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## Evaluating the toxicity and antioxidant activity of Oligopeptide with remineralizing potential on Zebrafish larvae

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**Abstract: Aim:** The aim of the study is to assess the ability of self-assembling peptide (p11-4) with varying concentrations based on diffusion and remineralization potential on early carious lesions.

**Objectives:** To evaluate the antioxidant property of p 11 4 self-assembling peptide and to determine the toxicity level of the p 11 4 peptide in zebrafish larvae and embryo .

**Materials and Methods:** Adult zebrafish (4 months old) were purchased from a local aquarist. 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. The fishes were acclimatised for 1 month, later the fishes were utilised for breeding, and embryos were collected and used for the following experiments. The collected embryos were further analysed under microscope.

**Results:** The survival rate of zebrafish larvae was investigated after being exposed with different groups of P114 peptide for 24hrs. ( $p > 0.001$ ) indicates the significant difference between the control (untreated larvae) and treated group.

**Conclusion:** According to this study, it indicates that P114 peptide has antioxidant activity. It helps to maintain the antioxidant enzyme level during the oxidative stressed condition. The SOD and CAT level are upregulated during P114 peptide.

### 1. Introduction

The tooth enamel is defined as a complex structure that comprises organic and inorganic components that produces the strongest remineralized structure. The composition of enamel which comprises calcium phosphate salts and hydroxyapatite crystals that results in enamel prisms (1,2). The self-assembling was introduced in 1989. The peptide p11-4 is considered as the best biomimetic alternative for providing remineralising properties of enamel. The self-assembling peptide p-11 4 which diffuses into subsurface lesions assembles into aggregates throughout the lesion process, thereby supporting the nucleation of de novo hydroxyapatite nanocrystals resulting in increased mineral density.(3) assessed the interaction of p11-4 with organic dentin components and also its impact on the proteolytic activity, mechanical aspects of bond interface and nanoleakage evaluation in simulated caries-affected dentin. P11-4 also interacts with the collagen type 1 fibres thus improving the integrity of hybrid layer which is been generated by the artificial caries -affected dentin and enhancing collagen fibre resistance to proteolysis.(Burton PA ; Burke JL et al 2013) The uses of P11-4 peptide (1) Early Enamel caries remineralisation (2) Dentinal caries (3) Hypersensitivity of dentin (4) Erosion of the Enamel .(4) Zebrafish have become a popular animal model for studying drug toxicity due to their genetic similarity to humans, rapid development, and transparent embryos, which allow for easy visualisation of developmental processes (5)4-(4–6). In addition, they have a well-characterised genome, making it easier to study the effects of drugs on specific genes (7),(8). To use zebrafish as a model for drug toxicity, researchers typically expose embryos or adult fish to various concentrations of the drug of interest and then monitor for any adverse effects (9). This can include observing changes in behaviour, growth, development, or organ function. Researchers can also use molecular

biology techniques to analyse changes in gene expression or protein levels (10),(11). One advantage of using zebrafish for drug toxicity studies is that they are relatively inexpensive to maintain and can be kept in large numbers. In addition, zebrafish embryos are permeable to many drugs, allowing researchers to easily administer compounds in a controlled manner. This can be particularly useful for screening large numbers of compounds for toxicity (3).

Overall, the zebrafish model for drug toxicity offers a powerful tool for preclinical drug testing, allowing researchers to assess the safety of potential therapeutics before testing in higher-order animals or humans (8),(12). In this study, we synthesised the P114 peptide and their toxicity level with different concentration was tested in the zebrafish larvae. Also, the p114 peptide was taken for the antioxidant activity in larvae whether it can upregulate the superoxide anion and catalase enzyme level.(13)

## 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 (13). The fish were fed three times a day, with live brine shrimp (*Artemia salina*). The fishes were acclimated for 1 month, later the fishes were utilised for breeding, and embryos were collected and used for the following experiments. The collected embryos are further analysed under a microscope, unfertilized embryos are discarded, whereas the fertilised 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, and P114 peptide with different concentration (5µM, 25µM, and 50µM). 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 (14).

### 2.3. Enzyme activity assays in zebrafish larvae

Zebrafish larvae were exposed to H<sub>2</sub>O<sub>2</sub> of 100µM for inducing the oxidative stressed condition. Then the larvae were treated with P114 peptide of different concentrations. These larvae are anaesthetised with tricaine and were homogenised and centrifuged at 5000 rpm for 15 min (9),(15). The supernatant was used to determine the biological activities of the enzymes (including superoxide dismutase (SOD), catalase (CAT), SOD activity of the larvae group was determined by the pyrogallol auto-oxidation method (Marklund and Marklund 1974). The supernatant of the larvae was added to the reaction mixture of pyrogallol (2.64 mM) and 1 mM EDTA in the Tris-HCl buffer. The absorbance was measured at 420 nm and the SOD activity was calculated. One SOD unit (U) is the amount of SOD that inhibits 50% auto-oxidation of pyrogallol.

To calculate the CAT activity in the supernatant of the larvae (16), 0.05 M of phosphate buffer and 0.03 M of H<sub>2</sub>O<sub>2</sub> solution were added. The change in the absorbance was recorded for 1 min at 240 nm. One unit of catalase activity is defined as the amount of enzyme that decomposes 1 mM of H<sub>2</sub>O<sub>2</sub> in 1 min.

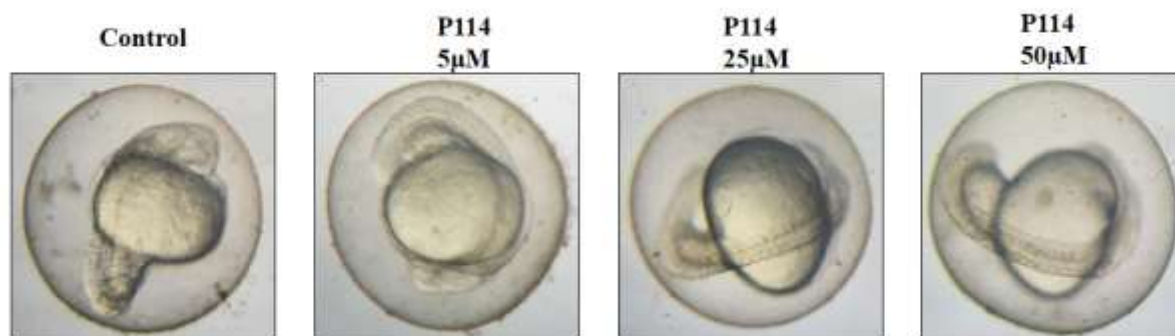
### 2.4. Statistical analysis

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 levels of significance between control and other groups.

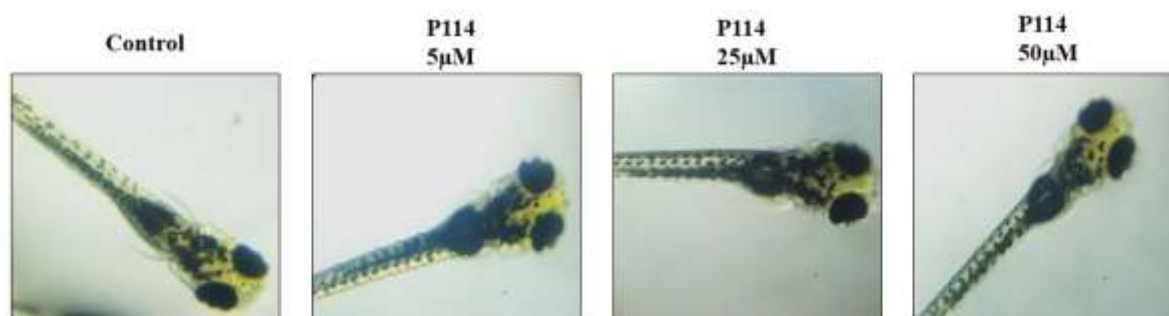
## 3. Result And Discussion

### 3.1 Malformation in zebrafish embryos and larvae

The P114 peptide of different concentration was treated to the larvae and embryos to analyse their toxic effect. The P114 treatment with different concentration was incubated with the larvae and embryos for 24 hrs. The results showed that P114 treatment from the lower to higher concentration didn't affect the embryos or larvae. The treated larvae and embryos were observed with normal morphology when compared to the control (Fig. 1&2).



**Fig. 1. Embryos were observed with normal morphology without any deformities exposed to different groups. Control were untreated embryos.**



**Fig. 2. Zebrafish larvae were observed with normal morphology without any deformities exposed to different groups. Control were untreated embryos.**

### 3.2 Survival, Hatching and Heart rate

Zebrafish larvae were widely used in the toxicity experiment and based on their survival, hatching and heart rate the toxicity effect was calculated (17). In this experiment, the untreated larvae were used as control and then treated with different groups of P114 peptide concentration (5 $\mu$ M, 25 $\mu$ M, and 50 $\mu$ M). The P114 peptide treatment showed slight changes in the survival rate of larvae when compared to control. The survival rate was observed to be 92%, 94% , and 95% for the concentration of 5 $\mu$ M, 25 $\mu$ M, and 50 $\mu$ M

Meanwhile, the hatching rate of all the groups showed no significance compared to the control group. The normal heart beat rate of 167 was observed in the control larvae group without treatment. Similar heart beat rate was observed in other groups of peptide concentration. These results suggest that P114 are not toxic.

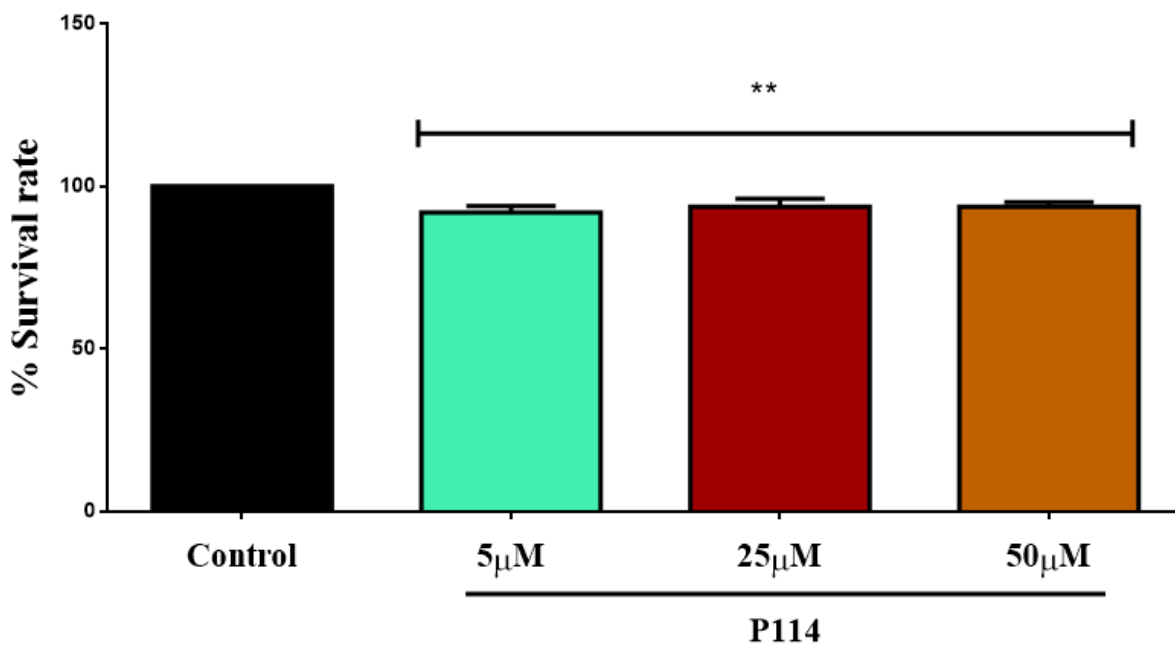


Fig. 3. The survival rate of zebrafish larvae was investigated after the exposed with different groups of P114 peptide for 24hrs. The \*\* ( $p > 0.001$ ) indicates the significant different between the control (untreated larvae) and treated group

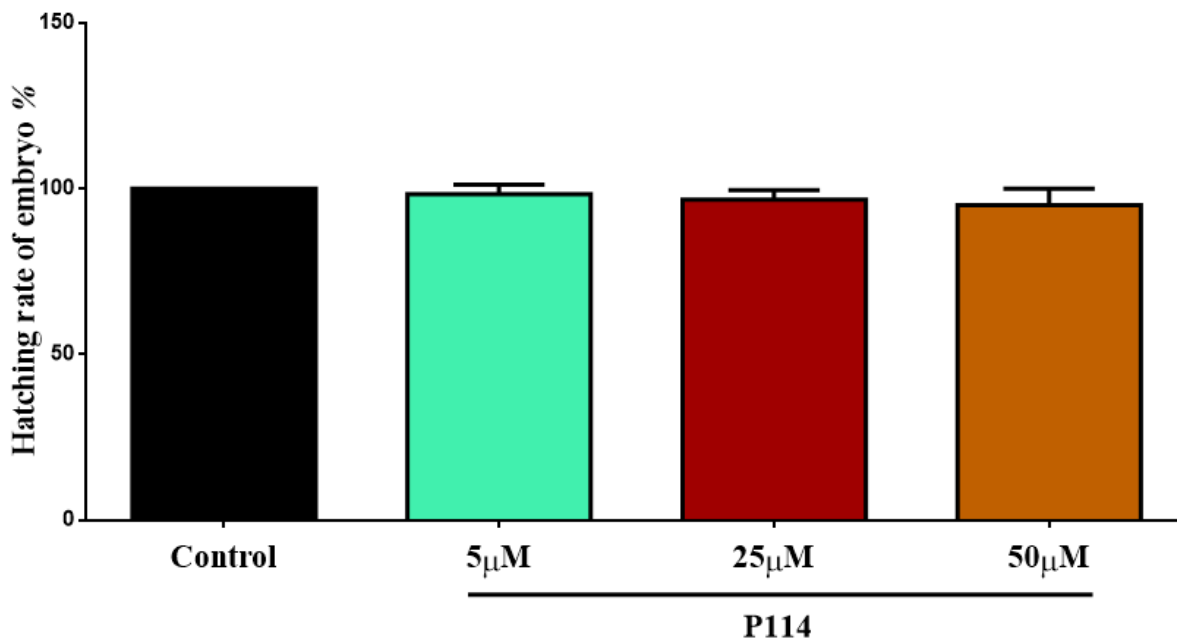
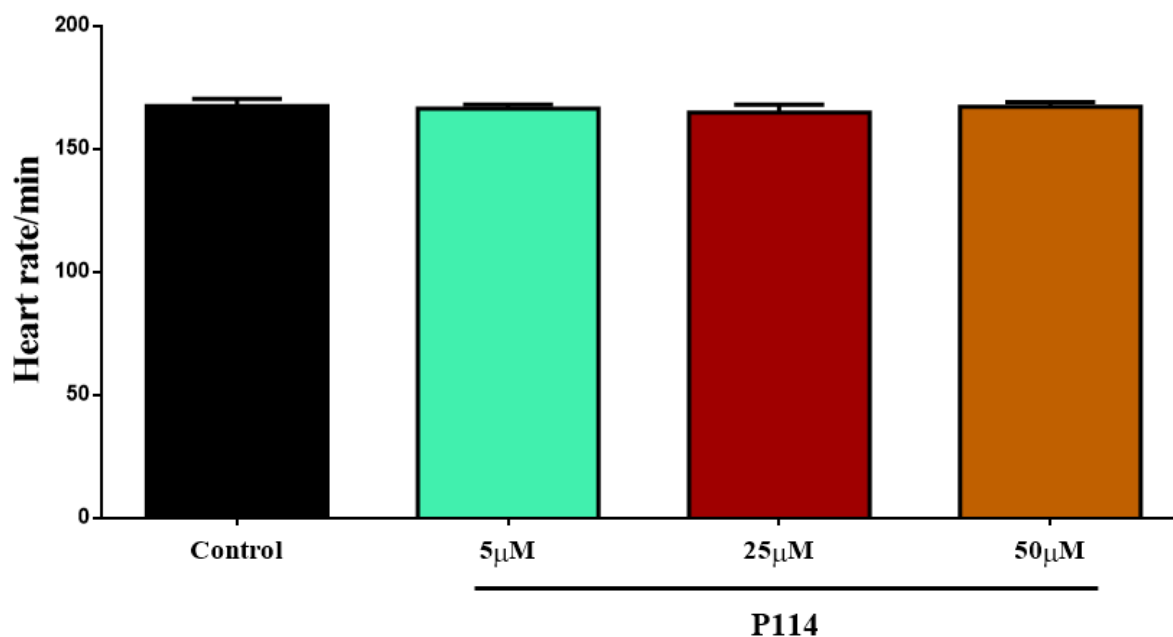


Fig. 4. The hatching rate of zebrafish embryos was investigated after the exposed with different groups of P114 peptide for 24hrs. The significant difference was not observed between the control (untreated larvae) and treated group

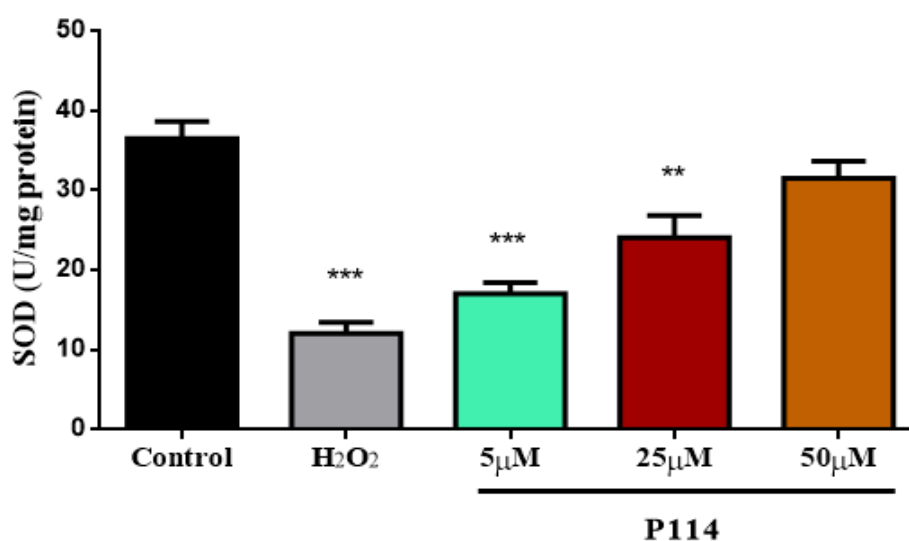


**Fig. 5.** The heart rate of zebrafish larvae was investigated after the exposed with different groups of P114 peptide for 24hrs. The significant difference was not observed between the control (untreated larvae) and treated group

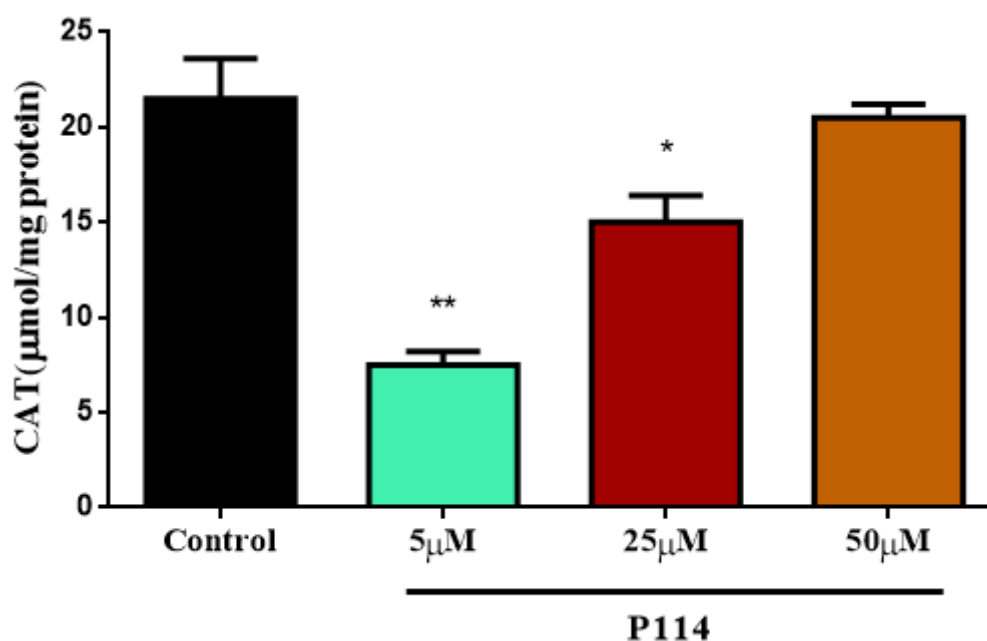
### 3.3 Antioxidant enzymes level

$H_2O_2$  is of considerable importance for its ability to penetrate biological membranes.  $H_2O_2$  is not very reactive in itself, but can also be harmful to cells because it can induce hydroxyl radicals in cells. In this study, enzymatic antioxidants (SOD and CAT) decreased after exposure to  $H_2O_2$  (100  $\mu M$ ) in zebrafish larvae. Excessive ROS is likely to inhibit the development of antioxidant enzymes.

In this study, significant ( $p < 0.05$ ) increases in total SOD and CAT activity was observed in 96 hpf zebrafish larvae treated with P114 peptide (5, 25, and 50  $\mu M$ ) compared to the control (Fig. 7 and 8). Zebrafish larvae treated with high concentration (50  $\mu M$ ) of P114 peptide, showed elevated SOD (32 U/mg protein) and CAT (21  $\mu mol/mg$  protein) when compared to other groups. The SOD (12 U/mg protein) and CAT (6  $\mu mol/mg$  protein) activity was significantly inhibited in the  $H_2O_2$  (100  $\mu M$ ) treated group.



**Fig. 6.** The SOD activity in  $H_2O_2$  induced oxidative stressed zebrafish larvae (96 hpf) treated with P114 peptide. Data expressed as mean  $\pm$  standard deviation ( $n = 15$ /group). Values are statistically significant at \*\*\*  $p < 0.01$  & \*\*  $p > 0.001$  compared to the control.



**Fig. 7.** The CAT activity in H<sub>2</sub>O<sub>2</sub> induced oxidative stressed zebrafish larvae (96 hpf) treated with P114 peptide. Data expressed as mean  $\pm$  standard deviation (n = 15/group). Values are statistically significant at \*  $p < 0.05$  & \*\* ( $p > 0.001$ ) compared to the control.

#### 4. Conclusion

From this study, it indicates that P114 peptide has antioxidant activity. It helps to maintain the antioxidant enzyme level during the oxidative stressed condition. The SOD and CAT level are upregulated during P114 peptide. Also, the P114 peptide didn't show any toxic level in the tested concentration.

#### Financial Support And Sponsorship

Nil

#### Conflict Of Interest

There are no conflicts of interest.

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