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Investigation of the effect of the curcumin component as an alternative to the local treatment of nasal diseases

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Abstract. – OBJECTIVE: This study aimed to define the impacts of curcumin on nasal cell viability and proliferation.

MATERIALS AND METHODS: Specimens of healthy primary nasal epithelium were collected and incubated in cell culture during septorhinoplasty from people who signed a consent form. After implementing 2.5 μM curcumin in cultured cells, cell viability was defined *via* trypan blue assay, and proliferation was defined *via* the XTT method. The number of total cells, viability, and proliferation was defined. XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) experiments can be used to evaluate cellular toxicity.

RESULTS: The results revealed no harm to nasal cells after the topical implementation of curcumin. There was no significant change in the proliferation of the cells related to 24 hours of implementation. There was no adverse effect of using curcumin on the cell viability, either.

CONCLUSIONS: No cytotoxic effect on nasal cells has been observed after applying topically implemented curcumin. Curcumin could be used topically for an alternative treatment for allergic rhinitis as it has anti-inflammatory and immune response modulatory effects if clinical trials will confirm experimental data.

Key Words:
Curcumin, Nasal cells, Potential way of treatment, Cytotoxic effect, Cell viability, Allergic rhinitis.

Introduction

Curcumin, which is a yellow-flowered plant with large leaves from the turmeric family, is an Indian spice. The homeland of turmeric, which can be grown in the tropics, is South Asia. It is a plant that helps to prevent many diseases when consumed consciously. Turmeric, which we can easily reach, is a spice full of healing components attracting the culinary and medical world. It is known to help inflammation and muscle pain in individuals, and its functions are associated with antioxidant and anti-inflammatory effects[1-3].

This traditional Chinese medicinal function food is used for inflammation in China and Southeast Asia[4]. It is a curry spice known[5] to be effective in protecting health in terms of body inflammations and direct benefits to joint pains and cell growth and apoptosis with its constituents of curcumin (demethoxycurcumin and bisdemethoxycurcumin). Turmeric is known[6] to be very effective in diseases such as cancer, fatty liver, osteoporosis, flu, tuberculosis, respiratory problems like asthma. As it has precious components and functions, its products, such as creams, extracts, sprays, and tablets, have been provided by many manufacturers for medical reasons[7,8].

Turmeric is also known to soothe oxidative stress and the problems induced by chronic illnesses through the Nrf2-keap1 track. It restrains pro-inflammatory tracks relevant to several illnesses and blocks the production of tumor necrosis factor (TNF) and the cell signaling mediated by TNF. It is a TNF blocker from *in vitro* and *in vivo* studies by binding to TNF in a direct way[8-11].

This study aims to define the impacts of curcumin on nasal cell viability and proliferation. If clinical trials will confirm the experimental data, an alternative way of treatment can be suggested.

Materials and Methods

This research has been conducted by the Medical Biology and Otorhinolaryngology De-

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partments of Eskisehir Osmangazi University, Faculty of Medicine, before starting the study, the volunteers signed a consent form to allow us to use their tissue samples for scientific reasons. Then nasal epitheliocytes for cell culture were collected from surplus tissue after septorhinoplasty.

Methods

Just after tissues were brought to the lab in transport solution, they were dissected into smaller bits in a sterile petri dish. Then, they were transmitted into sterile centrifuge tubes, which included a washing solution. 4 ml of solution that included Dulbecco's Phosphate Buffered Saline was put in, and they were transmitted to trypsin/EDTA centrifuge tubes and centrifuged at 1,000 rpm. Following the centrifugation process, the supernatant was separated, and 4 ml of solution was put into the pellet to bind at the base and was washed twice. The pellet remaining at the base was taken into T25 petri dishes, including Dulbecco's modified Eagle medium (DMEM) medium consisting of 1% penicillin-streptomycin solution, and placed in a 37°C CO_2 incubator.

Cell Treatment

Tissue specimens included a mixture of epithelial and fibroblast cells. To be able to decrease the number of invasive fibroblast cells holding the petri dish faster, the culture was incubated with trypsin/EDTA solution for 4 minutes at 37°C after the cells reached 80% majority at the bottom. Fibroblasts, which stuck to the petri dish surface, stayed stuck to the base with no effect of trypsinization phases. Furthermore, the culturing of epithelial cells separated from the medium by trypsinization went on.

Afterward, the remaining pellet was transmitted to T25 petri dishes with DMEM medium and put into a 37°C CO_2 incubator. Then the cells were grouped as control and experimental cells to deal with curcumin. After the cells at the base got 80% majority, culture was implemented. 5 µM curcumin was also implemented in the cells[13,14]. Viability was defined *via* trypan blue assay, and proliferation was defined *via* XTT[12].

Cell Viability

After trypsinizing the cells, they were taken away from the bottom of the flask. Then, 10 µl cell specimen was mixed with 10 µl trypan blue solution. To define the amount of dead or live cells, measurement was done *via* Neubauer slide utilizing trypan blue staining[15].

Proliferation Analysis

Evaluating cellular toxicity, XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) analysis could be utilized[16]. The XTT Kit (Beit, Haemek, Israel) has been utilized to define how effective curcumin is on cell proliferation. To summarize, 96-well plates were formed for the cells, and all wells were filled with XTT solution. Then, they were cultured at 37°C with 5% CO_2 for 2 hours. To check the absorbance at 450 nm, a microplate spectrophotometer was utilized.

Results

In this study, cells were exposed to curcumin to observe whether it has anti-proliferative and cytotoxic effects. The XTT test shows whether the substance to be applied is cytotoxic. Therefore, the most important criterion was to be able to determine these non-cytotoxic concentrations of curcumin on cells, whose appropriate concentration was determined. The IC_{50} concentration of curcumin, initially applied at various concentrations, such as 0.5, 5, and 10 µM, was 5 µM. The XTT experiment shows that after the implementation of curcumin for 24 hours, there was no cytotoxic or anti-proliferative to the nasal cells. Also, there was no significant change in the proliferation of the cells after 24 hours of application (Figure 1). Another critical parameter is that this concentration, which is not cytotoxic in cells, does not affect the viability percentages of the cells.

Additionally, curcumin had no adverse effect on the % cell viability (Figure 2). Moreover, cell viability continued for a long time after the application. These positive findings regarding the viability and proliferation of cells show that curcumin has the potential to be applied as an alternative active ingredient in the topical treatment of nasal diseases since it does not harm normal cells when applied at appropriate concentrations.

Discussion

The components of curcumin make it a perfect free radical scavenger. It reduces various interleukins *via* nuclear factor kappa B (NF-κB). It can help to bind amyloid, acting directly on the misfolded cascade[17]. It causes free radical increase and lipid peroxidation formation. It reacts quickly

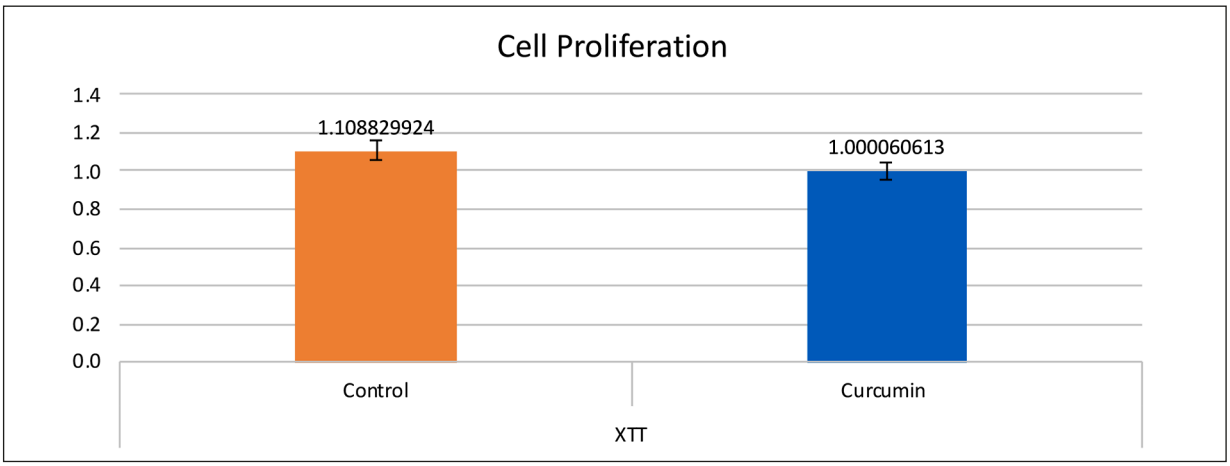

Figure 1. The results of nasal cells exposed to 5 µM of curcumin for 24 hours in terms of cell proliferation were obtained by XTT.

against harmful oxidants and eliminates them from the metabolism. It is a powerful antioxidant responsible for removing harmful toxicity based on the abstraction of the H-atom from the phenolic group and not the major CH_2 group in the heptadienone linkage. Curcumin, methyl curcumin, and semi-curcumin, having almost the same form of hydroxylated polybrominated diphenyl ethers (OH-BDEs), showed that the phenolic groups are independent[18,19].

This study aimed to define the impacts of curcumin on nasal cell viability and proliferation. The results revealed no harm to nasal cells after the topical implementation of curcumin. There was no significant change in the proliferation of the cells related to 24 hours of implementation. There was not any side effect on cell viability, either.

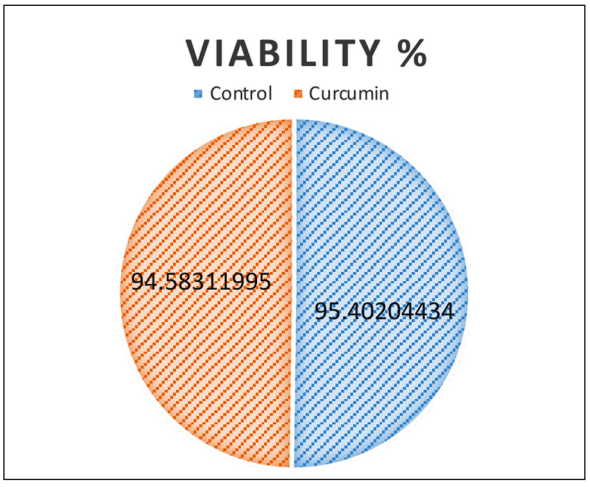

Figure 2. Trypan blue was used to measure the effects of nasal cells exposed to curcumin for 24 hours in terms of cell viability.

Ammar et al[20] conducted further research to see the inhibitory effects of curcumin on asthma-related biological changes and studied the effects on serum. This study showed that curcumin quenched inflammation by inhibiting NF-κB and the downstream transcription factor GATA3.

Chong et al[21] conducted another study to see curcumin's anti-inflammatory impact on acute allergic asthma. The Notch1 receptor is explicitly essential in developing allergic airway inflammation. They concluded that curcumin use in healing is essential. It helps airway inflammatory cell infiltration and downregulated Notch1/2 receptors and GATA3 levels. Curcumin also inhibits the Notch1-GATA3 track and avoids the formation or deterioration of allergic inflammation.

Acar et al[22] researched and tested allergy symptoms and histopathological components of the nasal mucosa. The research revealed that sneezing and nasal congestion scores were lower in the group with curcumin supplements. The histopathological check revealed a decline in goblet cell metaplasia in the epithelium, less inflammatory cell infiltration, and vascular proliferation in the lamina propria. Their results say that using curcumin helps treat allergic rhinitis in rats.

Thakare et al[23] showed that curcumin could control the growth of eosinophil peroxidase in nasal homogenate and serum IgE, NO, and IL-4 in nasal lavage with allergic rhinitis[23]. Curcumin

prevents allergic airway inflammation in asthma, putting Treg/Th17 balance in order[24]. Curcumin may inhibit CD4+ T cell proliferation[25].

Chung et al[26] concluded that using curcumin supplements is vital in allergic asthma treatment. The use of curcumin also significantly affects the production and inhibits iNOS, which helps and eases conjunctiva. It creates a pharmacokinetic impact to inhibit NF-κB, eIF-2α dephosphorylation, proteasome, and COX2[27].

Wu and Xiao[28] studied 241 people with AR. Some patients had a placebo, and others got curcumin for two months. They found that the group receiving curcumin successfully inhibited IL-4, IL-8, and tumor necrosis factor, and they augmented the production of IL-10 and soluble intercellular adhesion molecule. Their study showed curcumin's capability of healing nasal airflow and modulating immune response for people with AR.

Conclusions

No cytotoxic effect on nasal cells has been observed after applying topically implemented curcumin. Curcumin could be used topically for an alternative treatment for allergic rhinitis as it has anti-inflammatory and immune response modulatory effects if clinical trials will confirm experimental data.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

This is a cell-culture study, therefore, Ethics Committee approval was not needed.

Informed Consent

Human primary nasal epithelium was obtained from healthy tissue removed routinely as part of surgery (septorhinoplasty) from individuals who gave written consent for their tissue to be used in scientific research.

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There are no funds for this study.

Authors' Contribution

Emrah Ceylan: Planning, designing, literature survey, active intellectual support. Didem Turgut Cosan: Planning, designing, data collection, literature survey, interpretation of the results, active intellectual support. Nuray Bayar Muluk: Planning, designing, literature survey, interpretation of the results, active intellectual support, writing, submission. Cemal Cingi: Planning, designing, literature survey, data collection, interpretation of the results, active intellectual support, English editing.

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