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Toxicity analysis of Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement in in-vivo zebrafish model

[1]Tanvi Deepak Shah

Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Mail Id : tanvis20597@gmail.com

[2]Sanyukta Singh

Assistant Professor, Department of Conservative Dentistry and Endodontics Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Mail Id: singhsanyukta95@gmail.com

[3]Surendar Sugumaran

Assistant Professor, Department of Conservative Dentistry and Endodontics Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Mail Id: surendars.sdc@saveetha.com

Corresponding Author

Dr. Surendar Sugumaran

Assistant Professor, Department of Conservative Dentistry and Endodontics Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Mail Id: surendars.sdc@saveetha.com

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Abstract:

Zebrafish (*Danio rerio*) is a commonly used model organism in various fields of research, including dental studies. Zebrafish possess several advantages as a model organism, such as their small size, rapid development, transparency of embryos, and genetic tractability. These characteristics make them particularly suitable for studying various aspects of dental research, including tooth development, regeneration, and oral disease modelling. Here are some ways in which zebrafish can be utilized in dental studies. Zebrafish continuously develop replacement teeth throughout their lifespan, which makes them an excellent model for studying tooth development and regeneration. Researchers can investigate the genetic and molecular mechanisms underlying tooth formation by manipulating specific genes and observing the resulting phenotypes. This can provide insights into the molecular pathways involved in human tooth development and regeneration.

Keywords: Zebrafish (*Danio rerio*), microscope, unfertilized embryos, molecular pathways, regeneration

1. Introduction

Zebrafish (*Danio rerio*) is a commonly used model organism in various fields of research, including dental studies [1–4]. Zebrafish possess several advantages as a model organism, such as their small size, rapid development, transparency of embryos, and genetic tractability. These characteristics make them particularly suitable for studying various aspects of dental research, including tooth development, regeneration, and oral disease modelling [5–8]. Here are some ways in which zebrafish can be utilized in dental studies. Zebrafish continuously develop replacement teeth throughout their lifespan, which makes them an excellent model for studying tooth development and regeneration [9, 10]. Researchers can investigate the genetic and molecular mechanisms underlying tooth formation by manipulating specific genes and observing the resulting phenotypes [11–13]. This can provide insights into the molecular pathways involved in human tooth development and regeneration. Dental regeneration. Zebrafish have the remarkable ability to regenerate lost or damaged teeth throughout their lives. By studying the regenerative processes in zebrafish, researchers can gain insights into the cellular and molecular mechanisms that enable tooth regeneration [14–17]. This knowledge can potentially be applied to enhance the regenerative capacity of human teeth, which could have implications for dentistry and oral health treatments.

Oral disease modeling: Zebrafish can be used to model various oral diseases, including dental caries, periodontal disease, and oral cancer. By introducing specific genetic mutations or exposing zebrafish to disease-inducing agents, researchers can study the pathogenesis of these oral diseases and test potential therapeutic interventions [18–21]. Zebrafish's optical transparency during early developmental stages allows for real-time visualization of disease

progression and the effects of treatments. Zebrafish can serve as a screening platform for identifying potential drug candidates for dental-related conditions [22–25]. By using zebrafish models of oral diseases, researchers can screen libraries of chemical compounds to identify molecules that can modulate disease progression or promote tooth regeneration [26–29]. Zebrafish-based drug discovery approaches can offer a cost-effective and efficient way to identify promising therapeutic candidates. Overall, zebrafish provide a valuable model system for dental research due to their genetic amenability, regenerative capacity, and optical transparency [30–33]. By utilizing zebrafish, researchers can gain insights into tooth development, regeneration, oral disease pathogenesis, and potentially discover new therapeutic approaches for dental-related conditions.

2. Materials and Methods

2.1. Origin and maintenance of zebrafish

Adult zebrafish (Wild type – AB strain, 4 months old) were purchased from a local aquarist (NSK aquarium, Kolathur, Tamil Nadu, India). The male and female fishes were separated, maintained in our facility under the following condition in a 10 L glass tank: 28.5˚C, with a 14/10 h light/dark cycle [34–37]. The fish were fed three times a day, with live brine shrimp (*Artemia salina*). The fishes were acclimatized for 1 month, later the fishes were utilized for breeding, and embryos were collected and used for the following experiments [38]. The collected embryos are further analyzed under a microscope, unfertilized embryos are discarded, whereas the fertilized embryos are taken in a six-well plate and incubated in E3 medium.

2.2. Zebrafish embryo toxicity test

For the developmental toxicity assessment studies, 4 hpf embryos were used, the exposure was carried in a 6 well plate containing untreated larvae as the control, Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM. Around 15 embryos/well were used with 3 mL of E3 medium. The exposure was non-static and renewed every 24 h with the fresh treatment solution throughout the exposure period (4 hpf to 96 hpf). All the experiments were carried out in triplicates. Parameters such as survival and malformation were observed during this period, and calculations were presented at the end of 96 hpf [39].

2.3. Intracellular ROS assay

The DPPP (2,2-diphenyl-1-picrylhydrazyl) fluorescent assay is used to measure the antioxidant capacity or radical scavenging activity of a sample [40]. It is based on the principle that DPPP, a stable free radical, is reduced to a non-fluorescent compound upon interaction with antioxidants [41–43]. If you are interested in conducting a DPPP fluorescent assay in zebrafish larvae, here's a general outline of the procedure. Preparation of DPPP solution: Prepare a stock solution of DPPP by dissolving it in an appropriate solvent, such as ethanol or methanol, to a concentration of typically 1 mM. Protect the stock solution from light and store it at -20°C. Larval zebrafish handling: Obtain zebrafish larvae at the desired developmental stage (e.g., 3-5 days post-fertilization). Handle the larvae with appropriate care and follow ethical guidelines for animal experimentation. Keep the larvae in an appropriate fish facility under controlled conditions (temperature, lighting, water quality, etc.) [34]. Treatment groups: Design your

experiment with appropriate treatment groups. For example, you may have control larvae and experimental groups exposed to specific conditions or treatments that affect antioxidant activity.

Incubation with DPPP: Prepare a working solution of DPPP by diluting the stock solution in an appropriate buffer or medium [44]. The concentration of DPPP in the working solution will depend on the specific requirements of your experiment. Typically, concentrations between 10-100 μM are used. Incubate the zebrafish larvae in the DPPP working solution for a defined period, often 1-2 hours, at an appropriate temperature (typically 28-32°C). Wash and homogenization: After the DPPP incubation period, carefully remove the larvae from the DPPP solution and wash them with fresh buffer or medium to remove any excess probe. Transfer the larvae to a suitable homogenization buffer or medium. Homogenization and fluorescence measurement: Homogenize the zebrafish larvae using a suitable method to extract the cellular contents. Centrifuge the homogenate to remove debris if necessary. Measure the fluorescence intensity of the resulting supernatant using a fluorometer or spectrofluorometer. DPPP exhibits fluorescence emission at specific wavelengths, typically around 420-430 nm, when excited at an appropriate wavelength (around 340-360 nm) [45–47].

2.4. Statistical analysis

The data were presented as the mean of triplicates with a standard deviation. GraphPad Prism software (Ver 5.03, CA, USA) was used for statistical analysis. One-way ANOVA was performed and Tukey's post-hoc test was used to find level of significance between control and other groups.

3. Results and Discussions

3.1 Zebrafish embryo toxicity test

3.1.1 Mortality and hatching rate

The Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement did not cause mortality in zebrafish embryos. As shown in Fig. 1, the Survival rate was found to be more than 90% in all the group. Additionally, the hatching rate was calculated at 48 hpf. It was observed that 100% of the zebrafish embryo emerged out of their chorions in the control group. However, a similar in the hatching rate was observed all the other treated group.

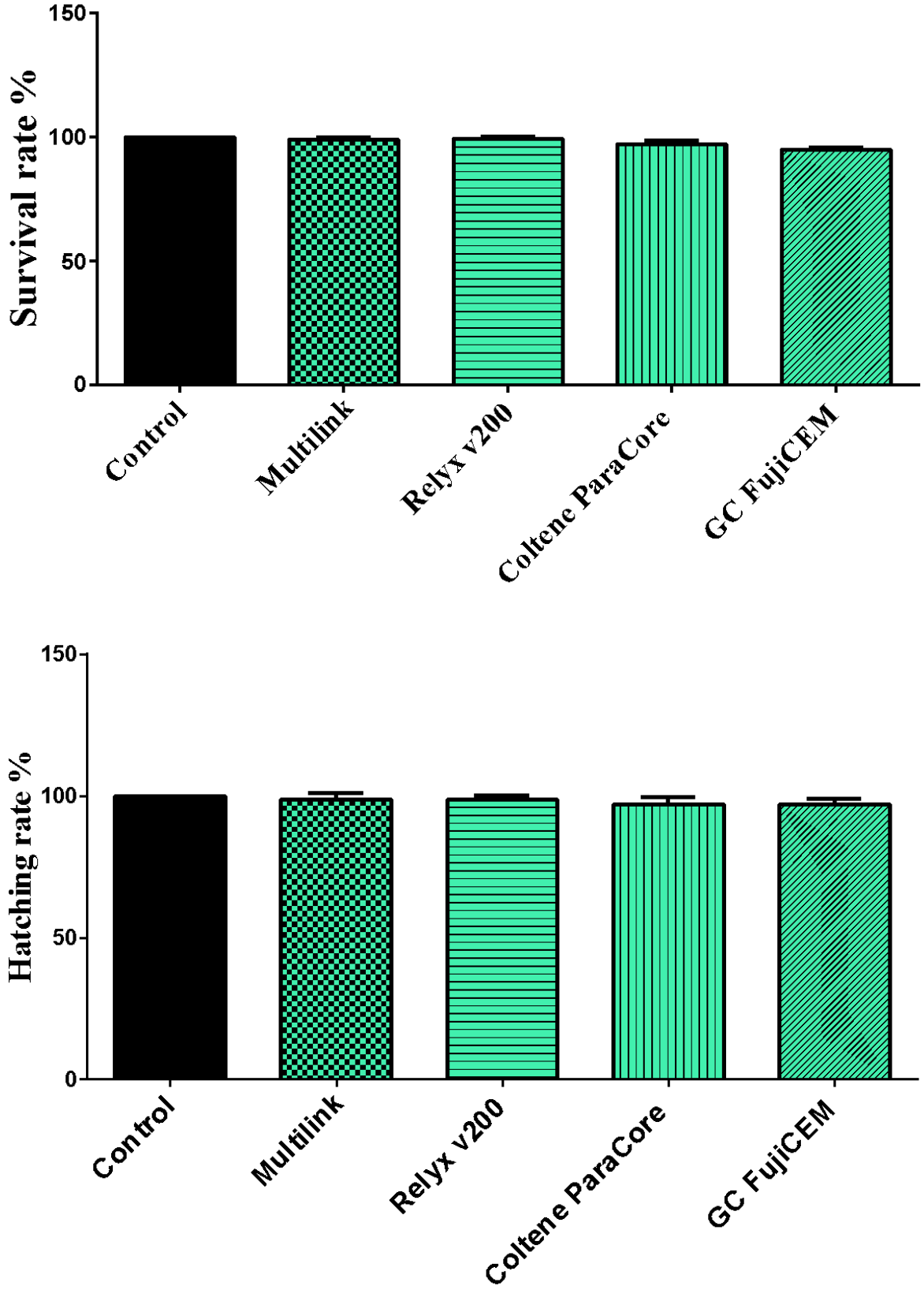

Figure.1. Survival and Hatching rate of the zebrafish larvae exposed to Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement.

3.2 Measurement of heart rate

The heart rate of zebrafish embryos was evaluated at 72 hpf, to assess the toxicity of treatment group. The atrial and ventricular contractions were counted and recorded under a microscope for 1 min and average heart beats per minute were reported. The result shows that all the group of Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement did not significantly alter the heart beat rate of the zebrafish embryos when compared to the embryos from the control (untreated) group (Fig. 2).

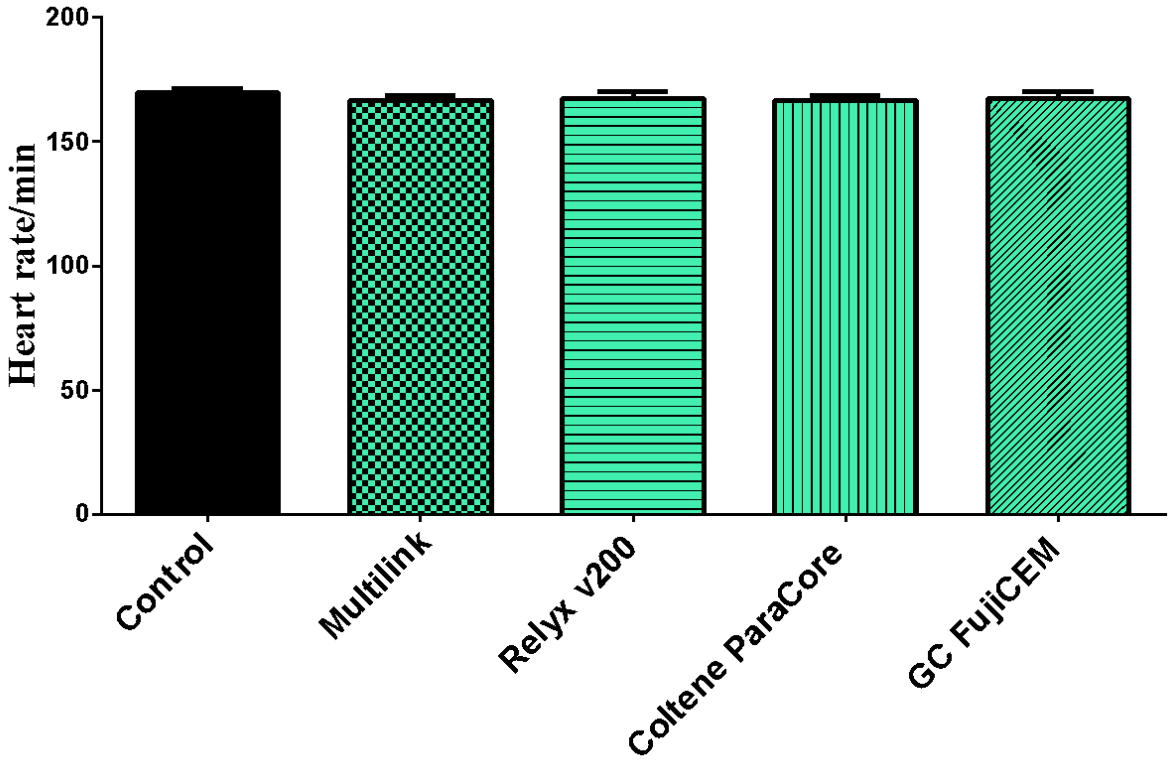

Figure.2. Heart rate of the zebrafish larvae exposed to Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement

3.3. Morphological malformation

The Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement treated zebrafish embryos exhibited normal morphological architecture under the microscope (Fig. 3). No malformations such as yolk sac edema and bent spine are formed in the larvae.

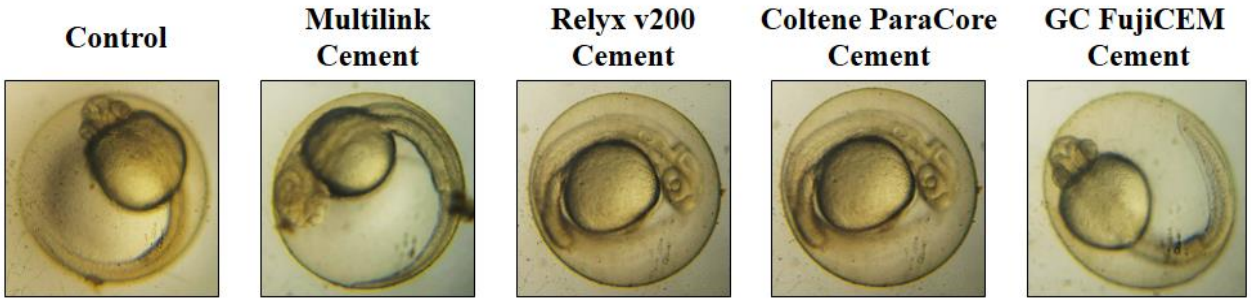

Figure 3: Morphology of the zebrafish larvae exposed to Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement

3.4 Lipid peroxidation detection

Using the fluorescent stain, the level of lipid peroxidation was visualized in the larvae. The increase in fluorescent intensity indicates the increase in lipid peroxidation level in larvae. But this condition was effectively low when the larvae were treated with Coltene ParaCore, and GC FujiCEM cement. The fluorescent intensity was noted as similar to the control group. Meanwhile, Multilink and Relyx v200 cement increased the fluorescent intensity which indicates the lipid peroxidation level. These results suggest that with Coltene ParaCore, and GC FujiCEM cement induces no oxidative stress condition compared to the Multilink and Relyx v200 cement. The **Figure 4 shows the** DPPP experiment in the zebrafish larvae exposed to Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement. The blue fluorescent indicates the presence of lipid peroxidations in zebrafish larvae.

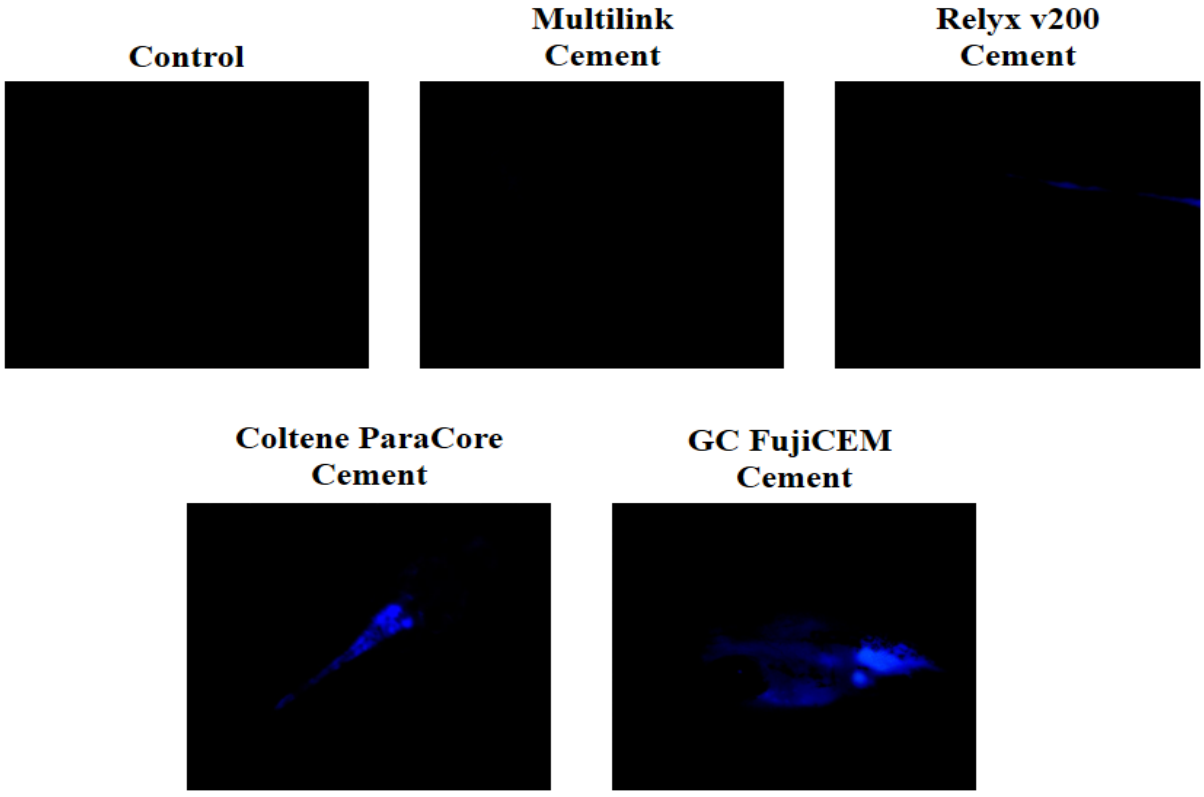

Figure 4. The DPPP experiment in the zebrafish larvae exposed to Multilink, Relyx v200, Coltene ParaCore, and GC FujiCEM cement. The blue fluorescent indicates the presence of lipid peroxidations in zebrafish larvae.

4. Conclusion

The results shows Coltene ParaCore, and GC FujiCEM cement showed oxidative stress condition to the larvae compared to the Multilink and Relyx v200, when exposed to the zebrafish larvae

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