

Niosome Encapsulated Bromelain Reduced IL-6 and TNF- α in LPS Induced Human Skin Fibroblast Cell Line

Siavash Hosseinpour Chermahini ^{1,2*}, Fadzilah Adibah Abdul Majid ¹, Azila Abdul Aziz ¹ and Roya Anvari ²

¹Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi, Malaysia, 81310 Johor Bahru, Johor, Malaysia.

²The University of Georgia, Kostava St. 77a, 0171 Tbilisi, Georgia.

***Corresponding Author:** Siavash Hosseinpour Chermahini, Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi, Malaysia, 81310 Johor Bahru, Johor, Malaysia.

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Abstract

The topical delivery of bromelain as an anti-inflammatory solution for skin inflammation has attracted the attention of researchers. Due to the skin barrier issue, a new method was designed for the effective delivery of specific doses of bromelain to the desired action sites. A niosome was selected as a novel and practical transdermal vehicle for the delivery of bromelain to inflamed sites. In this regard, a lipopolysaccharide (LPS)-induced human skin fibroblast (HSF1184) cell line was assembled *in-vitro* as a simulated model. The levels of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α), the two immune-modulatory regulators of cell responses to inflammation, were measured to determine the response towards the niosome-encapsulated bromelain treatment. The results showed that the niosome-encapsulated bromelain significantly reduced the levels of IL-6 and TNF- α compared to the non-encapsulated bromelain, the vehicle (niosome) and the control.

Keyword: niosome, human skin fibroblast, inflammation, IL-6, TNF- α , bromelain

Introduction

Bromelain has demonstrated many beneficial properties *in-vitro* and *in-vivo* such as anti-oedematous, anti-thrombotic, and fibrinolytic properties. Most importantly, clinical trials of bromelain have confirmed its anti-inflammatory properties, which include, but are not limited to, breast engorgement during lactation [1]; osteoarthritis of the knee and hip [2, 3]; rhinosinusitis [4]; sepsis in children [5]; and urogenital inflammation [6]. Experimental evidence also exists on its effects on blood coagulation, where increases in the serum fibrinolytic activity and prostaglandin levels have been recorded due to a decrease in PGE2 and thromboxane A2. Essentially, this phenomenon is important for reducing inflammation [7]. However, there are many internal and external factors that trigger the transcription of pro-inflammatory cytokines in the human body, for instance, viral and bacterial infections, cuts, wounds, and obesity. Bromelain inhibits bacterial endotoxin LPS-induced NF- κ B activity as well as the expression of PGE2 and Cox-2 [8]. To explain this mechanism, it was hypothesized that bromelain induces the cleavage of cell surface markers such as CD14 [9]. Among the secreted regulators of inflammation that are connected to the NF- κ B pathways and that respond to bromelain are IFN- γ , TNF- α , IL-1 β , and IL-6. Depending on the context and micro-environment, these regulators can either stimulate tumour growth and invasion or activate immune responses and cause tumour regression [10–13]. Experimental evidence derived from an

analysis of peripheral blood mononuclear cells (PBMC) from healthy volunteers as well as mouse macrophages suggest that bromelain can activate TNF- α , IL-1 β and IL-6 secretions in an IFN- γ -dependent mechanism [14,15]. However, the IFN- γ production is also encouraged by the presence of bromelain [16]. These data led to the hypothesis that bromelain has the potential to activate a healthy immune system to ensure a rapid response to pathogens and cellular stress. Nevertheless, in situations when immune cells have already been stimulated, bromelain reduces the secretion of TNF- α and IL-6 [17]. According to the summary by Chobotova et al. (2010) [18] on the influence of bromelain on inflammation regulation in relation to the cancer network, an over-production of cytokines is expected in inflammation-induced cases. A study has also found that in the presence of LPS, which can stimulate an acute inflammatory reaction, bromelain reduces elevated TNF- α , IL-1 β , and IL-6 expressions in human PBMC [19]. The reduction of TNF- α and IFN- γ expressions has also been observed in inflamed tissues of patients with inflammatory bowel disease (IBD) that have been treated with bromelain [20]. The described data demonstrate that the effects of bromelain on cytokine expressions depend on the presence of inflammation-inducing conditions. This underlines the potential of bromelain for the treatment of inflammation-based pathologies. However, the challenge of the transdermal therapeutic system (TTS) is the stratum corneum (SC). The stratum corneum (SC) is the main barrier to the transportation of nutritional compounds via the skin [21]. In this regard,

the delivery and release of bioactive materials like bromelain to different parts of the body are practically controlled by nanocarriers [22]. Several types of nanocarrier systems are available for TTSs such as vesicular phospholipid gels (VPG) [23], microspheres [24], nanospheres [25], nanoliposomes [26] liposomes [27] archaeosomes [28], complexes [29], ethosomes [30], dendrimers [31] nanoemulsions [32], and niosomes [33]. The effective delivery of bromelain to the affected sites depends on the delivery system used in the topical formulation [34]. Niosomes have specific characteristics for topical delivery [35] as they possess high chemical long-term stability; high solubility for lipophilic and hydrophilic components; complete drug delivery; and large membrane flexibility to be effective against the SC barrier [36]. Niosomes are thermodynamically stable because they contain two volumes of liquids, namely water and oil, which are combined into a single phase by means of a surfactant to produce a non-ionic surfactant. Moreover, niosomes are highly compatible with biological systems and have no toxicity because of their non-ionic nature [37]. Due to the technological and physicochemical characteristics of niosomes, researchers have been motivated to consider them as ideal for carrying drugs for topical administration [38, 39]. For instance, Bouwstra and Hofland (1996) [40] loaded ellagic acid (EA) onto a niosome as an antioxidant drug application by transdermal administration. LPS is a suitable agent in stimulating inflammation since it can be used to induce inflammation both *in-vitro* and *in-vivo*. [41]. In order to detect LPS-induced inflammation, 2 cytokines, namely IL-6 and TNF- α , were selected in this study. IL-6 and TNF- α are immunomodulating agents that act as regulators of host responses to infection, immune responses, inflammation, and trauma. They include various groups of soluble proteins, peptides, or glycoproteins which act as hormonal regulators or signalling molecules from nanomolar to picomolar concentrations [42]. Some of them are pro-inflammatory, being necessary for initiating the inflammatory response that is needed for the recruitment of granulocytes and, later on, lymphocytes, to fight disease. However, excessive inflammation is sometimes the pathogenicity of certain diseases. Other cytokines are anti-inflammatory and serve to reduce inflammation and promote healing [43]. Essentially, the definition of inflammation is that the cells are alive up to a certain point but will die subsequently. In this study, niosome-encapsulated bromelain was shown to be an effective compound that reduces the levels of IL-6 and TNF- α in LPS-induced inflammation of the human skin fibroblast (HSF1184) cell

line. This is the first study to use niosome-encapsulated bromelain as an anti-inflammatory application. In order to determine the effectiveness of the treatment in HSF1184, the LPS-induced inflammation was treated with niosome-encapsulated bromelain after four hours of induction. The inflammation responses were measured after four hours and 24 hours.

Materials and Methods

2.1 Chemicals and Cell Culture

All the chemicals used in this study were purchased from Sigma Aldrich and Merck, unless noted otherwise. These included Span (40, 60, 80), Labrasol, dicetyl phosphate, chloroform, DMEM, trypsin, penicillin, streptomycin, MTT solution, and DMSO (sterile and non-sterile). The bromelain was purchased from Merck, while the LPS from *Escherichia coli* (0111:B4) was purchased from Sigma Aldrich. The TNF- α kit (Catalogue Number RAB0476) and IL-6 kit (Catalogue Number RAB0307) were purchased from Sigma Aldrich (USA). Acetaminophen tablets (80 mg) were purchased from Guardian Pharmacy. HSF1184 was purchased from ATCC (USA) (Catalogue No. 107-75a). The cell culture grade chemicals and analytical grade chemicals used in this study were from Sigma Aldrich.

2.2 Preparation of Niosome-Encapsulated Bromelain

The niosome was created based on the protocol of Arora and Sharma (2010) [44] with minor modifications. First of all, a mixture of the vesicle-forming agents, i.e., the surfactant (40, 60, 80) and Labrasol, was dissolved in chloroform (a volatile organic solvent) in a round-bottom flask at a mole ratio of 1:1. The organic solvent was removed at a temperature of 45°C using a rotary evaporator (40 rpm), leaving a thin film of solid mixture deposited on the wall of the flask. This dried surfactant film was then rehydrated with 100 mL of aqueous-phase distilled water and was then agitated gently for an hour to yield multi-lamellar niosomes. The mixture was subjected to probe sonication using a Fisher Scientific sonicator at an amplitude of 40 m, energy of 2000 J, and power of 30 W for two minutes to yield uniform nanoparticles of niosomes. After selecting a span of 60 (40, 60, 80), different concentrations of bromelain (10, 30, 50, 70, 90) with different ratios of span/labrasol (50/50, 60/40, 70/30) were tested in this study.

NO.	Surfactant	Span (mg)	Bromelain (mg)	Labrasol (mL)	Dicetyl Phosphate (mg)	Chloroform (mL)	Distilled Water (mL)
1	Span 60/Labrasol (50/50)	47.5	10	47.5	5	100	100
2			30				
3			50				
4			70				
5			90				
6	Span 60/Labrasol (60/40)	57.5	10	37.5	5	100	100
7			30				
8			50				
9			70				
10	Span 60/Labrasol (70/30)	67.5	10	27.5	5	100	100
11			30				
12			50				
13			70				
14			90				
15							

Table 1. Different concentration of bromelain with different ratio of Span60/Labrasol

2.3 Cell Line Culture and Maintenance

The cell culture and maintenance followed the protocol of Freshney (2008) [45]. The cells were cultured between passages 7 and 9. A water bath at 37°C was used to pre-warm all the media and solutions before use. The cells were maintained routinely in a 75-cm² flask. Moreover, the cells that had been cultured in Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 units/ml), streptomycin (100 lg/ml), and foetal bovine serum (10% v/v) were also incubated in a humidified incubator with 5% CO₂/ 95% air at 37°C. The cells took between 24-48 hours to reach a confluent state. Cells from the exponential growth phase were used for the experimental work, where 2 x 10⁵ cells/mL were loaded into

each well and incubated for 24 hours for cell attachment before the start of the experiment.

2.4 Inflammation Induction of HSF1184 with Serial Dilution of LPS

The serial dilution of LPS was modified from the protocol of Freshney (2008) [45]. After mixing 1mg of LPS with 1 mL of deionized water (DIW) according to the manufacturer's instructions (stock LPS solution), serial dilutions of LPS were performed from 12 µg/mL to 1.5 µg/mL (1.5 µg/mL, 3 µg/mL, 4.5 µg/mL, 6 µg/mL, 7.5 µg/mL, 9 µg/mL, 10.5 µg/mL, and 12 µg/mL) by diluting the solution in an appropriate volume of DMEM.

LPS Conc.(µg/ml)	LPS(µg)	DMEM(µl)
1	1.5	1498.5
2	3	1497
3	4.5	1495.5
4	6	1494
5	7.5	1492.5
6	9	1491
7	10.5	1489.5
8	12	1488

Table 2. LPS serial dilution

200 µl of cells at a concentration of 2 x 10⁵ cells/well were seeded in a 96-well plate and incubated (5% CO₂ and 37°C) for two days to reach a confluent state. Then, the cells were exposed to different concentrations of LPS (1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12 µg/mL) for four hours or 24 hours using separate plates. The arrangement of the different concentrations of LPS on the plate is presented in Figure 3. DMEM was used as the blank, while cells without the LPS induction were used as the control. The cell induction was terminated accordingly after four hours and 24 hours.

2.5 Quantification of Cell Viability Using MTT Assay

The cell viability after the LPS induction was quantified using an MTT assay, as recommended by Freshney (2008) [45]. The assay was used to investigate the effects of niosome-encapsulated bromelain, niosome without bromelain (vehicle), and bromelain alone on the viability of HSF1184 cells treated with 1.5 µg/mL of LPS-induced inflammation. The HSF1184 cells were seeded at 2-4 x 10⁴ cells per/mL on two 96-well plates (4hrs and 24hrs) and were left to grow for two days to a confluent state, with media changes being made once per day. On completion of the confluence cells, inflammation was triggered in the plates treated with 1.5 µl/mL of LPS and the cells were incubated for four hours at 37°C in a humidified atmosphere with 5% CO₂. Then, they were treated with acetaminophen (2 µg/mL), bromelain (25 µg/mL), niosome with 10% bromelain (20 µl/mL), and niosome as a vehicle (20 µl/mL) before being incubated for either four or 24 hours. The induced cells were washed twice with PBS, and 20 µl of MTT solution (5mg/1ml PBS) was added to each experimental well. The plate was wrapped with aluminium foil and incubated for four hours. In continue, the MTT solution was carefully removed from all the wells using fine tips before being replaced with 200 µl of non-sterile DMSO and mixed well with MTT formazan. A model ELX 808 BIO-TEK plate reader was used to measure the optical density of the colour formations of each sample at a wavelength of 570 nm and reference wavelength of 630nm. The procedure for 24 hours was similar to that for four hours. The average of six repetitions taken thrice from

each sample was taken as the result. The OD was changed to a percentage of cell viability. Since the control (cells and DMEM) had 100% viability, the other treatments were compared to the control.

2.6 Quantification of IL-6 and TNF-α Concentrations

In accordance with Freshney (2008) [45] protocol, after each culture medium had been collected and centrifuged for three minutes at 3300 rpm and the supernatant had been separated, the resulting cells in the well plates (supernatant) were processed for IL-6 and TNF-α according to the manufacturer's instructions for the ELISA protocol. The standards and samples were pipette into the wells of the kits. The IL-6 and TNF-α presented in the sample were bound to the wells by the immobilized antibody. The wells were then washed and biotinylated with the added anti-human IL-6 and TNF-α antibodies. After washing away the unbound biotinylated antibodies, HRP-conjugated streptavidin was pipette into the wells. The wells were again washed before a TMB substrate solution was added to the wells. The colour that developed was in proportion to the amount of IL-6 and TNF-α that had been bound. The colour of the stop solution changed from blue to yellow. The intensity of the colour was measured at 450 nm, and the result was based on pg/mL. From the standard curve equation ($y = a * \ln(x) + b$) of both cytokines (IL-6 and TNF-α), since y (OD) = 450 nm and x is in pg/mL, then with various amounts of y in the above stated equation, various amounts of x in pg/mL were obtained.

2.7 Treatment of LPS-Induced Inflammation using Niosome-Encapsulated Bromelain

The LPS induction was carried out according to the protocol by Zdarilova et al. (2009) [46] with minor modifications, as described above. 2 – 4 x 10⁴ cells/mL were prepared at the exponential growth phase, and 200 µL of cell suspension was transferred to each well on two 96-well plates according to the plate design. The plates were then incubated in 5% CO₂

at 37°C for two days with a daily change of medium until the cells reached a confluent state. Then, 1.5 µg/mL of LPS was added to each well and the plates were incubated for another four hours. After four hours of post induction incubation, the designated wells in both the four-hour and 24-

hour plates were treated with acetaminophen (2µg/mL), bromelain (25µg/mL), niosome with bromelain 10% (20µl/mL), and niosome as a vehicle (20µl/mL), and then incubated again for another four hours or 24 hours.

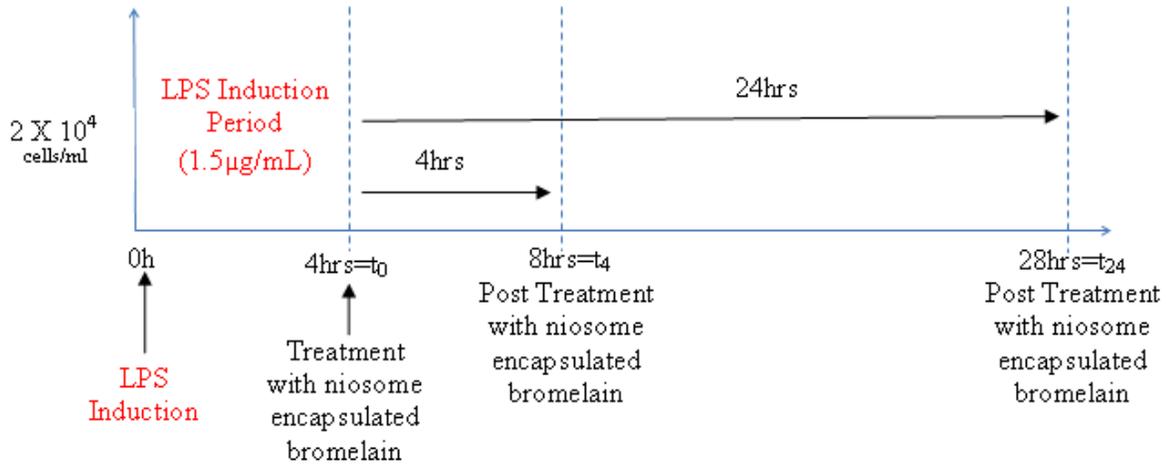


Figure 1. LPS induced inflammation after 4hrs, treated with niosome encapsulated bromelain for 4hrs and 24hrs post treatment.

The summary of the LPS induction and treatment with the niosome-encapsulated bromelain for both plates is presented in Figure 1. The cell viability was determined using the MTT assay as described above. The levels of both the IL-6 and TNF-α cytokines present in the wells were measured accordingly based on the above protocol.

Statistical analysis

In this experiment, two pairs of samples were compared using the t-Test because these two groups contributed a pair of scores. This statistical technique is often called a paired sample t-test or correlated t-test that tells whether there is a statistically significant difference in the mean scores

for niosome-encapsulated bromelain and the other treatments. When *P*<0.05, it means that there is a significant difference.

Results and Discussion

4.1 Determination of suitable LPS Concentration to Induce Inflammation in HSF1184

A suitable concentration of LPS to induce inflammation in HSF1184 was determined based on the cell viability and IL-6 response. The purpose was to select the lowest concentration of LPS that would cause inflammation on the cells without causing cell death.

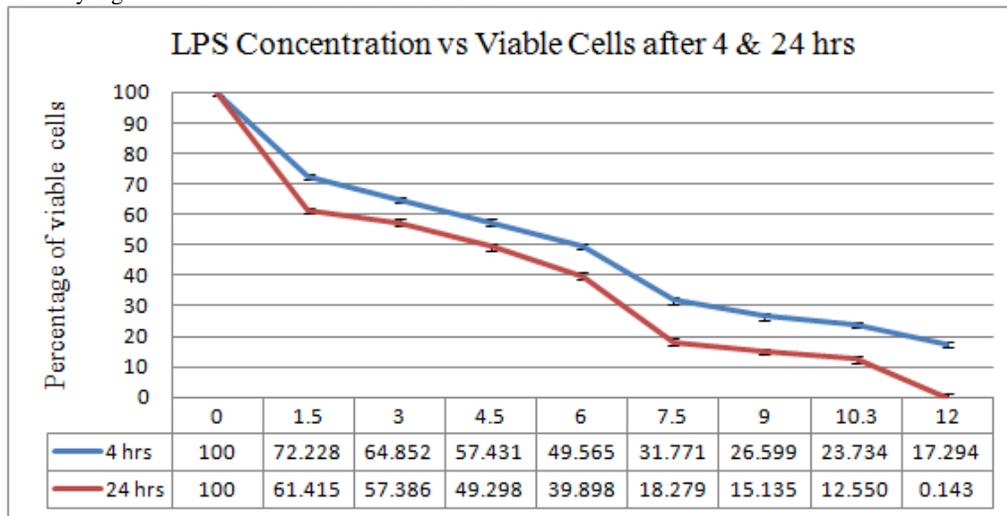


Figure 2. LPS concentration vs viable cells after 4 hrs and 24 hrs.

As shown in Figure 2, the HSF1184 cell line was treated with different concentrations of LPS to induce inflammation after 4 hours and 24 hours. Then, the percentage of viable cells was calculated. By using the paired sample t-Test in the analysis and comparing the LPS concentration of 1.5µg/mL with other concentrations, the difference was most significant for the 1.5µg/mL LPS. The interleukin-6 (IL-6) and tumour necrosis

factor alpha (TNF-α) were used as indicators for the host’s responses to the inflammation. The suitable concentration of LPS-induced inflammation in the HSF1184 cell line was determined based on the response of the cytokine. The determination of the inflammation-inducing concentration of LPS was based on the production of TNF-α and IL-6 by the cells.

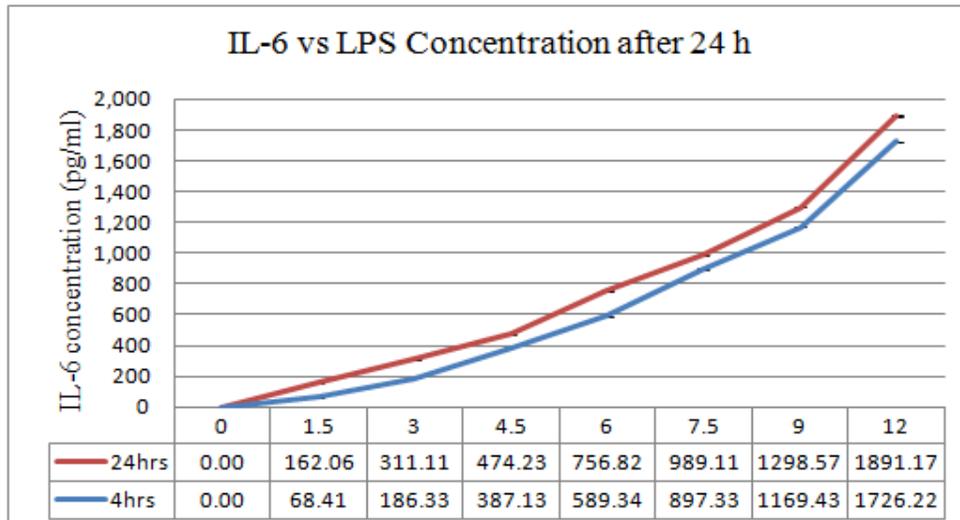


Figure 3. IL-6 Concentration vs LPS concentration after 4 hrs and 24 hrs.

In Figure 3, the HSF1184 cell line was treated with different concentrations of LPS-induced inflammation for four hours and 24 hours. The supernatant of the HSF1184 cell line that was inflamed by the LPS was measured by the response of the cytokine (IL-6). By using the paired sample t-Test to compare the concentration of 1.5µg/mL LPS with other concentrations, the 1.5µg/mL LPS showed a more significant difference than the other concentrations ($p < 0.05$). From the two graphs shown above, the suitable concentration of LPS to induce inflammation in the HSF1184 based on the cell viability and the IL-6 response was 1.5µg/mL. Although many different parameters could have been used as the point of reference in this study, the concentration of 1.5 µg/mL had induced inflammation for the MTT assay, IL-6, and TNF- α , and thus, their responses toward the HSF1184 cell line were surveyed. Previous studies have found that an effective dose of 1µg/mL of LPS induced inflammation [47]; however, this study achieved a profound physiological response at 1.5µg/mL. Subsequently, a concentration of 1.5µg/mL of LPS was used to induce inflammation in the HSF1184 cell line before treatment with niosome as an *in-vitro* simulated model. This LPS

concentration was determined using quantitative experiments such as quantitative cell viability using an MTT assay and quantitative cytokines concentration. Thus, 1.5µg/mL of LPS was selected to induce inflammation in the HSF1184 cell line in the following experiments.

4.2 Cell Viability of LPS-Induced HSF1184 Treated with Niosome-Encapsulated Bromelain

In the MTT assay, inflammation was induced in the HSF1184 by the 1.5 µg/mL LPS. It was treated with the niosome-encapsulated bromelain, non-encapsulated bromelain, and the vehicle (niosome). In this experiment, acetaminophen at a concentration of 2 µg/mL was considered as the positive control, LPS at a concentration of 1.5 µg/mL was the negative control, and DMEM-plus cells with a volume of 1 mL was the control specimen. The concentrations for the other treatments involving niosome-encapsulated bromelain, bromelain alone, and niosome (vehicle) were 20 µg/mL, 25 µg/mL, and 20 µg/mL, respectively, as determined from previous experiments.

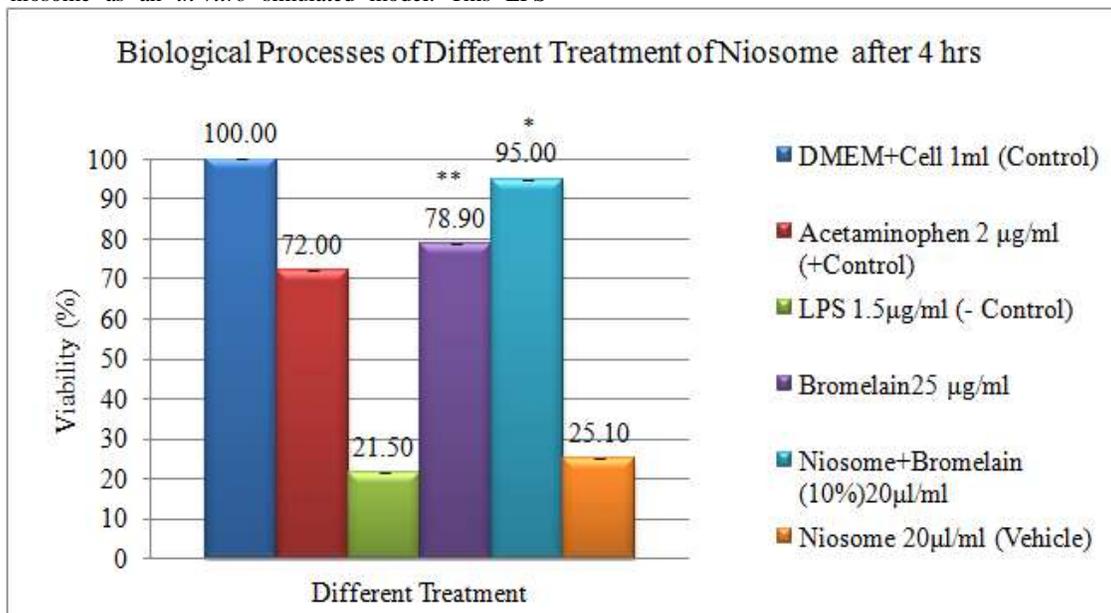


Figure 4. Percentage viability of LPS induced HSF1184 with different treatment after 4 hrs.

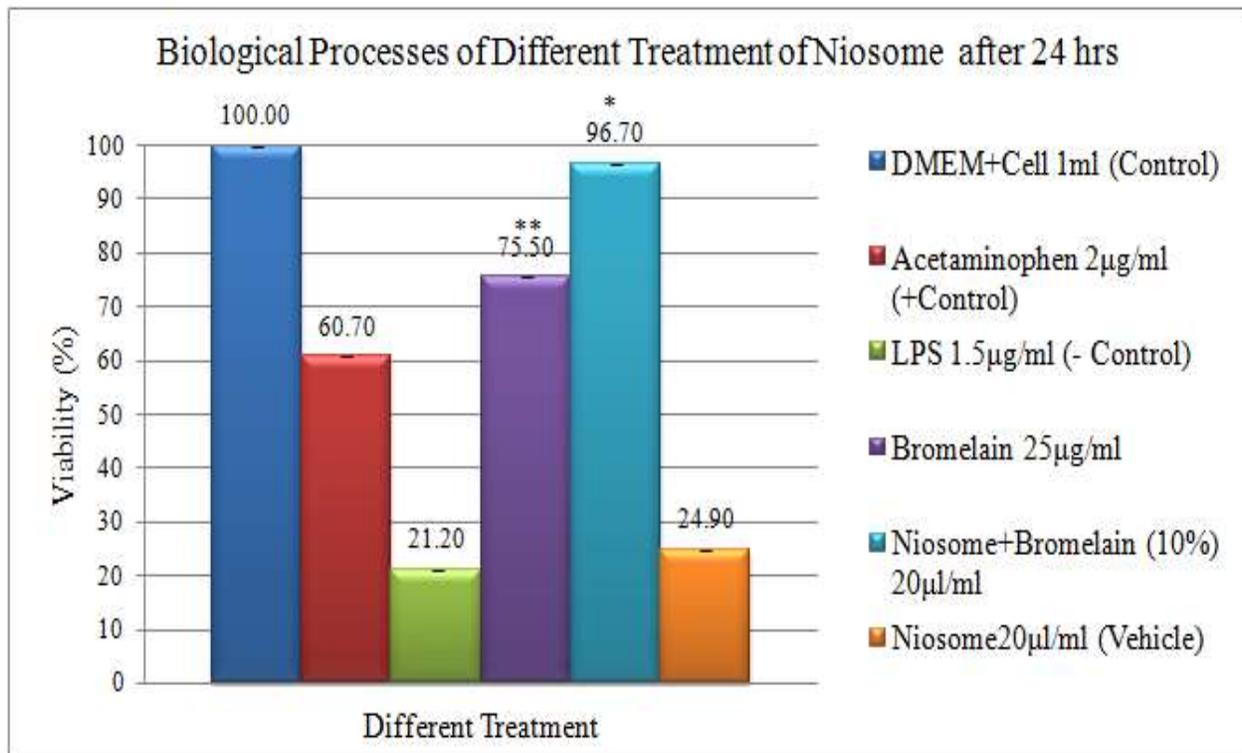


Figure 5. Percentage viability of LPS induced HSF1184 with different treatment after 24 hrs.

Figure 4 and Figure 5 show the differences in the results for various treatments of niosome after four hours and 24 hours. Based on the presented figures, the biggest difference in the percentage of viability was related to the niosome-encapsulated 10% bromelain, which had a viability of about 96.70 percent in Figure 5 and a viability of 95 percent in Figure 4. On the contrary, the vehicle had the lowest viability at 24.90 percent in Figure 5 and 25.10 percent in Figure 4. However, the viability of bromelain alone was 78.90 percent in Figure 4 and 75.50 percent in Figure 5; this was the second highest after the niosome-encapsulated 10% bromelain (Figure 4 and Figure 5). As a positive control, acetaminophen ranked third with 72 percent viability in Figure 4 and 60.70 percent in Figure 5. The free and encapsulated bromelain were evaluated by MTT assays using four different cell lines, which were HeLa, HEK293, MCF-7 and A549, for 24, 48 and 72 hours, respectively [48]. It was found that the encapsulated bromelain needed significantly ($p < 0.05$) lower doses than the free bromelain alone. On the other hand, cells treated with the encapsulated bromelain did not show any significant decrease in cell viability. Despite some differences, such as in the cell line, period of time and treatments, the results were similar to the findings of this study [49]. In this study, the highest percentage of cell viability of HSF1184 inflamed by the 1.5 µg/mL LPS was achieved by the treated niosome-encapsulated bromelain compared to the non-encapsulated bromelain and the vehicle. The results of this study have shown that when the niosome alone acted as the vehicle, it did not affect the level of viable cells in the culture, thus suggesting that the anti-inflammatory effect of the niosome-encapsulated

bromelain was not due to the presence of the vehicle [50]. The statistical analysis of the niosome-encapsulated 10% bromelain showed a significant difference in comparison with the other niosome treatments, where $P < 0.05$.

4.3 IL-6 and TNF α Response in LPS Induction of HSF1184 Treated with Niosome-Encapsulated Bromelain

Interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α), the two immune-modulatory regulators of cell responses to inflammation, were measured in response to 1.5 µg/mL LPS-induced inflammation and were then treated with niosome-encapsulated bromelain, non-encapsulated bromelain, and the vehicle. The cytokines, IL-6 and TNF- α , were used because these pro-inflammatory cytokines play a key role in the inflammatory response and can be easily quantified in the supernatant.

4.3.1 Determination of Inflammation Stage Using the IL-6 Response to Different Niosome Treatments:

Interleukin-6 (IL-6) was measured in response to 1.5 µg/mL LPS and was then treated with niosome-encapsulated bromelain at a concentration of 20 µg/mL, bromelain alone with a concentration of 25 µg/mL, and niosome (vehicle) with a concentration of 20 µg/mL. 1 mL DMEM was used as the blank sample and 1 mL of DMEM-plus cells was used as the control. All the concentrations resulted from previous experiments in this study.

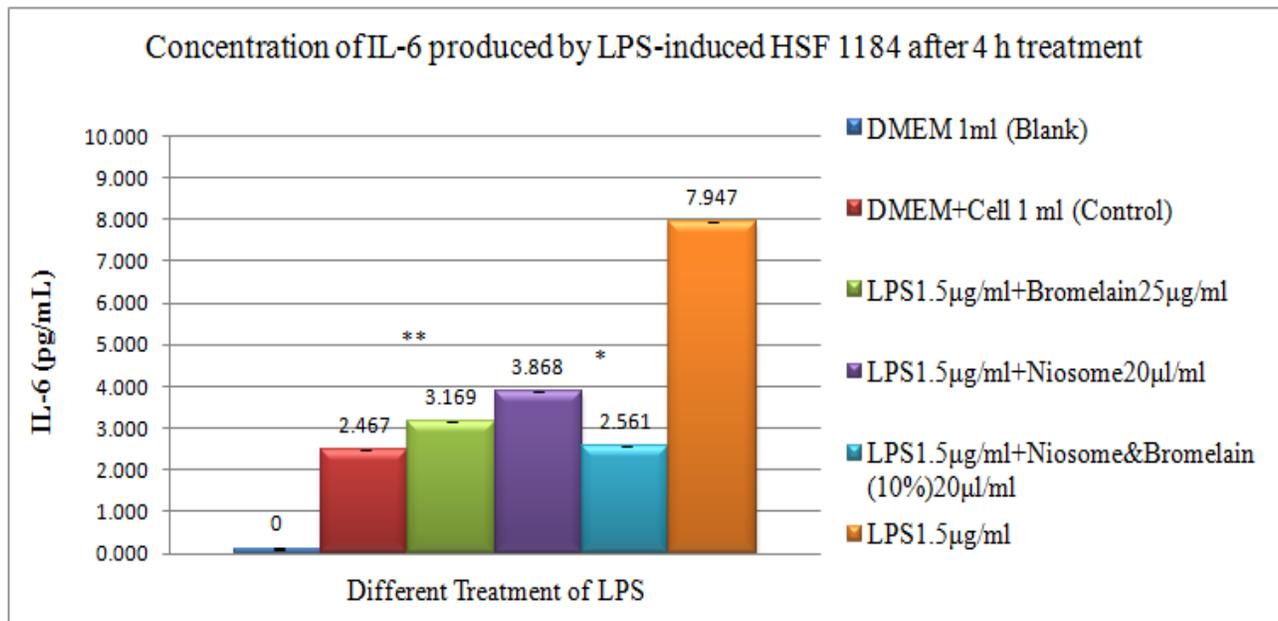


Figure 6. Concentration of IL-6 produced by LPS-induced HSF 1184 after 4 hrs.

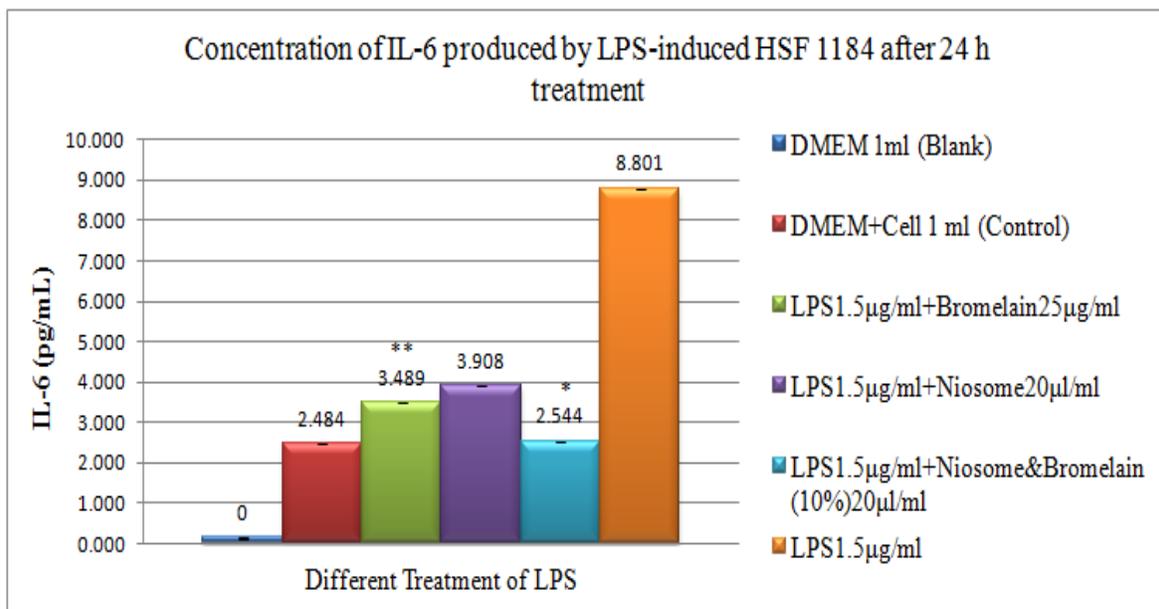


Figure 7. Concentration of IL-6 produced by LPS-induced HSF 1184 after 24 hrs.

Figure 6 and Figure 7 show that the lowest amounts of 2544 pg/mL in Figure 7 and 2561 pg/mL in Figure 6 belonged to the niosome-encapsulated bromelain. The highest amounts, marked at 3908 pg/mL in Figure 7 and 3868 pg/mL in Figure 6, were related to the vehicle. However, bromelain alone, reported at 3169 pg/mL in Figure 6 and 3489 pg/mL in Figure 7, was ranked second. Strong evidence on the anti-inflammatory effect of theniosome-encapsulated 10% bromelain was found when the result of the quantitative cytokines concentration showed the highest induction for LPS without any treatment and vehicle. The lowest induction of inflammatory cytokines was observed in the niosome-encapsulated bromelain and the bromelain-treated cells, as previously mentioned by Mosmann (1983) [51]. The statistical analysis of the

niosome-encapsulated 10% bromelain showed a significant difference compared to other treatments of niosome, where $P < 0.05$.

4.3.2 Determination of Inflammation Stage Using TNF-α Response to Different Niosome Treatments

The tumour necrosis factor alpha (TNF-α) was measured in response to 1.5 µg/mL LPS, and then to treatments with niosome-encapsulated bromelain at a concentration of 20 µg/mL, bromelain alone at a concentration of 25 µg/mL, and niosome (vehicle) at a concentration of 20 µg/mL. The setup for the control and blank samples was similar to that of the IL-6.

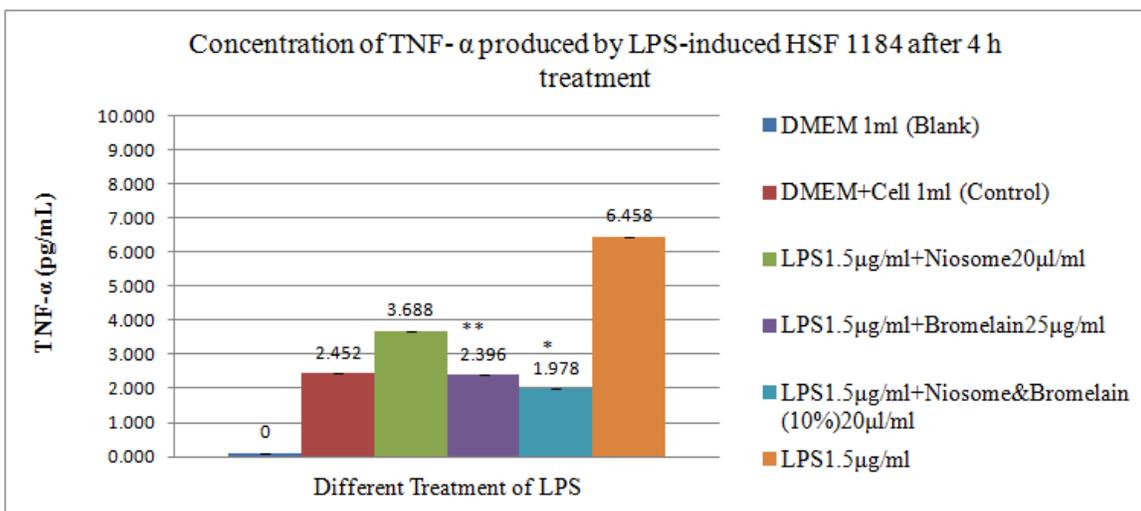


Figure 8. Concentration of TNF-α produced by LPS-induced HSF 1184 after 4 hrs.

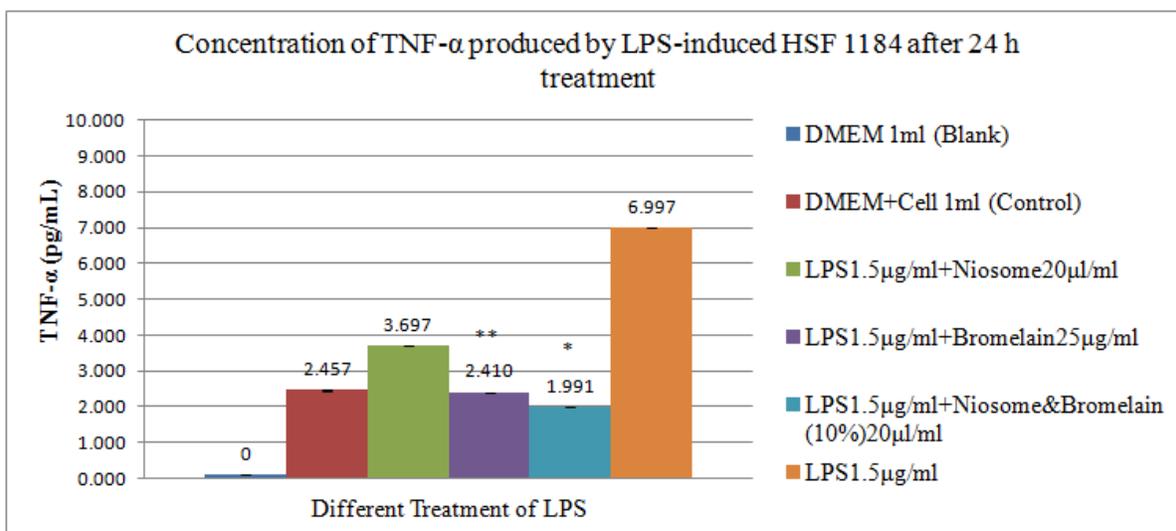


Figure 9. Concentration of TNF-α produced by LPS-induced HSF 1184 after 24 hrs.

Figure 8 and Figure 9 show that the lowest amounts, marked at 1978 pg/mL in Figure 8 and 1991 pg/mL in Figure 9, were from the niosome-encapsulated bromelain. The highest amounts, reported at 3697 pg/mL in Figure 9 and 3688 pg/mL in Figure 8, were related to the vehicle. However, bromelain alone was ranked second since the reported readings were 2396 pg/mL in Figure 8 and 2410 pg/mL in Figure 9.

Unlike IL-6, strong evidence on the anti-inflammatory effect of niosome-encapsulated 10% bromelain was from the low induction of inflammatory cytokines with the niosome-encapsulated 10% bromelain and bromelain. The highest induction was observed in the LPS without any treatment and the vehicle-treated cells (Mosmann, 1983) [51]. Again, the niosome-encapsulated 10% bromelain was statistically significant. The efficacy of the bromelain-niosome system on skin inflammation around the knee was evaluated. The potential anti-inflammatory properties of the niosome-encapsulated bromelain were investigated in the HSF1184 cell line because this model produces high concentrations of IL-6 and TNF-α in cultures upon activation by LPS. Studies on the effects of bromelain on pro-inflammatory cytokines are limited. Moreover, no previous studies have evaluated the effects of niosome-encapsulated bromelain on IL-6 and TNF-α cytokines. Greenspan et al. (2005) [52] stimulated human endothelial cells with TNF-α and found that co-treatment with bromelain

inhibited the production of TNF-α for LPS induction. However, in another study evaluating the inhibitory effects of bromelain on TNF-α production, Mastuda et al. (2012) [53] showed that bromelain inhibited the antigen IgE-mediated TNF-α secretion in RBL-2H3 mast cells. A potential reason for the differences in the results may be due to differences in the stimuli and cell types used. These findings are partly coherent with the results of this study, which show that niosome-encapsulated bromelain significantly impaired LPS-induced IL-6 and TNF-α production. Apart from this finding, in different comparisons of niosome encapsulation, the niosome-encapsulated bromelain could be affected in a higher range compared to other treatments like bromelain alone or a vehicle. Moreover, the results showed that niosome alone as a vehicle did not affect the IL-6 and TNF-α levels in the cultured supernatant and suggest that the immunostimulatory properties of the bromelain were not due to the presence of the vehicle [54]. On the other hand, during the post treatment, a pattern was observed where the anti-inflammatory cytokines peaked at four hours and 24 hours. A similar time period has been reported in numerous studies on humans [55]. Other key factors are the biological conditions of the HSF1184 cell line, for instance, inflammation induced by other components. Future perspectives should include an understanding on the molecular basis for the inhibitory effects of niosome-encapsulated bromelain on the HSF1184 cell line. The importance of niosome-

encapsulated bromelain on the elimination of inflammation in the HSF1184 cell line infected by LPS-induced inflammation was evaluated. The result showed that niosome-encapsulated 10% bromelain significantly reduced the production of IL-6 and TNF- α in the LPS-induced human skin fibroblast cell line (HSF1184) after four hours post treatment as compared to the non-encapsulated bromelain and vehicle.

Conclusion

This study set out with the aim of assessing the importance of niosome-encapsulated bromelain in the elimination of inflammation in the HSF1184 cell line infected by LPS-induced inflammation. The results showed that niosome-encapsulated 10% bromelain significantly reduced the production of IL-6 and TNF- α in the LPS-induced human skin fibroblast cell line (HSF1184) after 4 hours of post treatment as compared to the non-encapsulated bromelain and vehicle.

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