Effect of bromelain encapsulated in niosome on IL-6 respond in mice infected by LPS induction

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Abstract: Skin inflammation is a pathogenic factor for ectodermal tissue, but applicable prescriptions like NSAIDS (non-steroidal anti-inflammatory drugs) gives serious side-effects. As an alternative, bromelain is a more natural, safer, and more effective remedy that has no side-effects. On the other hand, existence of stratum corneum (SC) as the skin barrier needs a novel method to deliver specific doses of bromelain to desired site of action. In this case, niosome is the practical system that delivers bromelain to inflamed skin. To this regard, Lipopolysaccharide (LPS) induced inflammation in mice was assembled as an *in-vivo* simulated model. Interleukin-6 (IL-6) was measured in response to noisome encapsulated bromelain treatment. Base on the results, noisome encapsulated bromelain has significantly reduced IL-6 compared to other treatments such as bromelain alone and vehicle (noisome alone).
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Introduction

Bromelain has been shown to demonstrate many potentially beneficial properties, both *in vitro* and *in vivo*, including those which are anti-edematous, anti-thrombotic, fibrinolytic and profoundly anti-inflammatory. Clinical trials of bromelain have shown its effectiveness for treating various inflammation-based conditions. These include breast engorgement during lactation [1], osteoarthritis of the knee and hip [2,3], rhinosinusitis [4], sepsis in children [5], and urogenital inflammation [6].

There is experimental evidence to suggest that its effects on blood coagulation is promising where an increase in serum fibrinolytic activity and prostaglandin levels has been noted when levels of PGE2 and thromboxane A2 have been decreased, and this finding is crucial for inflammation receding [7]. Bromelain has also demonstrated its ability to inhibit bacterial endotoxin LPS-induced NF-kB activity and the expression of PGE2 and Cox-2 [8, 9]. To explain this mechanism, we hypothesized that bromelain induces cleavage of cell surface markers such as CD14 [8].

Among the secreted regulators of inflammation that are connected to NF-kB pathways and respond to bromelain are IFNγ, TNF-α, IL-1β, and IL-6. Depending on the context and micro-environment, these regulators can either stimulate tumor growth and invasion or activate immune responses and cause tumor regression [10–13].

Experimental evidence derived from analyzing peripheral blood mononuclear cells (PBMC) from healthy volunteers as well as mouse macrophages suggested that bromelain can activate TNF-α, IL-1β, and IL-6 secretion in an IFNγ-dependent mechanism [14–17]. IFNγ production, in turn, has also increased in the presence of bromelain [17]. These data allow us to hypothesize that bromelain has the potential to activate healthy immune system to ensure rapid response to pathogens and cellular stress.

However, in situations when immune cells have already been stimulated, bromelain reduces TNF-α and IL-6 secretion [18]. Chobotova et al (2010) summarized that bromelain's in inflammation regulation of cancer network occurs when there is an inflammation induced over-production of cytokines. For instance, in the presence of LPS that stimulates acute inflammatory reaction, bromelain has decreased the elevation of TNF-α, IL-1β and IL-6 expression in human PBMC [8].

Reduction of TNF-α and IFNγ expression have also been observed in bromelain-treated inflamed tissues obtained from patients with inflammatory bowel disease (IBD) [19]. The described data demonstrated 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 that researchers are facing in terms of transdermal therapeutic system (TTS) is stratum corneum. The stratum corneum (SC) has been shown as the main barrier for skin dermal transportation of nutrition compounds via the skin

[23]. Delivery of bioactive materials like bromelain to different parts of our body and the attitude of its releasing are practically carried out by nanocarriers [24].

Several types of nano carrier systems are available for transdermal drug delivery such as vesicular phospholipid gels (VPG) [25], microspheres [22], nanospheres [26], nanoliposomes [27] liposomes [28] archaeosomes [29], complexes [28], ethosomes [30], dendrimers [31] nanoemulsions [32], and niosomes [33]. Effectiveness of bromelain delivery to the affected sites depends on the delivery system used in the topical formulation [34].

Niosome has the specific characteristics for topical delivery [33] and its technological and physicochemical characteristics have motivated researchers to consider them as ideal for carrying drug for topical administration [35]. To simulate inflammation condition, LPS can be the suitable agent since it has been used to induce *in-vitro* and *in-vivo* inflammation [21].

To detect LPS induced inflammation, two cytokines 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 signaling molecules from nano to picomolar concentrations[20].

Some of them are proinflammatory, and this is necessary to initiate an inflammatory response necessary to recruit granulocytes and then 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 [34]. Inflammation definition is that, up to certain point, the cells are still alive but will die afterwards.

In this study niosome encapsulated bromelain has been the effective compound that has reduced IL-6 and TNF-α in LPS induced inflammation in mice's skin around knee. In order to determine the effectiveness of the treatment in mice, the LPS induced inflammation was treated with niosome encapsulated bromelain after 4 hrs induction. The inflammation response was measured at 4 hrs. This study showed that niosome encapsulated bromelain is effective for reducing inflammation after 4hrs post treatment.

Materials and Methods

Chemicals and Mice

All chemicals used in this study were purchased from Sigma Aldrich and Merck unless otherwise noted. Span, Labrasol, Dicetyl Phosphate, Chloroform, used were also purchased from Sigma Aldrich. Bromelain was purchased from Merck while the LPS from *Escherichia coli* (0111:B4) was purchased from Sigma Aldrich. The cytokine IL-6 kit (Catalog Number RAB0307) was purchased from Sigma Aldrich (USA).

The maintenance and Stabilization of Animal (Mice)

All procedures on animals followed the guidelines for animal treatment set by the Laboratory Animal Sciences Association of Malaysia (LASAM) Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. The ICR mice (8–10 week-old; male mice: 28–30 g; female mice: 24–26 g) were purchased from Universiti Kebangsaan Malaysia.

The mice were kept in pairs in small polypropylene cages (14×30×13cm) with wood shavings bedding. The animals were allowed free access to food and water at all times and were maintained on a 12-h light:12-h dark cycle in a controlled temperature (20–25°C) and humidity (50±5%) environment for a week before use.

Inflammation Induction of Mice 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 deionized water.

Then, the stabilized mice were injected intra-articular in to the skin area with different concentrations of LPS (1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12 µg/ml). Deionized water was used as blank while plasma without LPS induction was used as control. After 4 hrs, the blood was taken and plasma was analyzed for IL-6 and TNF-α respond. Three groups of mice were prepared for next experiment.

Results and Discussion

Selection of Suitable Concentration of LPS to Induce Inflammation in Mice

This experiment was carried out to evaluate appropriate concentration of LPS to induce inflammation based on IL-6 respond. Based on *in-vitro* study, 7.5µg/mL and 9 µg/mL LPS were selected for inflammation induction.

Figure 1 shows the respond of IL-6 with different LPS concentrations. The response for 7.5 µg/mL LPS is 6656 pg/mL IL-6 and for 9 µg/mL LPS, it is 7384 pg/mL IL-6. Both concentrations induced inflammation. However 7.5µg/mL was chosen

because at this low concentration it already induced inflammation compare to 9µg/mL. Previous studies have shown that an infusion of 9 µg/mL LPS (*E. coli* 0114) in mice produces, on average, 7,510 pg/mL of IL-1 and 5,980 pg/mL of TNF-α (Suffredini et al., 1995). Therefore, 7.5µg/mL LPS was selected for further experiment.

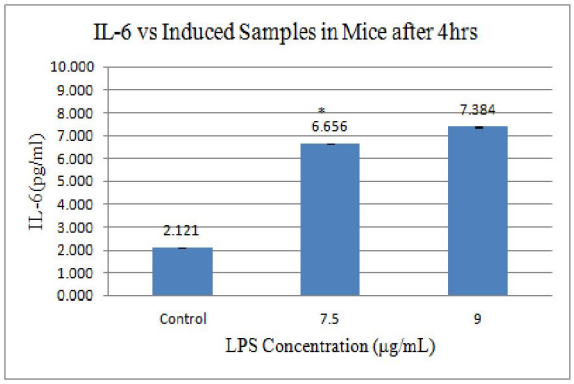

Figure1. IL-6 responds after 4 hours LPS induction in mice

Determination the Effectiveness of Niosome Encapsulated Bromelain on LPS Induced Inflammation

This study was carried out to assess the effectiveness of niosome encapsulated with 10% bromelain in treating inflammation in mice's skin around knee when induced by 7.5µg/mL LPS. To this regards, treatment with niosome encapsulated with 10% bromelain, bromelain alone, base cream, and hydrocortisone as the Nonsteroidal Antiinflammatory Drugs (NSAIDS) was performed.

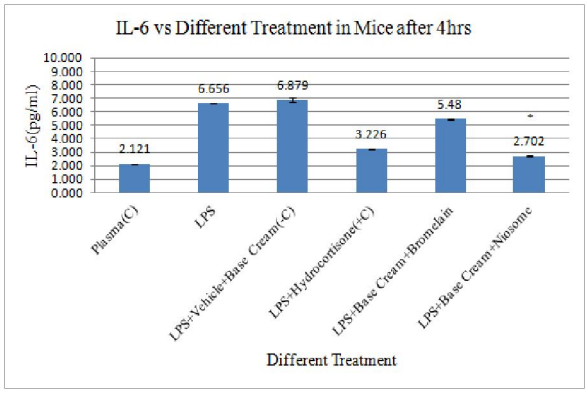

Figure2. IL-6 responds of different treatment after 4h in mice.

Figure 2 shows the IL-6 response against the treatments of niosome encapsulated with 10% bromelain, without bromelain (vehicle), and bromelain alone. As indicated, the responses of niosome encapsulated with bromelain was 2702 pg/mL, bromelain (vehicle) was 6879 pg/mL, and bromelain was 5480 pg/mL. Hydrocortisone gave better result than bromelain since the response was 3226 pg/mL.

The results showed that niosome encapsulated with 10% bromelain had the best treatment on LPS induction and even better than hydrocortisone as a well-known anti-inflammatory drug. On the other hand, experimental effect of niosome alone suggested that the anti-inflammatory properties of bromealain are not dependent on the presence of vehicle (Heuschkel et al., 2008).

There were no significant differences among the replications in different groups. Although preliminary results showed that administration of low dose LPS had no obvious inflammation, it was detected by immune-modulatory regulators. This finding is identical to that reported by Godin et al. (1996) and Van Zee et al. (1995).

Other proinflammatory mediators including chemokines (MIP-2), plasma enzyme mediators, and lipid mediators (COX, PG, and platelet activating factor) may be affected rather than cytokines (Nelson et al., 2002). This mice model is identical to other studies of LPS and thrombotic disease (Weiler et al., 2001).

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 or not. When $P<0.05$, it means that there is a significant difference.

Conclusion

This study has been set out with the aim of assessing the importance of niosome encapsulated with bromelain to eliminate inflammation in mice's skin around knee infected by LPS induce inflammation. The result showed that niosome encapsulated with 10% bromelain has significantly reduced IL-6 in LPS induced in mice after 4hrs post treatment as compared to non-encapsulated bromelain and vehicle.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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