

An *in vitro* evaluation of cytotoxicity of curcumin against human periodontal ligament fibroblasts

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Abstract

Introduction: Curcumin, a component of turmeric (*Curcuma longa* L.), is a molecule of multitude of medicinal properties. Although curcumin has found a place in the treatment of gingival and periodontal diseases, there are no reported cytotoxicity studies on the cells of clinical significance (i.e., periodontal ligament [PDL] fibroblasts). **Aims:** The objective of this research was to assess the *in vitro* cytotoxicity of curcumin against human PDL fibroblasts. **Materials and Methods:** Human PDL fibroblasts from premolar teeth were cultured and used for cytotoxicity tests from healthy children presented for orthodontic extractions. Test concentrations of curcumin (100%, 50%, and 25%) were prepared by diluting 95% curcumin with di-methyl-sulfoxide and added to 96-well microtiter plate (in triplicate) containing the fibroblast culture (approximately 2×10^4 cells/well). Fibroblast cells without treatment (without curcumin) acted as a control group. The viability of cells after 48 h of incubation at 37°C in a humidified atmosphere of 5% CO₂ and 95% air was ascertained by the 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. The viability of PDL fibroblast cells of experimental wells was expressed relative to that of control, in terms of change in the color intensity. Absorbencies were recorded at 450 nm on a microplate reader with background subtraction at 620 nm. The cell viability at various concentrations of curcumin against the PDL fibroblasts was calculated as mean absorbance (optical density) and percentage values. **Results:** Cell viability of PDL fibroblasts to 100%, 50%, and 25% curcumin concentration was 111.75%, 112.50%, and 114.40%, respectively. **Conclusions:** No *in vitro* cytotoxicity was detected for curcumin against human PDL fibroblasts, at any of the concentrations used (100%, 50%, and 25%) by MTT assay at the end of 48 h.

Keywords: *Curcuma longa*, fibroblasts, toxicity tests, wound healing

Introduction

Conventional medicine is a huge reserve for the development of contemporary medicines. One medicine in that category is turmeric (*Curcuma longa* L.) that belongs to the ginger (*Zingiberaceae*) family. The constituents of turmeric are called as curcuminoids. The three primary curcuminoids are curcumin (diferuloyl-methane), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin, a bright yellow phenolic pigment, is the most significant constituent and is responsible for the important biological actions of turmeric.^[1] Various research studies on curcumin have demonstrated a wide spectrum of therapeutic actions such as anti-inflammatory, antibacterial, antiviral, antifungal, antidiabetic, anticoagulant, hepatoprotective, antiulcer, hypotensive, and hypocholesteremic.^[1,2] Curcumin is a molecule of multitude of medicinal properties.^[3] Curcumin

is an attractive agent to investigate further, as it has desired pharmacological properties and is economical.

It is important to evaluate the biologic and toxicologic properties of biomaterials before their clinical usage. Cytotoxicity screening assays provide a measure of cell death caused by materials or their extracts. The effect a material may have on cell survival, is an apparent determinant of

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biocompatibility. *In vitro* cytotoxicity screening as a most important aspect of biocompatibility is determined by cell culture.^[4]

The selection and evaluation of any material or device intended for use in humans requires a structured assessment.^[4] A three-stage process has been recommended for testing dental materials in order to evaluate their biocompatibility: Stage I – preliminary evaluation of unspecific toxicity which includes (a) evaluation of cytotoxicity in cell lines; (b) evaluation of growth inhibition of microorganisms and evaluation of genotoxicity, mutagenicity, and carcinogenicity in bacteria; (c) evaluation of systemic toxicity by oral ingestion or respiratory uptake in mice or rats; and (d) sensibility testing and skin irritation testing in mice or rats; Stage II – evaluation of clinical application(s) by animal experiments on rats, pigs, and monkeys (e.g., implantation in connective tissue and bone; histopathological and histobacteriological tests); and Stage III – clinical controlled studies in humans.^[5]

Although curcumin has found a place in the treatment of gingival and periodontal diseases,^[6-10] there are no reported cytotoxicity studies on the cells of ultimate clinical relevance (i.e., periodontal ligament [PDL] fibroblasts). The reason for the development of new materials for clinical use, is to augment successful applications. Therefore, research studies must be carried out to assess cytotoxicity. Hence, the aim of this study was to evaluate the cytotoxicity of curcumin on cultured human PDL fibroblasts.

Materials and Methods

The present *in vitro* study was carried out at the Department of Pedodontics and Preventive Dentistry and Department of Molecular and Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belagavi (Karnataka, India). The study was approved by the ethical committee of the institute (IRB O. No. 415). Figure 1 illustrates the methodology of the study.

Fibroblast isolation and harvesting

Human PDL cells were obtained from two healthy patients who reported to the Department of Pedodontics and Preventive Dentistry and Department of Molecular and Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belagavi (Karnataka, India), for extraction of premolars for orthodontic treatment. Three premolar teeth, which were devoid of any caries, restorations, and periodontal disease, were included in the study after informed written permission of parents. After extraction, the teeth were immediately transported to the laboratory in Dulbecco's modified eagle's medium (HiMedia Laboratories, Mumbai, Maharashtra, India) for fibroblast culture.

In a laminar airflow chamber (York Scientific Laboratory Equipment, New Delhi, India), the teeth were carefully placed on sterile dishes with complete Dulbecco's modified eagle's medium supplemented with 10% fetal bovine

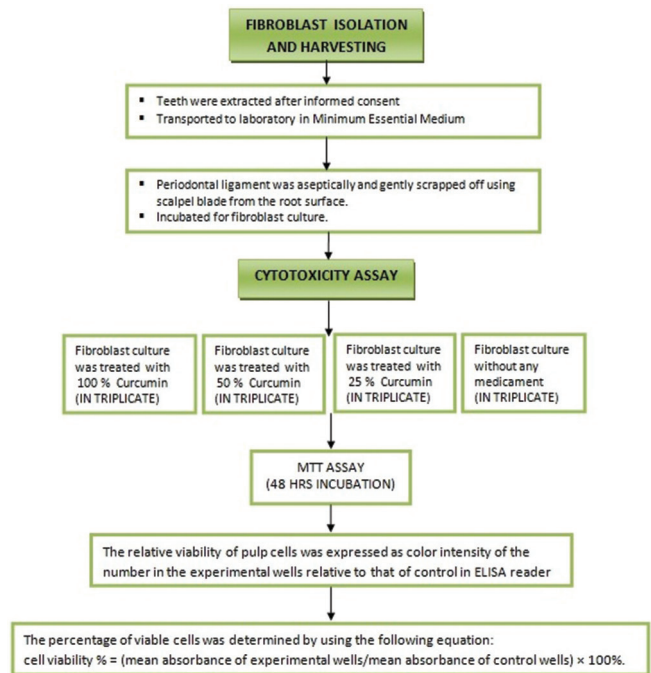


Figure 1: Flowchart showing the methodology of the study

serum (FBS) (HiMedia Laboratories, Mumbai, Maharashtra, India) to supplement the growth and $\times 100$ antibiotic antimycotic (HiMedia Laboratories, Mumbai, Maharashtra, India) to prevent bacterial and fungal contamination of the fibroblast culture. PDL was aseptically and gently scraped off using a scalpel blade from the root surface. The processed PDL was collected and seeded into a 24-well microtiter plate containing complete media and incubated at 37°C and 5% CO_2 with 98% humidity for 48 h (ThermoScientific, Massachusetts, USA) for fibroblast culture. Some of the harvested cells were stored in cryopreservation media (20% dimethylsulfoxide [DMSO], 10% FBS in minimum essential medium) and stored at -80°C for further use. The fourth subculture [Figure 2] was used for cytotoxicity assay.^[11]

Cytotoxicity assay

Ninety-five percent of curcumin (HiMedia Laboratories, Mumbai, Maharashtra, India) [Figure 3] was diluted with DMSO (HiMedia Laboratories, Mumbai, Maharashtra, India) in the following three concentrations: 10 mg in 1000 μL (100%), 5 mg in 1000 μL (50%), and 2.5 mg in 1000 μL (25%) in sterile Eppendorf tubes. Each concentration of curcumin was added in triplicate into 96-well microtiter plate containing the fibroblast culture (approximately 2×10^4 cells/well). Cells without any medication served as a control group.

The number of viable cells after 48 h incubation at 37°C in a humidified atmosphere of 5% CO_2 and 95% air was determined by the MTT assay.^[11] For this, 100- μL 5-mg/ml MTT (HiMedia Laboratories, Mumbai, Maharashtra, India) was added to all the 12 wells (10 mg in 1000 μL , 5 mg in 1000 μL , and 2.5 mg in 1000 μL and control, in triplicate) in 96-well microtiter plate and incubated at 37°C and 5% CO_2 with 98% humidity

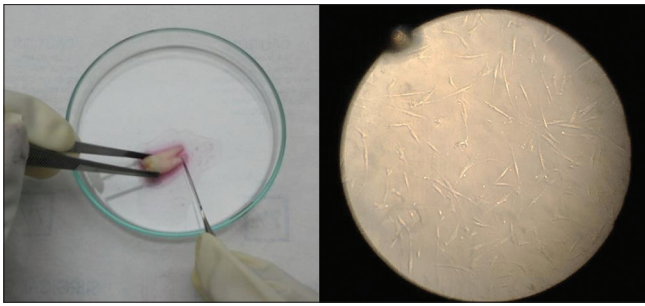


Figure 2: Cultured human periodontal ligament fibroblasts as seen under inverted microscope TCM 400 Labomed, USA ($\times 20$)

for 4 h. At the end of the incubation period, the medium with MTT was removed, and 100- μ l DMSO was added to each well. The plate was shaken on the microplate shaker to dissolve the blue MTT-formazan. The viability of PDL fibroblast cells of experimental wells was expressed relative to that of control, in terms of change in the color intensity. Absorbencies were recorded at 450 nm on a microplate reader with background subtraction at 620 nm (Lisaplus, Aspen Diagnostics Pvt., Ltd., Mumbai, Maharashtra, India).^[5,11,12]

The percentage of viable cells was determined by using the following equation.^[11]

Cell viability % = (mean absorbance of experimental wells/ mean absorbance of control wells) \times 100%.

Results

All the tests were done in triplicate. The mean absorbance (optical density [OD]) and percentage values of cell viability at various concentrations of curcumin to human PDL fibroblasts are shown in Table 1 and Figure 4.

Discussion

Many types of biomaterials are in clinical use for the treatment of dental diseases. Assessing cytotoxicity based on several cytotoxicity testing methods is a necessary step in evaluating the biocompatibility of biomaterials.^[4] The present study investigated the *in vitro* cytotoxicity of curcumin against human PDL fibroblasts. No cytotoxicity was detected (fibroblast cells were viable) for curcumin at any of the concentrations used (100%, 50%, and 25%) by MTT assay. However, a combination of two or more sensitive and quantitative methods for cytotoxicity evaluation is necessary to avoid false-positive results at the preclinical stage.

Therapeutic materials during their clinical use should have desirable action, maintain maximal tissue vitality, and at the same time should have negligible or no cytotoxic effects. *In vitro* cytotoxic screening as a primary factor of biocompatibility is determined by cell culture, and MTT assay is one of the most frequently used tests for evaluating the cytotoxicity of materials from different origins on cell cultures. It is recommended that the cells of eventual clinical relevance should be selected



Figure 3: Ninety-five percent of curcumin (HiMedia Laboratories, Mumbai, Maharashtra, India)

Table 1: The mean survival of human periodontal ligament fibroblasts as measured by methyl-thiazol-diphenyl-tetrazolium assay after exposure to various dilutions of curcumin for 48 h

Concentration (%)	Optical density at 450 nm	Cell viability (%)
100	1.75367 \pm 0.04	111.75
50	1.76567 \pm 0.07	112.50
25	1.79533 \pm 0.07	114.40
Control	1.56933 \pm 0.25	100

for *in vitro* toxicity tests.^[13] Hence, the present cytotoxicity assay was carried out on human PDL fibroblasts. Although curcumin is currently advocated as an adjunct to scaling and root planing in the treatment of chronic periodontitis,^[6-10] there are no reported studies conducted to evaluate the effect on the PDL fibroblasts.

Ideally, a medicament intended for the treatment of wounds should improve one or more phases of healing without producing harmful adverse effects. Curcumin has been shown to have significant wound-healing properties. It acts on various stages of the natural wound-healing process to hasten healing. It has been shown to improve granulation tissue formation, collagen deposition, tissue remodeling, and wound contraction.^[2,14] Curcumin-treated wounds present not only a greater number of fibroblasts but also more infiltrating macrophages and neutrophils compared with untreated wounds. As curcumin has low water solubility, it is a medicament suited for topical applications.^[2,14]

Various *in vitro*, animal and clinical research studies have demonstrated the utility of curcumin in the treatment of gingivitis and as an adjunct to scaling and root planing in the treatment of chronic periodontitis.^[6-10,15-20] Because of its favorable action on collagen, curcumin can also be explored for its potential use as a transportation medium and in the treatment of re-implantation of avulsed teeth. More number of

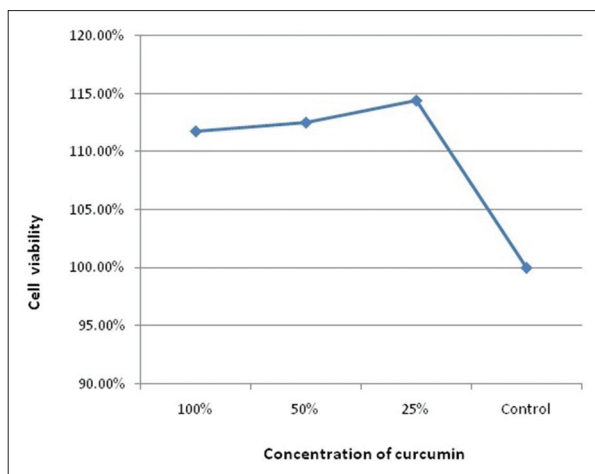


Figure 4: The mean survival of human periodontal ligament fibroblasts as measured by MTT assay after exposure to various dilutions of curcumin for 48 h

clinical studies are needed in this regard. Furthermore, in an earlier study by the same authors, curcumin has been shown to promote cell viability and induce the proliferation of primary dental pulp fibroblasts.^[21] Hence, it can also be explored for potential uses in vital pulp therapy procedures for primary and permanent teeth.

Few limitations of this study may be noted: (1) MTT assay is simple and inexpensive; it could be used as a preliminary method for screening. It should not be used as the primary evidence for cytotoxicity or antiproliferative activity. However, a combination of two or more sensitive and quantitative methods for safety evaluation is necessary to validate the results; (2) MTT assay is a “relative test,” in which the OD values of a treated cell population are compared to the OD values of a control condition (untreated cells) arbitrarily scaled at 100%; (3) The cell viability values were consistently above 100% for all the test concentrations of curcumin in this study. This could be either because of the action of curcumin alone or because of the color of curcumin, which might have added to the OD values obtained.

Conclusions

Curcumin was able to maintain the viability of PDL fibroblasts at 100%, 50%, and 25% concentrations, by MTT assay. It has proven properties such as anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, immunostimulant, antiseptic, and antimutagenic. It has a role in the treatment of periodontal diseases and oral cancers. Turmeric can also be used as a mouth wash, as a subgingival irrigant in different preparations, and as a component in local drug delivery system in gel form. Curcumin truly holds a promising future in therapeutic applications in dentistry. Therefore, further research is required to determine the optimal dosage and bioefficacy of curcumin-based drugs.

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Conflicts of interest

There are no conflicts of interest.

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