

Efficacy of butterbur in allergic rhinitis: a cell culture study

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Abstract. – OBJECTIVE: The study aims to define butterbur's impact on nasal cells' viability and proliferation. After topically administering butterbur to the nasal epithelial cells, research has been done to see if butterbur has any harmful effect on the nasal cells.

MATERIALS AND METHODS: Specimens of healthy primary nasal epithelium were collected from the subjects and incubated in cell culture in due course of septoplasty. After implementing 2.5 µM butterbur 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 findings of the XTT experiment reveal no harm to nasal cells after topical implementation of butterbur. No significant change in the proliferation of the cells, no matter what the doses are. There was no cytotoxic effect on the primary nasal cells at the end of 24 hours of implementation, and no side effects were found. There was no difference in cells' viability between the experimental group with butterbur application and the control group.

CONCLUSIONS: Cytotoxicity on nasal cells was not observed after the butterbur application. Even if there have been some indications of liver toxicity, butterbur can be suggested as a safe option for seasonal allergic rhinitis. Further studies related to the toxicity of topical butterbur are also recommended, even though this study indicates no cytotoxicity from the topical application on nasal cells.

Key Words:

Butterbur, Nasal cells, Cytotoxic effect, Cell viability.

Introduction

Butterbur (any plant of the *Petasites* genus), also known as coltsfoot, is a flowering plant native

to Asia, Europe, and North America¹. It has been used in traditional medicine for thousands of years to treat various ailments, from high blood pressure (hypertension) and asthma to tumors². The results of some research indicate that the components of the plant have antispasmodic activity and anti-inflammatory action in type I hypersensitivity³⁻⁶.

The plant also has analgesic, antioxidant, diuretic, antispasmodic, and antitumor activities⁷. It is most commonly used for migraine and allergy relief. It contains beneficial chemicals that can reduce inflammation, urinary tract irritation, ulcers, difficulty in breathing, oxidation, and pain⁸. Tys et al⁹ reported that butterbur positively affects bronchial asthma and allergic rhinitis.

PA-free coltsfoot products are safe and well tolerated on condition that they are consumed orally at recommended doses for up to 16 weeks. PA means pyrrolizidine alkaloids, chemicals found in raw, unprocessed butterbur plants. If it is processed, there are a few adverse effects of using butterbur, such as gastrointestinal upset, rash, diarrhea, etc. The so-called effects have yet to be seen in placebo groups¹⁰.

Recently, butterbur has been used in the treatment of cough, and it has been known to have spasm-solving and pain-relieving properties. The root and leaves are analgesic, cardiostimulant, diaphoretic, and diuretic. The use of this herbal function herb is getting more and more common^{3,11-13}. It should also be noted that the allergen hypersensitivity of butterbur is also known to increase substantially¹⁴⁻¹⁷.

This study aims to investigate the potential use of butterbur as an alternative way to treat patients with nasal diseases. The research has been done to see if there is any harmful effect on the nasal cells after applying butterbur topically to the nasal epithelial cells.

Materials and Methods

This research has been conducted by the Medical Biology and Otorhinolaryngology Departments 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. The nasal epithelium was collected from their healthy tissues and removed routinely as a part of surgery (septorhinoplasty). Then collected strips of mucosa were transferred to the Eskisehir Osmangazi University, Faculty of Medicine, Medical Biology Lab in preservative conditions appropriate for cell culture.

Cell Culture

Specimens of healthy primary nasal epithelium were collected from the subjects and incubated in cell culture in due course of septoplasty. Just after tissues were brought to the lab in penicillin-containing transport solution, they were dissected into smaller bits in a sterile petri dish in a laminar flow cabinet.

Then the pieces of tissues were processed with trypsin, and they were incubated at 37°C for 10 minutes with 5% carbon dioxide. 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 washed twice. The pellet remaining at the base was taken into T25 petri dishes, including Dulbecco's Modified Eagle's Medium (DMEM) consisting of 1% Penicillin-streptomycin solution, and placed in a 37°C CO₂ incubator¹⁸.

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, and the culturing of epithelial cells separated from the medium by trypsinization went on¹⁹.

Afterward, the remaining pellet was transmitted to T25 petri dishes with DMEM medium and put into a 37°C CO₂ incubator. Then the cells were grouped as control and experimental cells to deal with butterbur. After the cells at the base got 80% majority, culture was implemented. 520 µg/ml of butterbur⁷ was also implemented in the cells. Viability was defined *via* trypan blue assay, and proliferation was defined *via* the XTT method.

Viability of the Cells

To determine the viability of the cell, trypan blue was utilized. The cells which were treated with butterbur got exposed to trypan blue (Gibco, UK). Once the necessary amount of the cells was attained in the flask, they were collected using trypsin. The necessary amount of the cells was prepared for measurement *via* the Neubauer slide using trypan blue staining. Counting was done regarding total cell, viability, and proliferation²⁰.

Proliferation Analysis

Evaluating cellular toxicity, XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) analysis was utilized²¹. XTT kit was the tool that was used to define the effects of butterbur for proliferation. To check the proliferation, 96-well plates were formed for the cells, and all wells were filled with XTT solution and cultured at 37°C with 5% CO₂ for 2 hours. Proliferation analysis was done *via* a test of the absorbance at 450 nm by a microplate spectrophotometer.

Results

The XTT experiment shows that after the implementation of butterbur for 24 h, there was no harm to the nasal cells. Also, there was no significant change in the proliferation of the cells, no matter the doses. The substance was not cytotoxic to primary nasal cells after 24 hours of administration (Figure 1). There was no significant difference between the experimental and control groups in terms of the viability of the cells (Figure 2).

Discussion

The accumulation of allergens on mucosa causes intermittent or persistent allergic rhi-

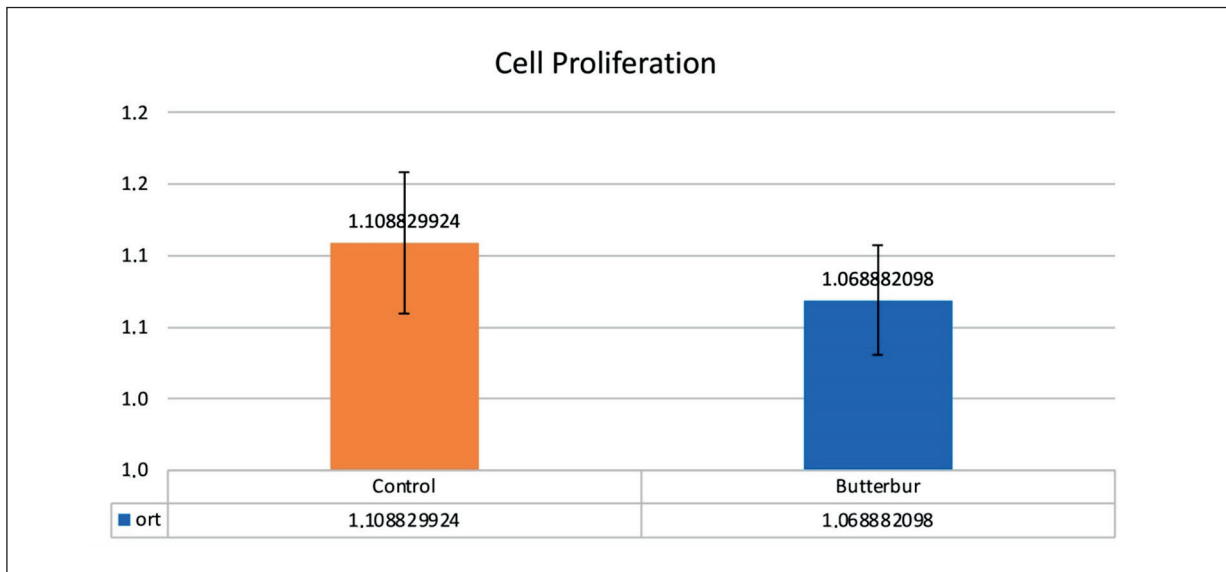


Figure 1. The results of nasal cells exposed to 50 µg butterbur for 24 hours in terms of cell proliferation were obtained by XTT.

nitis, and a hypersensitivity reaction follows it²². The two types differ for the duration and the reasons. Intermittent one is triggered by grass, tree, and weed pollens, whereas house dust mites and domestic animals cause the latter. Common symptoms include sneezing, rhinorrhea, nasal obstruction, lacrimation, and conjunctival itching²³.

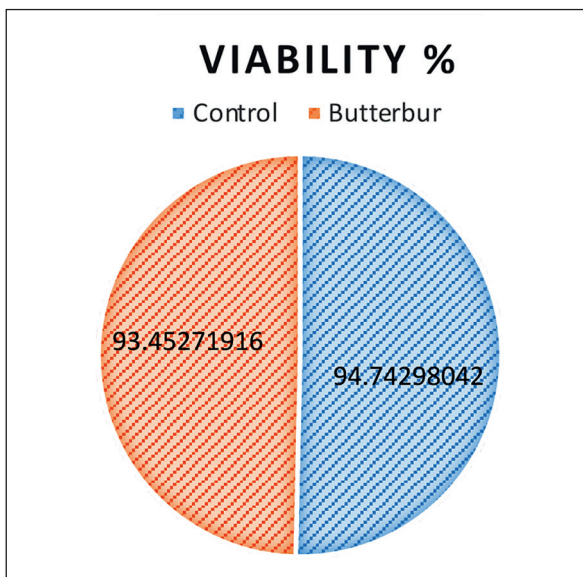


Figure 2. Trypan blue was used to measure the effects of nasal cells exposed to butterbur for 24 hours in terms of cell viability.

Butterbur (any plant of the *Petasites genus*), also known as coltsfoot, is a flowering plant native to Asia, Europe, and North America²⁴. To wrap the butter taken from the churn in the summer, people used the herb leaves, which explains the name of the plant. However, one of its names is “cough grass”, as it has been used for millennia to cure coughs and many other respiratory problems. It has also traditionally been used to reduce fevers, muscle spasms and headache²⁵. Some researchers²⁶⁻²⁸ claim that petasins inhibit the biosynthesis of leukotrienes, which is related to hypersensitivity¹⁴.

This study has been conducted to define the impact of butterbur on nasal cells’ viability and proliferation. Research has been done to see if butterbur has any harmful effect on the nasal cells after topical administration to the nasal epithelial cells. Our results show no harm on nasal cells after topical implementation of butterbur. No significant change in the proliferation of the cells, no matter what the doses are. Related to 24 hours of implementation, there was no cytotoxic effect on the primary nasal cells, and no side effect was identified. The viability of the cells had no difference between the experimental group, which had butterbur application, and the control group.

Schapowal et al³ claim that butterbur can be seen as an alternative for patients with seasonal allergic rhinitis if the sedative effects of antihistamines have to be refused. They¹⁴ also reported

that extracted butterbur Ze339 from the herb's leaves works for the symptoms of intermittent allergic rhinitis if the correct dose is used. Butterbur Ze339 is a practical choice when the sedative effects of antihistamines have to be considered.

Although butterbur is a popular herb that supports minimizing respiratory problems such as asthma and bronchitis. Butterbur may trigger liver problems and have a carcinogenic effect¹⁰. However, there are no precise results to prove the adverse effects due to residual pyrrolizidine alkaloid contamination¹⁰. It would be better not to use this plant, especially for people with a weak liver.

In the study conducted by Käufeler et al²⁹, Ze339, the extract from butterbur is found to be safe and effective for patients with seasonal allergic rhinitis. The study got 580 patients with seasonal allergic rhinitis as subjects, and they were given two tablets of Ze339 every day for 15 days. Symptoms of rhinorrhea improved in 90% of patients.

Conclusions

Cytotoxicity^{30,31} on nasal cells was not observed after the butterbur application. Even if there have been some indications of liver toxicity, butterbur can be suggested as a safe option for seasonal allergic rhinitis. Further studies related to the toxicity of topical butterbur are also recommended, even though this study indicates no cytotoxicity from the topical application on nasal cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

This is a cell-culture study. 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.

Funding

There were no funds for this study.

Authors' Contribution

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

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Availability of Data and Materials

Data cannot be shared as no data sets were generated for this study.

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