

Prophylactic Aloe Components on Autoimmune Diseases: Barbaloin, Aloe-Emodin, Emodin, and Fermented Butyrate

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ABSTRACT

The involvement of *Aloe* plants in the remedy of chronic diseases has been well publicized in the past. In this review paper, we attempted to focus on the major *Aloe* ingredients on their known efficacy related to the immune modulating activities. Several bioactive phytochemicals present in *Aloe barbadensis* (*vera*) are known to relieve rheumatoid arthritis (RA)-related pains by suppressing chronically over-activated inflammatory process. *Aloe*-emodin has been shown to promote natural killer cell and macrophage phagocytic activities in the tumor, and emodin, an anthraquinone found also in other plants, appeared to have some protective anti-inflammatory effect by showing its inhibition of IL-1 β and LPS-stimulated

proliferation of RA synoviocytes in a dose-dependent manner under hypoxic condition. Another putative immune modulator we reviewed in this paper is *Aloe*'s butyrate fermented by the dietary *Aloe* in the gut. Its beneficial effects are based on the host immune metabolism via the activation of intestinal mucosal functions on the suppression of obesity and inflammation. It is hoped that elaboration on butyrate may provide fresh insights as to *Aloe*'s immune modulation and chronic inflammation. Potential efficacious role of barbaloin, *Aloe*-emodin, emodin and fermented butyrate of *Aloe vera* needs further scrutiny and evidence-based documentation through carefully designed research.

Key words: *Aloe* barbadensis; Barbaloin; *Aloe*-emodin; Emodin; Fermented butyrate; Autoimmune-diseases

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic, systemic inflammation which primarily effects on synovial joints. A good number of anti-inflammatory or immunomodulatory plant extracts and phytochemicals were studied in the conditions related to RA. One of the good examples in this regard is barbaloin in *Aloe barbadensis* (*A. vera*). Barbaloin (Bar), a mixture of barbaloin and isobarbaloin, is metabolized into *Aloe*-emodin anthrone and *Aloe*-emodin dianthrone by intestinal bacteria under anaerobic conditions^[1]. Bar is inactive as a laxative but activated to *Aloe*-emodin anthrone to transform to a strong purgative component by a human intestinal anaerobe *Eubacterium* sp. Bar^[2]. Based on "*Aloe vera* bioassay"^[3,4], International *Aloe* Science Council has established the quality standard of < 10 mg of Bar showing carcinogenic activity per liter of *Aloe vera* juice products for safe oral consumption. The intestinal uptake and metabolism of physiologically active Bar and

Aloe-emodin was demonstrated using the Caco-2 and everted gut sac model. The % absorption of Bar and *Aloe-emodin* was ranged from 5.51% to 6.60%, 6.60% to 11.32%, respectively. Up to 18.15% of Bar and 18.11% of *Aloe-emodin* are absorbed as a glucuronidated or sulfated form. These results suggest that a significant amount is transformed during absorption^[5]. Bar content of the leaves of *A. barbadensis* and *A. arborescens* were determined by HPLC. Results showed that the Bar content was high in *A. arborescens* (0.602%) and low in *A. barbadensis* (0.266%)^[6]. For the removal of Bar, various treatments are utilized: the activated charcoal adsorption process and cellulase process for viscosity reduction. Distinct biological and chemical changes are taken place by going through both the charcoal and cellulase processes. The activated charcoal adsorption process that prevents color changes and reduces Bar content can inadvertently cause the loss of some loss of bioactive compounds. In addition, cellulase is routinely used for viscosity reduction. Although it is well known that extracts of *Aloe* leaf gels can exert significant activities for macrophages and other defensive immunologic cells, unfortunately, the *Aloe* preparations used for many previous studies poorly defined mixtures, making difficult to pinpoint active ingredients and elucidate the biological pathways involved. The status of bacterial growth on *Aloe* plant and products needs a careful consideration for the preparation, sanitization, and pasteurization processing, because of their influence on the efficacy of the finalized products. On the microbiology of *Aloe vera*, one should be cognizant of two sources: endogenous and exogenous microbiota. Exogenous microbiota can be picked up from the environment during industrial processing. Endophytes, endogenous microorganisms include bacteria and fungi living within plant tissues without causing any overt negative effects. It is important to note that endophytic bacteria in *Aloe vera* gel provided butyric acid in *in vitro* fermented broth extract^[7,8], which will be described in the later section.

This present review focuses on descriptions of the potential efficacious role of barbaloin, *Aloe-emodin*, emodin, and fermented butyrate of *Aloe vera* based on what have been documented by previously published data.

IMMUNE MODULATION BY BARBALOIN, ALOE-EMODIN, AND EMODIN IN TREATMENT OF RHEUMATOID ARTHRITIS (RA)

It has been reported that emodin in *Aloe vera* latex inhibits various inflammation kinases and signaling pathways *in vitro* and *in vivo* conditions as an immunomodulatory agent. The potential immunosuppressive mechanism of emodin may be the suppression of lymphocyte proliferation and influences on cytokines. The putative therapeutic benefits of *Aloe vera* in treatment of RA and the efficacy of a dietary plant-derived polysaccharide supplement in patients have been reviewed^[9]. For the suppression of inflammation, the *Aloe vera*-mediated reduction of IL-1 β was shown to be the consequence of the reduced expression of both pro-IL-1 β as well as the most versatile and important NLRP3 inflammasome (more details on inflammasome later) via suppressing the pro-inflammatory signaling transduction pathway. The downregulated expression of the ATP sensor, P2X7R by *Aloe vera* may contribute to the attenuated IL-1 β cytokine secretion. These data could serve as a basis for new therapeutic opportunities on the modulation of inflammasome-mediated responses^[10]. Emodin, one of *Aloe vera* and *A. arborescens* ingredient, significantly inhibited IL-1 β and LPS-stimulated RA synoviocytes in a dose-dependent manner

under hypoxic condition. Furthermore, emodin is known to inhibit histone deacetylase (HDAC) as well as suppresses the expression of HDAC1, but not HDAC2 in IL-1 β , and LPS-treated synoviocytes under hypoxia. These findings strongly indicate that emodin inhibits pro-inflammatory cytokines and production of vascular endothelial growth factor, and HDAC1 activity in hypoxic RA synoviocytes^[11]. Recently, Han et al reported that emodin ameliorated the severity of NLRP3 inflammasome-mediated symptoms in LPS-induced endotoxin mouse models. The anti-inflammatory effect of emodin through attenuation of NLRP3 inflammasome activation was determined as the underlying mechanism. This is the first report providing scientific evidence substantiating the use of emodin in medicine for the treatment of various inflammatory diseases through the regulation of inflammasome activation. This approach may well provide a plausible therapeutic strategy for controlling other related inflammasome-mediated pathological conditions^[12].

It is known that *Aloe-emodin* inhibits dose-dependently inducible nitric oxide synthase (iNOS) mRNA expression and nitric oxide (NO) production at 5-40 μ M. In addition, the levels of COX-2 mRNA and PGE₂ production are also suppressed by 40 μ M *Aloe-emodin*. Bar (barbaloin, aloin) also suppresses the production of NO at 5-40 μ M, while no PGE₂ production is affected. These results indicate that aloin and *Aloe-emodin* may suppress the inflammatory responses by blocking iNOS and COX-2 mRNA expression. *Aloe-emodin* was shown as a potential key constituent responsible for the anti-inflammatory activity of *Aloe*. *Aloe-emodin* suppresses the production of NO, IL-6, and IL-1 β in LPS-stimulated RAW264.7 cells with no apparent cytotoxicity^[13]. These data indicate that *Aloe-emodin* is a bioactive component that confers an anti-inflammatory effect through the mechanisms that modulate the pro-inflammatory cytokine production via inhibition of NF- κ B, MAPK, and PI3K pathways in LPS-induced RAW264.7 macrophages^[14]. IL-1 β activation involves reactive multiprotein complexes called inflammasomes. One of the most intensively studied inflammasome complexes is the NLRP3 inflammasome. Its activation requires two signals in that one signal primes the cells, inducing the expression of NLRP3 and pro-IL-1 β , while the other signal, leading to the assembly and activation of the complex. The effect of plant-derived natural compounds including *Aloe-emodin* and emodin was demonstrated on suppressing NLRP3 inflammasome-mediated IL-1 β production^[15].

RA is a chronic inflammatory disease characterized by synovial hyperplasia. Methotrexate (MTX), an anti-folate derivative, is used for the treatment of RA, as it exerts anti-proliferative effects on lymphocytes and synovial cells. The effect of *Aloe-emodin* on the proliferation and apoptosis of MH7A human RA synovial cells was examined by comparing with that of MTX^[16]. These results indicate that ≥ 10 μ M *Aloe-emodin* and ≥ 0.05 μ M MTX effectively decreased the numbers of viable MH7A cells. In addition, 40 μ M *Aloe-emodin* and 1 μ M MTX induced apoptosis in MH7A cells. Cell cycle analysis revealed that ≥ 20 μ M *Aloe-emodin* induced G2/M phase arrest, whereas ≥ 0.1 μ M MTX induced S phase arrest. These observations suggested that *Aloe-emodin* treatment inhibited the growth of MH7A cells by arresting the cell cycle at a different checkpoint in contrast to MTX treatment. These data support the notion that *Aloe-emodin* could be used as a potential therapeutic agent for the treatment of RA, and may be used in conjunction with MTX, based on its anti-proliferative effect on synovial cells^[16].

The endochondral bone formation is the process by which mesenchymal cells condense into chondrocytes, which are ultimately responsible for new bone formation. Interestingly, the effect of *Aloe-emodin* on chondrogenic differentiation in clonal mouse

chondrogenic ATDC5 cells was demonstrated. *Aloe-emodin* induced the synthesis of matrix proteoglycans and increased the activity of alkaline phosphatase. *Aloe-emodin* also enhanced the expressions of chondrogenic marker genes such as collagen II, collagen X, BSP, and RunX2 in a time-dependent manner, and increased the activation of the extracellular signal-regulated kinase (ERK), and enhanced the protein expression of the bone morphogenetic protein (BMP-2) in a time-dependent manner. These results showed that *aloe-emodin* exhibits chondro-modulating effects via the BMP-2 or ERK signaling pathway^[17]. Several publications reported the therapeutic possibility of *Aloe-emodin* as well in neurodegenerative conditions^[18,19].

Amyloid aggregation is linked to a number of neurodegenerative syndromes, the most prevalent one being Alzheimer's disease (AD). The β -amyloid peptides (A β) aggregate into oligomers, protofibrils, and fibrils and eventually into plaques, which constitute the characteristic hallmark of AD. The *in silico* and *in vitro* results provide useful insights for the design of small-molecule, 9, 10-anthraquinone inhibitors of aggregation with therapeutic potential in AD^[18]. *Aloe-emodin* acts as an inhibitor of hemoglobin (Hb) aggregation. Increasing concentration of *Aloe-emodin* partially reverses the aggregation of the model hemeprotein (hemoglobin). The study serves as baseline for translatory research and development of *Aloe-emodin* based therapeutics for diseases attributed to protein aggregation^[19]. Protein fibrillation is the pathological hallmark of several neurodegenerative diseases such as Alzheimer's and Parkinson diseases. In a case study, the inhibitory activity of barbaloin against insulin fibrillation was investigated. The degradation products of barbaloin formed over weeks of storage, in particular oxidation products, were able to significantly inhibit insulin-fibrillation^[20].

ROLES OF BUTYRATE AND GUT MICROBIOTA IN COMPROMISED IMMUNE SYSTEM AND AUTOIMMUNE DISEASES

Gut microbiota-derived short-chain fatty acids (SCFAs) regulating T cells

Gut commensal microbes shape the mucosal immune system by regulating the differentiation and expansion of several types of T cell. Treatment of naive T cells under the regulating T cell (Treg)-polarizing conditions with butyrate enhanced histone H3 acetylation in the promoter and conserved non-coding sequence regions of the Foxp3 locus, suggesting a possible mechanism for how microbial-derived butyrate regulates the differentiation of Treg cells. A new insight into the mechanisms by which host-microbe interactions establish immunological homeostasis in the gut was revealed^[21]. Treg cells (Tregs) that express the transcription factor Foxp3 are critical for regulating intestinal inflammation. It was shown that SCFAs, fermentation products from gut bacteria regulate the size and function of the colonic Treg pool and protect against colitis in a Ffar2-dependent manner in mice. A class of microbial metabolites underlies adaptive immune microbiota's coadaptation and promotes colonic homeostasis and health^[22]. Various factors such as antigens, co-stimulation signals, and cytokines regulate T cell differentiation into functionally specialized effector and Tregs. Other factors such as nutrients and microbial products provide important environmental cues for T cell differentiation. A mounting body of evidence indicates that the microbial metabolites, SCFAs have profound effects on T cells and directly and indirectly regulate their differentiation. A review by Kim *et al.*^[23] summarized well about the current understanding of SCFA functions in regulation of peripheral T cell activity on tissue inflammation^[23]. Metagenomic shotgun sequencing

and a metagenome-wide association study of fecal, dental and salivary samples from a cohort of RA subjects and healthy controls were carried out. Results revealed specific alterations in the gut and oral microbiomes in individuals with RA, suggesting feasible ways of using microbiome profile for prognosis and diagnosis^[24]. Another interesting aspect of SCFAs is the inhibition of histone deacetylase (HDAC) in T cells by increasing the acetylation of p70 S6 kinase and phosphorylation rS6, to regulate the mTOR pathway for the generation of Th10, Th1, and IL-10 (+) cells. Butyrate promotes T cell differentiation into both effector and regulatory T cells for boosting either immunity or immune tolerance depending on immunological milieu^[25]. The interaction between genetic predisposition and environmental factors are of great significance in the pathogenesis and development of autoimmune diseases (AIDs). Epigenetic modification triggered by environmental factors is an important mechanism that leads to altered gene expression. Studies showed that human microbiota and their metabolites can regulate immune cells and cytokines via epigenetic modifications. SCFAs produced by gut microbiota promote the differentiation of naive T cell into Treg by suppressing HDACs. Dysbiosis and resulting metabolites may cause aberrant immune responses in AIDs via epigenetic modifications, thereby leading to AIDs. The studies aiming at the crosstalk between human dysbiosis and epigenetic modifications and their influences on AIDs will facilitate the understanding and better managing of these debilitating AIDs^[26].

Effects and mechanisms underlying oral supplementation with butyrate on experimental murine colitis were evaluated. Butyrate inhibited pro-inflammatory cytokine production in RAW 264.7 cells. Butyrate attenuated both the LPS-induced degradation/phosphorylation of I κ B α and DNA binding of NF κ B and enhanced histone H3 acetylation. Oral supplementation with butyrate suppressed colitis, even in IL-10^{-/-} mice. Furthermore, butyrate attenuated I κ B α phosphorylation and histone H3 deacetylation in the colon. The anti-inflammatory effect of butyrate is IL-10 independent, and butyrate may therefore be a therapeutic agent for colitis^[27]. The microbiota in multiple sclerosis patients, an autoimmune disease targeting the myelin sheath of the central nervous system, is characterized by a reduction of bacteria belonging to *Clostridium clusters* IV and XIV-a, which are potent producers of butyrate through fermentation of indigestible carbohydrates. Oral administration of SCFAs ameliorated the disease severity of systemic autoimmune inflammatory conditions mediated by lymphocytes such as experimental autoimmune encephalitis and collagen-induced arthritis. Amelioration of disease was associated with a reduction of Th1 cells and an increase in regulatory T cell. In contrast, SCFAs contributed to the exaggeration of K/BxN serum transfer arthritis, representing the effector phase of inflammation in rheumatoid arthritis. Further understanding of the effects of microbiota metabolites will lead to prevention of systemic inflammatory disorders^[28]. Better understanding is needed for defining roles of individual HDAC isozymes and effects of HDAC inhibitors on bone cells, like osteoblasts, osteoclasts, and osteocytes, in bone remodeling due to pathological bone loss, including periodontitis, rheumatoid arthritis and myeloma bone disease^[29]. HDAC inhibitor can regulate bone remodeling by suppressing osteoclasts and promoting osteoblasts. Novel isozyme-specific HDAC inhibitors are critical to elucidate HDAC's role in bone remodeling and communication among bone cells. The adaptive immune response in RA is influenced by an interaction between host genetics and environment, particularly the host microbiome. Chen *et al.* identified the gut microbiota profile in patients with RA and found decreased

species richness (α -diversity) that positively correlated with increased rheumatoid factor levels and disease progression^[30]. The rheumatoid factor, C-reactive protein, disease progression and methotrexate treatment correlated with β -diversity found in the gut microbiota of patients with RA, suggesting that these clinical outcomes might play an important role in gut microbiota modulation^[31].

PROTECTIVE ROLE OF INTESTINAL MICROBIOTA IN GOUT, AMYOTROPHIC LATERAL SCLEROSIS, ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

1. NLRP3 inflammasome in gout:

Recent studies have implicated IL-1 β as a key regulatory pro-inflammatory cytokine in gout, by promoting a neutrophil influx into the synovium and joint fluid, which is the pathological hallmark of an acute inflammatory attack. This IL-1-dependent innate inflammatory phenotype, which is observed in a number of diseases in addition to gout, is now understood to rely on the formation of the macromolecular NLRP3 inflammasome complex in sensing to the MSU (monosodium urate) "danger signal". Current understanding of the NLRP3 inflammasome and its critical role in MSU-crystal induced inflammatory gout attacks was highlighted by Kingsbury *et al*^[32]. Butyrate exhibit its anti-inflammatory effects mainly through the underlying mechanisms of inhibition of HDACs. The additional suppression action of butyrate was reported on MSU-induced cytokine production and its inhibition of specific HDAC. These novel HDAC inhibitors could even improve the efficacy as well as reducing adverse effects^[33]. More recently, Vieira *et al*^[34] reported that the effects of a high-fiber diet and acetate generated from the metabolism of fiber by gut microbiota, promote resolution of neutrophilic inflammation in an experimental model of gout in mice. It was also found that acetate is effective, even when given after injection of MSU crystals at the peak of the inflammatory response and induced caspase-dependent apoptosis of neutrophils that accounted for the resolution of inflammation^[34].

2. Butyrate as a therapeutic agent for restoring Amyotrophic lateral sclerosis (ALS)–related dysbiosis:

ALS is a serious neurodegenerative disease characterized by the progressive loss of motor neurons. It was shown recently by Zhang *et al*^[35] that G93A transgenic mice fed with butyrate, intestinal microbiota homeostasis was restored, gut integrity was improved, and lifespan was prolonged compared with those in control mice. At the cellular level, abnormal Paneth cells-specialized intestinal epithelial cells that regulate the host-bacterial interactions-were significantly decreased in the ALS mice treated with butyrate. In both ALS mice and cultured human intestinal epithelial cells, butyrate treatment decreased aggregation of the G93A superoxide dismutase 1 mutated protein. These findings highlight the complex role of the gut microbiome and intestinal epithelium in the progression of ALS and present butyrate as a therapeutic potential for restoring ALS-related dysbiosis^[35].

3. The role of SCFAs in Alzheimer's disease (AD)-type β -amyloid (A β) aggregation:

The presence of the individual differences in the human gut microbiota may lead to interpersonal variation to benefit from the protective effects of dietary fiber and polyphenols in AD. Such findings provide critical information for developing probiotics

to help prevent and/or treat of AD^[36]. Multiple complementary assays were used to investigate individual SCFAs for their responsive effects in interfering with the assembly of A β 1-40 and 1-42 peptides into soluble neurotoxic A β aggregates. It has been proposed recently that intestinal microbiota may help protect against AD, in part, by supporting the generation of selective SCFAs interferes with the formation of toxic soluble A β aggregates^[37]. Aggregation of proteins that is an aspect of the physiological process is considered as the major causative culprit underlying pathophysiology of several maladies including diabetes mellitus, Huntington's disease and AD. *Aloe-emodin* and *emodin*, which are active components of *Aloe vera* and *A. arborescens*, act as inhibitors of hemoglobin aggregation^[18,19]. Combination of *Aloe-emodin* and *emodin* together with butyrate fermented in *Aloe vera* show intriguing insights into the design of small molecule inhibitors of aggregation with therapeutic potential for AD.

4. The contribution of SCFAs to gastrointestinal dysmotility in Parkinson's disease (PD):

Investigation of anti-parkinsonian effect of *Aloe vera* revealed the possibility of *Aloe vera's* efficacy on haloperidol-induced experimental animal model, suggesting *Aloe vera* could serve as a good alternative in improving to dysmotility of PD^[38]. α -Synuclein (α -Syn) damage and its deposition cause neurodegeneration in the brain resulting in PD. The abnormal α -Syn accumulation has been identified in the biopsies within colon and rectum of PD patients. Colonization of α -Syn-overexpressing mice with microbiota from PD-affected patients enhances physical impairments compared to microbiota transplants from healthy human donors. α -Syn is considered a major culprit in PD pathophysiology^[39]. Recent evidence suggests that α -Syn may play a role in transcription regulation, possibly by modulating the acetylation of histones. As turn out, increased α -Syn expression led to reduced levels of acetylated histone 3 in dopaminergic neuronal cells. Importantly, treatment with sodium butyrate rescued wild-type α -Syn-induced DNA damage, possibly via upregulation of genes involved in DNA repair. These findings provide novel insights into the mechanisms associated with α -Syn neurotoxicity in DNA repair^[40]. Patients with PD frequently have gastrointestinal symptoms (e.g. constipation) and exhibit the PD-typical pathologic histology in the enteric nervous system. Recent reports show the association between PD and the abundance of certain gut microbiota. Alterations in gut microbiota might be accompanied by altered concentrations of SCFAs. The reduction in fecal SCFA concentrations might induce alterations in the enteric nervous system, contributing to gastrointestinal dysmotility in PD^[41].

IMPORTANCE OF GUT MICROBIAL METABOLITES BUTYRATE AND HOST IMMUNE RESPONSES

Gut butyrate has been suggested to promote the development of the intestinal barrier. The molecular mechanisms underlying this interesting butyrate's action on the intestinal barrier were demonstrated by the regulation of the assembly of tight junctions involving the activation of the AMP-activated protein kinase^[42]. The effects of butyrate on colonic mucus glycoprotein (mucin) synthesis have been assessed using tissue from colonic resection samples. The marked increase in mucin synthesis by butyrate suggests its important role affecting the rate of mucin synthesis *in vitro* and may also explain the therapeutic effect of butyrate in colitis^[43].

Lin *et al*^[44] recently reviewed the findings on the mechanisms

underlying the interaction between microbiota and products with host immunity and regulating imbalanced gut microbiota (dysbiosis) which often lead to autoimmune disorders, like inflammatory bowel disease, type-1 diabetes and systemic immune syndromes such as RA^[44]. SCFAs were shown to directly activate G-protein-coupled-receptors, and inhibit HDACs, and also serve as energy substrates. Butyrate, acting as a HDAC inhibitor and ligands for G-protein-coupled receptors, is considered as a crucial signaling molecule affecting host immune responses^[45]. The immunological data in the literature strongly implicate a close association between gut microbiota and RA. It became increasingly clear that gut microbiota contributes to the pathogenesis of RA via multiple molecular mechanisms. A better understanding of the dynamic nature of the interaction between gut microbiota and their host will help greatly in establishing a highly individualized management RA patient^[46]. The protective effects of SCFAs on bone mass are associated with inhibition of osteoclast differentiation and bone resorption *in vitro* and *in vivo*, while bone formation is not affected. Mechanistically, propionate and butyrate induce metabolic reprogramming of osteoclasts resulting in enhanced glycolysis at the expense of oxidative phosphorylation, thereby downregulating essential osteoclast genes such as TRAF6 and NFATc1. These data pinpoint SCFAs as potent regulators of osteoclast metabolism and bone homeostasis^[47].

PUTATIVE ANTI-INFLAMMATORY ACTION OF ALOE VERA SUPPLEMENTATION

In our previous study, we found that the inner gel of *Aloe vera* facilitates fermentation with endophytic bacteria in bacterial growth promotion, and butyrate was identified by GC/MSD analysis from ether extract of the gel fermentation broth^[8]. Furthermore, the prebiotic activity of *Aloe vera* juice (AVJ) with *Lactobacillus fermentum* was experimented in *in vitro* fermentation. Acetic, propionic and lactic acids were identified from the fermentation medium. The prebiotic activity of AVJ can be identified by the participation of SCFAs during 24h-incubation with *L. fermentum*. An innovative concept of symbiosis is perspective for future intestinal health claims to target health and possible anti-inflammatory effects of AVJ supplementation^[7]. It is interesting to speculate that such a rather simple dietary intervention might have the prophylactic effect on inflammatory diseases and autoimmunity.

The potential benefits, the antioxidant effects and the antibacterial effects on food-borne pathogens of *Aloe vera* fermentation supernatant, were evaluated *in vitro*. The *Aloe vera* fermentation supernatant fermented by *Lactobacillus plantanum* HM218749.1 had very strong scavenging capacities of the DPPH (86%), O₂^{•-} (85%), [•]OH (76%), and Fe²⁺ chelation (82%) and reducing powers (242.5 mg/L), and the inhibiting zones for *Salmonella spp.* and *Escherichia spp.* The antioxidant and antibacterial effects on food-borne pathogens of *Aloe vera* fermentation supernatant were evaluated *in vitro*. Moreover, the low concentration of *Aloe vera* fermentation supernatant had significantly reduced the production of IL-1 β , TNF- α , and IL-6 in both mRNA and protein levels ($p < 0.001$). Therefore, the *Aloe vera* fermentation supernatants indicate its potential use as functional beverage, foods to guard human intestinal health and preventing chronic diseases. The anti-inflammatory effects of the *Aloe vera* fermentation supernatant were evaluated^[48]. In fermentation study by endophytic bacteria in *Aloe vera* gel, the anti-inflammatory activity of the fermented supernatant containing butyrate was identified. The finding of anti-inflammatory as well as

anti-oxidant activities of butyrate in the fermented supernatant may help explain the known beneficial effects of butyrate in intestinal colon and on colitis^[49].

Gut microbial dysbiosis is linked to aberrant immune responses, which are often accompanied by the abnormal production of inflammatory cytokines. As part of the Human Functional Genomics Project (HFGP): Genomics, gut microbiome, and host-environment shape human immune-cytokine responses, Schirmer *et al* investigated how differences in composition and function of gut microbial communities may contribute to inter-individual variation in cytokine responses to microbial stimulation in healthy humans. Microbiome-cytokine interaction patterns that are stimulus specific, cytokine-specific, and cytokine and stimulus-specific were aimed at understanding the interplay between microbial, genetic, and environmental factors in the regulation of the immune response in humans. Many previous studies provided a comprehensive resource on how immune response vectors are determined by environmental, genetic, and microbial factors. The impact of host environmental factors, host genetics, and the gut microbiome on stimulus-induced cytokine responses was assessed in complementary studies of the HFGP^[50]. Accumulating data provide the basis for future clinical studies aiming to elucidate the potential preventive and therapeutic roles of butyrate in human health.

SUMMARY

The present review provides that *Aloe vera* gel containing *Aloe* components: barbaloin, *Aloe*-emodin, emodin and fermented butyrate are fully expected as prophylactic immune-stimulants on auto-immune diseases. *Aloe vera* inhibited the expression of pro-IL-1 β , NLRP3, and caspase-1 as well as that of the P2X7 receptor in the LPS-induced primary macrophages. Furthermore, LPS-induced activation of signaling pathways, such as NF- κ B, p38, JNK, and ERK, was inhibited by *Aloe vera* in these cells^[9]. Chronic inflammation of rheumatoid arthritis is promoted by pro-inflammatory cytokines and closely linked to angiogenesis.

Barbaloin is inactive as a laxative itself but is activated to *Aloe*-emodin anthrone, a genuine purgative component by *Eubacterium sp.* strain BAR^[2], and *Aloe*-emodin was produced from the oxidation of *Aloe*-emodin anthrone. *Aloe*-emodin on chondrogenic differentiation in clonal mouse chondrogenic ATDC5 cells showed that *Aloe*-emodin enhanced the expressions of chondrogenic marker genes such as collagen, collagen X, BSP and RunX2 in a time-dependent manner. *Aloe*-emodin may have potential future applications for the treatment of growth disorder and new bone formation^[15]. Furthermore, *Aloe*-emodin acts as an anti-aggregatory agent to thermally aggregated hemoglobin. The development of *Aloe*-emodin based therapeutics for diseases attributed to protein aggregation is expected^[19].

Emodin, an active constituent of oriental herbs, which is widely used to treat allergy and inflammation, provided the scientific basis for the anti-inflamasome effects of the substantiating traditional claim^[12].

Microbiota and products thereof: short-chain fatty acids and quorum sensing signal molecule, are indispensable for shaping the development and function of host innate immune system, thereby exerting the multifaceted impact on gut health. Butyrate was found to be anti-inflammatory mainly through the suppression of NF- κ B, be capable of altering the composition of the mucus layer by inducing mucin synthesis and of exerting anti-cancer activities. Multiple beneficial effects of butyrate at an intestinal and extra-intestinal level have been demonstrated and the mechanisms of action of butyrate are different and many of these involve an epigenetic regulation of gene

expression through the inhibition of histone deacetylase^[51].

Although immunity influenced by heritability and the environment is well-known, but how these factors synergize to influence immune function is not well characterized. Autoimmune diseases present an especially relevant context to study the intersection of these factors. Extensive genomic and epidemiological studies suggest that autoimmunity results from the coincident interaction of an environmental trigger with a genetically predisposed individual.

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