1089/zeb.2007.0508.
36. Duan J, Yu Y, Shi H, Tian L, Guo C, Huang P, et al. Toxic effects of silica nanoparticles on zebrafish embryos and larvae. *PLoS One*. 2013;8(9):e74606. doi: 10.1371/journal.pone.0074606.
37. Bereznowski Z. Effect of methyl methacrylate on mitochondrial function and structure. *Int J Biochem*. 1994;26(9):1119–27. doi: 10.1016/0020-711x(94)90134-1.
38. Chen MS, Wu JN, Yang SN, Hsieh WY, Liu JC, Fu E, et al. Free radicals are involved in methylmethacrylate-induced neurotoxicity in human primary neocortical cell cultures. *Chin J Physiol*. 1998;41(4):203–9.
39. Lu Z, Li CM, Qiao Y, Yan Y, Yang X. Effect of inhaled formaldehyde on learning and memory of mice. *Indoor Air*. 2008;18(2):77–83. doi: 10.1111/j.1600-0668.2008.00524.x.
40. Li Y, Song Z, Ding Y, Xin Y, Wu T, Su T, et al. Effects of formaldehyde exposure on anxiety-like and depression-like behavior, cognition, central levels of glucocorticoid receptor and tyrosine hydroxylase in mice. *Chemosphere*. 2016;144:2004–12. doi: 10.1016/j.chemosphere.2015.10.102.
41. Liu Y, Ye Z, Luo H, Sun M, Li M, Fan D, et al. Inhalative formaldehyde exposure enhances aggressive behavior and disturbs monoamines in frontal cortex synaptosome of male rats. *Neurosci Lett*. 2009;464(2):113–6. doi: 10.1016/j.neulet.2009.06.037.

42. Tkachenko H, Grudniewska J. Evaluation of oxidative stress markers in the heart and liver of rainbow trout (*Oncorhynchus mykiss walbaum*) exposed to the formalin. *Fish Physiol Biochem.* 2016;42(6):1819–32. doi: 10.1007/s10695-016-0260-0.
43. Tong Z, Han C, Luo W, Wang X, Li H, Luo H, et al. Accumulated hippocampal formaldehyde induces age-dependent memory decline. *Age (Dordr).* 2013;35(3):583–96. doi: 10.1007/s11357-012-9388-8.
44. Tong Z, Han C, Qiang M, Wang W, Lv J, Zhang S, et al. Age-related formaldehyde interferes with DNA methyltransferase function, causing memory loss in Alzheimer's disease. *Neurobiol Aging.* 2015;36(1):100–10. doi: 10.1016/j.neurobiolaging.2014.07.018.
45. Zendejdel R, Fazli Z, Mazinani M. Neurotoxicity effect of formaldehyde on occupational exposure and influence of individual susceptibility to some metabolism parameters. *Environ Monit Assess.* 2016;188(11):648. doi: 10.1007/s10661-016-5662-z.
46. Kopperud HM, Kleven IS, Wellendorf H. Identification and quantification of leachable substances from polymer-based orthodontic base-plate materials. *Eur J Orthod.* 2011;33(1):26–31. doi: 10.1093/ejo/cjq020.
47. Uren-Webster TM, Lewis C, Filby AL, Paull GC, Santos EM. Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. *Aquat Toxicol.* 2010;99(3):360–9. doi: 10.1016/j.aquatox.2010.05.015.
48. Xu H, Shao X, Zhang Z, Zou Y, Chen Y, Han S, et al. Effects of di-n-butyl phthalate and diethyl phthalate on acetylcholinesterase activity and neurotoxicity related gene expression in embryonic zebrafish. *Bull Environ Contam Toxicol.* 2013;91(6):635–9. doi: 10.1007/s00128-013-1101-9.
49. Xu H, Shao X, Zhang Z, Zou Y, Wu X, Yang L. Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. *Ecotoxicol Environ Saf.* 2013;93:39–44. doi: 10.1016/j.ecoenv.2013.03.038.
50. Hoshi H, Ohtsuka T. Adult rats exposed to low-doses of di-n-butyl phthalate during gestation exhibit decreased grooming behavior. *Bull Environ Contam Toxicol.* 2009;83(1):62–6. doi: 10.1007/s00128-009-9729-1.
51. Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, et al. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. *Environ Health Perspect.* 2010;118(4):565–71. doi: 10.1289/ehp.0901470.
52. Song JE, Si J, Zhou R, Liu HP, Wang ZG, Gan L, et al. Effects of Exogenous Carbon Monoxide Releasing Molecules on the Development of Zebrafish Embryos and Larvae. *Biomed Environ Sci.* 2016;29(6):453–6. doi: 10.3967/bes2016.059.
53. Koda T, Tsuchiya H, Yamauchi M, Hoshino Y, Takagi N, Kawano J. High-performance liquid chromatographic estimation of eluates from denture base polymers. *J Dent.* 1989;17(2):84–9. doi: 10.1016/0300-5712(89)90137-1.
54. Ramlan NF, Sata NSAM, Hassan SN, Bakar NA, Ahmad S, Zulkifli SZ, et al. Time dependent effect of chronic embryonic exposure to ethanol on zebrafish: Morphology, biochemical and anxiety alterations. *Behav Brain Res.* 2017;332:40–9. doi: 10.1016/j.bbr.2017.05.048.

Figures

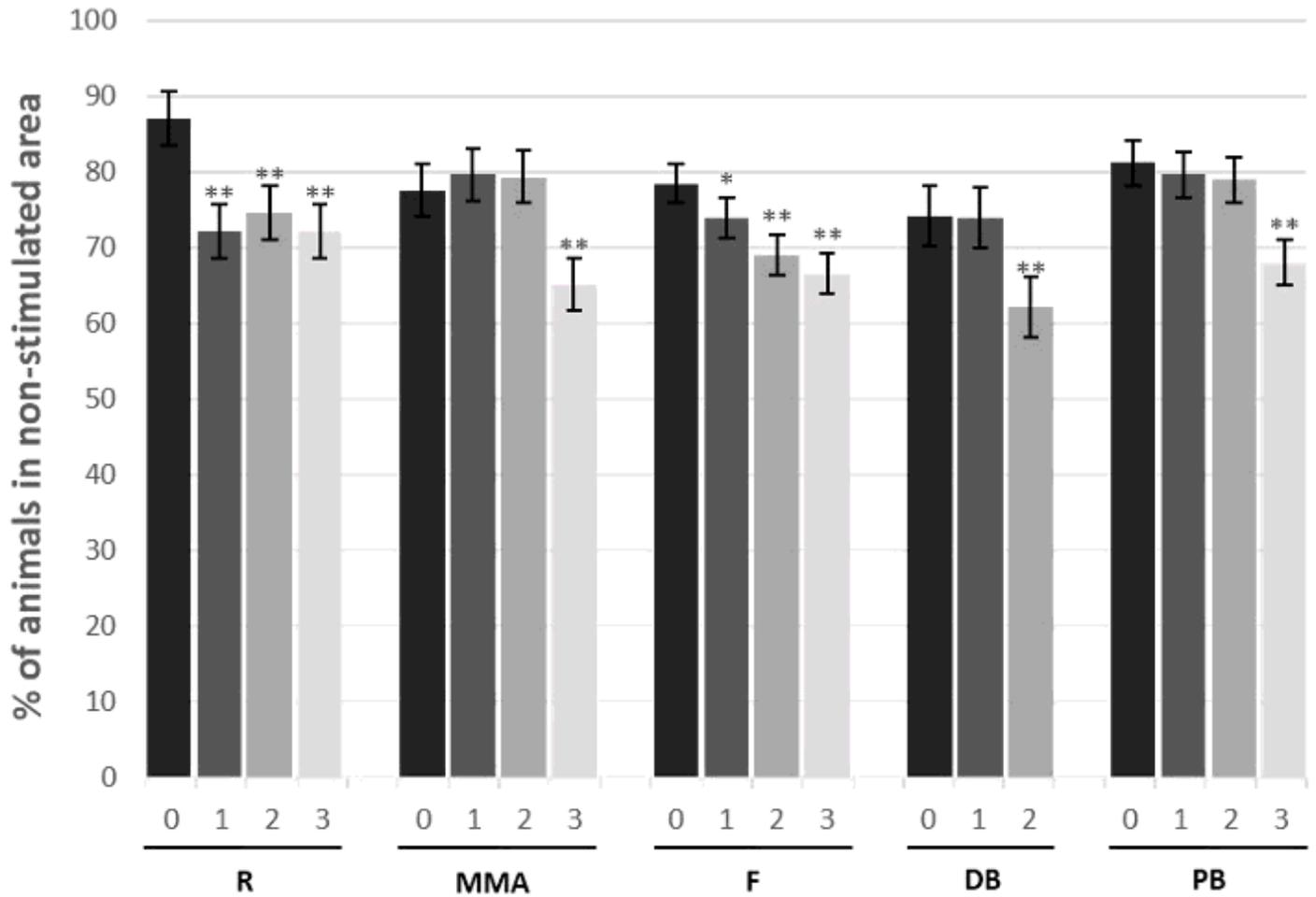


Figure 1

Effects of the resin and its components on the cognitive escape response in the 5 dpf. The bars expressed the mean \pm standard error (n = 10, in triplicate). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one-way ANOVA, followed by the Tukey test). The animals exposed to resin and their components separately showed a decrease in the escape response when compared to their respective control. The R and F group showed a significant decrease in the escape response even at its lowest dose.

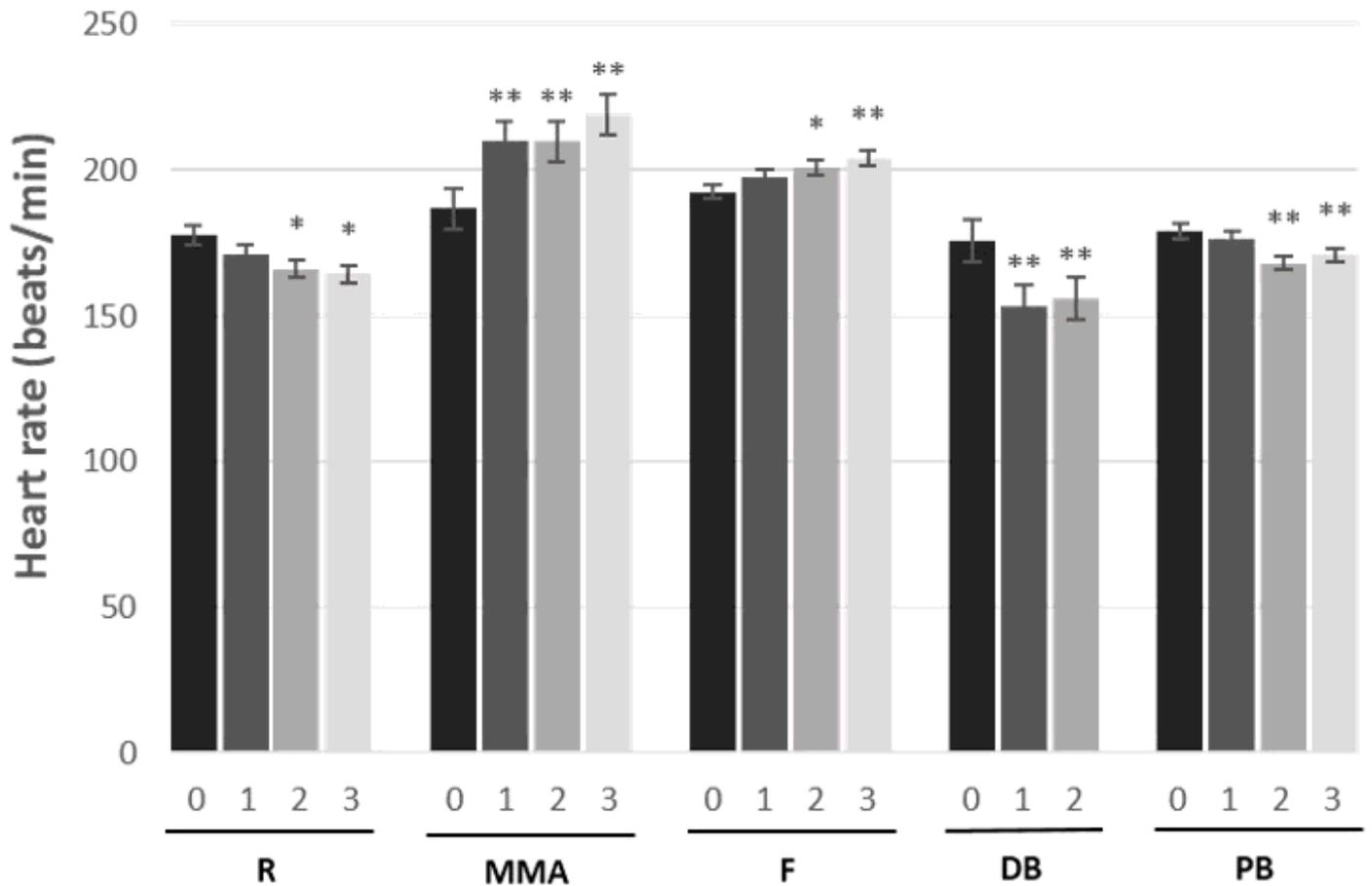


Figure 2

Effects of resin and its components on the heart rate of zebrafish larvae at 5 dpf. The bars expressed the mean \pm standard error (n = 10, in triplicate). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one

way ANOVA, followed by the Tukey test). The heart rate was counted with a stereomicroscope (Nikon, Melville, USA) for 10 seconds and then multiplied by 6 to obtain the average heart rate per minute. All groups tested showed changes in heart rate in relation to their respective controls.

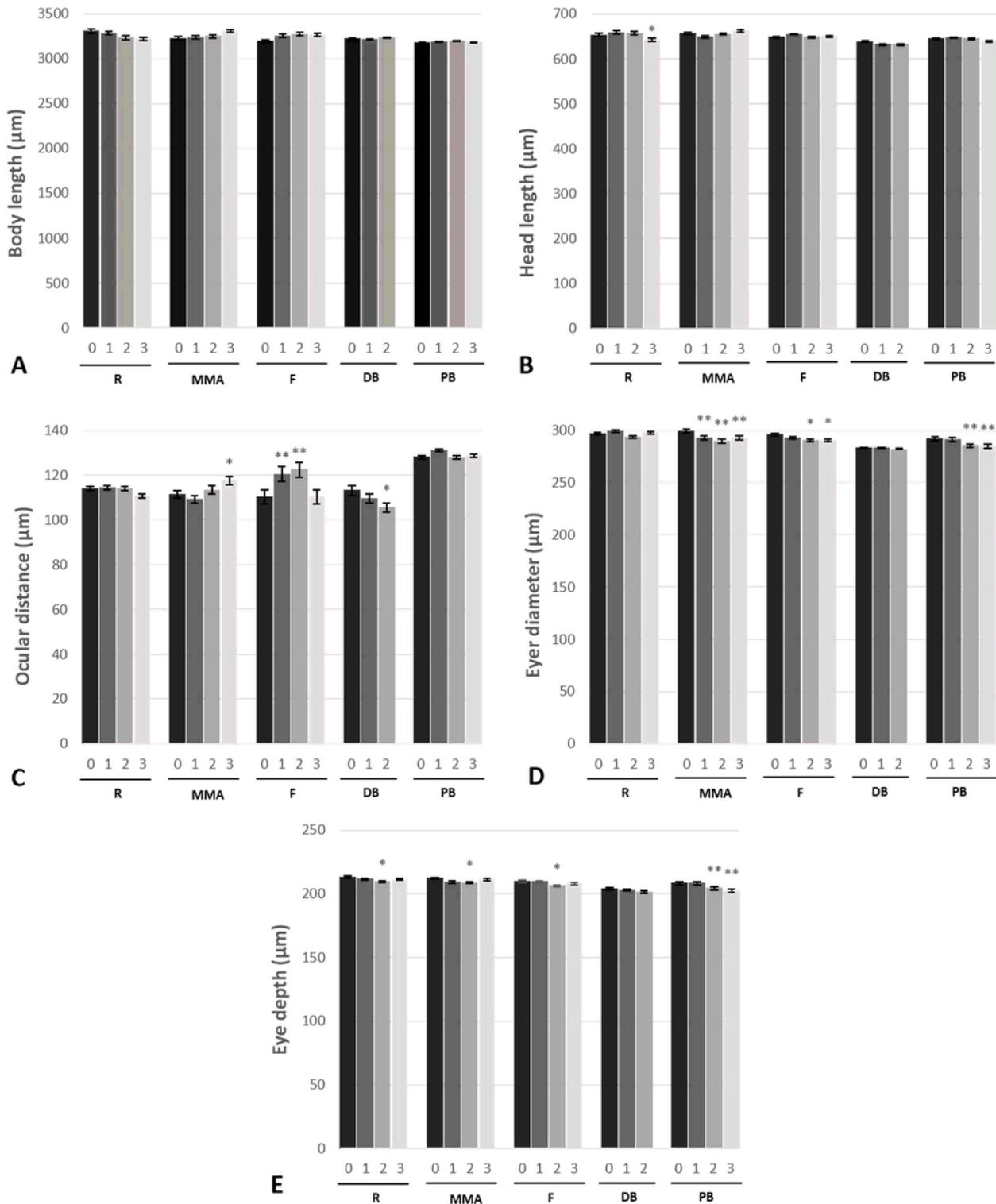


Figure 3

Morphological effects of acrylic resin and its components in 5° dpf larvae. A) Body length; B) Head length; C) Interocular distance; D) Eye diameter and E) Eye depth. The bars expressed the mean \pm standard error ($n = 10$ animals in triplicate analyzed individually for each subgroup). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one-way ANOVA, followed by the Tukey test).

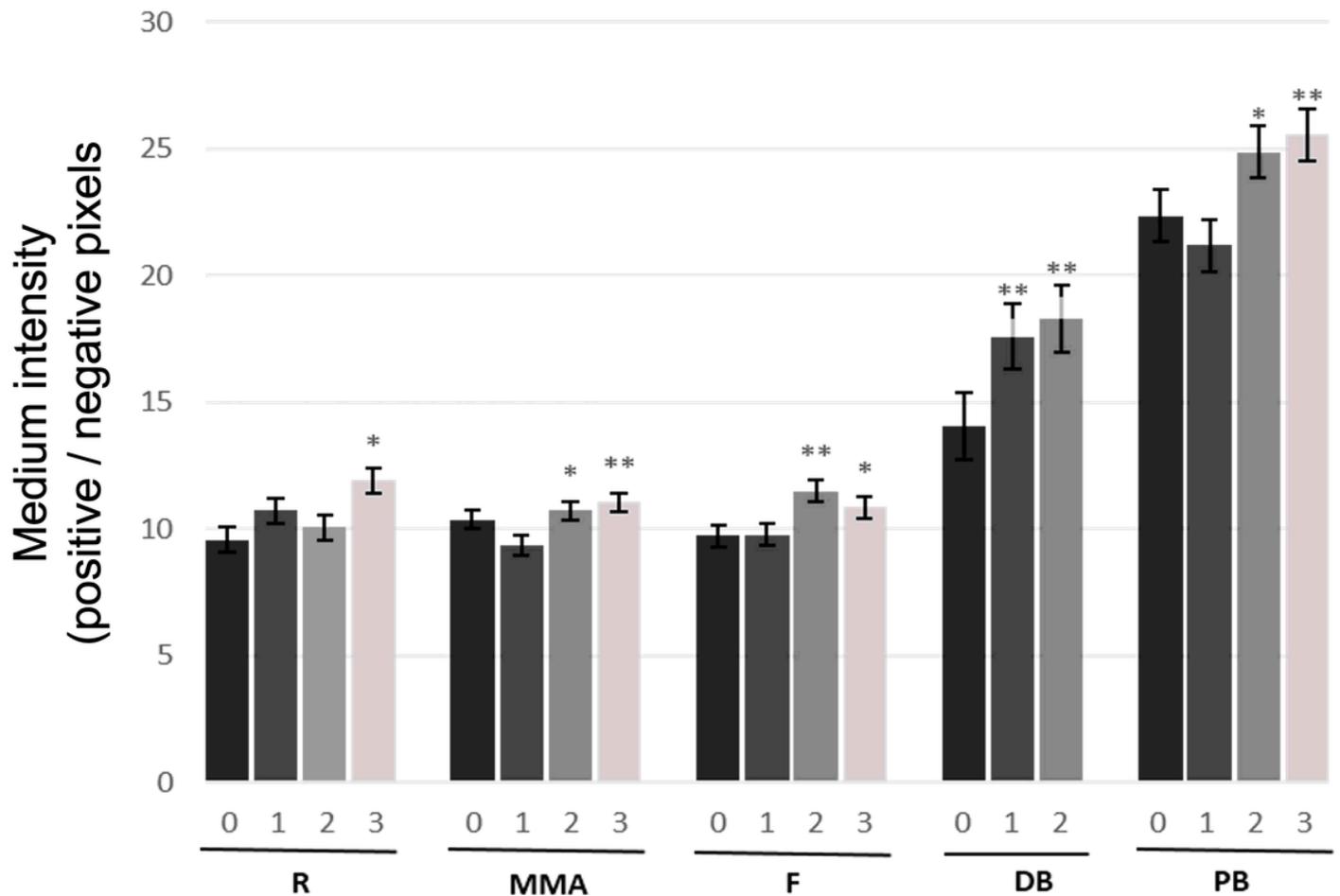


Figure 4

Apoptosis analysis by means of the acridine orange technique in zebrafish with 5 dpf. The bars expressed the mean \pm standard error (n = 10 animals in triplicate analyzed individually for each subgroup). * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$ in relation to the respective control of each group (one-way ANOVA, followed by the Tukey test). All groups showed significant increase of apoptotic cells relative to their respective controls.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AppendixFig1.tiff](#)