Cytotoxicity of acrylic-based resin compounds and clove oil in a human gingival fibroblast cell line

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ABSTRACT

Cytotoxicity is the ability of being toxic to cells. Gingival fibroblast is the most profusely found cell in periodontal ligament. Acrylic based compounds are widely being used in dentistry which is used for the fabrication of denture base. Monomers formed during the polymerization of acrylic resin which are in contact with the prosthetic and orthodontic devices often cause allergic reactions in the patient’s mouth. Clove oil is widely being used in Ayurvedic medicine. The aim of the study is to estimate the cytotoxic effects of acrylic-based resin compounds and that of clove oil in the human gingival fibroblast cell line.

KEY WORDS: Human gingival fibroblast, Acrylic-based resin, Clove oil

INTRODUCTION

Cytotoxicity is the ability of being toxic to cells. Cells on treating with the cytotoxic compound can cause a variety of cell fates. These cells may undergo necrosis, where they might lose their membrane integrity and die rapidly due to cell lysis. These cells will exhibit rapid swelling and shut down their metabolism.[1] Chemotherapy which is the treatment of cancer often depends on the ability of cytotoxic agents to kill the cells which are reproducing and this often targets the fast-dividing cancerous cells.[2-3]

Gingival fibroblast is the most profusely found cell in periodontal ligament.[4] The gingival fibroblast cells are used in regeneration and help in repair of periodontal tissues and also in inflammatory periodontal disease.[5-9]

Acrylic based compounds are widely being used in dentistry which is used for the fabrication of denture base. However, during these fabrications, the polymerization of the monomer methyl methacrylate is not achieved.[10] These monomers which are in contact with the prosthetic and orthodontic devices often cause allergic reactions in the patient’s mouth.[11] It also causes contact dermatitis and occupational respiratory hypersensitivity.[12]

Clove oil is widely being used in Ayurvedic medicine. This oil is used to relieve pain and initiates healing and also used as a flavoring and fragrance agent. The phenolic components present in clove essential oil has been found to change certain physical properties present in resin compounds such as surface roughness,[13-15] transverse strength,[16] and surface hardness.[14,17] Clove oil has eugenol and beta-caryophyllene as its major components and these components are said to be safe, but they do exhibit some cytotoxic properties at different concentrations toward human gingival fibroblast cells.

Hence, the goal of the study is to estimate the untransformed human gingival fibroblast and cytotoxic effects with respect to the increase in the concentration of acrylic-based resin compounds and that of clove oil in the human gingival fibroblast cell line.

MATERIALS AND METHODS

The clove oil was purchased from Aromazotika, India, and ethyl methanesulfonate (EMS) from HiMedia Laboratories, India.

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Hepatocyte growth factor (HGF) cell line was obtained from the National Center for Cell Science, Pune – 411007. HGF cells were seeded in α-minimal essential medium (α-MEM) containing 10% fetal bovine serum, 100 IU/ml penicillin, 2.5 μg/ml streptomycin, 2.5 μg/ml amphotericin B, and 50 μg/ml ascorbic acid, which was replaced twice a week, and incubated in a 5% CO$_2$ humidified atmosphere at 37°C.

Cytotoxicity Assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium umbromid (MTT) test was performed in three independent experiments. During the exponential growth phase of HGF, the cultures were seeded in 96-well plates in 100 μl of complete α-MEM. HGF was seeded with a cell suspension of 3 × 10^4 cells/cm$^2$. After 24 h of incubation, the EMS (0, 400, 1200, and 2400 μM/ml) was diluted in fresh complete α-MEM, just before replacing the initial culture medium with 100 μl of treatment medium. 10 μl of the sample stock clove oil was taken and then serially diluted from (25, 50, and 100 μg/ml) and from each concentration 100 μl was added to the wells. Subsequently, 24 h later, 10 μl of the MTT solution was added to each well and incubated for 3–4 h in standard conditions. Then, the culture medium was removed and 100 μl of dimethyl sulfoxide (DMSO) was added to each well, even as two blanks of DMSO in each plate. The plates were agitated for 5 min before being introduced in a microplate reader. The absorbance was read at a wavelength of 550 nm. The average absorbance values of controls were taken as 100% cell viability. IC$_{50}$ values were measured as the concentration of test sample which decreased the absorbance of the treated cells up to 50% of that of the control cells (DMSO treated).

Percentage of viable cell concentration was calculated thus:

$$\text{Viability (\%)} = \left[\frac{\text{Test sample OD}}{\text{Control OD}}\right] \times 100$$

RESULTS AND DISCUSSION

Cytotoxicity against human gingival fibroblast cell lines was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. In this study, induction of in vitro cytotoxic effects of clove oil and acrylic based resins were compared. The effect of concentration variations on the cytotoxicity were also observed. The absorbance of the solution in the wells were noted and the % of cell viability was calculated. The clove oil showed significant dose-dependent cytotoxicity towards HGF cells compared to untreated DMSO (0.1%) control [Figure 1] [Table 1]. The lethal concentration needed to kill 50% cells (LC50) was found to be respectively (25, 50, 100 μg/ml). Our studies showed that cytotoxicity actually increased in the presence of a clove treatment in comparison with the EMS treatment, [Figure 2] suggesting a potential therapeutic candidate for further preclinical studies.

CONCLUSION

Clove oil is generally recognized as ‘safe’, but the in-vitro study here demonstrates cytotoxic properties of clove oil towards human fibroblasts cells. Cytotoxicity of Acrylic based resion used in dentistry was also studied and compared with the positive control.
REFERENCES


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