Animal Platform

Acemannan in animals and possible veterinary applications

Scientific Review

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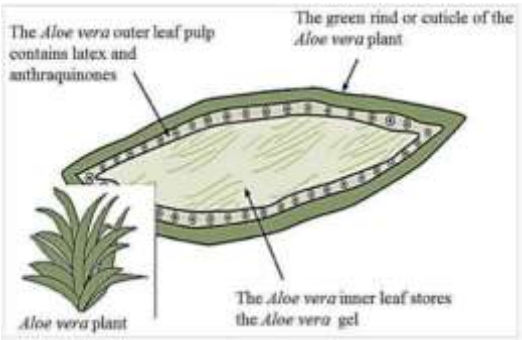
INTRODUCTION

Aloe Vera

Aloe-Vera is a plant widely used in the biomedical, pharmaceutical, food and cosmeceuticals industry. There are at least 420 different plant species of Aloe. Aloe-Vera specifically refers to the *Aloe barbadensis* Miller plant, which is the most common form used in Aloe-based products [1].

Aloe-Vera is a stemless or very short-stemmed plant. The plant is considered a succulent species due to its thick fleshy leaves that help it to retain water (in the form of the gel) in hot, arid climate. Aloe species prefer semi-desert regions with warm climates and grow best on dry, sandy and calcareous terrain [2].

The transverse section of the leaf exhibiting three cells layers: the protective layer, middle layer and colorless inner layer [3].



The plant leaves contains over 75 biologically active and naturally-occurring compounds including polysaccharides, vitamins, minerals, enzymes, amino acids and natural sugars that function in association with other compounds of the human body to deliver numerous health benefits [1,4]. The bioactive components in the Aloe-Vera have been reported to, antiseptic, antiviral,

antibacterial, antidiabetic, anti-inflammatory, antioxidant, immune-modulatory, gastro protective and wound healing properties.

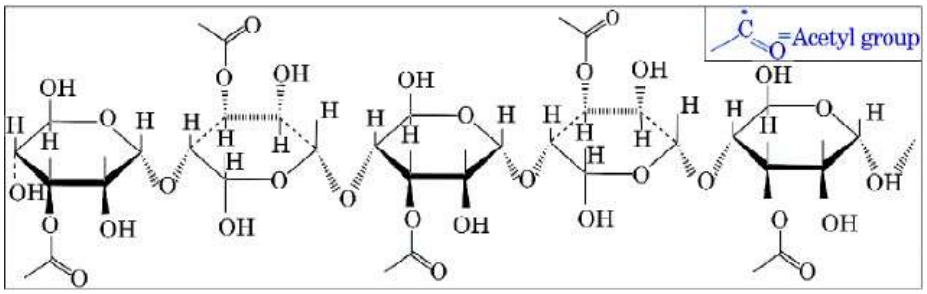
Biologically active components in Aloe-Vera may be labile, varied, or modified since the chemical composition of the Aloe-Vera varies and depends on source, climate, region, and the processing method [4,5].

Acemannan

Aloe is a rich source of polysaccharides [4], these polysaccharides are responsible for the majority of the biological activities observed from the use of the Aloe-Vera plant [6].

Acemannan is a predominant component polysaccharide of the Aloe-Vera. This polysaccharide constituted by acetyl-mannose unities united by β-(1,4) glycosidic bonds. It is a hydrophilic molecule with an average molecular weight of approximately 1-2 Million Daltons.

Basic molecular structure of acetylated mannan or Acemannan is shown below [7]:



Acemannan is obtained from the Aloe-Vera leaves and it is considered the main functional component of Aloe-Vera[3].

As mentioned, biologically active components in Aloe-Vera may be labile, varied, or modified since the chemical composition of the Aloe-Vera gel varies and that even within the same species, the plant depends on source, climate, region, and processing method [4,5]. For this reason, there is a big advantage of being able to use a stable commercially available purified active ingredient rather than relying on freshly harvested material.

It is also important to note that the processing steps can alter the properties of polysaccharides by affecting their original structure, which may bring some changes in the proposed physiological and pharmaceutical properties of these constituents[1]. This is why the manufacturing process is a key factor for obtaining an Extract that remain active, keeping all the beneficial properties of the plant.

Acemannan has many nutritional qualities and is largely responsible for the benefits Aloe-Vera offers [8]. It has been extensively studied in the last 30 years and was shown to have many biological activities including anti-cancer, anti-inflammatory, antibacterial, antiviral, cell proliferation, immunomodulatory and wound healing effects.

Most of the beneficial effects of Acemannan are derived from its effect on the immune system. **The immune system** role is to defend the organism from diseases such as infectious diseases (such as viral, bacterial etc.), neoplastic, metabolic, and more.

The immune system is subdivided into the innate and adaptive immune system. Both subsystems use humoral immunity and cell-mediated immunity. In order to function, the immune system components communicate and

work together. The immune system is composed of different types of cells, proteins, and organs. The fundamental cells are the white blood cells; those cells can communicate with each other by labyrinth language of cytokine and chemokine secretion and receptors [9].

One of the main pillars of the immune system is specialized cells; Antigen-presenting cells (APC) such as dendritic cells and macrophages. APC's are capable of recognizing pathogenic agents that have penetrated the body, swallow it (phagocytosis), break it into small pieces (antigens) and present it to other immune cells such as B and T lymphocytes. The lymphocytes "learn" the antigen presented to them by the APC, and they can develop a specific and more efficient immune reaction. All of this process is regulated by receptors, hormones, cytokines, chemokines, and more[9].

Immunomodulatory activity of Acemannan

The effect of Aloe-Vera extracts and specifically Acemannan were extensively investigated as immune system modulators 1,3. Those studies show that Acemannan has an augmenting effect on the innate and adaptive immune system 3,10.

It was shown that Mannan rich Acemannan activates Macrophages by binding to macrophage-mannose receptor (MMR). The binding of mannose to MMR induces macrophage activity, increases cytokines secretion, nitric oxide release, enhance phagocytosis, and intracellular killing [11–15]. The macrophages have an essential role in initiating the immune response. The macrophages are also responsible for mediating between the innate and the adaptive immune system.

Cell to Cell communication- Immune system recruitment and activation

Another *In-vitro* experiment showed that Acemannan have increased lymphocytic alloantigen response in a dose response fashion. The same study also found that Acemannan helps the white blood cells to communicate; Acemannan permitted monocytes to enhance signals to T- Cells due to IL-1 release and induce cytotoxic T- Lymphocyte [16,17]. Furthermore, another study was set to find the effect of Acemannan on dendritic cells. Dendritic cells are important parts of the immune system, they are mostly located in the skin and are responsible for initiation of the immune response and for presentation of pathological agent antigens to the adaptive immune system. It was shown that exposure of immature Dendritic cells to Acemannan increased their MHC class II expression suggesting induced maturation and functionality [18].

It was also shown that Acemannan effect other cell factors; stimulate interactions between macrophages, T-lymphocytes and B-lymphocytes; enhance the generation of cytotoxic T-lymphocytes; induced B- and T-lymphocyte activation and upregulate phagocytic activity [4,19].

Figure 2- augmentation activity of Acemannan on the immune system [20]

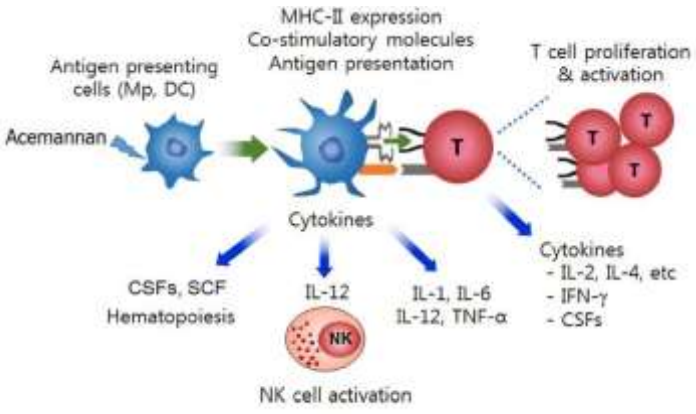


Fig. 2. Major mechanisms of the immunoaugmenting activities of acemannan.

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Anti-bacterial activity

Aloe-Vera gel was bactericidal against *Pseudomonas aeruginosa* and Acemannan prevented it from adhering to human lung epithelial cells in a monolayer culture [3]. Another *in vitro* study shows that Acemannan has anti-bacterial effect. In this study, Acemannan show antibacterial activity against four different bacteria stains. Two Gram-positive bacteria *S. aureus* and *Enterococcus faecalis* and two Gram-negative strains *E. coli*, and *P. aeruginosa.* Against those bacteria Acemannan showed inhibition greater than 98%. Furthermore, Acemannan had a strong *E.Coli* and *E.faecalis* biofilm inhibition effect and biomass eradication effect when used higher concentrations [21].

Anti-tumor activity

Acemannan and phenolic anthraquinones of the aloe vera gel are considered to be prophylactic against certain types of cancer and many chronic degenerative diseases being the potential antioxidants or pro-oxidants with unique DNA breakage preventing potential. Acemannan also prevent malignancy by its potential immune-stimulant activity [7]. In a study on mice that had previously been implanted with murine sarcoma cells, Acemannan stimulates the synthesis and release of interleukin-1 (IL-1) and tumor necrosis factor from macrophages in mice, which in turn initiated an immune attack that resulted in necrosis and regression of the cancerous cells[3].

Acemannan is licensed by the USDA for the treatment of sarcomas in dogs and cats[22] . Sarcomas are malignant tumors that are common in companion animals such as dogs and cats. Injection of Acemannan pre and post-surgery combined with radiation therapy increased the animal's mean survival time and tumor free interval compared to animals with the same condition that received the same therapy without Acemannan injections[23,24].

Anti-fungal activity

Candida Albicans is commonly used as biological model, it is an opportunistic pathogenic organism that can cause a superficial and local infections but also life threatening systemic infections[25]. In- vitro study showed that Macrophage that were exposed to Aloe-Vera Acemannan had a 98% killing and phagocytosis of candida albicans compared to the control group that had only 25% killing[11]. *In Vivo* mice study revealed that feeding Aloe-Vera extract increase resilience to C.*Albicans* challenge [26].

Anti-Viral activity

Acemannan was proven as an antiviral agent in both *in vitro* and *in vivo* studies. It was shown to reduce herpes simplex infection in two cultured target cell lines, to have *in vitro* activity against HIV-1 and a dose dependent synergistic effect with AZT on HIV-infected cells (Kahlon JB et al., 1991; Sahu et al., 2013).

Sun et al., showed *in vitro* inhibition of influenza replication. Recorded by Transmission Electronic Microscopy, the long chain saccharide of Acemannan appears to interact with the influenza virus particles. Furthermore, in the same study, the researcher challenged mice that received oral administration of Acemannan with Influenza virus [28]. The results showed lower mortality rate, less clinical signs, less body weight reduction, less lung tissue damage and reduced viral load.

In other study on cats with Feline immunodeficiency virus oral administration of Acemannan improved their quality of life and life span[29].

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Feline immunodeficiency virus (FIV)

FIV is a retrovirus similar to the human immunodeficiency virus (HIV). The FIV virus attack T- lymphocyte and weaken the immune system to and make the cat more susceptible to other pathogens.

Acemannan can help to treat FIV cats by immunostimulation. A pilot study was undertaken to determine Acemannan's effect in 49 feline immunodeficiency virus (FIV) infected cats with clinical signs of disease. The cats received Acemannan in different routes (IV, SC, 2 [mg] once weekly, or 100 [mg] PO daily). The results show higher mean survival time to cats treated with Acemannan compared to historical control FIV- infected populations. Furthermore, the survival curves do suggest, that IV administration may provide an advantage in the short term by enhancing survival within the first year of treatment[29].

Feline leukemia virus (FELV)

Feline leukemia virus is an oncogenic virus that effect cats. It is a severe disease that cause neoplastic disease and death, about 30% of cats that died from cancer are FeLV positive[30]. Not all of the cats that are infected with the virus develop the disease, most cats have a successful immune response that eliminate the virus. Kittens younger than 4 months old and FIV or other immunocompromised are at higher risk to develop FeLV symptoms, adult and healthy cats usually overcome the virus[31]. the age related immune response to FELV susceptibility is due to different macrophages activity, an in-vivo and in-vitro experiment show that kittens macrophages were 3 fold more susceptible for FeLV infection than adult cats[32].

There isn't an effective treatment and the infected cats usually die 4-8 weeks post diagnosis[33]. In-Vivo study on 50 FELV positive cats showed that cats that were treated with Acemannan significantly lived longer with better quality of life[34].

Wound healing

FDA approved product *CarraVet* Acemannan hydrogel for the treatment of wounds cuts abrasions, post-surgical incisions, first- and second-degree burns, radiation dermatitis and other skin irritations in companion animals- dog cats and horses. Application of the Acemannan hydrogel on the wound promote healing and pain relief by coating, cooling and protecting the wound. Acemannan hydrogel is designed to maintain the correct moisture level in the wound to optimize natural healing by the body[35]. Furthermore, it was shown that Acemannan stimulate fibroblast and connective tissue formation, stimulate repair processes and epithelial cell growth[36], these results suggests that using Acemannan as part of the dressing gel can be beneficial for wound healing process.

Acemannan as a vaccine adjuvant

NDV- Newcastle dieses virus and IBVD - infectious bronchitis disease virus.
Some of the vaccines used today need an adjuvant ingredient in them, the adjuvant helps to create a stronger immune response in order to develop adequate vaccination.
Acemannan as adjuvant can improve NDV and IBVD titer those improve the vaccine efficacy.
Evaluation of Acemannan as a vaccine adjuvant for NDV and IBVD was researched using 1-day old broilers. The broilers were antibody negative for both NDV and IBDV. The hatchlings were randomly assigned into 4

different treatment flocks. Flock 1 was shame vaccinated using saline, flock 2 was vaccinated using oil-based vaccine (the common ingredient in the market), flock 3 was vaccinated with vaccine+ ACE-M mixture and the 4 flock was vaccinated with vaccine and ACE- M on different anatomical locations. The Acemannan treatment groups were injected with 0.5mg of Acemannan suspended in 0.5ml breedervac (containing the antigens for the viruses).

IBDV and NDV IgG titers were determined using commercially available ELISA kits.

The results show that the immune response to **NDV** at 21 days post vaccination (PV) was significantly enhanced ($P \sim< 0.05$) by the addition of ACE-M to the vaccine, compared with vaccination without ACE-M. At day 35 Post Vaccination, 95% of the vaccine + Acemannan treatment group was protected compared to 90% of the flock vaccinated with oil-based adjuvant. In another study it was found that feeding broilers with aloe vera gel supplement increased NDV titer[37].

The effect of adjuvant Acemannan on IBDV was different from the NDV, only after 28 days post vaccination there was a significant different showed higher antibody titer to the treatment group received Acemannan as an adjuvant[38].

Gut microbiome

There is an utmost importance in maintaining steady population of gut microbiome in all animals and especially in productive animals such as poultry, cattle and swine. For years the primer technique in order to maintain stable gut microbiome was extensive use of AGP's (antibiotic growth promoters). But in the last decade the use of AGPs decreased drastically with the EU ban of using it for farm animal and because of public awareness. Stop

using AGPs caused reduction in animal performance especially in the poultry and swine industry. Therefore, there is a need in finding products that will help the animal maintain stable gut microbiome, severe changes lead to the proliferation of pathogens, diarrhea, reduction in food intake and more.

The effect of Aloe Vera on chicken's microflora population

Aloe-Vera and Acemannan can play a crucial role in preserving heterogeneous and healthy microbiome population. In *in-vivo* study on broilers the results show a significant reduction in the number of E-coli colonies and increase in the number of lactobacillus colonies in broilers that were fed with Aloe-Vera gel compared to virginiamycne (antibiotic). In the same study they also found that broilers that fed with 2.5% Aloe-Vera gel had a higher antibody titer for new castle virus[37]. These results suggests that Acemannan works at the intestine lumen and also ingested and enhances the immune system to produce more antibodies.

The effect of pre-biotic Mannan saccharides on farm animals Mannan oligo saccharides-(MOS)

It was shown in numerus research and meta- analysis that Mannan Oligo Saccharide, showed beneficial effects is various species of animals – Swine, Poultry, Turkeys, Cattle and Fish[39]. MOS as food supplement increase productivity, improves FCR (feed conversion ratio), increase Body weight and improve animal health and strengthen their immune system. This polytrophic effect derived from the saccharide structure, long chain Mannan saccharides. When it supplemented in the animal feed MOS reduces pathogenic bacteria colonization in the gastrointestinal tract and it effects intestinal structure and function[39].

Summery

Modern livestock animals are under enormous stress due to high metabolic demand, high density, intensive breeding, and more. This constant stress makes them more susceptible to different illnesses and reduces the function of their natural immunity and their ability to protect themselves. A safe and natural product that enhances immunity can be beneficial for the modern intense animal husbandry. Acemannan as a daily feed supplement or as an injection may help the animals to hold a better immune system, reduce diseases, reduce the use of AGP's, improve the animal's general health, wellbeing and productivity.

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