Bromelain Encapsulated in Niosome Reduced IL-6 and TNF-α LPS Induction

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Abstract: Due to Skin inflammation is a pathogenic factor for ectodermal tissue, and NSAIDS (non-steroidal anti-inflammatory drugs) as applicable prescription have serious side-effects, so bromelain as a natural, safe, and effective remedy without any side-effects would offer a welcome alternative treatment. On the other hand, existence of stratum corneum (SC) as skin barrier, needed a novel method for delivery of specific doses of bromelain to desire site of action. Niosome as practical system was selected to deliver bromelain to inflamed skin. To this regards, Lipopolysaccharide (LPS) induced inflammation in mice was assembled as an *in-vivo* simulated model. Interleukin-6 (IL-6) and Tumor Necrotic Factor–alpha (TNF-α), were measured in respond to noisome encapsulated bromelain treatment. Base on the results, noisome encapsulated bromelain significantly reduced IL-6 and TNF-α in compare of bromelain alone, vehicle and control.
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1. Introduction

Bromelain has demonstrated many *in vitro* beneficial properties such as anti-edematous and fibrinolytic. Most importantly, clinical trials of bromelain has 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]. However, there are many internal and external factors that trigger the transcription of pro-inflammatory cytokines in human body, for instance, viral and bacteria infection, cut, wound, and obesity.

The described data demonstrate that the effects of bromelain on cytokine expression depend on the presence of inflammation-inducing conditions. This underlines the potential of bromelain for treatment of inflammation-based pathologies. However, the challenge of the transdermal therapeutic system (TTS) is stratum corneum (SC). The stratum corneum (SC) is the main barrier for skin dermal for the transportation of nutrition compounds via the skin [4].

In this regard, the delivery of bioactive materials like bromelain to different parts of the body and the attitude of its releasing are practically controlled by the nanocarriers [5]. Several types of nano carrier systems are available for TTS such as vesicular phospholipid gels (VPG) [6], nanospheres [6], liposomes [7], and niosomes [8]. The effectiveness of bromelain delivery to the affected sites depends on the delivery system used in the topical formulation [9].

Niosome has the specific characteristics for topical delivery [8]. Niosomes are stable from the thermodynamical point of view because they contain two volumes of liquids water and oil which come to a single phase by means of a surfactant that makes non-ionic surfactant. Moreover, niosomes have high compatibility with biological systems and have no toxicity because of their non-ionic nature [10].

Some cytokines are proinflammatory, which are necessary for initiating an inflammatory response needed to recruit granulocytes, and later on, lymphocytes to fight disease. Excessive inflammation, however, is sometimes the pathogenicity of certain diseases. Other cytokines are anti-inflammatory and serve to reduce inflammation and promote healing[9]. 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 has been shown to be an effective compound that reduces IL-6 and TNF-α in LPS induced inflammation for human skin fibroblast (HSF1184) cell line. This is the first study that uses niosome encapsulated bromelain as the anti-inflammatory application. In order to determine the effectiveness of the treatment in HSF1184, LPS induced inflammation has been treated with niosome encapsulated bromelain after four hours induction. The inflammation responses were measured after 24 hours.

2. Materials and Methods

2.1 Chemicals and Cell Culture

All chemicals used in this study were purchased from Sigma Aldrich and Merck unless noted

otherwise. These include 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 cytokines TNF-a kit (Catalog Number RAB0476) and IL-6 kit (Catalog Number RAB0307) were purchased from Sigma Aldrich (USA). Acetaminophen tablet (80 mg) was purchased from the 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 Inflammation Induction of HSF1184 with Serial Dilution of LPS

The serial dilutions of LPS were modified from the protocol of Freshney (2008). After mixing 1mg LPS with 1 mL deionized water (DIW) according to the manufacturer's instructions (stock LPS solution), serial dilution of LPS were performed from 12 µg/mL to 1.5µg/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. 200µl of cells at the concentration of 2 x10^5 cells/well were seeded in 96 wells plate and incubated (5% CO_2 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 different concentrations of LPS on the plate is presented in Figure 3. DMEM was used as blank while cells without LPS induction was used as control. Cells induction was terminated after four hours and 24 hours accordingly.

3 Results and Discussion

3.1 Determination of Inflammation Stage Using Different Niosome Treatment by IL-6 Respond

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

Figure 1 shows that the lowest amounts belonging to niosome encapsulated bromelain was 2544 pg/mL. The highest was related to the vehicle, marked at 3908 pg/mL. However, bromelain alone was ranked second, reported at 3489 pg/mL. Strong evidence on anti-inflammatory effect of niosome encapsulated with 10% bromelain was found when the result from quantitative cytokines concentration showed the highest induction for LPS without any treatment and vehicle. Lowest induction of inflammatory cytokines was observed in niosome encapsulated bromelain and bromelain treated cells as previously mentioned by Mosmann (1983).

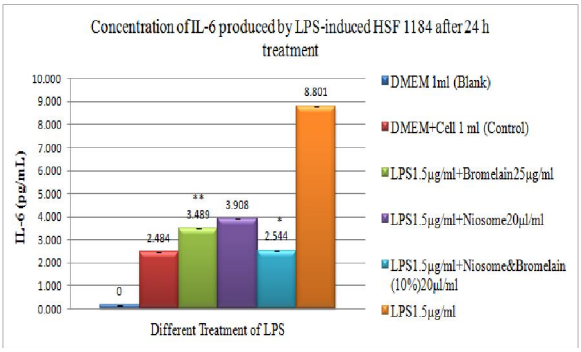

Figure 1. Concentration of IL-6 produced by LPS-induced HSF 1184 after 24 hrs.

3.2 Determination of Inflammation Stage Using Different Niosome Treatment by TNF-α Respond

Tumor Necrotic Factor–alpha (TNF-α) was measured in respond to 1.5 µg/mL LPS and then treated with niosome encapsulated bromelain with concentration of 20 µg/mL, bromelain alone with concentration of 25 µg/mL, and niosome (vehicle) with concentration of 20 µg/mL. Setup for the control and blank samples were similar to those of IL-6.

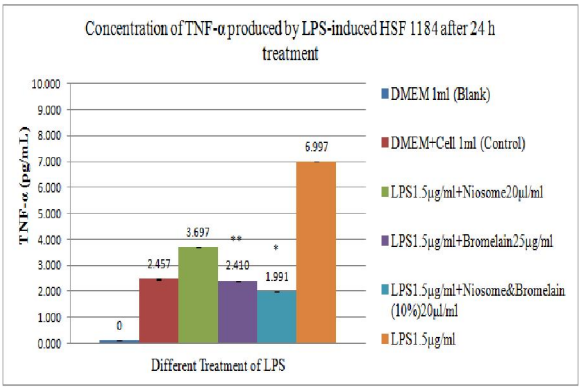

Figure2. Concentration of TNF-α produced by LPS-induced HSF 1184 after 24 hrs.

Figure 2 shows that the lowest amounts were from the niosome encapsulated bromelain, which were marked at 1991 pg/mL. The highest was related to vehicle, which was reported as 3697 pg/mL. However, bromelain alone was ranked second since the reported reading was 2410 pg/mL. Unlike IL-6, the strong evidence on anti-inflammatory effect of noisome encapsulated with 10% bromelain was from the low induction of inflammatory cytokines with

niosome encapsulated with 10% bromelain and bromelain. The highest induction was observed in LPS without any treatment and vehicle treated cells (Mosmann, 1983).

4. Conclusion

This study set out with the aim of assessing the importance of niosome encapsulated bromelain to eliminate inflammation in HSF1184 cell line infected by LPS induce inflammation. The result showed that niosome encapsulated bromelain10%, significantly reduced IL-6 and TNF-α in LPS induced human skin.

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