SUPPRESION EFFECT OF THE INNER GEL COMPONNENT OF ALOE VERA ON BACTERIAL INDUCED TNF-α AND IL-1β RELEASE FROM PERIPRHERAL BLOOD LEUKOCYTES

***El-Sakka Mazen** ****Valerie A. Ferro**
***Department of pharmacognosy - Al-Azhar University – Gaza – Faculty of Pharmacy**
****Department of Immunology, University of Strathclyde, SIBS Bulding, 27 Taylor Street, Glasgow, G4 0NR, UK**

Corresponding author: 00972 8 2824020
herbalcenter@hotmail.com

Abstract: *In light of the growing popularity of Aloe vera in complementary medicine, the present study was carried out to examine the anti-inflammatory activity of the inner leaf gel component of Aloe barbadensis Miller. A simple in vitro assay was used to determine the effect of the inner gel on bacterial-induced TNF-α and IL-1β concentrations. The organism used for evaluation was Shigella flexneri, as this is a significant world –wide causative agent of gastrointestinal disease. In addition, bacterial lipopolysaccharide was examined. The results showed that these pro-inflammatory cytokines were suppressed to significant ($p<0.05$) levels using 45mg/ml freeze-dried inner gel. A commercial product, containing 10% (v/v) of the inner gel component had a similar effect. The action of Aloe vera differed from a non-steroid anti-inflammatory drug (ibuprofen), which caused a significant increase ($p<0.05$) in TNF-α and IL-1β production. We therefore propose a significant basis for the therapeutic use of Aloe vera in inflammatory conditions.*

Keywords: Aloe vera / leukocytes/ pro-inflammatory cytokines/ Shigella flexneri/ TNF-α / IL-1β

INTRODUCTION

The aim of this work was to establish the potential anti-inflammatory effectiveness of the inner gel component.

Pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α),

the interleukins (IL-1β, IL-6, IL-8, IL12) and interferon gamma (INF-γ) are systemically elevated during bacteria invasion[1]. In Particular, TNF-α and IL-1β have been shown to play an important role in the inflammatory process in shigellosis caused by *Shigella flexneri* and septic shock.[2-4] *Shigella flexneri* is a non motile rod belonging to the enterobacteriaceae family and the species is regarded as an important cause of gastrointestinal illness, which is manifest by watery diarrhea that progresses to mucoid bloody diarrhea and shigellosis. A histological feature of *Shigella* infection is an increased infiltration of inflammatory cells into the colonic mucosa, particularly of cells producing pro-inflammatory cytokinase.[5-7] Infection is usually restricted to the top layer of colonic mucosa, where it causes ulceration, leading to severe tissue damage.[8] We describe recently the significant inhibition of growth of *Shigella flexneri* using an extract from plant *Aloe vera*.[9] In addition to its anti-microbial properties, *Aloe vera* is believed to have anti-inflammatory activity[10-12] and the aim of this study was to investigate these properties relative to systematic cytokine production.

Aloe vera has been used therapeutically for many centuries and *Aloe barbadensis* Miller or *Aloe vera* L *Burma flexneri*. is of particular interest due to its lengthy historic reputation as a curative agent and its widespread use in complementary therapies.[13] It is a cause –like plant belonging to the family Liliacea family and out of 360 species, only three have so far been attributed to having medicinal properties. Once the outer green cuticle is removed, a preserved but otherwise untreated inner gel is commonly used as a therapeutic agent or as a food supplement.

This has made it difficult to correlate therapeutic benefits to individual active components, even though over 75 active ingredients have been identified from the inner gel, including vitamins, enzymes, mineral, lignin, saponins, sugars, sterols, amino acids, salicylic acid and anthraquinones.[13] Some anti-inflammatory properties have been attributed to salicylates, magnesium lactate acting as an anti-histamine, bradykinin and thromoxane and inhibitors and sterols.[11,14] In addition, the polysaccharide acemannan (a beta linked acetyl mannan) is believed to have immunomodulatory properties, which stimulate or dampen the immune system in response to stimulus.[15,16]

RESULTS

Effect of Aloe vera on TNF-α levels induced from blood cells

Live bacteria (*Shigella flexneri*, 1×10^9 organisms/ml) and LPS from *Shigella* (2μg/ml final concentration and induced TNF-α concentrations of 99.7 ± 17.6ng/ml (figure 1a) and 52.3 ± 4.5ng /ml (figure 1b), respectively.

Untreated control samples showed levels around 2.0 ± 07ng/ml. Freeze-dried *Aloe vera* (examined at 4.5mg/ml and 45mg/ml final concentrations), significantly ($p<0.05$) reduced bacterial induced TNF-α to this base-line level, whereas only 45mg/ml significantly ($p<0.05$) reduced LPS- induced TNF-α to 30.1 ± 6.2ng/ml. *Aloe vera* Gel significantly ($p<0.05$) reduced both LPS and bacterial-stimulated cytokine production to base line levels, at 10% (v/v) final concentrations. Although 5% (v/v) *Aloe vera* Gel reduced the cytokine production significantly ($p<0.05$), it was not to base-line concentrations (25.0 ± 5.2ng/ml and 20.5 ± 0/7ng/ml, in the presence of bacterial and LPS, respectively). In contrast, ibuprofen (50μM) significantly ($p<0.05$) increased the production of TNF-α by 2.4 fold in the presence of LPS and 1.8 fold with live bacteria.

Effect of Aloe vera on IL-1β concentration induced from blood cells

IL-1β concentrations of 26.07 ± 1.4ng/ml (figure 2a) and 19.5 ± 2.23ng/ml (figure 2b) were induced by live bacteria (1×10^3 organisms/ml) and LPS (2μg/ml final concentration), respectively, compared with untreated controls (2.4 ± 0.3ng/ml). AV at 45mg/ml final concentration significantly ($p<0.05$) increased the levels by 1.3 and 1.6 fold with LPS and live bacteria, respectively and only 10% (v/v) *Aloe vera* Gel reduced the cytokine to base-line production.

DISCUSSION

The key cytokines, which mediate this tissue damage include TNF-α and IL-1β. These pro-inflammatory cytokines further activate leukocytes leading to the release of IL-8, which recruits circulatory neutrophils to the site of inflammation[17]. While neutrophils are essential for the innate immune response to bacterial infection, they promote the destruction of the mucosal epithelium via indiscriminate release of respiratory burst component such as H_2O_2 and O^-.[18] Thus, their activation and recruitment to site of inflammation and bacterial colonization must be tightly regulated. Infection with gram-negative bacterial can often results in the overproduction of these pro-inflammatory cytokines, which can eventually lead to septic shock and death.[1] Therefore, in this study, we chose to examine the production of these two cytokines by leukocytes stimulated with bacterial and LPS.

Aloe vera has been described previously as having anti-inflammatory properties. However, controversies have arisen due to the use of variable sources of material and different processing methods of the leaf. In view of the complexities of examining the pharmacology of this plant, simple assays which can be easily replicated are critical to test multiple fractions. This

study has shown clearly that both pro-inflammatory cytokines raised through bacterial stimulation can be suppressed with at least 45mg/ml unfiltered and undiluted inner gel. A ten-fold dilution was only effective against TNF-α. A similar trend was observed with the *Aloe vera* Gel, whereby both concentrations (5% v/v and 10% v/v) tested effectively suppressed TNF-α, but only the higher one showed significant activity against IL-1β.

Overall, the pro-inflammatory response to bacterial stimulation was greater than to the bacterial cell wall component (LPS) and the trends observed were different. Both concentrations of AV were effective against IL-1β and LPS, but only the high concentration suppressed TNF-α in the presence of LPS. Similarly, only the high concentration of AV significantly suppressed IL-1β in the presence of live bacteria, but both concentrations were affective against TNF-α It is conceivable that the multiple components within the bacteria, such as LPS, outer membrane proteins, flagelin and peptidoglycan, activate the cells via a number of different mechanisms, leading to variations in pro-inflammatory response. In addition, the greater response to the whole organism is particularly pertinent to LPS derived from *Shigella* compared with other organisms.[4] The importance of LPS in *Shigella flexneri* infections, is that it is a dominate inducer of PMN transmigration, which helps to further destabilize the epithelium.[4] In addition, LPS activates the nuclear factor kappa B (NFχ B) pathway, an important transcriptional regulator of genes involved in inflammation.[17,19, 20]

While NSAIDS such as aspirin and ibuprofen function as local anti-inflammatory agent and provide pain relief, they actually increase the hosts levels of pro-inflammatory cytokines. This is because they function as cyclooxygenase inhibitors and one of their main effects is to inhibit prostaglandin E_2 (PGE_2) synthesis. Ordinarily, PGE_2 acts as a negative-feedback mechanism to down-regulate TNF-α and IL-1β production, therefore in its absence, pro-inflammatory cytokine levels rise.[21] This is demonstrated with the use of ibuprofen, where the level of TNF-α increased, while treatment of the blood with *Aloe vera* led to a significant reduction in both cytokine levels. Consequently, active ingredients within the inner gel must act via a different mechanism from NSAIDS.

In addition, it is possible that *Aloe vera* exerts its effects through inhibition of pro-inflammatory cytokine gene transcription. For example, the TNF-α promoter possesses NFχB, mitogen activated protein kinase (MAP Kinase) and c-Jun N-terminal kinase (JNK) binding sites[22, 23]. If components from the inner gel can down-regulate any of these pathways, then it would reduce the production of these cytokines. In support of this, recent work has

indicated the isolated compounds from a variety of plants have been able to down-regulate the activation of NFχB, JNK and MAP kinase[24, 25]. Although there has been anecdotal evidence for the anti-inflammatory activity of *Aloe vera*, there has been sparse examination of the mechanism of action, particularly with respect to the inner gel. The next stage of experimentation will be to identity the components within the inner gel with anti-inflammatory therapeutic potential and ascertain, through examination of various signal transduction pathway, how they achieve their effects.

MATERIAL AND METHODS

Materials:

Packed blood cells were obtained from the Scottish Blood Transfusion Service, UK and consisted of partial donation. *Shigella flexneri* (NCTC9950) was purchased from the Public Health laboratory Services (Proton Down, Salisbury, UK), maintained and harvested for experimentation as described previously[9]. Freeze-dried *Aloe vera* inner gel was obtained from the USA, consisting of inner gel, from plants that were approximately 2 years of age at the time of harvest. The lyophilized powder was reconstituted in phosphate-buffered saline (pH=7.5) at 37^{0}C for 30 min. and then sterile filtered through a 0.22μm pore size filter unit (Scheicher and Schuell, Dassel, Germany). A commercial preparation of AV (containing undiluted and unfiltered inner gel from USA) was used for comparison purposes (Forever Living Products, AZ, USA). This was filtered through a sterile Whatman no.54 filter and it was not possible to determine the amount of active material lost by this process. Ibuprofen, LPS and RPMI-1640 medium were purchased from Sigma-Aldrich Ltd, UK and filter sterilized through a 0.22 μm pore size filter unit. Cytokine ELISA kits (Diaclone Research) were kindly donated by IDS Ltd, UK. Plastic ware was obtained from Greiner Bio-one Ltd, UK, 96 well tissue culture plates from TRP, Finland and Immuno-Nunc strips from Nunc Ltd, UK. All reagents were obtained from BDH Ltd, UK.

In vitro blood assay to assess anti-inflammatory response:

Packed blood cells (900μl) were incubated with a range of bacterial numbers (100μl) to establish the optimum number of *S. flexneri* needed to give detectable levels of TNF-α and IL-1β in sterile Eppendorf tubes (n=3) for 21h at 37^{0}C, 100% humidity and 5% CO_2. Alternatively, LPS at 2μg/ml was incubated instead of the live organism. The samples were vortexes and centrifuged at 5000g for 4 min. and the plasma decanted into fresh tubes and stored at -20^{0}C until assayed by ELISA, following the manufacturer's

instructions. For the TNF-α and IL-1β assays, 1×10^9 and 1×10^3 respectively *S. flexneri* were consistently found to give detectable results, expressed as the mean of triplicate readings ± SD.

A preliminary ELISA was carried out with various plasma dilutions (using diluted buffer) for each new donation of blood. In general, for the TNF-α and IL-1β assays, a 1: 100 and 1: 200 dilution respectively were found to given values, which fell within the standard curve.

Assessment of Aloe vera on cytokine production

The assay was set up as above in sterile Eppendorf tubes with 100µl AV (4.5 or 45mg/ml) and *Aloe vera* Gel (5% v/v or 10% v/v) at 37ºC, 5% CO_2 and 100% humidity. At the end of the incubation period, the blood was vortexed and a drop smeared onto sterile glass slides. The morphology of the cells was examined by light microscopy in order to verify that the concentrations of *Aloe vera* did not cause osmotic stress, while the supernatant was assayed by ELISA for cytokine determination.

Statistical analysis:

A non-parametric Mann-Whitney U-test was carried out, performed on Stat view, v.5.1 software.

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Figure legends

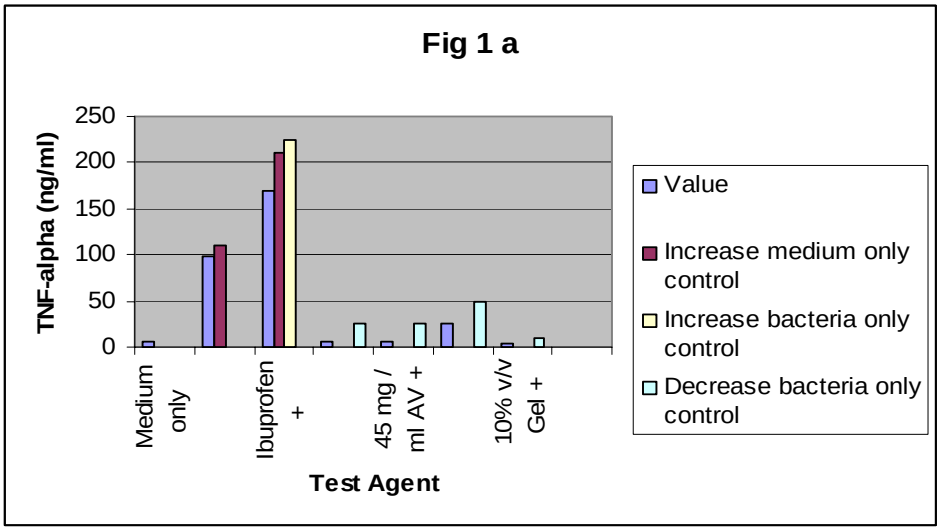

Figure 1a TNF-α (ng/ml)

Figure 1a: Production of TNF-α from peripheral blood leukocytes stimulated with 1×10^9 *S. flexneri* /ml for 21h at 37°C, 5% CO_2 and 100% humidity. Test agents consisted of 50μM ibuprofen, freeze-dried inner gel from *Aloe vera* (AV, 4.5 and 45mg/ml) and *Aloe vera* Gel (Gel, 5% v/v and 10% v/v). The plasma was assessed by ELISA and the concentration of cytokine calculated from the A_{450}. The results are expressed as the mean of triplicate readings ± S.D, n=3, *$p<0.05$ = significant increase in TNF-α compared with medium only control, **$p<0.05$ = significant increase in TNF-α compared with the bacteria only control, *** $p<0.05$ = significant decrease in TNF-α compared with the bacteria only control.

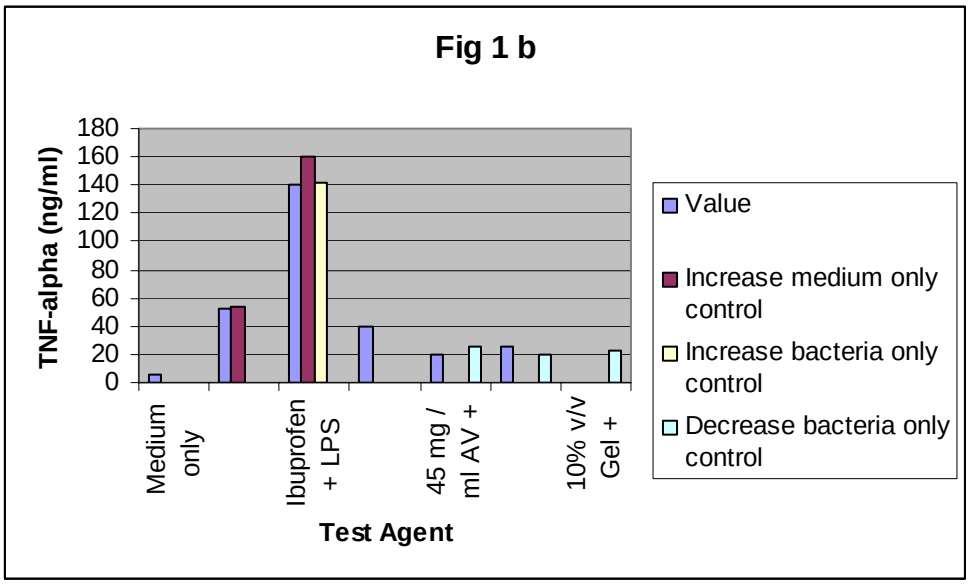

Figure 1b TNF-α (ng/ml)

Figure 1b: Production of TNF-α from peripheral blood leukocytes, stimulated with LPS (2μg/ml) for 21h at 37°C, 5% CO_2 and 100% humidity. Test agents consisted of 50μM ibuprofen, freeze-dried inner gel from *Aloe vera* (AV, 4.5 and 45mg/ml) and *Aloe vera* Gel (Gel, 5% v/v and 10% v/v). The plasma was assessed by ELISA and the concentration of cytokine calculated from the A_{450}. The results are expressed as the mean of triplicate readings ± S.D, n=3, *$p<0.05$ = significant increase in TNF-α compared with medium only control, **$p<0.05$ = significant increase in TNF-α compared with the LPS only control, *** $p<0.05$ = significant decrease in TNF-α compared with the LPS only control.

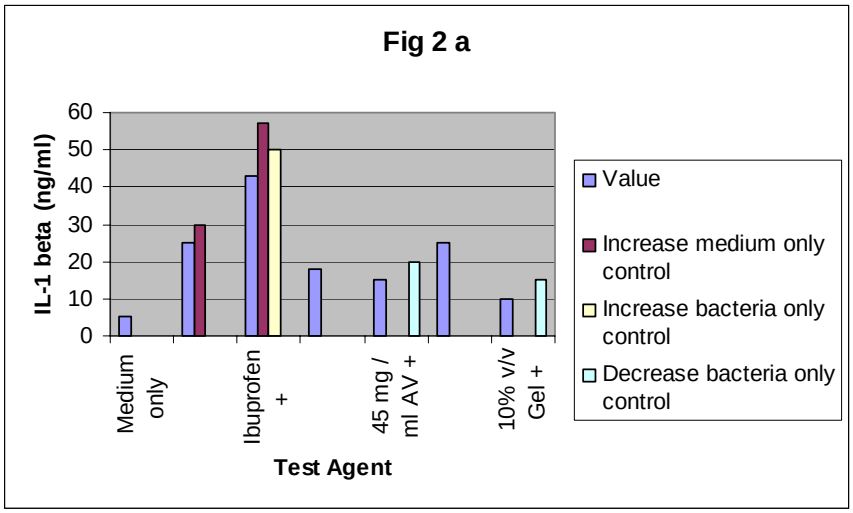

Figure 2a IL-1β (ng/ml)

Figure 2a: Production of IL-1β from peripheral blood leukocytes stimulated with 1×10^3 *S. flexneri* /ml for 21h at 37°C, 5% CO_2 and 100% humidity. Test agents consisted of 50μM ibuprofen, freeze-dried inner gel from *Aloe vera* (AV, 4.5 and 45mg/ml) and *Aloe vera* Gel (Gel, 5% v/v and 10% v/v). The plasma was assessed by ELISA and the concentration of cytokine calculated from the A_{450}. The results are expressed as the mean of triplicate readings ± S.D, n=3, *$p<0.05$ = significant increase in IL-1β compared with medium only control, **$p<0.05$ = significant increase in IL-1β compared with the bacteria only control, *** $p<0.05$ = significant decrease in IL-1β compared with the bacteria only control.

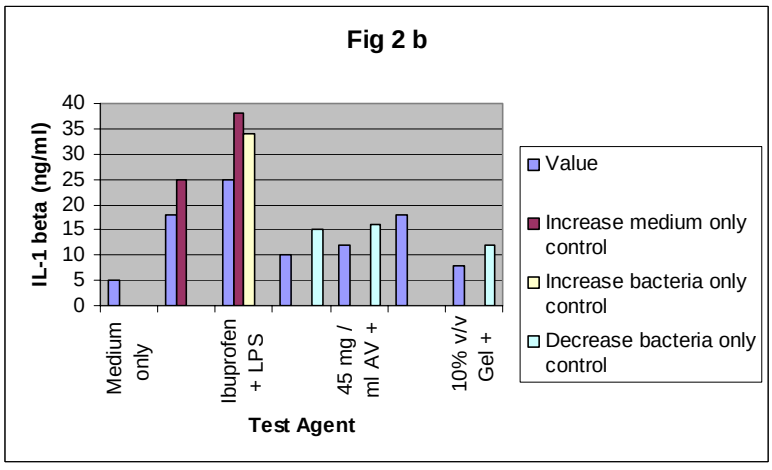

Figure 2b IL-1β (ng/ml)

Figure 2a: Production of IL-1β from peripheral blood leukocytes stimulated with LPS (2μg/ml) for 21h at 37^{0}C, 5% CO_2 and 100% humidity. Test agents consisted of 50μM ibuprofen, freeze-dried inner gel from *Aloe vera* (AV, 4.5 and 45mg/ml) and *Aloe vera* Gel (Gel, 5% v/v and 10% v/v). The plasma was assessed by ELISA and the concentration of cytokine calculated from the A_{450}. The results are expressed as the mean of triplicate readings ± S.D, n=3, *p<0.05 = significant increase in IL-1β compared with medium only control, **p<0.05 = significant increase in IL-1β compared with the LPS only control, *** p<0.05 = significant decrease in IL-1β compared with the LPS only control.

REFERENCES

Karima R, Matsumoto S, Higashi H, Matsushima K (1999) The molecular pathogenesis of endotoxic shock and organ failure, *Molecular Medicine Today* 5:123-132.

Luo G, niesel DW, shaban RA, Grimm EA, Kilmpel GR (1993) Tumor necrosis factor alpha binding to bacteria: evidence for a high-affinity receptor and alteration of bacterial virulence properties. Infect immune 61: 830-835.

Raqib R, Lindberg AA, Wretlind B, Bardlhan PK, Andersson U, Andersson J (1995) persistence of local cytokine production in shigellosis in acute and convalescent stages. Infect Immun 36:289-296.

Beatty WL, Sansonetti PJ (1997) Role of lipopolysaccharide in signaling to subepithelial polymorphonuclear leukocyets. Infect immun65: 4395-4404.

Raqib R, wrtlind B, lindberg AA, ljundahl A, Andersson j(1996) pathogenesis and immune responses in shigellosis.Ann NY Acad Sci 797:299-301.

Perdomo OJ, Cavaillon JM, Huerre M, OhayoH Gounnon P sansonetti PJ (1994) Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis.J Exp Med 180: 1307-1319.

Sansonetti PJ, Arondel J, Cavaillon JM, (1995)Role of interleukin-1in the pathogenesisof experimental shigellosis.J Clin Invest 96:884-892.

Islam MM< Azad AK, Bardhan PK, Raqib R, Islam D (1994) pathology of shigellosis and its complications. Histopathology 24:65-71.

Ferro VA, Bradbury F, Cameron P, Rahman SR, Sitmson WH (2003) in vitro susceptibilities of Shigella flexneri and streptococcus pyogenes to inner Gel of Aloe barbadensis Miller. Antimicrob Agents Chemother 47: 1137-.

DVIS RH, Donato JJ, Hartman GM,Haas RC (1994) Anti-inflammatory and wound healing activity of a growth substance in Aloe vera. J Am podiatr Med Assoc 84:77-81.

Vazquez B, Avila G, Segura D, Escalante B (1996) Antiinflammatory

activity of axtracts from Aloe vera gel. J Ethnopharmacal 55:69-75.
Yagi A, Kabash A, Okammura N, Haraguchi H, Moustafa SM, Khalifa TI (2002) Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in Aloe vera Planta Med 68:957-960.
Reynolds T, Dweck AC (1999)Aloe vera leaf gel: a review update. J Ethnopharmacol 68: 3-37.
Shelton RM (1991) Aloe vera. Its chemical and therapeutic properties.int J Dermatol 30: 679-683.
lee JK, lee MK, yun YP, Kim Y, Kim JS, Kim K, Han SS, Lee CK (2001) Accemannan purified from Aloe vera induces phenotypic and functional maturation of immature dendritice cells.Int Immunopharmacal1:1275-1284.
Robinson M (1997) Optimizing therapy for inflammatory bowel disease.Am J Gastroenterol 92:12S-17S.
Philpott DJ, Yamaoka S, Israel A, Sansonetti PJ (2000) Invasive Shigella flexneri activates NF-kappa B through a lipopysaride-dependent innate intracellular response and leads toIL-8 expression in epithelial cells.J Immuol 165:903-914.
varani J, Word PA (1994)Mechanisms of rndothelial cell injury in acoute inflammation. Shock 2:3 311-319.
shakhov AN, Collart MA, Vassali, P, nedospasov SA, Jongeneel CV (1990) Kappa B- type enhancers are involed inlipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. J Ezp Med 171:35-47.
Mastronarde JG, HR B, Monik MM Mukaid n, Matsushima k, Hunninghake GW (1996) Induction of intereleukin (IL)-8 gene expression by repiratory syncytial virus involves activation of nuclear factor (NF)-kappa B and NF-IL-6. J Infect Dis 174:262-267.
spinas GA, Bloesch D, Bloesch D, Keller U, Zimmerli W, Cammisuli S (1991) pretreatment with ibuprofen augments circulating tumor necroses factor-alpha, interleukin-6, and elastase during acute endotoxinemia.J infect Dis 163:89-59.
liu B, Whisler RL(1998) transcriptional activation and redox regulation of the tumor necrosis factor-alpha promoter in human T cells: role of the CRElkappa3 promter region interferon cytokine res18:999-1007.
newell CL, Deisseroth AB, Lopez-Berestin G (1994) interation of nuclear protins with an AP-1/CRE-like promoter sequence in the human TNF-alpha gene. JLeukoc Biol 56:27-35.
chainy GB, Manna SK, Chaturvedi MM.Aggarwal BB (2000) Anethole blocks both early and late celluar responees transduced by tumor necrosis factor on NF-kappaB, AP-1,JNK,MAPKK and apoptosis. Ooncogene 19:2943-2950.

Bremner P, Heinrich M (2002) Natural products as targeted modulators of the nuclear factor-kappaB pathway. J pharm pharmacaol 54:453-472.