

Immune Modulation of Aloe vera: Acemannan and Gut Microbiota Modulator

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Received: March 6, 2015

Revised: April 2, 2015

Accepted: April 5, 2015

Published online: August 21, 2015

ABSTRACT

Carbohydrate-based immune adjuvants are capable of enhancing the vaccine against various infectious disease. Several natural polysaccharides originating from plants and microbes have been tested for their adjuvant potential. Adjuvant potential of plant polysaccharides mainly depends upon their solubility, molecular weight, degree of branching and the conformation of carbohydrate structural backbone. Aloe polysaccharide or acemannan as an adjuvant may interact and activate various toll-like receptors and inflammasomes, involving several innate immune system players in the ensuing immune response. Aloe polysaccharide or acemannan plays critical roles in immune system function and has strong safety and tolerability records and is readily biodegradable. Antigen dependent adjuvant properties of acemannan were evaluated to both Newcastle disease virus and infectious bursal disease virus in day-old broiler chicks. Acemannan enhanced the immune response to both viruses. And a pilot study of the effect of acemannan in cats infected with feline immunodeficiency virus (FIV) demonstrated that acemannan therapy may be of significant benefit in FIV-infected cats exhibiting clinical signs of disease. Recent technology to study the gut microbiota have defined new milestones for understanding the microbial ecology of the gastrointestinal

ecosystem and assessing how the microbial world within us impacts our everyday life. A noble immune-enhancing polysaccharides and an importance of gut microbiota inducing gut immunity are discussed on the basis to apply the *Aloe vera* for dietary supplement. In the present review, the following topics are summarized. Introduction: immune modulation and its importance for health and disease, immune modulation of plant sterols and polyphenols in *Aloe vera* gel, known aloe's immune efficacy; Immune adjuvant of *Aloe vera*: general properties, *Aloe vera* gel: role of biological vehicle in drug delivery system, *Aloe vera* gel: role of an immune adjuvant; Immunomodulation of acemannan: acemannan properties, immunomodulation of acemannan and modified acemannan; Acemannan as an immune adjuvant: biological properties, acemannan as an immunomodulator in cats and chickens; Aloe sterols responsible for immunomodulator; Gut microbiota: importance of microbiota for health and disease, aloe's putative efficacy based on the modulation of gut microbiota status. Section of conclusion and future perspectives focuses on biological characteristics of acemannan, and an importance of diet and host-microbial crosstalk to ensure maintenance of homeostasis.

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Key words: *Aloe vera*; Acemannan; Immunomodulator; Biological vehicle; Adjuvant; Drug delivery system; Plant sterols; Gut microbiota modulator

Yagi A, Yu BP. Immune Modulation of *Aloe vera*: Acemannan and Gut Microbiota Modulator. *Journal of Gastroenterology and Hepatology Research* 2015; 4(8): 1707-1721 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/1332>

INTRODUCTION

Immune modulation and its importance for health and disease

Human body is protected by various nonspecific defence mechanisms. Pathogens that break through the mucosal surface

barrier encounter two additional levels of defence; the innate and acquired immune responses. The efficient cross-talk between innate and acquired immunity enables the powerful host defence protecting us from immune-related disorders and pathogenic invaders. The innate immune response is immediately activated after infection, while the acquired immune response takes at least several days for full development. It is important to maintain proper levels of immune defences through-out life as they are challenged daily. The aging processes induce multiple changes in metabolism, the hormones net work, the immune system, which can modulate the efficacy and effectiveness of the immune system, determining a response to stress/disease. Aging is normally associated with deregulated immune and inflammatory responses, which result in increased susceptibility toward infections in the elderly. Immune modulation is important to maintain health conditions in the development and deterioration of our immune defences throughout life.

Immune modulation of plant sterols and polyphenols in *Aloe vera* gel

Inflammation can best be described as a necessary series of cellular and immune responses to tissue insult caused by trauma, bacterial invasion, or immune problems. Inflammation arising from irritant, surgery, and arthritis can be treated with corticosteroids. Corticosteroids decrease edema by reducing capillary permeability and vasodilation as well as by stabilizing lysosomal membranes. Corticosteroids can increase the spread of infection by inhibiting connective tissue formation. *Aloe vera* not only aids the absorption of hydrocortisone but also contributes to biological activity as a biological vehicle or adjuvant. This effect should be enable the physician to lower the hydrocortisone dose to reduce the risk of side effects. β -Sitosterol (BSS) is the major phytosterol in higher plants, and is found in the serum and tissue of healthy individuals at concentrations 800-1000 times lower than that of endogenous cholesterol. Its glycoside, β -sitosterol glycosides (BSSG), is present in serum in even lower concentrations. These molecules are synthesized in plants; whereas animals obtain them through diet. Many epidemiological studies of groups consuming diets rich in vegetables and fruits have indicated a reduced incidence of various types of cardiovascular disease, diabetes and other chronic diseases. In animals, BSS and BSSG have been shown to exhibit anti-inflammatory, and immune modulating activity. The BSS:BSSG complex is a new type, natural immune modulator which has demonstrated promising results in a number of clinical trials. These important plant sterol constituents seem to specifically target T-helper cells, and may help to restore balance between TH1 and TH2 cells, helping normalize their functioning and resulting in improved T-lymphocyte and natural killer cell activity^[1]. Several polyphenolic compounds such as aloein derivatives in *Aloe vera* gel exerted the potent antioxidative and superoxide anion scavenging activity and the inhibitory effect for cyclooxygenase-2 and thromboxane A2 synthase. These findings may explain, at least in part, the wound healing activities of *Aloe vera* gel^[2]. Plant phytochemicals in *Aloe vera* gel as new potential drugs for immune disorders represent an exciting opportunity to maintain best health conditions through a balanced and properly administered daily nutrition.

Known aloe's immune efficacy

Specialized molecules in *Aloe vera* whole leaf extract interact with some 'receptor' substances that are embedded into the outer membrane of our immune system cells. The results is that the immune system cells are galvanized into action. In particular, the

class of cells known as 'phagocytes' increase the activities by which they attack and then engulf bacteria, waste products and debris. This increase in scavenging activities cleanses and protects the body, with knock-on benefits for a whole cascade of different medical conditions. This process of phagocytosis plays an important part of the overall process of immunity. The actual phagocytosis step is really a cleaning up operation after some of the earlier immune processes have taken place. The effect is therefore both protective and cleansing. Macrophages are large phagocytic cells that line blood passages and are found in connective tissue. They have mitochondria and lysosome enzymes that kill bacteria, and can influence every aspect of the immune and inflammatory responses. The literatures indicate that a common mechanism in this respect probably exists in both humans and animals and that both can benefit enormously from use of *Aloe vera*. The following references, for instance, related the effect of aloe extract to human adult bronchial asthma and identified positive effects of polysaccharide fraction and amino acids upon peripheral phagocytosis in these patients^[3,4].

IMMUNE ADJUVANT OF ALOE VERA

General properties

Aloe vera contains 99.5% water and 0.5% solids, and the water-soluble compounds, such as amino acids, enzymes and carbohydrates, and the oil-soluble compounds, such as vitamins, phytosterols and anthraquinones are originated from the leaf inner fillet gel and the leaf latex near the outer rind, respectively. A vehicle is defined as a medium, carrier or transporter that carries pharmacological agent, such as 1% DMSO, through a biological system. Most times, the vehicle has no biological activity of its own. It merely carries the pharmacological agent to the site where the biologic effect is to take place. A biological vehicle is one that acts as a physical or physiological carrier for active biological agents but also adds biological activity to the test agent. An adjuvant is defined as any compound, such as alum, that enhance the immune response against a vaccine antigen; the word adjuvant comes from *adjuvare*, Latin for help or to enhance. An adjuvant can be used for multiple purposes: to enhance immunogenicity, provide antigen-dose sparing, and to accelerate the immune response.

***Aloe vera* gel: role of biological vehicle in drug delivery system**

The plasma bioavailability of vitamin C and E, were determined in normal fasting subjects, with eight subjects for vitamin C and ten subjects for vitamin E. In a random crossover design, the subjects consumed either 500 mg of ascorbic acid or 420 mg of vitamin E acetate alone (control), or combined with 2 oz of two different aloe preparations (a whole leaf extract or an inner fillet gel). Blood was collected periodically up to 24 h after consumption. Plasma was analyzed for ascorbate and tocopherol by HPLC with UV detection. There was no significant difference in the areas under the plasma ascorbate-time curves among the groups sincerely due to large differences within the groups. For comparative purposes the control area was 100%. The aloe gel area was 304%, and aloe whole leaf 80%. Only aloe gel caused a significant increase in plasma ascorbate after 8 and 24 h. For vitamin E, the results for the relative areas were control 100%, gel 369%, and leaf 198%. Only the aloes produced a significant increase in plasma tocopherol after 6 and 8 h. Both aloes were significantly different from the control after 8 h. Aloe gel was significantly different from the baseline after 24 h. There was no difference between the two types of aloe. The absorption is slower and the vitamins last longer in the plasma with the aloes. Vinson JA.

group suggested that aloe is the only known supplement to increase the absorption of these vitamins and should be considered as a complement to them^[5].

The buccal permeability properties of didanosine (ddI; 2', 3'-dideoxyinosine, an anti-retroviral hydrophilic medicine to treat HIV/AIDS) and the potential of *Aloe vera* gel (AVg) as a novel buccal permeation enhancer were investigated. Permeation studies were performed using Franz diffusion cells, and the drug was quantified by UV spectroscopy. Histomorphological evaluations were undertaken using light and transmission electron microscopy. The permeability of ddI was concentration-dependent, and it did not have any adverse effects on the buccal mucosae. A linear relationship ($R^2=0.9557$) between the concentrations and flux indicated passive diffusion as the mechanism of drug transport. *Aloe vera* gel at concentrations of 0.25 to 2% w/v enhanced ddI permeability with enhancement ratios from 5.09 (0.25 % w/v) to 11.78 (2 % w/v) but decreased permeability at 4 and 6 % w/v. Ultra-structural analysis of the buccal mucosae treated with phosphate buffer saline pH 7.4 (PBS), ddI/PBS, and ddI/PBS/AVgel 0.5 % w/v showed cells with normal plasmalemma, well-developed cristae, and nuclei with regular nuclear envelopes. However, cells from 1, 2, and 6 % w/v AVg-treated mucosae showed irregular nuclear outlines, increased intercellular spacing, and plasmalemma crenulations. Ojewole E. group suggested that this study demonstrates the potential of AVg as a buccal permeation enhancer for ddI to improve anti-HIV and AIDS therapy^[6].

Methanol precipitable solids from whole leaves of *Aloe vera* were used as permeation enhancer. To achieve improved bioavailability of diltiazem, novel buccal adhesive tablets (NBATs) in cup and core fashion designed to achieve unidirectional release towards mucosa were prepared in a three-stage process using specially fabricated punches. Ex vivo permeation studies in a Fourier transform infrared spectroscopy studies and differential scanning calorimetry thermographs showed no remarkable interactions. Histopathological studies showed no remarkable damage of buccal mucosa by the NBATs. *In vivo* studies were conducted on anaesthetized male New Zealand albino rabbits, estimated by reversed-phase HPLC, and the pharmacokinetics were compared with the oral and intravenous bolus injection. Sudhakar Y. group demonstrated that NBATs exhibited a C_{max} 74.6 ng/mL, T_{max} 3.5 h, $t(1/2)$ 4.36 h. The NBATs prevented salivary scavenging effect and exhibited 82.1% bioavailability^[7].

The formulated piroxicam (NSAID)-*Aloe vera* gel (PAG), prepared by using carbopol 9 as gelling agent and methyl paraben as a preservative in an *Aloe vera* gel base, was evaluated physicochemical parameters like pH, viscosity, drug content, and *in vitro* diffusion assessment. Pharmacodynamic activity of the formulation was evaluated in Wistar albino rats. The formulated gel was compared with that of similar marketed gel (commercial piroxicam gel: CPG) against the same parameters. From *in vitro* studies, an effect drug release from PAG was observed to be 68.17% when compared with that of the CPG (62.71%) at 180 min indicated better drug release from the gel formulated in this study. Percentage inhibition of edema was greater for the preparation of PAG (29.57 mean percent inhibition after 60 min) compared to marketed gel which exhibited 18.3% after 60 min. Velam V. group concluded that the *Aloe vera* gel acts as an effective gel base to prepare piroxicam gel with high drug loading capacity and improved anti-inflammatory effect. From the statistical analysis the formulation of PAG showed better release than the CPG at $p<0.05$ level of significance^[8].

The transdermal delivery system of naproxen (NSAID) using *Aloe vera* as aqueous gel base and caropol 934 as a gelling agent was investigated by Thushara BD. group. The prepared gel was assessed

for pH, permeation parameters, drug content, skin irritation and *in vitro* diffusion. *In vivo* study was performed to characterize the efficacy of the prepared naproxen-*Aloe vera* transgel (NAG). The pH of formulation was found to be 6.98. The values of permeation data for flux, permeability coefficient and enhancement ratio were obtained as 9.07 $\mu\text{g}/\text{cm}^2/\text{h}$, 0.00453 cm/h and 5.96, respectively. The viscosity was found as 610 cps for NAG. The drug release kinetics followed Higuchi model. Stability study has proved the integrity of the formulation. No skin irritation was observed in Wistar albino rats. Anti-inflammatory assessment in Wistar rats showed significant effect in paw-volume reduction at $p<0.05$ in less than one hour for NAG compared to that of plain aloe gel and commercial naproxen gel (CNG). The prepared NAG could able to show better anti-inflammatory activity compared to the CNG. This could be attributed by synergistic effect of aloe^[9].

Biogenic gold nanotriangles and spherical silver nanoparticles were synthesized by Chandran SP. group, using *Aloe vera* leaf extract as the reducing agent. This procedure offers control over the size of the gold nanotriangle and thereby a handle to tune their optical properties, particularly the position of the longitudinal surface plasmon resonance. The effect of reducing agent concentration in the reaction mixture on the yield and size of the gold nanotriangles was studied using transmission electron microscopy. It is observed that the slow rate of the reaction along with the shape directing effect of the constituents of the extract are responsible for the formation of single crystalline gold nanotriangles. Reduction of silver ions by *Aloe vera* extract however, led to the formation of spherical silver nanoparticles of $15.2 \text{ nm} \pm 4.2 \text{ nm}$ size^[10].

The effect of whole leaf and gel materials from two aloe species (*Aloe vera* and *A. ferox*) was compared with that of the precipitated polysaccharides from these aloe materials on the trans-epithelial electrical resistance (TEER) as well as transport of a model compound (atenolol; a selective β_1 receptor agonist) in the apical-to-baso-lateral direction across rat intestinal tissue. All the aloe leaf materials and precipitated polysaccharides had a statistically significant effect of lowering the TEER ($p<0.05$) compared to the control group, which indicates their ability to open tight junctions between adjacent epithelial cells. In contrast to the expectation from the TEER results, only the precipitated polysaccharides from dehydrated *A. vera* gel (Daltonmax 700) had a statistically significant effect of enhancing the transport of atenolol ($p<0.05$). Beneke C. group suggested that these *in vitro* results indicate that *Aloe vera* gel polysaccharides have potential as drug absorption enhancing agent in novel pharmaceutical drug delivery system^[11].

Cimetidine (a histamine H-2 receptor antagonist) transport studies were performed across excised rat intestinal tissue mounted in Sweetana-Grass diffusion chambers in both the apical-to-basolateral and basolateral-to-apical directions. While *A. vera* gel and whole leaf materials did not inhibit the efflux of cimetidine, the polysaccharides precipitated from them did show a reduction of cimetidine efflux. On the other hand, both *A. ferox* and *A. marlothii* gel and whole leaf materials exhibited an inhibition effect on cimetidine efflux. Carien B. group identified a modulation effect of efflux transporters by certain aloe materials. This may cause herb-drug pharmacokinetic interactions when drugs that are substrates for these efflux transporters are taken simultaneously with aloe materials. On the other hand, these aloe materials may be used for drug absorption enhancement for drugs with low bioavailability due to extensive efflux^[12].

To examine the preventive effect of *Aloe vera* gel ethanolic extract using diabetic foot ulcer (DFUs) protocol in Wistar rats, Daburkar M. group divided male Wistar rats into untreated control (I), untreated

DFUs (II), DFUs treated with *Aloe vera* gel ethanolic extract (III), DFUs treated with topical *Aloe vera* gel (IV), DFUs treated with *Aloe vera* gel ethanolic extract and topical *Aloe vera* gel (IV). The rats in the treatment groups were daily administered the *Aloe vera* gel and ethanolic extract for 9 days. Fasting blood glucose levels and percentage of wound ulcer contraction were measured on day 3, 6, and 9. Data were analyzed using one-way analysis of variance (ANOVA) after Newman-Keuls test. ($p < 0.05$). Oral administration of *Aloe vera* gel ethanolic extract at a dose of 300 mg/kg body weight per day to diabetic rats for a period of 9 days resulted in a significant reduction in fasting blood glucose and a significant improvement in plasma insulin. Topical application of *Aloe vera* gel at a dose 30 mg/kg body weight per day to streptozotocin-induced diabetic rats for a period of 9 days resulted in no change in blood glucose and plasma insulin. Oral administration as well as topical application of *Aloe vera* gel ethanolic extract and gel significantly reduced the blood glucose, improved the plasma insulin, and significantly increased DNA and glycosaminoglycans to improve the wound ulcer healing as well as the breaking strength on day 9. In conclusion, present findings provide a scientific rationale for the use of *Aloe vera* gel ethanolic extract, and showed that the gel attenuated the diabetic foot wound in rats^[13].

Usual treatment for *Helicobacter pylori*-induced peptic ulcer includes a 14-day "triple therapy" of two antibiotics and a proton pump inhibitor. However, the current therapy has side-effects like stomach upset, non-compliance, incomplete absorption of drug and antibiotic resistance. To overcome these limitations, there is a need to suggest an alternative therapy. The best possible alternative is to deliver herbal constituents. Ranade AN. group examined to optimize the efficacy of herbal constituents by applying the concept of a novel drug delivery system, and designed to deliver and retain two herbal constituents in the stomach for better action against gastric ulcers. The objective was to develop a bilayer floating tablet of monoammonium glycyrrhizin (MG) and *Aloe vera* gel powder through rational combination of excipients to give the lowest possible lag time with maximum drug release in 7 h. Formulation of 2 containing hydroxy propyl methyl cellulose E5, crospovidone and effervescent agents in the ratio 1:2 gave 98% drug release with desired floating properties. Pharmacodynamic studies in rats showed that the combination of MG and *Aloe vera* gave 99% ulcer inhibition in comparison with 51% ulcer inhibition in the group administered with MG alone. X-ray studies in rabbits proved the gastroretention of the tablet for more than 6 h. This suggests relevance of novel drug delivery systems in delivery of herbal constituents in the treatment of gastric ulcer^[14].

The polymeric films containing *Aloe vera* and vitamin E was evaluated to treat wound caused by burns. Around 30% of vitamin E acetate was released from the polymeric films within 12 h. The *in vivo* experiments with tape stripping indicated an effective accumulation in the *stratum corneum* when compared to a commercial cream containing the same quantity of vitamin E acetate. Vitamin E was found in higher quantities in the deep layers of the *stratum corneum* when the film formulation was applied. The results obtained by Pereira GG. group show that the bioadhesive films containing vitamin E acetate and *Aloe vera* could be an innovative therapeutic system for the treatment of burns^[15].

Ascorbic acid is taken as a model drug for its high solubility. Different concentrations such as 30%, 40%, and 50% of matrix tablets of *Aloe vera* powder are made by wet granulation technique using starch paste as a binder. The formulated granules were further subjected to quality control test such as Angle of repose, Bulk density,

Carr's index and Hauser ratio. These matrix tablets are then subjected to *in vitro* drug release using USP dissolution apparatus. The amount of ascorbic acid released from the matrix is estimated by using UV spectrometer and this results is compared with marketed ascorbic acid tablets. Anurupa C. group demonstrated that formulation containing 40% matrix was found to be good as compared to other formulations and shows better controlled release of drug^[16].

Intra-peritoneal injection of cyclophosphamide (CP) significantly reduced the total number of lymphocytes and erythrocytes in the blood in mice. Oral administration of processed *Aloe vera* gel (PAG) quickly restored CP-induced lymphopenia and erythropenia in a dose-dependent manner. The reversal of CP-induced hematotoxicity by PAG was mediated by the functional preservation of Peyer's patch cells. Peyer's patch cells isolated from CP-treated mice, which were administered PAG, produced higher level of T helper 1 cytokines and colony-stimulating factors (CSF) in response to concanavalin A stimulation as compared with those isolated from CP-treated control mice. PAG-derived polysaccharides directly activated Peyer's patch cells isolated from normal mice to produce cytokines including interleukin (IL)-6, IL-12, interferon- γ , granulocyte-CSF, and granulocyte-macrophage-CSF. The cytokines produced by polysaccharide-stimulated Peyer's patch cells had potent proliferation-inducing activity on mouse bone marrow cells. In addition, oral administration of PAG restored IgA secretion in the intestine after CP treatment. From these results Im SA. group indicated that PAG could be an effective immunomodulator and that it could prevent CP-induced immunotoxic side effects^[17].

The preparation of nimesulide (a Cox 2 selective NSAID) emulsion for incorporation in *Aloe vera* gel base to formulate '*Aloe vera* transemulgel' (NAE) and to carryout in-vitro assessment and in-vivo anti-inflammatory studies of the product was investigated. Although the use of nimesulide is banned for oral administration, due to its potential for inducing hepatotoxicity and thrombocytopenia, the use of nimesulide for topical delivery is prominent in the treatment of many inflammatory conditions including rheumatoid arthritis. The drug loading capacity of transdermal gels is low for hydrophobic drugs such as nimesulide. Nimesulide can be effectively incorporated into emulgels (a combination of emulsion and gel). *Aloe vera* gel has a mild anti-inflammatory effect and in the present study *Aloe vera* gel was formulated and used as a gel base to prepare NAE. The emulgels thus prepared were evaluated for viscosity, pH, in-vitro permeation, stability and skin irritation test. In-vivo anti-inflammatory studies were performed using carrageenan induced hind paw edema method in Wistar rats. The results were compared with that of commercial nimesulide gel (CNG). From the in-vitro studies, effective permeation of nimesulide from NAE (53.04%) was observed compared to CNG (44.72%) at 30 min indicating better drug release from NAE. Topical application of the emulgel found no skin irritation. Stability studies proved the integrity of the formulation. The percentage of inhibition of edema was highest for the prepared NAE (67.4% inhibition after 240 min) compared to CNG (59.6%). From the results, Vandana KR. group concluded that the *Aloe vera* gel acts as an effective gel base to prepare nimesulide emulgel with high drug loading capacity (86.4% drug content) compared to CNG (70.5% drug content) with significant anti-inflammatory effect^[18].

Compounds contained within *Aloe vera* can cause a reduction in prostaglandin synthesis, which may inhibit secondary aggregation of platelets. Sevoflurane inhibits thromboxane A2 formation by suppression of cyclooxygenase activity, impairs platelet aggregation, and prolongs bleeding. Although the vascularity and size of the hemangioma were the most important factors for the massive

intraoperative blood loss, concomitant use of sevoflurane and *Aloe vera* played a contributory role. An objective causality assessment revealed that this adverse event was possible as a result of the sevoflurane and *Aloe vera* interaction. In conclusion, there is a potential herb-drug interaction between *Aloe vera* and sevoflurane based on the antiplatelet effects of these two agents. Lee A. group suggested that herbal medications with antiplatelet potential should be discontinued before anesthesia and surgery^[19]. The biological vehicle effect will reduce the amount of test agent needed to produce the therapeutic response.

***Aloe vera* gel: role of an immune adjuvant**

The efficacy of *Aloe vera* gel as an adjuvant treatment of oral submucous fibrosis (OSMF) was evaluated. A double-blind, placebo-controlled, parallel-group randomized controlled trial was conducted on 60 subjects with OSMF divided into medicinal treatment (submucosal injection of hyaluronidase and dexamethasone, $n=30$) and surgical treatment ($n=30$) categories. Each category was randomly divided into groups A (with *Aloe vera*, $n=15$ per category) and B (without *Aloe vera*, $n=15$ per category). Follow-up assessment for various symptoms was performed, and results were analyzed using paired and unpaired Student t test. The group receiving *Aloe vera* had a significant improvement in most symptoms of OSMF ($p<0.01$) compared with the non-*Aloe vera* group, in both the medicational and surgical categories. In conclusion, Alam S. group suggested that *Aloe vera* gel is effective as an adjuvant in treatment of OSMF^[20].

The skin hydrating and anti-erythema activity of gel materials from *Aloe marlothii* and *A. ferox* in comparison to that of *Aloe vera* in healthy human volunteers. Aqueous solution of the polysaccharide of the selected aloe leaf gel materials were applied to the volar forearm skin of female subjects. The hydration effect of the aloe gel materials were measured with a Corneometer CM 825, Visioscan VC 98 and Cutometer dual MPA 580 after single and multiple applications. The Mexameter MX 18 was used to determine the anti-erythema effects of the aloe material solutions on irritated skin areas. The *A.vera* and *A.marlothii* gel materials hydrated the skin after a single application, whereas the *A. ferox* gel material showed dehydration effects compared to the placebo. After multiple applications all the aloe materials exhibited dehydration effects on the skin. Mexameter reading showed that *A.vera* and *A. ferox* have anti-erythema activity similar to that of the positive control group (i.e. hydrocortisone gel) after 6 days of treatment. In conclusion, the polysaccharide component of the gel material from selected aloe species has a dehydrating effect on the skin after multiple applications. Fox LT. group suggested that both *A.vera* and *A.ferox* gel materials showed potential to reduce erythema on the skin similar to that of hydrocortisone gel^[21].

Herbal formulations having *Aloe vera* gel powder for its efficacy and activity on bed sores were tested. *Aloe vera* gel powder with high molecular weight (AHM) was prepared from the gel part, by washing with running water using the patented freeze-drying under micro wave and far infra red irradiations in which barbaloin content was less than 10 ppm in powder form. The treatment was given by applying the macromolecule aloe ointment for bed sores from 1 degree to 2 degree ulcer patients. AHM in the ointment form indicated a high possibility to cure bed sores. Being very difficult to cure, due to the patient's peculiar conditions such as old age, inability of the patient to turn by himself/herself and also due to complications caused by other symptoms. We were able to confirm the effectiveness of the macromolecule aloe ointment in four cases of bed sores with

two cases of positive control, using the Design Score and by checking the side effects. Matsuo K. group suggested the possible pre-clinical trials for bed sores by the external use of the AHM ointment^[22].

The effect of two different *Aloe vera* preparations (*Aloe vera* inner leaf gel:AG and *Aloe vera* whole leaf decolorized gel:AL), compared to placebo on the bioavailability of vitamin C and B₁₂, was examined in healthy human volunteers in a randomized crossover trial. Subjects ($n=15$) received in a random fashion either aloe whole leaf extract (AL with vitamin B₁₂, 1 mg and vitamin C 500 mg) or aloe fillet gel (AG with B₁₂ 1 mg and vitamin C 500 mg) or water (with vitamin B₁₂ 1 mg and vitamin C 500 mg). Blood was obtained fasting, followed by 2, 4, 6, 8 and 24 h post-ingestion of aloe/water. When given with vitamin C and B₁₂, AG significantly increased plasma oxygen radical absorbance capacity at both 4 and 24 h and AL at 4 h compared to baseline and placebo. AG significantly increased plasma vitamin C at 4, 6, 8, and 24 h and AL at 4 and 6 h compared to baseline and placebo ($p<0.01$). Also, both aloes significantly increased serum vitamin B₁₂ levels at 1 and 2 h compared to baseline and placebo ($p<0.01$). Thus, Yun JM. group suggested that AG and AL preparations are safe, well tolerated, and enhance the bioavailability of vitamin C and B₁₂ and antioxidant potential^[23].

Aloe fillet gel preparation are safe, well tolerated, and enhanced the bio-availability of medicines and vitamins. Pre-clinical trials of aloe high molecular weight fraction for bed sore provided a high competence of epithelialisation followed by tissue and texture restoration as an adjuvant. *Aloe vera* gel can act as an adjuvant to enhance the immune response to an antigen.

IMMUNOMODULATION OF ACEMANNAN

Acemannan properties

Acemannan is the name given to the acetylated mannan isolated from *Aloe vera*, and it consists of long chain polysaccharide β (1-4)-linked mannan polymers with random O-acetyl groups. There is considerable discrepancy in the literature as to the structure of the polysaccharide isolated from *Aloe vera* mucilaginous gel in cell walls. Acemannan hydrogel (Carrington Lab.) isolated by clarification and ethanol precipitation of the inner leaf gel of *Aloe vera* was dissolved in distilled water by gentle shaking overnight and vacuum filtrated through a 0.45 μ m nitrocellulose membrane to yield bulk water-soluble polysaccharide (BSW). BSW was found to contain 90% soluble carbohydrate, 1-2% protein, less than 1% insoluble materials, and the remainder organic salts (oxalate, malate). The polysaccharide had an average molecular weight of 1.1×10^6 , as determined by size exclusion chromatography. The polysaccharide isolated by alcohol precipitation of *Aloe vera* mucilaginous gel was found a Man:Glc:Gal:GalA:Fuc:Ara:Xyl ratio of 120:9:6:3:2:2:1 with trace of Rha and GlcA. Hydrolysis with strong acids produced a mixture of short oligosaccharides and acid-resistant fraction containing grater relative fractions of Manp-(1 \rightarrow), Araf-(1 \rightarrow), Xylp-(1 \rightarrow), and Xylp than the bulk polysaccharide. The data provided direct evidence of a previously proposed glucomannan backbone but draw into question previously proposed side-chain structures, based on NMR analysis of oligosaccharides and analysis of the samples with endo-(1 \rightarrow 4)- β -D-mannanase and α -D-galactosidase. In conclusion, Chow J T-N. group suggested that the polysaccharide has a β -glucomannan backbone with a Man:Glu ratio of $\sim 15:1$ and that branching occurs from the O-2, O-3, and O-6 of $\rightarrow 4$ - β -Manp- (1 \rightarrow residues to single α -Galp- (1 \rightarrow side chains^[24].

It has proven exceedingly difficult to separate acemannan from contaminating protein and lectin. As a result, it is by no means proven

that acemannan alone possesses the biological activities ascribed to it. Acemannan immunostimulant is a commercially available, partially purified carbohydrate preparation containing about 60% acetylated mannan together with other carbohydrates, especially aloe pectins, hemicelluloses, lectins and proteins. It should not be confused with the complex carbohydrate acemannan. Acemannan immunostimulant can activate macrophages. This macrophage activating ability is probably responsible for its activity as an adjuvant, its pro-wound healing activity and anti-viral activity. These findings showed that commercial acemannan is a mixture of polysaccharides having activities as adjuvants.

Immunomodulation of acemannan and modified acemannan

The study of Bhalang K. group elucidated the safety and effectiveness of acemannan in the treatment of oral aphthous ulceration. A skin patch test was performed on 100 healthy subjects, and 0.5% acemannan in commercial Carbopol 934P NF was applied to the oral mucosa of the lower lip of 50 healthy participants 3 times/day for 7 days. Oral examinations and blood tests measuring liver and kidney function were performed prior to, and following, 7 days of application to assess the side-effects of acemannan when used on oral mucosa. Another 180 subjects with recurrent aphthous ulceration randomly received one of three treatments: 0.1% triamcinolone acetonide, 0.5% acemannan in Carbopol 934P NF, or pure Carbopol 934P NF. Medications were applied to the ulcers 3 times/day for 7 days. Measurements of ulcer size and patient satisfaction ratings were performed on days 2, 5, and 7. Pain ratings were recorded daily. No subjects exhibited allergic reactions or side-effects to acemannan. There were no significant differences between the blood test values before and after 7 days of acemannan application. The effectiveness of acemannan in reducing ulcer size and pain was superior to that of control, but inferior to that of 0.1% triamcinolone acetonide. Patients were mostly satisfied with 0.1% triamcinolone acetonide and acemannan treatment. In conclusion, acemannan can be used for the treatment of oral aphthous ulceration in patients who wish to avoid the use of steroid medication, although the effectiveness was not comparable to that of 0.1% triamcinolone acetonide^[25].

A Baylor College of Dentistry group in Dallas, Tx in USA, found that acemannan hydrogel accelerated healing and reduced pain associated with aphthous ulcers. The Baylor research involved 90 patients with histories of recurrent oral ulcers. The patients were separated into three groups with each group given a different treatment to applied four times a day. Participants received either acemannan hydrogel, freeze-dried acemannan hydrogel or an active control, which was an over-the counter product. Those using acemannan hydrogel in either form healed faster than those using OTC remedy. Acemannan hydrogel found effective in treating oral ulcers has been approved by the US Food and Drug Administration for market distribution.

Canadian HIV Trials Network (Montaner JS. group) assessed the safety and surrogate markers' effect of acemannan as an adjunctive to anti-retroviral therapy among patients with advanced HIV disease receiving zidovudine (ZDV) or didanosine (ddI) in a randomized, double-blind, placebo-controlled trial of acemannan (400 mg orally 4 times daily). Eligible patients of either sex had CD4 counts of 50-300/ μ l twice within 1 month of study entry and had received 26 months of anti-retroviral treatment (ZDV or ddI) at a stable dose for the month before entry. CD4 counts were made every 4 weeks for 48 weeks. P24 antigen was measured at entry and every 12 weeks thereafter. Sequential quantitative lymphocyte cultures for HIV and ZDV pharmacokinetics were performed in a subset of patients. Sixty-

three patients were randomized. All were males (mean age 39 years). The mean baseline CD4 counts were 165 and 147/ μ L in the placebo and acemannan groups, respectively; 90 percent of the patients were receiving ZDV at entry. Six patients in the acemannan group and five in the placebo group developed AIDS-defining illnesses. There was no statistically significant difference between the groups at 48 weeks with regard to the absolute change or rate of decline at CD4 count. Among ZDV-treated patients, the median rates of CD4 change (ACD4) in the initial 16 weeks -121 and -120 cells per year in the placebo and acemannan groups, respectively ($p=0.45$), ACD4 from week 16 to 48 was 0 and -61 cells per year in the acemannan and placebo groups ($p=0.11$), respectively. There was no statistical difference between groups with regard to adverse events, p24 antigen, quantitative virology, or pharmacokinetics. Twenty-four patients, 11 receiving placebo and 13 receiving acemannan, discontinued study therapy prematurely, none due to serious adverse reactions. These results demonstrate that acemannan at an oral daily dose of 1600 mg does not prevent the decline in CD4 count characteristic of progressive HIV disease. Acemannan showed no significant effect on 24 antigen and quantitative virology. Acemannan was well tolerated and showed no significant pharmacokinetic interaction with ZDV^[26].

Acemannan, a major acetylated mannan fraction of *Aloe vera* inner gel, enhances immune responses and has antiviral and anti-tumor activities. Acemannan, an immune stimulant, increases the immune response to an antigen, and inhibits viral replication. The ability is important to the effectiveness of a vaccine, as antigens alone sometimes fail to produce an immune response. This immunostimulant and vaccine adjuvant ability would be extremely important in the fight against AIDS. Acemannan was shown to have positive *in vitro* results against HIV, and got FDA clearance as an oral ulcers remedy. Acemannan, however, showed no significant effect on p14 antigen and quantitative virology in AIDS-patients.

Neutral polysaccharides that inhibit carrageenin-induced edema in rats were isolated from the nondialysate of the pulp of *Aloe saponaria* by gel filtration. These were shown to be a linear polymer of a 1,4-linked β -D-mannopyranose (mol.wt. 15KD) containing 18% acetyl groups (As mannan 1, and a 1,4-linked α -D-mannopyranose polymer containing a single branch on the principal chain consisting of D-glucose residues linked at C-2 and C4 (mol.wt. 66KD), with 10% acetyl groups (As mannan 2)) on the basis of ¹H/¹³C-NMR physico- and chemical analyses. Neutral protein-free polysaccharide, As mannan 1 inhibited carrageenin-induced hind paw edema at 50 mg/kg intraperitoneally in rats. As mannan 2 was not tested for pharmacological activity. A crude preparation of both As mannans was effective when given i.p., but was ineffective when given orally. This may be due to poor absorption of As mannan when give orally^[27]. This *in vivo* study indicates that oral administration of neutral polysaccharide may not be directly efficacious to carrageenin-induced edema in rats. It is speculated that water soluble polysaccharide containing 90% soluble carbohydrate (acemannan), in contamination of 1-2% protein, and less than 1% insoluble materials, in *Aloe vera* gel may exhibit some biological activities^[24].

The ability of aloe gel to prevent suppression of contact hypersensitivity responses to hapten decayed rapidly after manufacture. In contrast, agents that protected against systemic suppression of delayed type hypersensitivity (DTH) responses to *Candida albicans* were stable over time. Oligosaccharides (MW 1-5KD) obtained from *Aloe vera* polysaccharide (MW >2,000KD) by cellulose cleavage, prevented suppression of DTH responses *in vivo* and reduced the amount of IL-10 observed in UV irradiated murine epidermis. To assess the effect of aloe extract on keratinocytes, Pam

212 cells were exposed *in vitro* to UV radiation and treated for 1 h with aloe oligosaccharides. Culture supernatants were collected 24 h later and injected into mice. Supernatants from UV irradiated keratinocytes suppressed the induction of DTH responses, whereas aloe oligosaccharide treatment reduced IL-10 and blocked the suppressive activity of the supernatants. Byeon SW. group suggested that aloe contains multiple immunoprotective factors and that aloe oligosaccharides may prevent UV induced suppression of DTH by reducing keratinocyte derived immunosuppressive cytokines^[28].

Aloe vera polysaccharide was partially digested with cellulase and further purified by dialysis, stepwise ethanol precipitation, and size exclusion chromatography. Crude modified aloe polysaccharide (MAP) activated macrophage cells and stimulated fibroblast growth. Under the same conditions, native *Aloe vera* gel had no effect on macrophage activation. MAP prevented UVB irradiation-induced immune suppression as determined by contact hypersensitivity (CHS) response in C3H/HeN mice. This *in vivo* activity was correlated with the activity of MAP to inhibit UVB irradiation-induced tumor necrosis factor α (TNF- α) release from human epidermoid carcinoma cells. MAP with an average molecular weight of 80 KD contained Man, Gal and Glu in a ratio of 40:1.4:1.0. MAP was likely a linear, highly acetylated molecule^[29]. Qiu Z. group indicated that this oligosaccharide obtained from acemannan by cellulase hydrolysis contains the restorative activity in immunomodulation together with the original polysaccharide, suggesting an important role of gastrointestinal microbiota.

There is no doubt that extracts of *Aloe vera* gel can exert significant anti-inflammatory activity. At the same time similar gel materials can activate macrophages and other cells involved in the defense of the body. Unfortunately, the aloe gel preparations used for these studies have tended to be complex, containing lectin and pectin moiety and it is therefore difficult to elucidate the biochemical pathway involved. Furthermore, gut microbiota participate with the catabolism of acemannan when administered orally. Two features of acemannan, the β -1-4 linkage and acetylation, are highly conserved among *Aloe* species. Considerable variations exist regarding to the presence of other sugars, primarily glucose (glucomannan) and the degree of acetylation. Besides, contamination of aloe pectin, lectin and peptide moiety, having the differences in chemical and biological properties were identified in acemannan which is commercially available, partially purified carbohydrate preparation containing about 60% acetylated mannan together with other carbohydrate, especially pectins and hemi-celluloses. Acemannan with immunostimulation can activate macrophages and this macrophage activating ability may be responsible for its activity as an adjuvant.

ACEMANNAN AS AN IMMUNE ADJUVANT

Biological properties

The study by Tizard I. group was undertaken to determine if acemannan could influence the rate of wound healing in rats. When a solution containing 30 μ g acemannan was injected into tissues surrounding biopsy punch wound in young (3-month old) rats, a reduction in wound healing time of approximately 4 days when compared to untreated wound was observed. When used to treat biopsy punch wound in 2-year old rats, it resulted in a reduction in wound healing time from 21 days in untreated wound to 14 days in treated wound. This enhancement in the rate of wound healing was observed in both ad *libitum*-fed and calorie-reduced aged rats. Acceleration in wound healing was also observed when acemannan was administered by either the ip or intracardiac routes. Acemannan

is believed to exert its effects on the wound healing process by two mechanisms. First, it is a potent macrophage-activating agent and may therefore stimulate the release of fibrogenic cytokines. Macrophage function is known to be a limiting factor in the healing of wound in aged animals. Alternatively, growth factors may bind directly to acemannan, promoting their stability and possibly prolonging their stimulating effects on granulation tissue formation^[30]. Tizard I. group reported acemannan can cause a significant increase in the rate of wound closure in rats. The effects of acemannan claimed to have several important therapeutic properties including acceleration of wound healing and immune stimulation, were investigated on the mouse macrophage cell line, RAW264.7 cells. It was found that acemannan could stimulate macrophage cytokine production, nitric oxide release, surface molecule expression, and cell morphologic changes. The production of the cytokines IL-6 and TNF- α were dependent on the dose of acemannan provided. Nitric oxide production, cell morphologic changes and surface antigen expression were increased in response to stimulation by a mixture of acemannan and IFN- γ . These results suggest that acemannan may function, at least in part, through macrophage activation^[31].

In the *in vitro* experiments the activity of various concentrations of vitamin C (ranging from 3 mM to 3 nM) and *Aloe vera* juice (50 μ l) were examined in the modulation of the natural killer cells (NKC) functionality, taken from blood samples collected from 12 healthy volunteers. The *in vivo* experiments were performed on 15 healthy volunteers, who took supplements of a combination of 1g/day of vitamin C and 50 mL of *Aloe vera* juice for 45 consecutive days. The *in vitro* results showed that both substances increased NKC cytotoxicity against K562 cancer cell line. Furthermore, in the *in vivo* experiment the cytotoxicity of the NKC was significantly increased compared to the pre-supplementation values ($p < 0.05$) under all three conditions tested. These results indicate that vitamin C and *Aloe vera* juice can modulate NKC cytotoxicity and has the potential to enhance the immune system^[32]. Toliopoulos I. group indicated that the *in vitro* and *in vivo* effects in a pilot study of vitamin C and *Aloe vera* juice were evaluated on NK activity.

To this day, alum-based adjuvants, alone or combined with additional immune activators, remain the only adjuvants approved for use in the USA. A relative lack of sources and funding for adjuvant development has only helped to maintain alum's relative monopoly. To seriously challenge alum's supremacy a new adjuvant has many major hurdles to overcome, not least being alum's simplicity, tolerability, safety record and minimal cost. Carbohydrate structures play critical roles in immune system function and carbohydrates also have the virtue of strong safety and tolerability record. A number of carbohydrate compounds from plant, bacterial, yeast and synthetic sources have emerged as promising vaccine adjuvant candidates. Carbohydrate are readily biodegradable and therefore unlikely to cause problems of long-term tissue deposits seen with alum adjuvants. Carbohydrate-based compounds have many favorable properties that could place them in a unique position to challenge alum's monopoly over human vaccine usage^[33].

By using the patented hyper-dry system after washing out coloured material with running water, aloe high molecular fractions (AHM) were obtained in original and natural form containing less than 10 ppm of barbaloin. AHM mainly contained high molecular fractions, such as polysaccharide (acemannan) and glycoprotein (verectin) showing immunomodulatory and anti-inflammatory activities. On the basis of chemical and biochemical properties, AHM were examined for the therapy designed by the implementation of well-controlled pre-clinical trials, and exhibited the efficacy as immunomodulators

for viral infection-induced hepatic periportal fibrosis and type 2 diabetic patients, and also wound and ulcer-healing ointment to patients suffering from bed sores. Therefore, Yagi A. group demonstrated that AHM are considered to be a novel low-cost and safe natural original adjuvant^[34].

Acemannan as an immunomodulator in cats and chickens

Acemannan was tested for *in vitro* and *in vivo* activities against human immunodeficiency virus type 1 and feline immunodeficiency virus. *In vitro* antiviral efficacy of acemannan was evaluated in a variety of cell lines including human peripheral mononuclear, CEM-SS1 and MT-2(2) cells. Suppression of syncytia formation was observed at an acemannan concentration of 31.25 µg/mL, and complete inhibition was observed at 62.5 µg/mL^[35].

A pilot study was undertaken by Yates KM. group to determine acemannan's effect in 49 feline immunodeficiency virus (FIV) infected cats with clinical signs of disease (Stage 3, 4 or 5), 23 of which had severe lymphopenia. Cats received acemannan either by intravenous (group 1) or subcutaneous (group 2) injection once weekly for 12 weeks, or by daily oral (group 3) administration for 12 weeks. Upon entry into the study, cats were randomly assigned to one of the three groups. Laboratory analyses were performed at the beginning of the study and at weeks 6 and 12. Cats were allowed to continue with a predetermined maintenance regimen of acemannan after completing the 12-week study. Thirteen cats died during the course of treatment. Upon necropsy, the most frequent histopathologic findings were neoplastic, kidney and pancreatic disease. Friedman's two-way ANOVA test showed no significant differences in efficacy among groups administered acemannan by the different routes. Therefore, groups were combined and a signed-ranks test was used to determine changes over time. A significant increase was seen in lymphocyte counts ($p < 0.001$). Neutrophil counts decreased significantly ($p = 0.007$), as did incidence of sepsis ($p = 0.008$). When cats entering with lymphopenia were analyzed separately, a much greater increase in lymphocyte counts was noted (235%) compared with non-lymphopenic cats (42%). A survival rate of 75% was found for three groups. Thirty-six of 49 animals are alive 5-19 months post-entry. These results suggest that acemannan therapy may be of significant benefit in FIV-infected cats exhibiting clinical signs of disease^[36].

The adjuvant properties of a polydispersed β-(1,4)-linked acetylated mannan, acemannan (ACE-M), were evaluated by Chinnah AD, group. Day-old broiler chicks were randomly selected and allocated to four flocks (Vac 1-4). The Vac 1 flock was sham vaccinated with saline. The Vac 2 flock was vaccinated with an oil-based vaccine (Breedervac III; Newcastle disease virus (NDV), infectious bursal disease virus (IBDV) and infectious bronchitis virus). The Vac 3 flock was vaccinated with a vaccine-ACE-M mixture, and the Vac 4 flock was vaccinated with vaccine and ACE-M at separate anatomical sites. ELISA titres to NDV and IBDV were determined. The immune response to NDV at 21 days postvaccination (PV) was significantly enhanced (P less than or equal to 0.05) by the addition of ACE-M. Subsequently, the vaccine-ACE-M mixture appeared to suppress the immune response to NDV. However, at day 35 PV, 95% of the Vac 3 chicks compared with 90% of the Vac 2 and 89% of the Vac 4 chicks exhibited protective titres. The response to IBDV differed from that to NDV. At day 21 PV the immune response to IBDV was essentially the same for all flocks that received vaccine, i.e. addition of ACE-M to the vaccine did not significantly enhance the immune response; however, it did significantly (P less than or equal to 0.05) sustain the immune response at days 28 and 35. In addition to the observed

effect on titres to NDV and IBDV, ACE-M also had an effect on flock immunity^[37]. Acemannan adjuvant had mixed results when added to an oil-based vaccine for immunization of chickens, suggesting insufficient potency to justify human vaccine development.

Cultures of normal chicken spleen cells and HD 11 line produce nitric oxide (NO) in response to acemannan. Neither cell type produced detectable amounts of NO in response to similar concentrations of yeast mannan. NO production was dose dependent and inhibitable by the NO synthase inhibitor N^G-methyl-L-arginine. In addition, the production of NO was inhibited by preincubation of acemannan (ACM) with concanavalin A in a dose-dependent manner. These results suggest that ACM-induced NO synthesis may be mediated through macrophage mannose receptors, and macrophage activation may be accountable for some of the immunomodulatory effects of ACM in chickens^[38]. Karaka K. group have demonstrated that acemannan activates macrophages *in vitro*.

Djeraba A. group demonstrated a quick and lasting *in vivo* priming effect of acemannan (ACM) on macrophage response after intramuscular inoculation in chickens (500 µg per 2-month-old bird). In response to IFN- γ *in vitro*, monocytes from ACM-treated chickens exhibited a strong enhancement of NO production from 3 to 9 days intramuscular injection (p.i.) but a weaker effect on MHC II cell surface antigen expression on days 3 p.i. A stimulating effect of ACM treatment was also observed on spontaneous and inducible NO production for splenocytes only on days 3 p.i.. By that time, splenocytes exhibited a strong higher capacity to proliferate in response to the T cell-mitogen PHA. At the same time, the *in vivo* capacity to produce NO, measured by the (NO₂⁻+NO₃⁻) serum level after intravenous LPS injection, increased greatly from 3 to 9 days p.i.. In conclusion, ACM was able efficiently and durably to increase the activation capacity of macrophages from the systemic immune compartment (in particular from the blood and spleen after an intramuscular injection) in chickens, especially for NO production. These findings provide a better understanding of the adjuvant activity of ACM for viral and tumoral disease^[39]. *Aloe vera* gel may contribute the activity to the test agent in synergistic fashion in increasing drug penetration. And *Aloe vera* gel fraction and acemannan are a modulator capable of blocking the immune system observed in the adjuvant arthritis animals and pre-clinical trials, and can block the mediators responsible for inflammation. A single injection of ACM to chickens appeared able to prime systemic macrophages efficiently and durably better to respond to stimulatory signals. This could account for the very effective adjuvant activity of avian vaccination against viral and tumoral diseases. Acemannan was approved by the United States Department Agriculture for use as a vaccine adjuvant in chickens, cats and dogs.

ALOE STEROLS RESPONSIBLE FOR IMMUNOMODULATOR

Aloe vera may be powerful, non-toxic, plant-derived treatment of both inflammation and wound healing. Hydrocortisone and its related drug, are most effective treatment for inflammation. However, while inhibiting inflammation, hydrocortisone blocks wound healing. phytosterols in *Aloe vera*, such as β-sitosterol, stigmasterol, campesterol, and lupeol, may decrease the intestinal absorption of cholesterol, and also block cholesterol synthesis within subcutaneous cells by inhibiting HMG-CoA reductase. To wound healing, anti-inflammation and LDL-cholesterol- lowering effect, a thorough discussion of the role of phytosterols in *Aloe vera* is presented. Macrophages are essential for proper wound healing to

occur. Stimulation of the macrophages will increase cell and tissue growth, fibroblast proliferation, and fibroblast activity. Furthermore, acemannan may also promote wound healing in this way.

A comparative study by Malini T. group was made of the effects of β -sitosterol, estradiol-17 β and progesterone, individually and in combinations, on certain biochemical parameters important to carbohydrate metabolism in the uteri of adult ovariectomised rats. β -Sitosterol (SITO), estradiol (E₂) and combined treatment (SITO+E₂) induced significant increases in glycogen concentration and the activities of glucose-6-phosphate dehydrogenase (G6PDH), phosphohexose isomerase (PHI) and total lactate dehydrogenase (LDH). Progesterone (P) administration however, raised only the uterine PHI and LDH activities. Co-administration of P with β -sitosterol (P+SITO) suppressed the SITO-induced increase in glycogen concentration and G6PDH activity. On the other hand, combined treatment (P+SITO) augmented total LDH activity^[40]. Administration of estradiol/progesterone to ovariectomized animal significantly increased the uterine weight, RNA, DNA and protein concentrations. Similarly, administration of β -sitosterol alone or in combination with estradiol caused a marked increase in the above parameters and the maximum influence was evident only median and high dose treatments. However, administration of median/high dose of β -sitosterol along with progesterone accentuated only the RNA and protein concentrations but exerted an inhibitory effect on β -sitosterol-induced increment in uterine weight and DNA concentrations^[41].

For the oral study, experimental ICR mice received *Aloe vera* in their drinking water for 2 months, whereas the control animals received only water. In the topical study, experimental animals were given 25% *Aloe vera* in Eucerin cream topically. The control animals received cream only. A 62.5% reduction in wound diameter was noted in mice receiving 100 mg/kg/day oral *Aloe vera* and a 50.8% reduction was recorded in animals receiving topical 25% *Aloe vera*. Davis RH. group suggested that *Aloe vera* is effective by both oral and topical routes of administration^[42].

Aloe vera, as a biological vehicle for hydrocortisone 21-acetate, was tested topically and systematically against acute inflammation. Systemically, the combination of *Aloe vera* and hydrocortisone produced a maximum 88.1% inhibition of edema. Polymorphonuclear leukocyte infiltration was reduced 91.1%. The topical inhibition of edema peaked at 97%. The possibility that *Aloe vera* has significant potential as a biologically active vehicle for steroids is discussed by Davis RH. group^[43]. *Aloe vera* gel at dose of 100 and 300 mg/kg daily for 4 days in subcutaneous injection, blocked the wound healing suppression of hydrocortisone acetate up to 100% using the wound tensile strength assay. This response was because of the growth factors present in *Aloe vera* masking the wound healing inhibition such as sterols and certain amino acids. The sterols showed good anti-inflammatory activity (-36%) in reducing the croton oil-induced ear swelling. This activity displayed a dose-response relationship^[44]. The topical and systemic anti-inflammatory activity of *Aloe vera* alone and in combination with hydrocortisone acetate revealed that *Aloe vera* contributed in an additive way to the activity of the steroid, suggesting that *Aloe vera* may be useful as a biological vehicle for hydrocortisone. In Davis RH. group studies, the significance of this finding was suggested that if used in combination with *Aloe vera*, the dosage of hydrocortisone can be reduced, while maintaining its biological activity, thereby reducing or eliminating any toxic side effects associated with higher dosage.

Evaluation of bioactive potential of *Aloe vera* sterol extract was carried out by Bawankar R group. β -Sitosterol and stigmasterol were found to be 2.89% and 2.1% in the extract. HPLC analysis was

carried out to confirm the presence of stigmasterol. The concentration of sterol extract needed to scavenge DPPH free radical by 50% was calculated as 5.2 mg/mL. In the FRAP assay, the sterol extract showed significant hydroxyl radical scavenging in a dose-dependent manner (IC₅₀ value 1.17 μ g/mL). Concentration of the sample required to reduce lipid peroxidation was found to be 4.18 g/mL, and the extract also possessed acetylcholinesterase activity (IC₅₀: 5.26 μ g/mL). Catalase activity was 0.196 μ M/H₂O₂ decomposed/min/ μ g protein, whereas the peroxidase activity was 17.01 μ M of pyrogallol oxidized/min/ μ g protein. The extract recorded higher activity against growth of *S.greaseus* and *C.albicans* in the experiments carried out to determine antibacterial and anti-fungal activity, respectively^[45].

The effects of the oral administration of aloe sterols: lophenol and cycloartenol isolated from *Aloe vera* on glucose and lipid metabolism in Zucker diabetic fatty (ZDF) rats. Orally ingested aloe sterols altered the expressions of genes related to glucose and lipid metabolism, and ameliorated obesity-associated metabolic disorders in ZDF rats. These findings by Misawa E. group suggest that aloe sterols could be beneficial in preventing and improving metabolic disorders with obesity and diabetes in rats^[46].

The study was performed to quantify the blood triglyceride (TG)-lowering effect of plant sterol (PS) by pooling individual subject data from 12 randomised controlled trials that investigated the effect of PS on blood lipids. The main outcome variable was the control-adjusted PS effect on relative (%) and absolute (mmol/L) changes in TG. The relative and absolute changes in high-density lipoprotein cholesterol (HDL-C) were also assessed. Differences in changes of serum lipid concentrations between PS and control treatments were estimated by an ANCOVA using a random effect model which included PS intake (active or control), study and predefined subject characteristics. The twelve randomised controlled trials included in total 935 hypercholesterolaemic subjects not preselected based on their baseline TG concentrations. In most studies, the PS dose ranged between 1.6 and 2.5 g/day. PS intake significantly lowered serum TG by 6.0% (95% CI:-10.7, -1.2) or 0.12 mmol/L (95% CI:-0.20, 0.04). No significant interaction was observed between PS intake and baseline TG concentrations on relative changes, but, on absolute changes, interaction was significant with larger TG decreases observed with higher TG concentrations at baseline. No effects were observed on HDL-C concentrations. In conclusion, these results show that PS exert a modest TG-lowering effect which is dependent on baseline concentrations^[47]. Demonty I. group demonstrated that these effect may add to the overall benefit of using plant sterols-enriched foods as part of therapeutic lifestyle and diet changes for improving blood lipid profiles.

Ras RT. group aimed for a meta-analysis to investigate the combined and separate effects of plant sterols and stanols (PS) when classified into different dose ranges. Studies were searched and selected based on predefined criteria. Relevant data were extracted. Average LDL-cholesterol effects were calculated when studies were categorised by dose, according to random-effects models while using the variance as weighing factor. This was done for plant sterol and stanols combined and separately. In total, 124 studies (201 strata) were included. Plant sterols and stanols were administered in 129 and fifty-nine strata, respectively; the remaining used a mix of both. The average PS dose was 2.1 (range 0.2-9.0) g/d. PS intakes of 0.6-3.3 g/d were found to gradually reduce LDL-cholesterol concentrations by, on average, 6-12%. When plant sterols and stanols were analyzed separately, clear and comparable dose-response relationships were observed. Studies carried out with PS doses exceeding 4g/d were not pooled, as these were scarce and scattered across a wide range

of doses. In conclusion, the LDL-cholesterol-lowering effect of both plant sterols and stanols continues to increase up to intakes of approximately 3 g/d to an average effect of 12%^[48].

Based on plasma low-density lipoprotein-cholesterol (LDL-C) levels lowering and the absence of adverse signals, the European Atherosclerosis Society (EAS) concluded that functional foods with plant sterols/stanols may be considered 1) in individuals with high cholesterol levels at intermediate or lower global cardiovascular risk who do not qualify for pharmacotherapy, 2) as an adjunct to pharmacologic therapy in high and very high risk patients who fail to achieve LDL-C targets on statins or are statin-intolerant, 3) and in adults and children (>6 years) with familial hypercholesterolaemia, in line with current guidance. However, it must be acknowledged that there are no randomised, controlled clinical trial data with hard endpoints to establish clinical benefit from the use of plant sterols or plant stanols^[49]. The EAS consensus panel critically appraised evidence relevant to the benefit to risk relationship of functional foods with added plant sterols and/or plant stanols, as components of a healthy lifestyle, to reduce LDL-C levels and thereby lower cardiovascular risk.

When consumed in sufficient amounts, plant sterols, including phyto-sterols, -stanols and esterified sterols with fatty acids, are associated with reduced blood levels of cholesterol. Because elevated with reduced blood levels of cholesterol have been identified as a risk factor for coronary heart disease, there is considerable interest from the food industry in marketing the cholesterol-lowering effect by selling plant sterol-fortified products carrying health claims. The Food Directorate of Health Canada has assessed the validity of this claimed effect as well as the safety of plant sterol addition to food products in response to industry petitions^[50]. Health Canada concluded that acceptable scientific evidence exists in support of the claim about the relationship between the consumption of plant sterol-enriched foods and blood cholesterol lowering.

GUT MICROBIOTA

Importance of microbiota for health and disease

The use of prebiotics is a possible strategy to manage and steer the complex gut microbial community towards a health-promoting composition. Larch arabinogalactan is an excellent source of dietary fiber, and has been approved as such by the United State FDA. It has been shown to have the significant more-generating activity than either pectin or xylan in production of short-chain fatty acids, principally butyrate and propionate, and has been shown to decrease the generation and absorption of ammonia. Evidence also indicates human consumption of larch arabinogalactan has a significant effect on enhancing beneficial gut microbiota, especially increasing anaerobes such as Bifidobacteria and Lactobacillus. Larch arabinogalactan has several increasing properties which appear to make it an ideal adjunctive supplement to consider in cancer protocols. Experimental studies by Kelly GS. group have indicated larch arabinogalactan can stimulate natural killer (NK) cell cytotoxicity, enhance other functional aspects of the immune system, and inhibit the metastasis of tumor cells to the liver. The immune-enhancing properties also suggest an array of clinical uses, both in preventive medicine, due to its ability to build a more responsive immune system, and in clinical medicine, as a therapeutic agent in conditions associated with lowered immune function, decreased NK activity, or chronic viral infection^[51]. In the studies of Marzorati M. group the Simulator of the Human Intestinal Microbial Ecosystem was used to investigate the effects of two commercially- available

plant polysaccharide supplements on the structure, composition and metabolism of an in vitro cultured colon microbial community. Microbial analyses showed both a bifidogenic (up to +1.3 log cfu/mL) and a lactobacillogenic (up to +0.9 log cfu/mL) effect during treatment with the dietary supplements. Quantitative PCR confirmed that the increase of Bifidobacteria spp. was statistically significant ($p < 0.05$) in all of the colon compartments and showed a significant increase of the Bacteroides-Prevotella group concentration (+0.6 log cells/mL) in the compartment simulating the proximal colon. Denaturant gradient gel electrophoresis analyses and a relative ecological interpretation, in combination with sugar and short-chain fatty acids quantification, provided evidence of a positive effect of both the tested products. Overall, the treatment period was associated with (i) good and selective fermentability of the polysaccharide supplements along the entire colon; (ii) positive and selective bifidogenic effect; (iii) the possibility of enhancing species belong to Bacteroidetes, a phylum recently associated with body weight management^[52].

Prebiotic oligosaccharides, with a degree of polymerization (DP) of mostly less than 10, exhibit diverse biological activities that contribute to human health. Currently available prebiotics are mostly derived from disaccharides and simple polysaccharides found in plants. Soluble differences in the structures of oligosaccharides can cause significant differences in their prebiotic properties. Therefore, alternative substances supplying polysaccharides that have more diverse and complex structures are necessary for the development of novel oligosaccharides that have actions not present in existing prebiotics. Yoo HD. group showed that structural polysaccharides found in plant cell wall, such as xylans and pectins, are particularly potential resources supplying broadly diverse polysaccharides to produce new prebiotics^[53].

Udani JK. evaluated the ability of a proprietary arabinogalactan extract from the larch tree (ResistAid) to change the immune response in healthy adults to a standardized antigenic change (tetanus and influenza vaccines) in a dose-dependent manner compared to placebo. The randomized, double-blind, placebo-controlled trial included 75 healthy adults (18-61 years old). Subjects were randomized to receive either 1.5 or 4.5 g/day of ResistAid or placebo for 60 days.

At day 30, subjects were administered both tetanus and influenza vaccines. Serum antibody response [tetanus immunoglobulin G (IgG), influenza A and B IgG and immunoglobulin M (IgM)] was measured at days 45 (15 days after vaccination) and 60 (30 days after vaccination) of the study and compared to baseline antibody levels. Frequency and intensity of adverse events were monitored throughout the study. All 3 groups demonstrated an expected rise in tetanus IgG levels 15 and 30 days following the vaccine. There was a strong significant difference in the rise in IgG levels at 60 days in the 1.5 g/day group compared to placebo ($p = 0.008$). In the 4.5 g/day group, there was significant rise in tetanus IgG at days 45 and 60 compared to baseline ($p < 0.01$) but these values were not significant compared to placebo. Neither group demonstrated any significant elevations in IgM and IgG antibodies compared to placebo following the influenza vaccine. There were no clinically or statistically significant or serious adverse events. In conclusion, ResistAid at dose of 1.5 g/day significantly increased the IgG antibody response to tetanus vaccine compared to placebo. In conjunction with earlier studies, this validates the effect of ResistAid on the augmentation of the response to bacterial antigens (in form of vaccine)^[54].

Kukkonen K. group performed a nested unmatched case-control study of 237 infants participating in a randomized double-blind

placebo-controlled allergy prevention trial using a combination of four probiotic strains (*Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99, *Propionibacterium freudenreichii* ssp. *Shermanii*) prenatally and during 6 months from birth. The authors measured faecal IgA, α 1-antitrypsin (α 1-AT), tumor necrosis factor- α (TNF- α), and calprotectin at the age of 3 and 6 months. By age 2 yr, 124 infants had developed allergic disease or IgE-sensitization (cases) and 113 had not (control). In infants with high faecal IgA concentration at the age of 6 months, the risk of having any allergic disease before the age of 2 yr tended to reduce [odds ratio (OR): 0.52] and the risk for any IgE-associated (atopic) disease reduced significantly (OR:0.49). High faecal calprotectin at the age of 6 months associated also with lower risk for IgE-associated diseases up to age 2 yr (OR:0.49). All faecal inflammation markers (α 1-AT, TNF- α , and calprotectin) correlated positively with faecal IgA ($p < 0.001$). Probiotics combination of four strains, tended to augment faecal IgA in early life associates with significantly increased faecal α 1-AT ($p = 0.001$). High intestinal IgA in early life associates with minimal intestinal inflammation and indicates reduced risk for IgE-associated allergic diseases^[55].

In the randomized, double-blind, placebo-controlled trial (Clinical Trials. gov no. NCT 00167700), 94 preterm infants (gestational age, $\geq 32+0$ and $\leq 36+6$ weeks; birth weight, >1500 g) treated at Turku University Hospital, Turku, Finland, were allocated to receive oral prebiotics (galacto-oligosaccharide and polydextrose mixture, 1:1), a probiotic (*Lactobacillus rhamnosus* GG, ATCC 531039, or placebo (microcrystalline cellulose) between days 3 and 60 of life. The primary outcome was the incidence of clinically defined virus-associated respiratory tract infections (RTI) episodes confirmed from nasal swabs by using nucleic acid testing. Secondary outcomes were the severity and duration of RTIs. A significantly lower incidence of RTIs was detected in infants receiving prebiotics [ratio (RR), 0.24; 95% CI, 0.12-0.49; $p < 0.001$] or probiotics (RR, 0.50; 95% CI, 0.28-0.90; $p = 0.022$) compared with those receiving placebo. Also, the incidence of rhinovirus-induced episodes, which compared 80% of all RTI episodes, was found to be significantly lower in the probiotic (RR, 0.31; 95% CI, 0.14-0.66; $p = 0.003$) and probiotic (RR, 0.49; 95% CI, 0.24-1.00; $p = 0.051$) groups compared with the placebo group. No differences emerged among the study groups in rhinovirus RNA load during infections, duration of rhinovirus RNA shedding, duration or severity of rhinovirus infections, or occurrence of rhinovirus RNA in asymptomatic infants. In conclusion, Luoto R. group demonstrated that gut microbiota modification with specific prebiotics and probiotics might offer a novel and cost-effective means to reduce the risk of rhinovirus infections^[56].

Kostic AD. group examined the relationship between human gut microbiome dynamics throughout infancy and type 1 diabetes (T1D) on a cohort of 33 infants genetically predisposed to T1D. Modeling trajectories of microbial abundances through infancy revealed a subset of microbial relationships shared across most subjects. Although strain composition of a given species was highly variable between individuals, it was stable within individuals throughout infancy. Metabolic composition and metabolic pathway abundance remained constant across time. A marked drop in α -diversity was observed in T1D progressors in the time window between seroconversion and T1D diagnosis, accompanied by spikes in inflammation-favouring organisms, gene functions, and serum and stool metabolites. This works identified trends in the development of the human infant gut microbiome along with specific alterations that precede T1D progressors from nonprogressors^[57]. T1D onset is preceded by a drop in community diversity and a spike in

inflammation-associated species and metabolic pathways.

There is increasing evidence that genetics of the host influence and interact with gut microbiota. Moreover, aging-associated oxidative stress may cause morphologic alterations of bacterial cells, thus influencing the aggressive potential and virulence markers of an anaerobic bacterium and finally the type of interaction with the host. At the same time, microbiota may influence host gene expression and it is becoming apparent that it may occur through the regulation of micro RNAs. They are short single-stranded noncoding RNAs that regulate post-transcriptional gene expression by affecting mRNA stability, and/or translational repression of their target mRNAs. The introduction of -omics approaches (such as metagenomics, metaproteomics, and metatranscriptomics) in microbiota research will certainly advance the knowledge of this area. Patrignani P. group suggested that these will lead to greatly deepen the understanding of the molecular targets in the homeostatic interaction between the gut microbiota and the host and, thereby, promise to reveal new ways to treat diseases and maintain health^[58].

The event (A conference: Human microbiome science: vision for future) brought together experts in the field of human microbiome research, and aimed at providing a comprehensive overview of the state of microbiome research and understanding the functional roles of microbiota in health and disease^[59]. This report summarizes what is needed for human microbiome research to move forward and deliver medical translational applications.

Aloe's putative efficacy based on the modulation of gut microbiota status

Sinnott RA. group hypothesized that a small subset of colonic flora would be able to utilize complex plant polysaccharides (CPPs). Feces from 4 healthy humans were sequentially passed three times over 9 days in growth media composed of either *Aloe vera* gel polysaccharides, larch arabinogalactan, or a dietary supplement including these two ingredients suspended in a minimal salt solution. CPPs were first dialyzed to eliminate polymers and sugars $< 8,000$ MW, forcing bacteria to consume only higher MW components. Isolates were obtained on rich media and then 16SDNA sequenced for species identification. API strips were in some cases used to confirm identification. A total of 6 species were identified; however, 90% of isolates were Enterococci. Supernatant analysis showed variable consumption of the entire MW range of polysaccharide components. These findings suggest that enterococcus species play an important role in the utilization of CPPs^[60].

The metabolism of fluoresceinyl isothiocyanate labelled aloemannan (*Aloe vera* freeze-dried powder supplied by Aloecorp Inc: FITC-AM) was examined by p.o. and i.v. administration in mice at a dose of 120 mg/kg. Analysis of FITC-AM in urine and feces showed that FITC-AM (MW 500KD) was metabolized into smaller molecules that mainly accumulated in the kidneys. Aloemannan (AM) was catabolized by the human intestinal microflora to catabolites 1 and 2 with molecular weights of 30 and 10KD, respectively. Hydrolysis of AM showed hexosamine peaks on high performance anion exchange chromatography. The findings suggest that the immunomodulation of AM may come from not only neutral polysaccharides but also contaminated hexosamine in AM. The restorative activity of catabolite 1 and 2 in immunomodulation (UVB-induced immunosuppression or contact hypersensitivity) showed 3.5% and 29.5%, respectively, together with that of AM (41.7%) and that of the lyophilized powder (60.8%) from *Aloe vera* gel. No significant difference between catabolite 2 and AM was observed. Yagi A. group exhibited that participation of gut microbiota to *In*

in vivo metabolism of aloe polysaccharide (FITC-labelled acemannan, MW:500 KD) was noteworthy in immuno-modulation^[61].

Pogribna M. group investigated the effect of *Aloe vera* whole leaf extract on short chain fatty acids production. *Bacteroides fragilis*, *Bifidobacterium infantis*, and *Eubacterium limosum* were incubated with *Aloe vera* extracts [0%, 0.5%, 1%, 1.5%, and 2%; (w/v)], obtained by treating the slurry with 23mg/L cellulase 4000 to reduce viscosity and maximize yields, for 24 and 48 h. Short chain fatty acids (SCFA) production was measured by GC/MS analyses. A significant linear increase in growth response to *Aloe vera* supplementation was observed at 24 h for each of the bacterial culture; however, only *B.infantis* and a mixed bacterial culture showed a significant positive linear dose response in growth at 48 h. In pure bacteria cultures, a significantly enhanced dose response to *Aloe vera* supplementation was observed in the production of acetic acid by *B.infantis* at 24 h and of butyric acid by *E.limosum* at 24 and 48 h. In the mixed bacterial culture, the production of propionic acid was reduced significantly at 24 and 48 h in a dose-dependent fashion, whereas butyric acid production showed a significant linear increase. In conclusion, the results indicated that *Aloe vera* possessed bacteriogenic activity *in vitro* and altered the production of acetic, butyric and propionic acids by micro-organisms selected for the study. The results of the study suggest that consumption of a dietary supplement, *Aloe vera*, may alter the production of SCFA by human intestinal microflora^[62].

The evaluation of the prebiotic potential of *Aloe vera* gel was carried out by *in vitro* fermentation using intestinal microbiota from six healthy donors as the inoculum. The prebiotic activity was assessed through the quantification of short chain fatty acids and the evaluation of dynamic bacterial population in mixed faecal cultures by fluorescence *in situ* hybridization. The findings support the possible incorporation of *Aloe vera* gel in the development of a variety of food products known as prebiotics aimed at improving gastrointestinal health. Gullon B. group's findings support the possible incorporation of the *Aloe vera* gel in the development of a variety of food products known as prebiotics aimed at improving gastrointestinal health^[63].

The non-starch dietary fiber in *Aloe vera* whole leaf extract may alter the growth and short chain fatty acids production of intestinal microbial *in vitro*. *B.fragilis*, *B.infantis* and *E.limosum* as the representative anaerobic bacteria of the human gut ecosystem showed the effect of *Aloe vera* on SCFA production. Further studies are needed to assess the impact of *Aloe vera* on SCFA production in the human intestinal environment.

CONCLUSION AND FUTURE PERSPECTIVES

Aloe vera inner leaf latex and rind contain many complex organic compounds such as chromones, flavonoids and anthraquinones. Some of these molecules can have significant anti-inflammatory activity. Unfortunately *Aloe vera* extract preparations used for studies have tend to be complex and it is difficult to elucidate the biological pathways involved. Most studies that have been performed have focused on mesophyll gel of *Aloe vera* leaf and on its major storage acemannan, a complex carbohydrate with both immunostimulatory and anti-viral properties, but unique aloe pectin, lectin and protein having important properties, have been isolated from the mesophyll cell wall gel. There are several widely available plant β -1,4 linked mannan (galactomannan, Konjac mannan, and ivory mannan), but they are not acetylated. Thus, 1H-NMR analysis to identify the acetylated mannan in the aloe products was performed for product identification and prevention of falsification. The two

features of the mannan, the β 1-4 linkage and acetylation, are highly conserved among *Aloe vera* pulp. Significant variations exist regarding the presence of other sugars, primarily glucose and the degree of acetylation. Several researches strongly suggest that there are two type of mannans in the pulp, a pure mannan that is essentially free of glucose^[27] and another mannan (glucomannan) that contains various amounts of glucose, may be along with other sugars^[24]. Evidence has suggested that the mannan or glucomannan with a low glucose content is more heavily acetylated than the glucomannan with a high glucose content. Aloe mannan or acemannan is a unique polysaccharide. Future studies on the structure-function relationship in light of the increasing number of biological activities that have been attributed to the pulp carbohydrates; acemannan, aloe pectin, lectin (verectin)^[64], and protein in purified form, will certainly yield more insight into biochemical and functional properties.

An importance for understanding the microbial ecology of the gastrointestinal ecosystem and assessing how the microbial world within us impacts on our every day life. Long-term dietary intake influences the structure and activity of the trillions of micro-organisms residing in the human gut, but it remains unclear how rapidly and reproducibly the human gut microbiome responds to short-term micro-nutrient change. The short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression. The findings by David LA. group demonstrated that the gut microbiome can rapidly respond to altered diet, potentially facilitating the diversity of human dietary lifestyles^[65]. A comprehensive review of the literature to consolidate all controlled studies assessing various roles of exogenous polysaccharide-rich extracts from plants (acemannan), fungi (β -glucan), and other natural sources on brain function, were performed with a significant focus on benefits derived from oral intake. Although the mechanisms by which exogenous saccharides can influence brain function are not well understood at this time, the review article of Nelson ED. group suggested that polysaccharide-rich extracts show positive effects on cognitive function and mood in healthy adults and promises, when taken orally, in supporting neurologic health and function^[66]. Furthermore, the human microbiome impacts human brain health in numerous ways, and gut microbes shape the architecture of sleep and stress reactivity of the hypothalamic-pituitary-adrenal axis. Human gut microbiome impacts human brain health in various ways influencing memory, mood and cognition, and is clinically and therapeutically relevant to a range of disorders, including chronic fatigue syndrome. Galland L. suggested that nutrition tools for altering the gut microbiome therapeutically include changes in diet, probiotics, and prebiotics^[67]. The gut microbiome has played a crucial role in the bidirectional gut-brain axis that integrates the gut and central nervous system (CNS) activities, and thus the concept of microbiome-gut-brain axis is emerging. Studies are revealing how diverse forms of neuro-immune and neuro-psychiatric disorders are correlated with or modulated by variations of microbiome, microbiota-derived products and exogenous antibiotics and probiotics. The microbiome poises the peripheral immune homeostasis and predisposes host susceptibility to CNS autoimmune diseases such as multiple sclerosis. Neural, endocrine and metabolic mechanisms are also critical mediators of the microbiome-CNS signaling, which are more involved in neuro-psychiatric disorders such as autism, depression, anxiety, and stress. Research on the role of microbiome in CNS disorders were exhibited by Wang Y. group^[68]. El Aidy S. group commented: What would be the molecular mechanisms underlying the intimate cross-

talk between the immune system and the microbiota-gut-brain axis at its various nodes of interaction? This is required to be answered in future^[69]. New insights have raised an interest in fecal microbiota transplantation (FMT) for the management of extra-intestinal disorders associated with gut microbiota. FMT achieved a successful cure rate in recurrent *Clostridium difficile* infection. The review article of Xu MQ. group revealed that FMT could be a promising rescue therapy in extra-intestinal disorders associated with gut microbiota, including metabolic diseases, neuropsychiatric disorders, autoimmune diseases, allergic disorders, and tumors^[70]. In view of personalized physiology and nutrition providing patient-tailored therapies, the homeostatic interaction between the gut microbiota and the host reveals new ways to maintain quality of life and health.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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