

Bromelain: a candidate to enhance wound healing after endonasal surgeries

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Abstract. – OBJECTIVE: In the present study, we investigated the topical bromelain's cytotoxic effects on mouse fibroblast NIH/3T3 cells *via* cell culture study.

MATERIALS AND METHODS: In this cell culture study, Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS, 10%) and penicillin/streptomycin (1%) was used as a cell growth medium for NIH/3T3 mouse fibroblast cells. MTT test was performed in 96-well plates seeded with NIH/3T3 cells 5×10^3 /well and under standard cell culture conditions. Bromelain doses of 3.13 to 100 μ M were administered to the wells and incubated for 24, 48, and 72 hours in the same cell culture conditions. For Confocal microscopic evaluation, NIH/3T3 cells were plated on cover slips in 6-well plates (105 cells/well) and treated with 100 μ M concentration of bromelain for 24 h. Untreated cells were used as controls.

RESULTS: MTT results showed that bromelain is not cytotoxic on mouse fibroblast NIH/3T3 cells. All three incubation times of 24, 48, and 72 hours bromelain initiated cell growth. A statistically significant rise in cell growth was detected in the only applied highest dose of 100 μ M bromelain for all incubation times except for 24 hours. The nontoxic effect was further investigated by using confocal microscopy by applying the highest bromelain dose of 100 μ M to NIH/3T3 mouse fibroblast cells. Confocal micrographs showed that bromelain did not change the morphology of mouse fibroblast cells at the incubation time of 24h. In untreated cells and bromelain-treated cells, the nucleus of NIH/3T3 cells was undamaged and compact, and the cytoskeleton was fusiform and non-fragmented.

CONCLUSIONS: Bromelain is not cytotoxic on mouse fibroblast NIH/3T3 cells and enhances cell growth. If clinical trials will confirm this, it is possible that bromelain will be used topically in humans to enhance wound healing, in rhinosinusitis and chronic rhinosinusitis with nasal polyps and endonasal surgeries due to its anti-inflammatory effects.

Key Words:

Bromelain, NIH/3T3 Cells, Cell culture, Cytotoxicity, Septorhinoplasty, Rhinosinusitis, Chronic rhinosinusitis with nasal polyps (CRSWNP).

Introduction

Several enzymes, notably bromelain, are produced by the pineapple plant (*Ananas comosus*) and utilized commercially for protein digestion¹⁻³. It is a nontoxic chemical with therapeutic effects for modulation. Although the ability of bromelain to reduce edema and inflammation is widely recognized, its list of benefits is growing. Since it is a naturally occurring anti-inflammatory enzyme, bromelain has several uses. Bromelain interacts with other enzyme systems to have an anti-inflammatory impact when treating soft tissue injuries. It can also reduce rats' inflammatory discomfort dose-dependently^{1,4}.

Analgesic and anti-inflammatory effects of systemic enzyme treatment for rheumatic disorders have been demonstrated in pre-clinical and clinical investigations^{1,5-7}.

It has been discovered that bromelain inhibits T-cell activation and removes CD44 molecules from T cells. The ability of peripheral blood lymphocytes (PBL) to adhere to human umbilical vein endothelial cells was examined using the highly purified bromelain protease F9 (HUVEC). Bromelain and protease F9 decreased CD44 expression, although F9 was ten times more active than bromelain and had a 97% suppression of CD44 expression. According to the findings⁸, F9 specifically reduces expression. Additionally, it suggests that F9 specifically reduces the CD44-mediated binding of PBL to HUVEC.

We investigated the topical bromelain's cytotoxic effects on mouse fibroblast NIH/3T3 cells

in the present study. This cell culture study, MTT colorimetric assay, and confocal microscopic evaluation were performed to evaluate the cytotoxic effects.

Materials and Methods

This study was undertaken at the ENT Department of Eskisehir Osmangazi University, working alongside the Department of Biology within the Faculty of Science at Eskisehir Technical University.

Cell Culture

Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS, 10%) and penicillin/streptomycin (1%) was used as cell growth medium for NIH/3T3 mouse fibroblast cells (Commercially available from <https://www.atcc.org/products/crl-1658>) (February 12, 2023). Cell culture was realized in incubator conditions with CO₂ (5%) and an adequate humidity in air. Cell passaging was realized twice in a week and confluent cells (85%) were used for all tests.

MTT Colorimetric assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was performed in 96 well plates seeded with NIH/3T3 cells 5x10³/well and under standard cell culture conditions. Bromelain doses of 3.13 to 100 µM were administered to the wells and incubated for 24, 48 and 72 hours in same cell culture conditions. After incubation, 20 µL of MTT (5 mg/mL in PBS) was added in each well and were incubated for 3 hours. Then, media of each well were removed, and formazans were dissolved by adding 200 µL of dimethyl sulfoxide (DMSO) per well. Plates were read with an ELISA reader (BioTek HTX Synergy, Winooski, VT, USA) at a wavelength 560 nm. Viability percentages were calculated from the absorbances.

Confocal Microscopic Evaluation

NIH/3T3 cell were plated on cover slips in 6-well plates (10⁵ cells/well) and treated with 100 µM concentration of bromelain for 24 h. Untreated cells were used as controls. All cell groups were fixed in glutaraldehyde and stained with phalloidin for 1 hour in room temperature in dark. Then, were washed with primary blocking solution. Followingly, acridin orange staining was performed to the cells for 10 minutes in room

temperature in the dark. Stained samples were photographed under a confocal microscope (Leica SP5-II, Wetzlar, Germany).

Statistical Analysis

Statistical evaluations were realized on Graph-Pad Prism 6 program (La Jolla, CA, USA) by using One-way ANOVA and post-Tukey test. *p*-values <0.05 were taken as significant.

Results

Bromelain is not cytotoxic to mouse fibroblast NIH/3T3 cells, according to MTT data. All three incubation periods of 24, 48, and 72 hours saw the onset of cell proliferation (Figures 1-3). For all incubation times, besides 24 hours, a statistically significant increase in cell growth was seen in the only application of the highest dose of 100 µM bromelain. With the applied dose, the range of bromelain between 3 and 50 µM statistically significant change in viability percentages was not detected for none of the application periods. In the application time of 48 hours compared to control cells a slight increase was recorded in the growth of NIH/3T3 cells. At a concentration of 100 µM, the growth of NIH/3T3 cells was significantly increased (*p* ≤ 0.0386). The long-term application of bromelain for 72 hours on NIH/3T3 mouse fibroblast cells indicated that lower doses of bromelain ranging

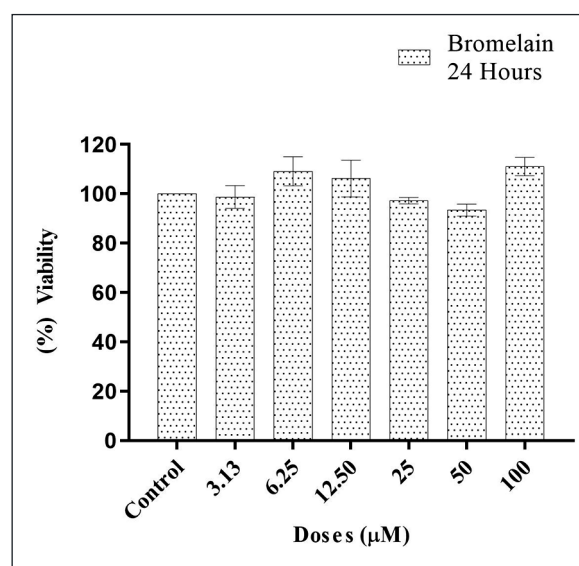


Figure 1. Viability percentages of NIH/3T3 cells treated with bromelain for 24 h.

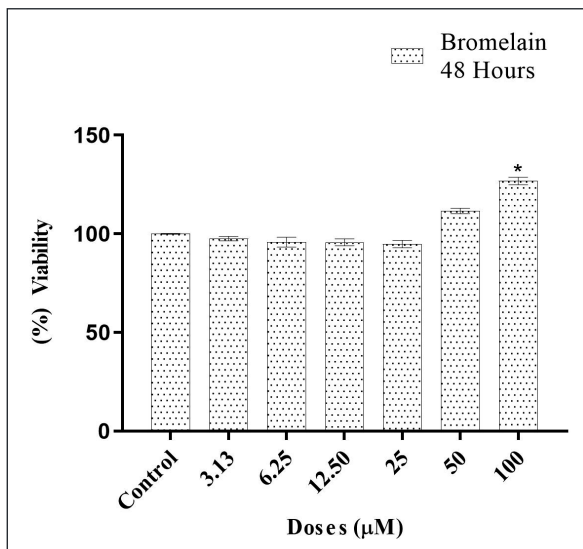


Figure 2. Viability percentages of NIH/3T3 cells treated with bromelain for 48 h (* $p \leq 0.0386$).

between 3-50 μM triggered cellular proliferation at a non-significant level, but at the highest bromelain concentration of 100 μM remarkably induced cell growth at a significance level of $p \leq 0.001$. Based on these results, long-term application of bromelain for 48 and 72 hours may be applicable and useful for growth-inducing and wound-healing. MTT results also indicated the non-toxicity of bromelain on mouse fibroblast

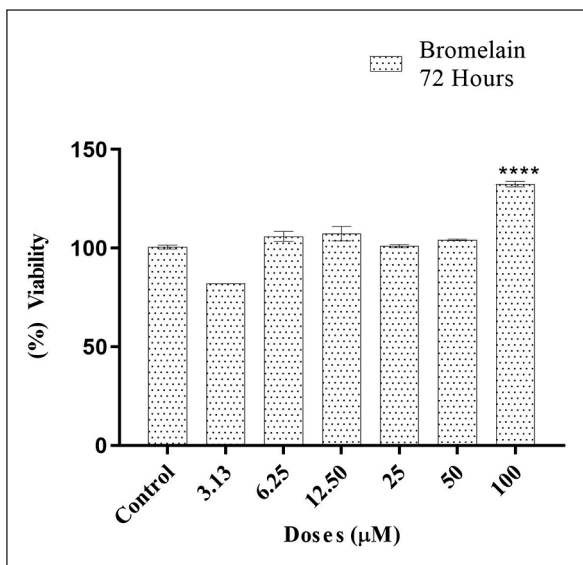


Figure 3. Viability percentages of NIH/3T3 cells treated with bromelain for 72 h (**** $p \leq 0.001$).

NIH/3T3 cells. The nontoxic effect was subsequently examined using confocal microscopy on NIH/3T3 murine fibroblast cells when the highest bromelain dose of 100 μM was applied. The analysis was performed based on the fluorescent dyes for cytoskeleton and DNA/nucleus, phalloidine and acridine orange, respectively.

Confocal images demonstrated that after 24 hours of incubation, bromelain did not affect the morphology of mouse fibroblast cells. Based on the staining with acridine orange, it was demonstrated that the NIH/3T3 cells' nucleus was unharmed and compact, or the chromatin was not condensed. Both in untreated and bromelain-treated cells, the cell shape was indicated to be fusiform as seen in Figures 4 and 5. These findings indicated that the highest concentration of bromelain applied for 24 hours to NIH/3T3 cells is non-toxic and help to keep the regular fusiform shape, compact cells skeleton as well as the nuclei of NIH/3T3 cells that means the viability and growth of fibroblast cells have been maintained with bromelain application of 100 μM .

Discussion

An extract of pineapple (*Ananas comosus*), bromelain is made up of various compounds, primarily proteolytic enzymes¹. It has various pharmacological effects and is utilized in therapy

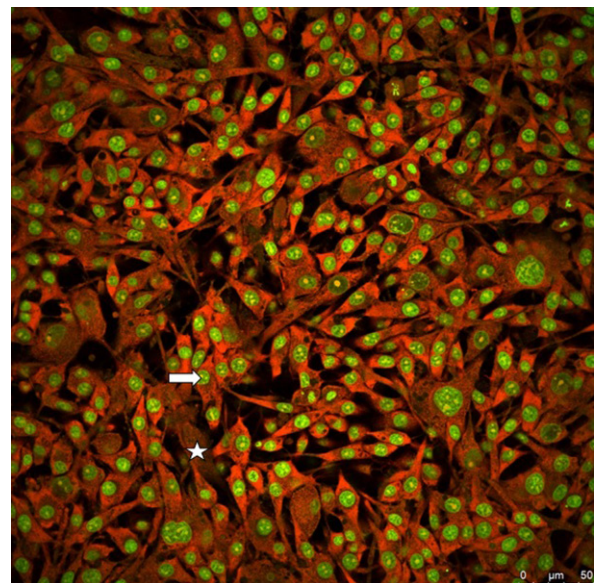


Figure 4. Confocal micrograph of NIH/3T3 cells applied to with bromelain for 24 h. Arrow-nucleus in green. Asterisk-cytoskeleton in red (Scale bar: 0-50 μm).

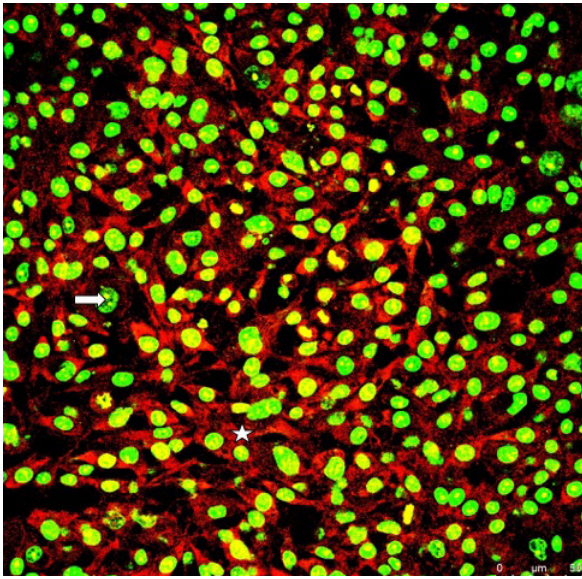


Figure 5. Confocal micrograph of NIH/3T3 control cells. Arrow-nucleus in green. Asterisk-cytoskeleton in red (Scale bar: 0-50 μm).

for many issues, but its exact mode of action is still unclear. Numerous investigations⁹ into its effectiveness and activity have found antithrombin, anti-edema, and fibrinolytic activity.

Bromelain may be used in conjunction with other medications to treat ulcerative colitis. On standard therapy, the two patients were unable to reach remission. There is documented evidence^{7,10} of the improvement from endoscopic and clinical studies. Bromelain, which is generated from pineapple stems, is thought to be a proteolytic enzyme^{7,10}. A more recent study¹¹ showing the use of bromelain in an IL-10-deficient mouse model of inflammatory bowel disease (IBD) showed that supplementation with bromelain reduced the severity of colonic inflammation on both the clinical and histological levels. The anti-inflammatory benefits of bromelain depend on its proteolytic ability, and some speculate¹¹ that bromelain may change cell surface molecules, which could affect T-cell activation.

In the current work, we examined the cytotoxic effects of topical bromelain on mouse fibroblast NIH/3T3 cells. Bromelain is not cytotoxic to mouse fibroblast NIH/3T3 cells, according to MTT data. Bromelain started cell proliferation when incubated for 24, 48, or 72 hours in all three cases. For all incubation times besides 24 hours, a statistically significant increase in cell growth was seen in the only application of the highest dose of 100 μM bromelain. The nontoxic

effect was subsequently examined using confocal microscopy on NIH/3T3 murine fibroblast cells when the highest bromelain dose of 100 μM was applied.

Confocal images demonstrated that after 24 hours of incubation, bromelain did not affect the morphology of mouse fibroblast cells. The cytoskeleton of NIH/3T3 cells was fusiform and non-fractured, also the nucleus was unharmed and compact in both untreated and bromelain-treated cells.

Clinical studies¹²⁻¹⁵ have demonstrated the value of bromelain in treating several conditions, including autoimmune illnesses and chronic inflammation¹², especially osteoarthritis and rheumatoid arthritis¹³⁻¹⁵. It has been shown¹⁶ *in vitro* that it can alter macrophages, NK cells, and T cells as well as the immune response to lessen allergic reactions. Additionally, it promotes the release of IL-1, IL-6, and TNF.

Bromelain penetration into the sinonasal mucosa was examined by Passali et al⁹ in comparison to a control group in individuals with chronic rhinosinusitis (CRS). Pineapple (*Ananas comosus*) is the source of the pharmacologically diverse substance bromelain. The study included 40 participants: 20 study participants and 20 control participants. For 30 days, a 500 mg tablet of bromelain was taken twice daily. They measured the amount of bromelain in both groups' serum and turbinate and ethmoid mucosa. The blood-to-rhinosinusal mucosa distribution of bromelain was quite good. They concluded that the anti-inflammatory medication bromelain's ability to diffuse could be used to treat sinus and nasal diseases. Due to its good tolerance and safety without any particular limits, the pharmacokinetics, pharmacodynamics, and safety profile of bromelain may make it a viable choice to achieve therapeutic effects in CRS.

According to Büttner et al¹⁷, patients with CRS, who have had prior sinus surgery, may benefit from further treatment with bromelain tablets (500 FIP). The treatment improved patient quality of life (QoL) and symptom reduction. As anticipated, CRS-NP patients showed a more remarkable average improvement in the symptom and rhinoscopy ratings on the QoL questionnaire than CRS+NP patients. Although CRS+NP is regarded as a subtype of CRS¹⁸, the two groups have diverse symptom patterns and treatment responses^{19,20}.

Topical or systemic corticosteroids, antibiotics, and saline irrigations²¹ are just a few options

for treating CRS^{22,23}. Even with comprehensive medical care, not all patients experience long-lasting symptom relief^{18,23}. Investigating alternative therapy modalities that can enhance symptoms and quality of life is crucial. It is ideal if these methods are well-tolerated by patients and, therefore, likely to result in high compliance.

The pineapple plant (*Ananas comosus*) produces a set of proteolytic enzymes, of which bromelain is one, in the stems and young fruit^{24,25}. Because of its anti-inflammatory and anti-edematous properties, bromelain can be used instead of glucocorticoids²⁵. Additionally, its extremely low toxicity can be used to treat chronic inflammatory illnesses²⁵.

Limitations

This is a study conducted with NIH/3T3 fibroblast cells. The results will be used if clinical trials will confirm experimental data that is the limitation of our study.

Conclusions

Bromelain stimulated cell proliferation in mouse fibroblast NIH/3T3 cells and was not cytotoxic²⁶ to them. If clinical trials will confirm this, it is possible that bromelain will be used topically in humans to enhance wound healing, in rhinosinusitis and chronic rhinosinusitis with nasal polyps and endonasal surgeries due to its anti-inflammatory effects.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

This is a cell-culture study conducted by commercially available NIH/3T3 cells. Therefore, Ethics Committee approval was not needed.

Informed Consent

This is a cell-culture study conducted by commercially available NIH/3T3 cells, and there is no need to take informed consent.

Funding

There are no funds for this study.

Authors' Contribution

Erkan Esen: Planning, designing, literature survey, active intellectual support. Nuray Bayar Muluk: Planning, designing, literature survey, interpretation of the results, active intellectual support, writing, submission. Canan Vejselova Sezer: Planning, designing, data collection, literature survey, statistical analysis, interpretation of the results, active intellectual support. Hatice Mehtap Kutlu: 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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