

# Protective effect of bromelain and pineapple extracts on UV-induced damage in human skin cells

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## SUMMARY

In Hawaii, people experience high sunlight exposure, contributing to an increased risk of sunburn. Additionally, most parts of pineapples are discarded, contributing to food waste and microbial spoilage, and we wanted to determine whether bromelain sourced from pineapple could be repurposed as a treatment to protect skin cells from UV damage. Bromelain from pineapple can be used to treat symptoms of sunburn, which occurs when UV rays damage the DNA in cells. Therefore, we hypothesized that bromelain extracted from various parts of the pineapple (crown, flesh, skin, and stem) would protect the skin cells from cell death caused by UV damage. Also, we expected bromelain from the stem, which is known to contain the highest concentration of bromelain, to be the most effective in protecting skin cells from UV-induced cell death. The effect of purified bromelain powder on skin cell survival was analyzed using the PrestoBlue cell viability assay to compare the protective effect of pineapple extracts on UV-induced cell death. We also examined the expression level of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which is a pro-inflammatory marker. The results indicated that bromelain protected skin cells from UV-dependent cell death and increased the mRNA expression level of TNF- $\alpha$ . Additionally, bromelain had a more protective effect when it was used as a pre-treatment method than post-treatment. Overall, this study suggests that bromelain may provide some protection to the skin from UV-induced cell death. Thus, further studies may investigate its application in sunscreen products to improve protection.

## INTRODUCTION

People in Hawaii experience higher sunlight exposure than those living in other regions because Hawaii is located on the equator (1). The average solar UV index of Hawaii ranges from high (6 to 8) to extreme (11+) (1). Compared to the mainland United States, Hawaii receives sun rays from directly above, which are consequently more intense (2). As a result of the increased sun intensity, residents and visitors are at increased risk of sunburn (3). Sunburn is an inflammatory reaction of the outermost layer of the skin that occurs when the skin is exposed to UV radiation from the sun or other UV light sources (4). Symptoms of sunburn include redness, painful swelling, and skin peeling. UV radiation damages the DNA inside the cells, and sunburn is caused by cellular

apoptosis from too much UV-induced DNA damage (5).

Apoptosis is a form of programmed cell death that occurs through a series of complex molecular mechanisms, including cell detachment, chromatin condensation, and DNA fragmentation (6). This process is tightly regulated by the balance between pro-apoptotic and anti-apoptotic signaling pathways. TNF- $\alpha$ , one of the major initiators of the inflammatory cascade, has a multifunctional effect of inducing cell death, thus contributing to the balance between pro-apoptotic and anti-apoptotic signaling (7). Though the body will attempt to eliminate DNA damage through apoptosis, repeated sunburns raise the risk of developing skin cancer when DNA damage accumulates from once healthy skin cells. The possibility of skin cancer underscores the importance of protection from direct sunlight (8).

Hawaii is a biologically rich area, and thus, we took advantage of our environment to find preventative measures in a plant that is prevalent in this locale (9). One of Hawaii's most commonly eaten and harvested fruits is *Ananas comosus*, commonly known as pineapples. Although they are not native to the region, Hawaii has a large pineapple industry, and the fruit is commercially produced on plantations. Pineapples in Hawaii can be traced back to the early 1900s (9). Pineapples contain bromelain, a group of proteolytic enzymes and non-enzymatic substances that is found in the fruit of the pineapple, but it is mainly concentrated in the stem of the pineapple (10). Today, bromelain is added to skincare formulations for its topical properties of exfoliation and removing damaged skin tissue (10). Many cosmetic brands are incorporating bromelain into formulations to exfoliate the skin and offer anti-inflammatory properties. Cell-based studies showed that bromelain decreases several proinflammatory mediators and can serve as an anti-inflammatory agent by reducing the secretion of proinflammatory cytokines, such as IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and TGF- $\beta$  (11). Therefore, we postulated that bromelain could reduce sunburn before and after the skin had been exposed to UV light. Additionally, the waste produced from eating pineapples makes it well-suited for our purposes, since the skin, crown, and stem are often disposed of after the flesh is eaten (12).

Even though bromelain has been extensively studied for anti-inflammatory and anti-microbial benefits, the protective effect of pre- or post-exposure treatment of bromelain on UV-damaged skin cells has not been investigated. Through our research, we sought to find accessible sources of sunburn treatment and UV light protection. It is important to explore alternate sources of UV protection and post-treatment because of the high rates of skin cancer and harmful chemicals found in sunscreens, such as oxybenzone and octinoxate. These chemicals can prevent the growth of green

algae, cause coral bleaching, decrease fertility of fish, etc (13). Skin cancer is the leading type of cancer in the United States, and 10,000 patients are diagnosed with skin cancer annually in Hawaii (14). Therefore, we investigated the cell viability of bromelain-treated UV-damaged Detroit 551 skin cells, comparing treated and control results, and quantified TNF- $\alpha$  expression to identify how bromelain protected the UV-damaged skin cells through TNF- $\alpha$  signaling pathway. Overall, this study found that bromelain may protect the skin from UV-induced cell death.

## RESULTS

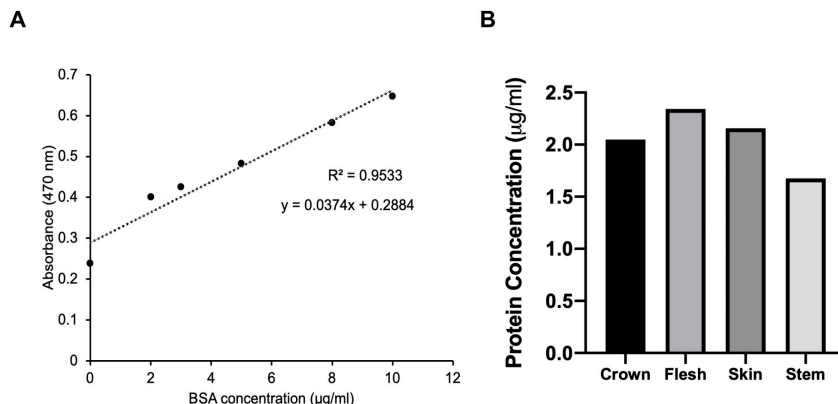
The goal of our study was to determine whether bromelain can protect skin cells from UV light or repair skin cells with UV-induced damage. First, we used a Bradford assay to quantify the protein concentration of crude extracts from pineapple crown, flesh, skin, and stem. We quantified protein concentration to ensure that skin cells in each well were treated with the same amount of protein regardless of the pineapple part the bromelain extract was taken from. We used

BSA standard curve to quantify the protein concentration of crude extracts from pineapple (Figure 1A). The protein concentration from pineapple extracts from crown, flesh, skin, and stem was 2.05, 2.34, 2.16, and 1.68  $\mu\text{g/mL}$ , respectively (Figure 1B). The highest total protein concentration was found in the flesh, then skin, crown, and stem.

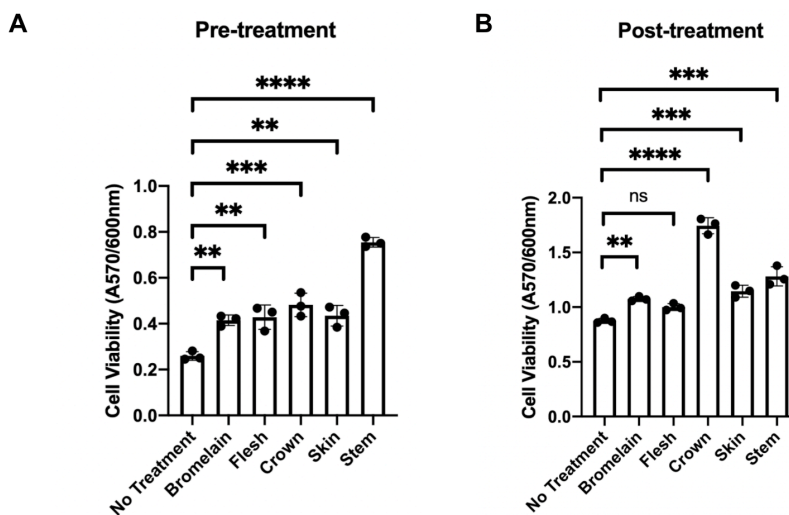
To determine if bromelain treatment before or after UV exposure could prolong skin cell viability, we pre-treated Detroit 551 skin fibroblasts with 30  $\mu\text{g/mL}$  of pure bromelain or crude extracts derived from the pineapple crown, flesh, skin, or stem or phosphate-buffered saline (PBS) before and after irradiation with UVB at 300  $\text{J/m}^2$ .

Compared to the untreated control sample, all five conditions pre-treated with bromelain increased cell viability (Figure 2A). Excluding the purified bromelain, the condition that had the highest cell viability compared to untreated cells was the stem crude extract treated cells (Figure 2A).

For the post-treatment, the bromelain source from crown significantly increased the cell viability compared to no treatment control (One-way ANOVA  $p = 0.00009$ , Tukey post



**Figure 1: Pineapple flesh had the highest concentration of protein compared to other pineapple parts.** (A) Bradford assay standard curve of concentration versus absorbance used for quantifying the protein concentration from pineapple extracts. (B) The total protein concentration of pineapple extracts from crown, flesh, skin, and stem.



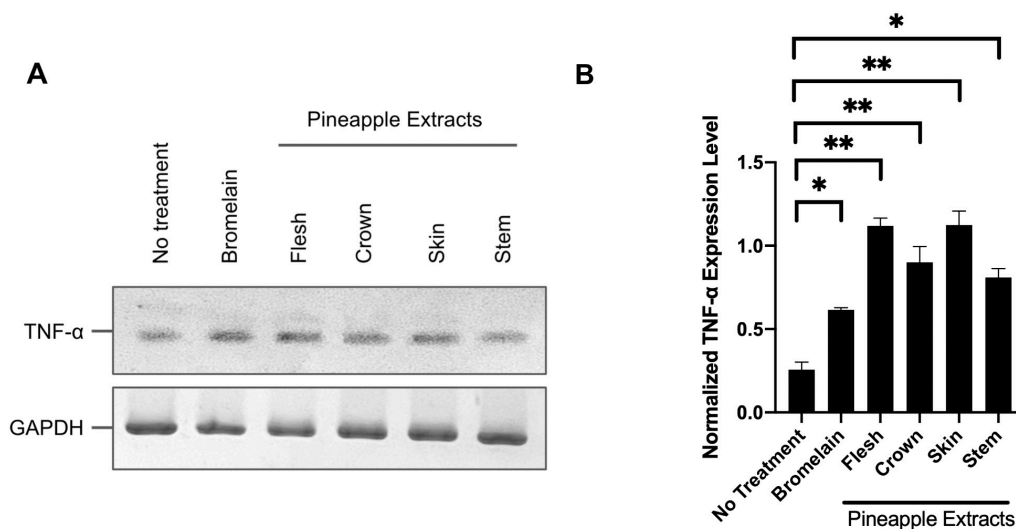
**Figure 2: Effect of pre-treatment and post-treatment of bromelain on the viability of UV-damaged skin cells.** (A) Bar graph showing mean  $\pm$  SD cell viability when skin cells were pre-treated with bromelain before UV exposure. (B) Bar graph showing mean  $\pm$  SD cell viability when skin cells were post-treated with bromelain after UV exposure. (n=3). One-way ANOVA and Tukey's post hoc test, ns  $p > 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

hoc  $p = 0.0001$ , **Figure 2B**). Despite the difference in the crown with the highest cell viability and that no significant difference was shown for the flesh extracted sample, the results still indicated that post-treatment of bromelain increased cell viability after UVB exposure compared to no treatment. In order to gain insight into the level of inflammation within the cells, we determined the levels of TNF- $\alpha$  in Detroit 551 cells that were cultured in bromelain solutions extracted from different sources. We hypothesized that bromelain would reduce inflammation and correlate with lower TNF- $\alpha$  levels.

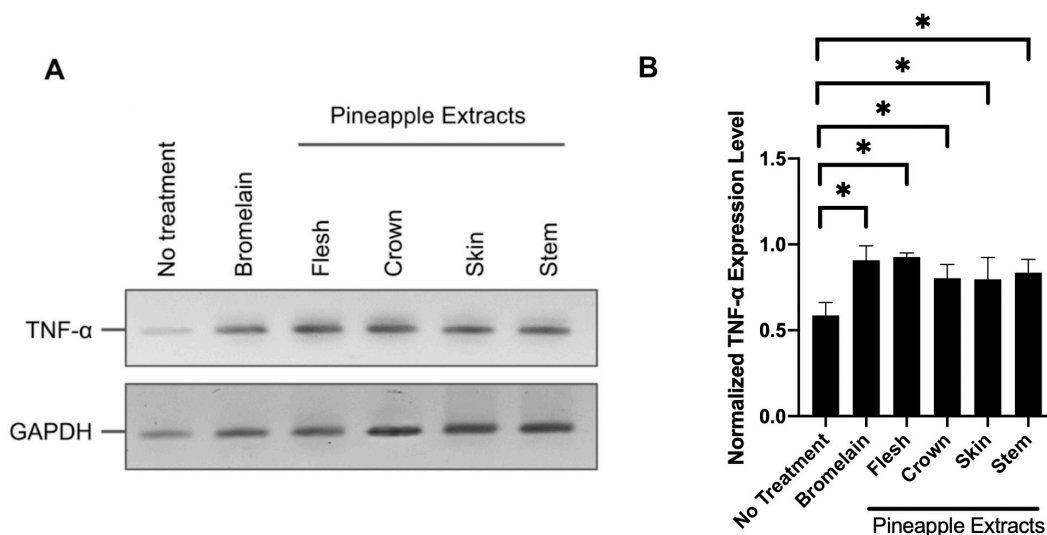
Our results showed that pre-treating skin cells with purified bromelain or bromelain from pineapple extracts (from flesh, crown, skin, and stem) increased TNF- $\alpha$  band intensity compared to the untreated control, which was contrary to our hypothesis (**Figure 3A**). Out of all the experimental conditions, the cells pre-treated with the bromelain from skin extract had

the greatest levels of TNF- $\alpha$  expression compared to no treatment control (One-way ANOVA  $p = 0.0012$ , Tukey post hoc  $p = 0.0017$ , **Figure 3B**).

Detroit 551 cells were then post-treated with different sources of bromelain solutions after exposure to UV light. We quantified TNF- $\alpha$  expression level to determine whether bromelain reduced inflammation from UV rays (**Figure 4A**). Like the pre-treatment procedure, post-treatment with both purified bromelain and bromelain from pineapple extracts (flesh, crown, skin, and stem) increased TNF- $\alpha$  expression level compared to the no-treatment control ( $p = 0.031$ , One-way ANOVA, **Figure 4B**). Our results showed that TNF- $\alpha$  expression levels are increased in Detroit 551 cells when treated with bromelain either before or after UV exposure.



**Figure 3: Effect of pre-treatment of bromelain and pineapple extracts on TNF $\alpha$  mRNA expression level in UV-irradiated Detroit 551 cells.** (A) Agarose gel displaying the amplification product of TNF- $\alpha$  and GAPDH cDNA. (B) Bar graph showing mean  $\pm$  SD of normalized TNF- $\alpha$  expression normalized to GAPDH (n=2). One-way ANOVA and Tukey's post hoc test, \*  $p < 0.05$ , \*\*  $p < 0.01$ .



**Figure 4: Effect of post-treatment of bromelain and pineapple extracts on TNF- $\alpha$  mRNA expression level in UV-irradiated Detroit 551 cells.** (A) Agarose gel displaying the amplification product of TNF- $\alpha$  and GAPDH cDNA. (B) Bar graph showing mean  $\pm$  SD of normalized TNF- $\alpha$  expression normalized to GAPDH (n=2). One-way ANOVA and Tukey's post hoc test, \*  $p < 0.05$ .

## DISCUSSION

Our research highlights bromelain as an enzyme that protects Detroit 551 fibroblasts from UV damage. Overall, we found that bromelain treatment both pre-treatment and post-treatment to UV exposure increases cell viability, indicating that bromelain may help alleviate the detrimental effects of UV rays on skin cells. Since TNF- $\alpha$  is a pro-inflammatory protein, we hypothesized that bromelain may decrease the expression level of TNF- $\alpha$  (15). However, in disagreement with our hypothesis, increased TNF- $\alpha$  expression was observed with both pre-treatment and post-treatment. TNF- $\alpha$  signaling may promote cell survival through NF $\kappa$ B signaling (16). A previous study indicates that TNF- $\alpha$  activates NF $\kappa$ B and suppresses apoptosis in corneal epithelial cells (16). In another study, an NF $\kappa$ B inhibitor, BAY11-7082, completely reversed the protective effects of TNF- $\alpha$  against oxygen-glucose deprivation ischemia-dependent apoptosis in brain cells (17). Therefore, bromelain may increase skin cell viability by inducing TNF $\alpha$  signaling, which reduces apoptosis following UV radiation. It is also possible that bromelain, as a foreign substance, could be inducing inflammation in the skin cells. This possibility warrants future research and discussion.

Overall, we found that bromelain and pineapple extracts protect skin cells from apoptosis that are exposed to UV rays in both pre-treatment and post-treatment. Bromelain had a greater cell-protective effect when it was used as pre-treatment instead of post-treatment (**Figure 2**). The cells submerged in cell media with bromelain before exposure to UV light were more likely to survive both the post-treatment and untreated cells. However, it is important to note that post-treatment was still beneficial; cell viability increased for most post-treatments when compared to no treatment (**Figure 2**). Activation of the NF $\kappa$ B - TNF- $\alpha$  pathway is one possible mechanism protecting the skin cells. However, TNF- $\alpha$  may cause the activation of inflammation in skin cells. The inflammation is involved in a complex mechanism with many different types of human cells, such as skin fibroblasts and immune cells. Therefore, the complex interaction between bromelain and other types of human cells should be investigated. If these cells have significant DNA damage still and are being allowed to survive, this can cause cancer. Therefore, investigating the amount of DNA damage in skin cells with and without bromelain treatment after UV exposure is also required for future studies.

Our research has some limitations. Since we only used one type of skin cell, different types of cells should be used to verify the protective effect of bromelain on UV irradiation-dependent apoptosis. The detailed mechanism of how bromelain activates TNF- $\alpha$  signaling should be addressed in future studies. We only used UVB (280–320 nm) to induce cell apoptosis. However, UV is divided into UVC (200–280 nm), UVB (280–320 nm), and UVA (320–400 nm) (18). Usually, UVA causes oxidative DNA damage that induces G to T transversions, UVB causes C to T transversions, and UVC causes dimerization of pyrimidines (18). Since the wavelength of UV may cause different DNA damage in skin cells, the protective effect of bromelain against skin cell apoptosis from UVA and UVC should be investigated in the future. Additionally, the exact quantity of bromelain in the pineapple extract was not analyzed, and there were many other proteins present in the extracts besides bromelain that could have influenced our experimental outcomes. Therefore,

an enzyme-linked immunosorbent assay (ELISA) measuring the concentration of bromelain in the pineapple extracts should be conducted in the future. An additional control is also needed to observe whether the pineapple extract induces cell death in the absence of UV. This observation may uncover the cell cytotoxicity of pineapple extract and bromelain without UV rays. In conclusion, our study suggests that bromelain and bromelain extracts from pineapple protect skin cells from UV irradiation-induced apoptosis. These results may provide the key to developing skin cell protection products against UV stress without using harmful chemicals in sunscreen products, such as oxybenzone and octinoxate.

## MATERIALS AND METHODS

### Crude bromelain extraction from pineapple

The pineapple was dissected into four parts: the crown, flesh, skin, and stem. Each part of the pineapple was further cut into smaller pieces using scissors. Then, each component of the fruit was weighed to be 0.3 g. Each 0.3 g sample was added to 1 mL of 50 mM sodium phosphate buffer. To perform the protein extraction, three rounds of freezing and thawing were performed. After the extraction, samples were filtered with a 4 mm diameter sterile syringe filter with a 0.45  $\mu$ m pore size hydrophilic PVDF membrane (Millipore). The samples were stored at -83°C until use.

### Preparation of bromelain stock solution

Purified bromelain powder (Sigma) was dissolved in Dulbecco's Phosphate Buffered Solution (DPBS) to prepare 200  $\mu$ g/mL stock solution. The stock solution was stored at -20°C before use.

### Protein quantification by Bradford assay

The Quick Start™ Bradford Protein Assay (BioRad) was used to determine the protein concentration in each pineapple extract using BSA standards according to manufacturer instructions. The well plate was incubated at RT for 10 min to ensure that the dye reagent bound to the protein. To determine the protein concentration of each well, the BioTek Epoch microplate spectrophotometer was used to measure 470 nm absorbance. To draw the standard curve using the BSA protein, we prepared six different concentrations of the BSA. Excel was used to calculate the fitting equation and R2 value for correlation. Based on the standard curve, we measured the absorbance of each pineapple extract using micro-spectrometry. The absorbance was used to calculate the protein concentrations in each pineapple extract.

### Cell culture and maintenance

Detroit 551 cells were cultured in RPMI160 media (Gibco) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). Fresh cell culture media was provided every two or three days to maintain healthy Detroit 551 cells.

### Treatment of cells with Bromelain and UV irradiation

In 12-well plates, 0.7 x 10<sup>6</sup> cells were seeded and maintained in media for 24 h. For bromelain pre-treatment samples, after removing the cell medium, the cells were treated with purified bromelain and bromelain extract suspended in sodium phosphate buffer (30  $\mu$ g/mL) for 24 h, followed by irradiation with UVB light emitted from the clean



bench at 300 J/m<sup>2</sup> for 30 min. Afterward, the cells were incubated for 72 h in feeding media for viability assays. For bromelain post-treatment samples, the cells were treated with bromelain through sodium phosphate containing the selected extract (30 µg/mL) for 72 h after the treatment of UVB light for 30 min.

#### Cell viability assay

PrestoBlue Assay (Invitrogen) was used to measure cell viability. After the treatment of bromelain and UV radiation on Detroit 551 cells, Presto blue reagent (10 % of culture media) was added to each well. The well plate was then incubated at RT for 10 min. The absorbance of the well plate was measured using the BioTek Epoch spectrometer with wavelengths 570 nm and 600 nm (standard). Then, the 570 nm absorbance was normalized to the 600 nm absorbance by dividing 570 nm by 600 nm.

#### RNA extraction and cDNA synthesis

To extract RNA from the cells, Total RNA Extraction Kit (Intron) was used. The cell samples that were exposed to bromelain pre-treatment or post-treatment were centrifuged at 13,000 rpm for no more than 10 seconds until the cells condensed into a pellet at the bottom of the microcentrifuge tubes. Once condensed, the cell culture media was removed. Then, R-buffer was added to the tubes and vortexed to induce cell lysis. To the R-buffer, 2-Mercapto-ethanol and 70% EtOH were added. The tubes were inverted carefully 10 times. The lysates were washed with Washing Buffer A then Washing Buffer B and centrifuged to purify the RNA. The elution solution was delivered to the tubes to release the RNA from the micro-centrifugal membrane. The master mix containing 1 µL of 10x Buffer, 1 µL of dNTP, 0.5 µL of DNA primer, and 0.5 µL of RT enzyme were added into six PCR tubes. Into the solution, 7 µL of extracted RNA was added into each PCR tube, and the tubes were centrifuged for a few seconds. The mixtures were then put in a Bio-Rad T-100 Thermal Cycler (Biorad) for a single cycle of 20 °C for 5 min, 42 °C for 1 hour, and 95 °C for 5 min.

#### Agarose gel-based RT-PCR

GAPDH and TNF-α genes were amplified by PCR PreMix kit (Bioneer). The following primer pair was used to amplify GAPDH: 5' - GGAGCGAGATCCCTCCAAAAT - 3' (forward) and 5' - GGCTGTTGTCACTTCTCATGG - 3' (reverse). The following primer pair was used to amplify TNF-α: 5' - CCTCTCTCTAATCAGCCCTCTG - 3' (forward) and 5' - GAGGACCTGGGAGTAGATGAG - 3' (reverse). PCR reactions (20 µL) were prepared with AccuPower® PCR PreMix solution (Bioneer). 1 µL of each primer (20 µM) and cDNA (10 ng) were added. The PCR reaction was performed using the following steps: 1) 95°C for 3 min, 2) 95°C for 30 sec, 3) 55°C for 30 sec, 4) 72°C for 1 min, 5) repeat steps two through four 34 times, 6) 72°C for 5 min, 7) held at 12°C until samples were collected for running on the gel. Then, an agarose gel (1.3%) made with Nucleic Acid Staining Solution from RedSafe (for clear visualization of DNA strands) was placed into the Mupid-2Plus submarine electrophoresis system filled with TBE Buffer. We loaded 6 µL of each sample and 3 µL of the ladder into individual wells of the gel mold. Then, the electrophoresis chamber was set to 100 V for 20–25 min. After, the Blue Light Transilluminator was used to view

the agarose gel and observe the migration of each nucleic acid stain down the gel.

#### Quantification of band intensity using ImageJ

ImageJ was used to quantify the TNF-α and GAPDH band intensity by densitometry. The Analyze and Gel function was used to select the area by drawing a rectangle. The selected area of the band intensity was measured by Plot Lane function. The background intensity was subtracted by drawing the line from the plot. The tracing tool was used to quantify the background-subtracted area. GAPDH intensity was used to normalize the TNF-α expression by dividing TNF-α intensity by GAPDH intensity.

#### Statistical tests

All statistical tests were performed using the Graphpad Prism 8 program. A one-way ANOVA followed by Tukey's posthoc test was used to analyze the statistical significance for each experiment.  $P < 0.05$  was considered statistically significant.

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