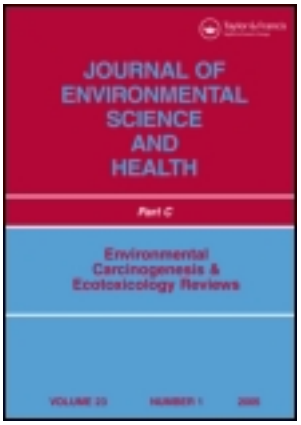

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An Evaluation of the Biological and Toxicological Properties of Aloe Barbadensis (Miller), Aloe Vera

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An Evaluation of the Biological and Toxicological Properties of *Aloe Barbadensis* (Miller), Aloe Vera

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Aloe barbadensis (Miller), Aloe vera, has a long history of use as a topical and oral therapeutic. The plant is the source of two products, gel and latex, which are obtained from its fleshy leaves. Aloe vera products contain multiple constituents with potential biological and toxicological activities, yet the active components elude definition. Ingestion of Aloe vera is associated with diarrhea, electrolyte imbalance, kidney dysfunction, and conventional drug interactions; episodes of contact dermatitis, erythema, and phototoxicity have been reported from topical applications. This review examines the botany, physical and chemical properties, and biological activities of the Aloe vera plant.

Key Words: Acemannan; Aloe vera; Aloe vera gel; Aloe vera latex; Anthraquinone; Polymannans; Toxicological effects

INTRODUCTION

More than one-third of the U.S. population uses some sort of alternative medicine, and the trend is increasing at a significant rate (1). Of the available alternative medicines, the most common are herbal remedies, which are taken by over 38 million individuals in the U.S., or approximately 20% of the U.S. adult population (2, 3). Herbal remedies are used either as topical agents or as dietary supplements for both general health promotion and the specific treatment of ailment symptoms (4, 5). Aloe vera has enjoyed a long history of

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lay acceptance as an herbal remedy and is perhaps the most popular herbal remedy employed today (6, 7). In fact, in a self-reporting survey that examined herbal use in clinical patients and U.S. residents, Aloe vera was the most frequently cited, accounting for 41.2% of the respondents who used herbs (8, 9).

The Aloe vera plant has been used in folk medicine for over 2000 years, and Aloe vera has remained an important component in the traditional medicine of many contemporary cultures, such as China, India, the West Indies, and Japan (10). The Aloe vera plant has a historical reputation as a topical healing agent for abrasions and burns, and as an emollient and moisturizer by the cosmetic industry. Different cultures use Aloe vera in much the same way. The latex, which drips from the plant when cut, is applied to the skin area, or the leaf is split lengthwise and either laid directly on the skin or the inner gel scrapped out and applied as an ointment. In Western societies, especially in the U.S., Aloe vera has been grown mainly to supply the latex component of the leaf to the pharmaceutical industry (11). However, over the last decade, Aloe vera has gained popularity as a therapeutic botanical and, consequently, a large industry has developed (12).

Today, Aloe vera is used as an ingredient in a myriad of health and cosmetic products that include numerous references to the healing properties of Aloe vera (12, 13). Aloe vera is available in a large range of skin moisturizers, face and hand creams, cleansers, soaps, suntan lotions, shampoos and hair tonics, shaving preparations, bath aids, makeup and fragrance preparations, and baby lotions and wipes (14). Topical preparations of Aloe vera have been used to treat frostbite (15), burns (16) radiation dermatitis (17, 18), ulcers (19), psoriasis (20), wounds (14, 21–25), and skin infections (26). Although much of the published evidence regarding the therapeutic value of Aloe vera is contradictory (27), the cosmetic industry has made claims of its rejuvenating, moisturizing, and healing properties, especially on the Internet (28–30).

In recent times, the use of Aloe vera has reached a level of concern, since some herbalists and organizations are promoting oral consumption of it as a prophylaxis and treatment to alleviate a variety of unrelated systemic conditions (31). Promoters offer a number of whole leaf formulations that are widely available for consumption at various concentrations in liquid, powder, and tablet form. Reports credit Aloe vera with anti-tumor (32–38), anti-arthritic (39–41), anti-rheumatoid (40, 42, 43), anti-cancer (36, 44), and anti-diabetic (23, 45–49) properties. In addition, Aloe vera is promoted for constipation and gastrointestinal disorders (50–52) and for immune system deficiencies (6, 53, 54). The scientific literature yields little to substantiate claims of usefulness for systemic conditions by the ingestion of Aloe vera (55, 56).

The popularity of Aloe vera has increased over the past decade, probably stimulated by the enactment in 1994 of the Dietary Supplement and Health Education Act (DSHEA). Aloe vera has multiple constituents possessing potential biological activities (57). Factors such as the species, growing and harvesting

conditions, plant components, and extraction and processing methods affect the concentration and potency of the various constituents of Aloe vera and their potential to exert adverse health effects (13, 58, 59). Despite the widespread use of Aloe vera in many cultures, evidence regarding its benefits is mostly anecdotal. Aloe vera is perceived as safe and "natural" and is marketed without mentioning any potential for harm. In 1998, the National Cancer Institute nominated Aloe vera as a high priority complex mixture for tumor promotion and carcinogenicity testing under the National Toxicology Program. The basis for its selection was the potential for widespread exposure of men, women, and children to oral and topical forms of the Aloe vera plant due to its level of market distribution (55), suspicion of carcinogenicity based on cell proliferation studies and functional comparison with croton oil (60, 61), and the lack of toxicity information. In this review, we briefly discuss the distinctive botany, examine the physical and chemical properties, and then address the biological activities and toxicities associated with the Aloe vera plant and Aloe vera plant products.

BOTANY OF THE ALOE VERA PLANT

Aloe barbadensis Miller (Aloe vera Linne), commonly referred to as Aloe vera, is one of approximately 420 species of Aloe belonging to the lily family (family *Liliaceae*, tribe *Aloineae*) that originated in South Africa, but are now indigenous to dry sub-tropical and tropical climates, including the southern United States (10, 62). Only a few species of Aloe are of commercial importance; Aloe vera is considered to be the most potent and, therefore, the most popular (63).

Aloes are perennial succulents or xerophytes; as such, they are adaptable to habitats with low or erratic water availability, are characterized by the capacity to store large volumes of water in their tissue, and are able to utilize crassulacean acid metabolism, which is an adaptation to the photosynthetic pathway in hot climates that involves the formation of malic acid (64, 65). Aloes have in common green fleshy leaves covered by a thick cuticle or rind and an inner clear pulp (Figure 1). The rind of the Aloe vera leaf accounts for approximately 20–30% by weight of the whole plant leaf, and the pulp represents approximately 65–80% (57). The rind lends turgidity to the leaf and consists of multiple layers of cells interspersed with chloroplasts, where the constituents (carbohydrates, fats, and proteins) are synthesized. The vascular bundles are located within the leaf pulp, but are just beneath and adjacent to the thick rind. The number of these bundles vary, depending on the size of the leaves and the age of the plant (65). The vascular bundles are composed of three types of tubular structures: the xylem—transports water and minerals from the roots to the leaves; the phloem—transports synthesized materials to the roots; and the pericyclic tubules—store and transport the Aloe vera latex along the margin of the leaf. The Aloe vera latex is also commonly referred to as "aloe juice," "aloe sap," or

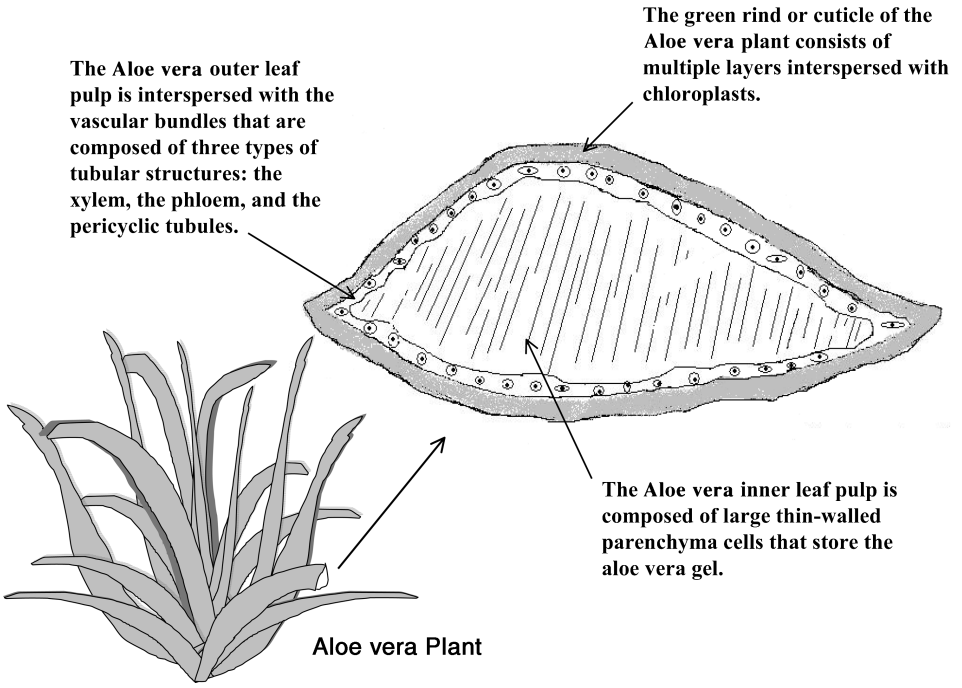

Figure 1: Schematic representation of the Aloe vera plant and a cross-section through an Aloe vera leaf.

simply "aloe." The Arabic word "alloeh" means shining and bitter, and likely refers to the bitter-tasting Aloe latex. When in its dried form, termed aloe, the latex is a drug that is regulated by the U.S. Food and Drug Administration (FDA) as a potent laxative and cathartic agent and is also used as a bitter agent in alcoholic beverages (4, 66). The leaf pulp of the Aloe vera plant, the major part of the leaf by volume, is the innermost portion of the leaf and is composed of large thin-walled parenchyma cells that contain Aloe vera gel. Aloe vera gel is the clear mucilaginous aqueous extract of the leaf pulp (67). Carbohydrates synthesized in excess of that needed for energy are transported by the phloem to the cells in the leaf pulp for storage (57). Water, minerals, and malic acid, a small organic acid formed by crassulacean acid metabolism, are also transported to the leaf pulp. Thus, the Aloe vera gel serves as the water and energy storage component of the plant (59). Among the Aloe species, Aloe vera is the most widely used both commercially and for its therapeutic properties (65).

PHYSICAL AND CHEMICAL PROPERTIES OF ALOE VERA

The physical and chemical composition of Aloes differs depending on the species, climate, and growing conditions (13, 56, 68). A two-year study of the Aloe

vera plant found fluctuations in several physical and chemical properties attributable to seasonal and grower influences (69). For example, the average leaf weight increased, and total and soluble solids decreased during the winter months, suggesting that water evaporation and longer daylight hours in the summer months may account for the lighter leaf weight. In contrast, decreased daylight hours during the winter months may reduce the amount of light energy needed to fix carbon, but may enhance hydration of the plants. Fluctuations in mineral concentrations were attributable to horticultural conditions, such as crop rotation and fertilization methods, rather than irrigation practices.

Limitation in light availability was found to affect primarily total dry mass production and carbon allocation to plant components, such as the number of leaves per plant (59). Surprisingly, light exposure had only minimal effects on the organic solute concentrations in the gel and latex. For example, the percentages of carbon distributed within plants grown in full sunlight were 53% in the leaves and 28% in the roots; while that of plants grown in partial shade was 70% in the leaves and only 13% in the roots. Genet and van Schooten (70) reported that an increase in hydration of the Aloe plant resulted in increases in leaf thickness and gel production; in contrast, overexposure to a combination of sunlight and drought conditions resulted in low gel yield.

The main feature of the Aloe vera plant is its high water content, ranging from 99–99.5% (51). The remaining 0.5–1.0% solid material is reported to contain over 75 different potentially active compounds, including water- and fat-soluble vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds, and organic acids. In compositional studies on the structural components of the Aloe vera plant leaf portions, the rind was found to compose 20–30% and the pulp 70–80% of the whole leaf weight. On a dry weight basis, the percentages of the rind and pulp represented as lipids (2.7% and 4.2%) and that as proteins (6.3% and 7.3%) only accounted for a minor fraction (57). The percentages of soluble sugars (11.2% and 16.5%), primarily as glucose, and the percentages of ash (13.5% and 15.4%), in particular calcium, were relatively high in the rind and pulp, respectively. However, the non-starch polysaccharides and lignin represented the bulk of each leaf fraction and was found to be 62.3% and 57.6% of the dry weight of the rind and pulp, respectively.

PHYSICAL AND CHEMICAL PROPERTIES OF ALOE VERA PLANT PRODUCTS

Aloe Vera Gel

Aloe vera gel is the clear jelly-like substance obtained from the Aloe vera leaf pulp. The mechanical extrusion of the mucilaginous gel from the fibrous

fraction of the pulp gives a 70% yield with a water content of 99–99.5% (57). The gel of field-grown Aloe vera is reported to have a pH of 4.4–4.7 and a total and soluble solids content of 0.56–0.66%; however, seasonal fluctuations and fluctuations due to water availability were noted (69). Others have reported similar findings (67, 71). The high acidity of the Aloe vera gel may be due to the accumulation of organic acids, such as malic acid, in the cells of the pulp.

Many investigators have endeavored to establish the active principles in Aloe vera gel. Chemical analysis of the gel revealed that, as with the rind and pulp, lipids and proteins were minor fractions of the dry weight, representing 5.1% and 8.9%, respectively; however, the amount of soluble sugars (27.8%) detected was substantially higher than that in the rind or pulp (69). The reported ash content was relatively high in all fractions of the plant, but in particular in the gel, where it accounted for 23.6% of the dry matter. Sodium, potassium, calcium, and magnesium were the predominant minerals detected in all leaf fractions; however, calcium was the main mineral detected in the rind and pulp fractions, while sodium and potassium were higher in the gel. The reasons for the predominance of these minerals in the gel is unclear, but sodium is known to have a role in water distribution and potassium is thought to improve tissue repair (72). Non-starch polysaccharides and lignin represented 35% of the dry mass of the gel (57).

The composition and structure of various polysaccharides of the pulp and gel have been described in numerous reports with differing results. Most agree that the Aloe vera gel polysaccharides consist of linear chains of glucose and mannose molecules, and because there is considerably more mannose present than glucose, the molecules are referred to as polymannans. These linear chains range in size from a few to several thousand molecules. By convention, the lower limit to qualify as a polysaccharide is usually a molecular weight of 1,000 Dalton. Different molecular-sized fractions may possess different physical characteristics and differing potential biological activities (73). The major polysaccharide, acemannan, is composed of one or more polymers of various chain lengths with molecular weights ranging from 30–40 kDa or greater, and consisting of repeating units of glucose and mannose in a 1:3 ratio (57, 67). Others have reported the ratio of glucose to mannose in acemannan to be 1:6 (74) , 1:15 (75), and 1:22 (76), discrepancies that may be the result of differences in the species or treatment of samples. The polysaccharide sugar moieties of acemannan are linked by beta (β) glycosidic bonds to form linear chains with random O-acetyl groups and a low degree of galactose side chain branching. The β-1$\rightarrow$4 glycosidic bond configuration of acemannan is an important consideration when examining the reported therapeutic effects of Aloe vera gel, since humans lack the capacity to enzymatically digest these bonds. Other smaller oligosaccharides in this carbohydrate fraction have been proposed to be absorbed in complete form via pinocytosis and enter the blood stream unchanged (51, 77, 78);

however, biochemical and carbohydrate research to support this concept are lacking.

The size and structure of the polysaccharide polymers result in the formation of a colloidal system within the leaf pulp tissue that increases the viscosity and opacity of the mostly aqueous solution (73). The chemical bonds within the carbohydrate polymers contribute to these qualities, but are susceptible to degradation by endogenous and exogenous bacteria (67, 71, 79). Chemically preserved fresh Aloe vera gel stored at room temperature or incubated at 40°C for 48 h exhibited degradation in its rheological properties, a decrease in the content and composition of polysaccharides, and a substantial increase in the mannose:glucose ratio, from 2.9 in the fresh gel to 13.4 in the incubated gel (67). Ross *et al.* (80) examined a number of commercial Aloe vera gel products using size exclusion chromatography and found a wide disparity in the levels acemannans; some products had levels below the detection limits. Similarly, Turner *et al.* (58) found significant variation in commercial product content when compared with plant-derived native Aloe vera gel.

Although acemannan is the most widely studied polysaccharide from the Aloe vera pulp, other polysaccharides have been detected. Some of the polysaccharides differed from acemannan in their degree of acetylation and branching, while others were neither linear nor acetylated and had much lower molecular weights (76, 81). Discrepancies in the composition of these polysaccharides were attributed to season of the year, geographical variations in the species, and sample extraction methods.

Femenia *et al.* (57) identified structural differences in the composition of polysaccharides isolated from the Aloe vera rind, pulp, and gel. β-(1→4)-Linked mannosyl residues were detected in all three fractions; however, significant differences were found in the average molecular weight, degree of acetylation, and abundance of side chains among the different fractions. In the rind, most of the mannose corresponded to structural polysaccharides located within the cell walls; whereas in the pulp and gel, mannose was the main component of the storage polysaccharide acemannan. Therefore, fluctuations in the polysaccharide composition of Aloe vera pulp found in the literature could be explained by the fact that most of the mannosyl residues arise from a storage type of polysaccharide. This would also explain the influence of seasonal and geographical variations in the amount and composition of mannose-containing polysaccharide present within the cells. The parenchyma cell wall and gel components of the Aloe vera leaf pulp were characterized further utilizing light and electron microscopy (65). Acemannan was the major component in the gel; the parenchyma cell wall accounted for 16.2% of the pulp on a dry weight basis and contained galacturonic-rich and galactose-rich polysaccharides that were distinct from acemannan.

Aloe Vera Latex

The restricted distribution of the latex within the margins of the leaves of the Aloe plant suggests that it is a source of secondary metabolites—compounds that do not function directly in plant growth and development, have restricted taxonomic distribution, and are often of unique chemotaxonomic significance (82). Studies have shown that the concentration of Aloe latex components depends on the leaf part, age, position of the leaf on the plant, leaf orientation, and season (83). Young leaves were found to have higher concentrations of latex components compared to older leaves, the terminal third of leaves had higher levels than the base third, and the adaxial part was higher compared with the abaxial part of the leaves (84, 85). In addition, repeated injury to the leaf increased the content of latex components in new growth from the remains of the leaf base on the plant (84). These findings lend credence to the occurrence and distribution of latex in the Aloe vera leaf as a defense strategy to deter it from being consumed.

A wide variety of secondary compounds have been isolated from the latex (86). The compounds are largely phenolic in nature, and chromatographic data of several species have distinguished approximately 80 main constituents (87). Of these, many remain unidentified (12). Smith and Smith (88) are credited with discovering the active purgative principle of the latex and for naming the crude crystalline substance aloin. The anthracene C-glycoside, barbaloin, was subsequently identified as the major component of the latex (89–91). The two names aloin and barbaloin have become synonymous and both currently refer to the compound, barbaloin, rather than the mixture, aloin.

The sugar moiety in barbaloin is D-glucose, and studies indicate that C1 of the D-glucose moiety is linked directly to C10 of the anthracene ring in a β-configuration. (Figure 2). The carbon–carbon bond is quite resistant to acid and alkaline conditions, and cleavage by oxidation, rather than hydrolysis, is achieved only under the drastic conditions of acid in combination with an oxidant (91). The β-(1$\rightarrow$10) C–C bond is also resistant to β-glycosidase of plants and most plant bacteria (92, 93); however, the intestinal micro flora of humans and animals have been shown to cleave the β-C-glucosyl bond, although considerable variation in response among animal species occurs (94–96). The anthracene C-glycosides, which are found in Aloe, are considered to be the most specific secondary products in thin layer chromatography screenings of Aloe plants (85, 97).

The distribution and content of barbaloin reflects that of the leaf latex itself. The percentage of barbaloin in the latex varies from species to species, from season to season, and plant or leaf age. The barbaloin content of latex from different Aloe species was assessed by a number of methods and found to be between 10–25% on a dry weight basis of the latex or approximately 1% on a leaf dry weight basis (98). The occurrence of barbaloin in various species of

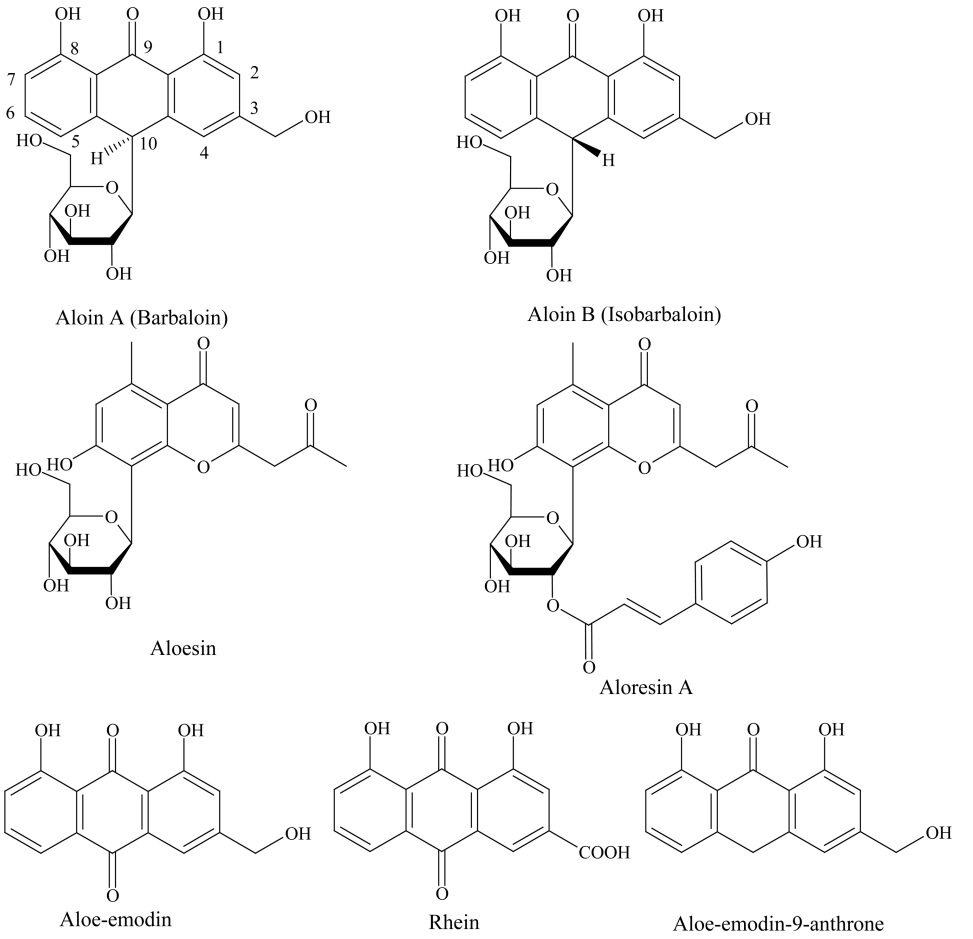

Figure 2: Structures of Aloe vera latex-derived anthraquinone C-glycosides, anthrones, and anthraquinones.

Aloe was analyzed by HPLC, and barbaloin was detected in 85 of 240 species of Aloe plants (98); however, a more recent chemotaxonomic survey of 380 species of Aloe indicated that only 36 (10%) species of Aloe shared similar latex chemistry profiles (99) In addition to barbaloin, Aloe latex was shown to contain a mixture of anthracene-based compounds including O- and C-glycosides of anthrones and anthraquinones, as well as free anthrones and dianthrones and a small amount of free anthraquinones (100). The occurrence of endogenous free anthraquinones and anthrones in Aloe latex results from oxidative processes rather than from metabolic synthesis (95, 97, 101). On a dry weight basis, the Aloe latex is reported to also contain an acid insoluble resin (16–63%), significant ash content (24.5%), and a small quantity of essential oil that is responsible for the odor of the latex (96).

The leaf latex obtained from Aloe vera contains four major C-glycosyl constituents (66). Barbaloin and its epimer, isobarbaloin (Figure 2), have a 9-anthrone skeleton and a β-D-glucopyranosyl substituent (102); aloesin, also known as aloeresin B, is a 5-methyl chromone with an 8-β-D-glucopyranosyl substituent (103); and aloeresin A is a 5-methyl chromone with an 8-β-D-glucopyranosyl -2-O-*trans*-*p*-coumarol substituent (104). Aloesin was suggested as the key component with respect to quality control for Aloe vera products since it shows less seasonal variation and is more stable against hydrolysis and heat than barbaloin (87). Barbaloin and its epimer currently serve as the key component to monitor the quality of latex products. Several other C-glycosylchromones and anthrones have been isolated from Aloe vera, including aloe-emodin, the anthraquinone of barbaloin and isobarbaloin (87, 101, 105–107). In addition, the latex from Aloe vera contains a number of aromatic compounds, such as aldehydes and ketones (66).

BIOLOGICAL AND TOXICOLOGICAL PROPERTIES OF ALOE VERA PLANT PRODUCTS

Aloe Vera Gel

Metabolism

Metabolic disposition studies, using FITC-labeled acemannan, showed that when administered to mice at a dose of 120 mg/kg by intravenous injection, acemannan (500 kDa molecular weight) was metabolized into lower molecular weight molecules, which mainly accumulated in the kidneys and were excreted in the urine within 24 h; however, the same dose of acemannan administered by gavage to mice resulted in low molecular weight substances appearing primarily in the feces (108). An intestinal bacterial mixture from human feces was shown to metabolize acemannan ($\geq$400 kDa molecular weight) to smaller components (30 and 10 kDa) in a 1% yield. Structural studies of the catabolites by ^{1}H-NMR and IR spectroscopy and HPLC analyses indicated the presence of sugar and peptide moieties. Since humans lack the capacity to digest enzymatically the β-1$\rightarrow$4 glycosidic bond configuration, these smaller components are likely segments of acemannan that lack the β-configuration or possibly mucin arising from the feces (108).

Endocrine Effects

Aloe vera is touted as a glucose-lowering agent in the treatment of diabetes. The administration of Aloe vera, either orally or by gavage, was shown to lower fasting blood glucose levels in chemically-induced diabetic animals and may exert other metabolic effects (45, 47). The administration of a plant-derived

Aloe vera gel extract by gavage to normal fasted rats did not alter blood glucose levels, but Aloe vera gel significantly enhanced glucose tolerance in glucose-loaded animals (109). Aloe vera caused a significant increase in body weight along with decreased blood glucose levels in streptozotocin-induced diabetic rats, which suggested that the Aloe vera extract may enhance glucose metabolism (109). Al-Awadi *et al.* (110) showed that the increased gluconeogenic activity in chemically-induced diabetic rats was inhibited significantly by the administration of Aloe vera gel. The glucose lowering effects of Aloe vera gel may also be mediated through an anti-oxidant mechanism since supplementation of Aloe vera plant extracts attenuated oxidative damage in the brain of streptozotocin-induced diabetic mice and lowered lipid peroxidation levels in the kidneys of streptozotocin-induced diabetic rats (111, 112). In addition, increased glutathione and decreased non-enzymatic glycosylation and lipid peroxidation were found in liver tissues of neonatal streptozotocin-induced diabetic rats treated by gavage with Aloe vera gel (113). Aloe vera also was shown to lower blood glucose levels of diabetic women, not on drug therapy, who were orally administered Aloe vera juice (80% Aloe vera gel, 20% flavorings) twice daily (49). Significantly lower blood glucose levels continued until the end of the study at 42 days, and the lower blood glucose levels were accompanied by significantly lowered triglyceride levels. Aloe vera ingestion had no effect on cholesterol levels.

The ability of Aloe vera to lower cholesterol and other risk factors of coronary heart disease was investigated. Sixty patients were enrolled in a controlled clinical trial and received daily drinks of either 10- or 20-ml of Aloe vera whole leaf extract or a placebo juice (114). After twelve weeks of daily administration, the 10- or 20-ml whole leaf Aloe vera extracts decreased total serum cholesterol by 15.4% and 15.5%, triglycerides by 25.2% and 31.9%, and low-density lipoproteins by 18.9% and 18.2%, respectively. Agarwal (115) also examined the effect of oral consumption of Aloe vera gel on risk factors of heart disease. Five thousand patients were selected for the study; all presented symptoms of heart disease. The patients consumed breads prepared with wheat flour and Aloe vera gel at two meals a day for a period of three months. There was a marked reduction in total lipids, serum cholesterol, and serum triglycerides. In addition, patients who also had diabetes showed reductions in fasting and post-meal blood glucose levels.

Cell Proliferation

There are several reports about the stimulatory effect of Aloe components on cell proliferation (24, 116); however, the identity of the substances responsible for influencing cell proliferation is currently not known. Since no single definitive active ingredient has been identified, some suggest that there may be synergism between the polysaccharides and other components in the Aloe

vera gel; others continue to isolate and examine the various polysaccharides, proteins, and numerous other components in the Aloe vera plant products for pharmacological and physiological activities.

There are numerous reports about stimulatory and inhibitory effects of Aloe vera lectin-like substances on cell proliferation. Lectins are glycoproteins of non-immune origin that are known for their ability to agglutinate (clump) erythrocytes *in vitro*. Reduced growth, diarrhea, and interference with nutrient absorption are caused by this class of toxicants. Different lectins have different levels of toxicity, though not all lectins are toxic. Lectins may bind with free sugar or with free or bound sugar residues of polysaccharides, glycoproteins, or glycolipids in cell membranes. When given orally to experimental animals, lectins interact with the mucosa of the gastrointestinal tract causing acute gastrointestinal symptoms, failure to thrive, and even death. Lectins can alter host resistance to infection or, more importantly, to tumors. Following the initial discovery of highly toxic ricin from castor bean, lectins have been detected in a number of edible plants. The toxic effects of lectins are dependent on source, species, dose, and route of administration (117).

The occurrence of lectin-like substances in Aloe vera was first described by Winters *et al.* (118), who reported that fractions prepared by differential centrifugation from fresh leaf and commercial Aloe vera gel extracts contained high levels of lectin-like substances. The fresh leaf fractions were found to promote the attachment and growth of normal human cells, but not tumor cells; while, the commercial Aloe vera gel fractions were found equally cytotoxic to normal human and tumor cells. Substances in fractions of Aloe vera whole leaf and gel extracts were also found to induce proliferation in fibroblast and neuron-like cells (60). Although the term lectin or glycoprotein was not mentioned, proteins in the Aloe vera extracts were measured and treatments assigned based on protein concentrations. The Aloe vera gel was found more potent in stimulating the growth of cells when treated prior to attachment than in the treatment of adherent cell cultures. Since the adherence of cells to a matrix is an essential factor for growth, the results suggested that Aloe vera gel may improve cell attachment. Subsequently, human fibroblast cells treated with fresh Aloe vera gel were shown to increase in a dose-dependent fashion, while cytotoxicity was observed in cells treated with Aloe vera latex (116). In contrast to the effects observed with treatment of native Aloe vera gel, a commercial gel was found to have differing effects, suggesting that substances were added during the processing that altered the lectin-like activities and resulted in the disruption of cell attachment and growth. However, when cytotoxicity assays were conducted with an *in vitro* system that mimicked human skin, the effective concentration to kill 50% of cells (EC50) could not be determined, since the Aloe vera gel at a 100% concentration was found essentially non-toxic and actually stimulated cellular activity (119).

Fractionated whole leaf and gel extracts of Aloe vera have been used to identify and characterize the Aloe vera lectin-like substances. Gel permeation was used to isolate three Aloe vera gel fractions (61). A glycoprotein fraction was found to promote cell growth, a colored glycoprotein fraction was found to inhibit cell growth, and a neutral polysaccharide fraction was found to neither promote nor inhibit cell growth. The colored glycoprotein fraction was found to contain phenolic components, and these components were thought to reduce the proliferative effect of the lectin-like substances in Aloe vera gel. Using HPLC analysis, small quantities of phenolic components, including barbaloin and aloesin, were detected in virtually all samples of Aloe vera gels tested (105). Although the phenolic substances were detected in negligible amounts, these results suggested that the variability observed in proliferation studies on Aloe vera gel may be explained by the presence of proliferative glycoproteins and inhibitory glycoproteins that also contain inhibitory phenolic substances.

Akev and Can (120) reported on the separation and purification of two leaf pulp lectins isolated from Aloe vera, aloctin I and aloctin II. The lectins had a glycoprotein structure and exhibited haemagglutinating activity against rabbit erythrocytes, but failed to agglutinate human erythrocytes and only weakly agglutinated rat erythrocytes. However, human foreskin keratinocytes and squamous cell carcinoma cells showed a significant proliferative response to an isolated glycoprotein fraction from Aloe vera gel, G1G1M1D12. Using a raft culture—a synthetic mono-layer culture of keratinocytes that mimics human epidermis—Choi *et al.* (121) demonstrated that G1G1M1D12 induced the migration of keratinocytes to restore wounded cell areas and stimulated the cells to express protein markers related to cell proliferation in a dose-dependent manner.

Burn or wound healing is a response to tissue injury resulting in the restoration of tissue integrity. The growth of endothelial, epithelial, and fibroblast cells plays a critical role in wound healing processes (122). Animal studies and clinical trials on the proliferative effects of Aloe vera gel have focused primarily on the duration to re-epithelialization of wounds, with few, if any, studies examining the effects of Aloe vera gel on healthy skin. The results from published studies are conflicting and likely reflect the use of different poorly characterized, complex commercial products, rather than native plant components, making comparisons difficult.

The wound healing effects of plant-derived Aloe vera gel were compared with that of a 1% silver sulfadiazine cream on second-degree burns in guinea pigs (17). The post-burn re-epithelialization, wound contraction, and hair follicle count were significantly lower in animals treated with Aloe vera gel compared with silver sulfadiazine treated animals; however, the thickness of tissue granulation was significantly higher, suggesting that topical application of Aloe vera gel hindered the wound healing process. Kaufman *et al.* (17) reported similar results in guinea pigs when Aloe vera gel was used to treat burn wounds. In

contrast, Rodriguez-Bigas *et al.* (123) found a commercial preparation of Aloe vera gel had healing effects on full-thickness wounds in guinea pigs when compared with other burn wound management modalities, including that of silver sulfadiazine, salicyclic acid cream, or a plain gauze occlusive dressing. The average complete healing time was substantially shorter in the Aloe vera-treated group, and it was the only treatment that had a significantly shorter healing time than the plain gauze occlusive dressing. The primary difference between these studies was the source and composition of the test material. In the former studies, the source of Aloe vera gel was Aloe vera plant-derived; whereas, a commercial Aloe vera gel product, Carrington Dermal Wound Gel, was used in the latter study.

When topical agents, including scarlet red ointment, benzoyl peroxide lotion, bacitracin ointment, silver sulfadiazine cream, plant-derived Aloe vera gel, tretinoin cream, capsaidin cream, and mucirocin ointment, were examined for their wound healing effects on full-thickness excision wounds in guinea pigs, the Aloe vera gel was found to have no effect on the rate of re-epithelialization of wound contraction (124). The effects of plant-derived Aloe vera gel in combination with anti-microbials on full-thickness excision wounds of rats were also examined (25). While the combination of Aloe vera gel and the anti-microbials reversed the retardation in wound healing caused by the anti-microbials alone, the wounds of the placebo group, which received an aqueous cream without active ingredients, healed more rapidly than did the wounds of other treatment groups. Conflicting results were observed by Heggers *et al.* (125), who found a commercial preparation of Aloe vera gel decreased the healing time of full-thickness excision wounds in rats. Subsequently, the commercial preparation of Aloe vera gel, either alone or in combination with anti-microbials, was examined for *in vitro* cell growth and wound healing of excision wounds in rats and was found to enhance cell proliferation and increase the tensile strength of wound scars (126).

As in animal models of the proliferative effects of Aloe vera, clinical trials for the most part have focused on time to wound healing. Fulton (127) examined the effects of two different dressings for wound-healing management on full-face dermabrasion in an intra-individual right/left comparison study in eighteen patients. One side of the face was treated with a polyethylene oxide gel wound dressing, while the other side received a polyethylene oxide dressing presoaked with commercially-stabilized Aloe vera gel. Vasoconstriction and a reduction in edema were observed with Aloe vera gel treatment.

The temporal and histological effects of Aloe vera gel were compared with Vaseline^{TM} gauze treatment in 27 male and female patients with partial thickness burn wounds (7). The source of the Aloe vera gel used in this study was from Thailand and the plant species was not specified. Aloe vera gel treatment enhanced angiogenesis and collagen formation and shortened healing times. In contrast, a significant delay in wound healing was associated with the use of a

commercial preparation of Aloe vera gel compared with a standard wound management procedure in 21 women with wound complications from gynecologic surgery (128).

Clinical trials have also examined the effectiveness of Aloe vera gel in the treatment of radiation-induced dermatitis and psoriasis. The ability of Aloe vera to heal ulcers, rashes, or poorly healed scars associated with radiation therapy was evaluated in two phase III trials in women who had undergone radiation therapy to the breast or chest wall (129). The plant-derived Aloe vera gel was incorporated into an inert gel. The first double-blind, placebo-controlled study examined the effect of Aloe vera gel versus a placebo gel on 194 patients undergoing radiotherapy for breast cancer. There was no difference between the treatment and placebo group. It was speculated that the inert carrier gel might have some beneficial effect, so a second trial study was conducted with 108 patients to compare Aloe vera gel with no treatment. The trial was not blinded; however, the results were identical on both treatment arms, with Aloe vera gel offering no benefit. The only reported toxicity was contact dermatitis. Similar effects were observed in a phase III study involving 225 patients who received either topical Aloe vera gel or aqueous cream applications three times a day throughout the radiation treatment and for two additional weeks after completion of radiation treatment (130). The aqueous cream was significantly better than Aloe vera gel in reducing treatment-related side-effects.

Olsen *et al.* (131) compared Aloe vera gel with mild soap cleansing to mild soap cleansing alone during radiotherapy. The Aloe vera gel was a commercial product that contained other ingredients, including vitamin E, carbomer, EDTA, triethanolamine, methylparaben, and imdazolidinyl urea. This study examined the time to first observed radiation-induced skin change in 73 patients. The investigators found that at low cumulative doses of radiation, no differences existed between the two study groups; however, at higher cumulative doses, the Aloe vera gel and mild soap group were lesion-free for a longer time than the mild soap alone group. Although the results indicated that, with increased doses of radiation, a protective effect was provided by adding Aloe vera gel to the skin care regimen, patient compliance was unclear and patient assessment was not included in the report.

The efficacy of Aloe vera gel in the treatment of psoriasis was examined in a double-blind, placebo-controlled, randomized, intra-individual right/left comparison study. The Aloe vera gel test agent was a commercial preparation that stated plant-derived and preserved, but otherwise untreated. The Aloe vera gel or a placebo gel was applied twice daily to symmetrical test lesions for a period of four weeks. The sum score of erythema, infiltration, and desquamation was measured and was found statistically significant in favor of the placebo treatment (132). Opposing results were observed by topical self-application of Aloe vera gel or placebo cream to 60 patients with mild to moderate psoriasis (133). The creams were applied thrice daily for four weeks, and the cure rate

in the Aloe vera-treated group was statistically significant, with no relapses reported in a 12-month follow up period.

Only a few studies have examined the influence of oral administration of Aloe vera gel on the wound-healing process. Davis *et al.* (134) evaluated the effects of food grade and colorized (with anthraquinones) Aloe vera on full thickness wounds induced in ICR mice. In an oral study, mice received the food grade Aloe vera (100 mg/kg/day) in the drinking water for two months. For a topical study, mice received 25% colorized Aloe vera in a vehicle cream or vehicle cream alone on each wound for 6 days. Wound diameter was used as a measure of healing in control and treated animals. In both studies, wounds healed more rapidly in Aloe vera treated mice compared to untreated or vehicle controls, suggesting that that food grade and colorized (with anthraquinones) Aloe vera were effective treatments in the healing of wounds.

Atherton (51) reported on a pilot study of six patients with chronic leg ulcers that had failed to heal using more conventional methods and one patient with a nondescript ulcer accompanied with lupus erythematosis. Each patient was subjected to a standard battery of tests to exclude allergic responses to the vehicle and preservatives in the topical Aloe vera product. Each patient was given an Aloe vera gel drink (98% stabilized Aloe vera gel) in the form of 60 ml fluid every day, in two divided amounts. The ulcer crater was also irrigated with tap water to remove debris and then filled with a topical Aloe vera jelly preparation containing 86% stabilized gel and bandaged with waterproof dressing and stretch bandaging. This process was carried out daily. Six patients completed the study: of these, the leg ulcers of three patients healed completely, one patient exhibited partial healing of the leg ulcer, and one leg ulcer showed no improvement. There was no mention of the duration of the study or the parameters used to measure healing. Aside from some initial discomfort caused by the application of the Aloe vera jelly, no adverse effects were mentioned. There were no controls in this study.

Hayes (117) described a case report of a patient who presented with oral lichen planus with severe systemic involvement. The patient had received treatments of Lidex cream and cortisone without relief of symptoms. The proposed therapy involved drinking 60 ml of stabilized Aloe vera juice daily for 3 months. The patient was also given Aloe vera lip balm and Aloe vera cream (75% stabilized Aloe vera) to help with the external eruptions and itching. After four weeks of therapy, the oral lesions had disappeared and eruptions on hands showed signs of improvement. The dose was then doubled to 120 ml per day, and seven months after the initial treatment, the patient noted all symptoms were gone. There were no controls in this study.

Angiogenesis

Angiogenesis is the growth of new capillaries from pre-existing vessels and is the summation of a multi-step process that involves the migration and

proliferation of capillary endothelial cells, tissue infiltration, and lumen formation (135). Capillaries provide the essential interface between the blood and the tissue for regulating nutrient delivery and for the transmigration of cells (136). Therefore, while angiogenesis is essential for normal tissue growth, it also occurs in many physiological and pathological conditions, including tumor growth (137) .

A number of potent angiogenic compounds have been identified in Aloe vera. Moon *et al.* (138) showed that the crude extract of Aloe vera gel actively induced neovascularization on the chorioallantoic membrane of chick embryo. Subsequently, the Aloe vera gel was separated into three fractions, which were tested *in vitro* and *in vivo* for angiogenic activity. Further fractionation showed that the angiogenic effect was mainly due to the plant sterol, β-sitosterol. Lee *et al.* (139) partitioned Aloe vera gel into three fractions and tested these fractions for *in vitro* angiogenic activity in calf pulmonary artery endothelial (CPAE) cells. One of the fractions was found to be active and induced the proliferation of CPAE cells, stimulated CPAE cells to invade the matrigel matrix, and enhanced the differentiation of CPAE cells to form capillary-like tubules. Furthermore, the CPAE cells were shown to have enhanced mRNA expression of several angiogenic activators (139). β-Sitosterol from Aloe vera gel was isolated and examined for its effect upon damaged blood vessels in ischaemic/reperfused brains of gerbils. β-Sitosterol from Aloe vera gel was shown to enhance new vessel formation in a dose-dependent fashion ($\geq$500 mg/kg) and to enhance the expression of several proteins related to angiogenesis, namely von Willebrand factors, vascular endothelial growth factor (VEGF), the VEGF receptor FLK-1, and blood vessel matrix laminin (140).

The effect of Aloe vera compared with saline treatment on angiogenesis and cutaneous microcirculation was also examined by Somboonwong *et al.* (16) on second degree burns in rats. Vasodilation and postcapillary venule permeability were reduced significantly in both the saline and Aloe vera treated rats compared with sham control animals. In addition, the wound healing area of the Aloe vera-treated rats was improved compared to the untreated and saline-treated groups.

Immunostimulation / Immunosuppression

Anecdotal reports describe both immunostimulating and immunosuppressing effects with use of Aloe vera plant components; however, there are few scientifically controlled studies examining these effects. Most of the reports focused on the immunomodulating effects of Aloe vera gel, with emphasis on acemmanan and other polysaccharides found in the gel. Although there is a general consensus among the studies that the polysaccharide fraction of Aloe vera gel has immunomodulating activities, the identity, size, and composition of the major immunomodulating polysaccharide are not known.

The immunostimulatory properties of commercial preparations of crude whole leaf Aloe vera were evaluated and characterized using a reporter-gene assay (141). A high molecular weight (4–7 $\times$ 10^6 Da) polysaccharide fraction, aloeride, induced the expression of mRNAs encoding interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in THP-1 human monocyte cells at levels equivalent to those of cells stimulated by lipopolysaccharide (LPS). The authors suggested that the reported immunomodulatory effects attributed by others to acemmanan were due to the presence of trace amounts of aloeride in the crude juice and Aloe vera gel. In contrast, Qui *et al.* (142) isolated a much smaller (80 kDa) polysaccharide, modified Aloe polysaccharide (MAP), from cellulose-digested Aloe vera gel that was found to activate macrophage cells and stimulate fibroblast growth. The native Aloe vera gel was found to have no effect on macrophage activation. Similarly, fractionated crude cellulose-digested Aloe vera gel was tested for *in vitro* and *in vivo* immunomodutory activities by Im *et al.* (143). Polysaccharides between 5- and 400-kDa were found to exhibit the most potent macrophage-activating activity, as determined by increased cytokine production, nitric oxide (NO) release, expression of surface markers, and phagocyte activity. Using differential centrifugation, ion exchange chromatography, and co-cultures of organ slices, Talmadge *et al.* (144) also purified a high-molecular-weight fraction that showed increased hematological and hematopoietic activity compared with the Aloe vera gel starting material. Increased hematopoietic activity was associated with increased mRNA levels for hematopoietic cytokines. This profile of activity differed from another purified polysaccharide fraction that had anti-inflammatory activities, suggesting that the conflicting results may be attributable to multiple and potentially conflicting activities of the Aloe vera extracts used in the studies.

The immunomodulating effects of Aloe-based carbohydrates are thought to function via activation of macrophage cells and stimulation of the antigen processing. Activated macrophage cells generate NO, secrete cytokines, such as TNF-α and IL-6, and present cell surface markers. While beneficial under certain circumstances, these agents are all known to cause inflammation and lead to subsequent pathology.

Several studies have examined the carbohydrates of Aloe vera gel for macrophage activation as well as the activation of other cell types that function in immune responses. Zhang and Tizard (145) examined the effects of a commercial preparation of acemannan, alone and in combination with interferon-gamma (INF-γ) on the activation of the murine macrophage-like cell line, Raw 264.7. Acemannan alone could activate the macrophages both directly and indirectly to release IL-6 and TNF-α. Acemannan also synergistically enhanced macrophage sensitivity to IFN-γ as reflected by increased NO release, enhanced surface molecule expression, and cell morphology changes. The same commercial preparation of acemannan was used by Ramamoorthy *et al.* (146) who demonstrated by northern blot analyses that the acemannan-induced increase

in NO production was preceded by increased expression of mRNA for the inducible form of macrophage NO synthase. Furthermore, the induction of NO synthase was inhibited by pre-incubation with pyrrolidine dithiocarbamate, an inhibitor of Nuclear Factor-kappa (NFκ), suggesting that acemannan causes the activation of macrophages by increasing the level of NO synthase at the level of transcription. In a subsequent experiment, Ramamoorthy *et al.* (147) showed that in the presence of IFN-γ, acemannan induced apoptosis in the RAW 264.7 cell line. The cells exhibited typical characteristics of apoptosis, including chromatin condensation, DNA fragmentation, and DNA laddering. Neither acemannan nor IFN-γ induced apoptosis alone; however, the induction of apoptosis appeared to be independent of NO production, since N-nitro-L-arginine methyl ester (L-NAME), a NO inhibitor, did not protect the cells. It was suggested that the induction of apoptosis by acemannan in combination with IFN-γ involved the inhibition of the expression of the apoptotic Bcl-2 proteins.

Other studies have focused on evaluating whether or not the activation of macrophage by acemannan occurs via mannose receptors on the cell surface of macrophage. Karaca *et al.* (148) demonstrated that normal chicken spleen cells and a chicken bone marrow macrophage cell line, HD11, produced NO and suggested that the acemannan-induced NO synthesis may be mediated through macrophage mannose receptors. In this study, HD11 or isolated chicken spleen cells were treated with serially diluted acemannan, yeast mannan, or LPS. Cells cultured in the presence and absence of concanavalin A (Con A) or N-methyl-DL aspartic acid (NMA) were used to evaluate the potential role for mannose receptors. Con A is reported to have a high affinity for terminal mannose residues and may serve to block acemannan. In contrast with results presented by Zang and Tizard (145), the NO-inducing effect of acemannan alone exhibited a dose-dependent relationship on spleen cells. Similarly, NO production was increased in HD11 cells in response to LPS and to a much lower extent by acemannan, but not to yeast mannan. The failure of yeast mannan to elicit a NO response was explained by involvement of acetylated mannose-specific receptors that may be present in macrophage activation. Con A was shown to inhibit acemannan- and not LPS-induced NO production by HD11 cells in a dose dependent manner; whereas, L-NAME, an inhibitor of NO synthase, inhibited both LPS and acemannan stimulated production of NO.

The immunomodulatory activity of acemannan on accessory cells has also been examined. Dedritic cells are accessory cells that function in the activation of T-cells and the generation of T-cell responses (149). Lee *et al.* (150) isolated immature dendritic cells from mouse bone marrow and stimulated the cells with acemannan, sulfated acemannan, or LPS. Acemannan induced functional and phenotypic maturation of dendritic cells *in vitro*; whereas, chemical sulfation of acemannan abrogated the differentiation-inducing and the mitogenic activities. The addition of mannose to culture medium along with acemannan failed to suppress cell differentiation by acemannan, suggesting that acemannan does not

bind to mannose receptors on cell surfaces. The differences in results obtained by Zang and Tizzard and these studies may be due to differential expression of mannose-specific receptors on the surfaces of HD11, RAW 264.7, and dendritic cells.

Aloe vera is also considered to have anti-oxidant potential and may exert immunomodulatory effects by inhibiting the generation of oxygen radicals. The anti-oxidant activity of Aloe vera plants extracts at different growth stages was compared with butylated hydroxytoluene (BHT) and α-tocopherol (151). Compared with younger plants, the anti-oxidant activity of mature three-year old Aloe vera extracts exhibited the strongest radical scavenging activity and was significantly higher than either BHT or α-tocopherol. t'Hart *et al.* (152) analyzed an aqueous extract of Aloe vera gel with regard to the *in vitro* activation of human polymorphonuclear leukocytes and isolated two fractions by ultrafiltration. The high-molecular-weight fraction depleted the complement activity pathway, while the low molecular weight fraction, possibly phenolic compounds, was found to inhibit the production of free oxygen radicals.

The release of arachidonic acid and other lipids from tissue and cell membranes results in the formation of lipid peroxides, the generation of free radicals, and the production of prostaglandins. The intragastric administration of an ethanol extract of Aloe vera whole leaves to streptozotocin-induced diabetic rats was shown to reduce lipid peroxidation and the formation of hydroperoxides, and resulted in increased levels of anti-oxidant enzymes, including reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase in the liver and kidney (153). A juice filtrate of Aloe vera whole leaves administered by gavage to whole body gamma-irradiated rats was also shown to reduce lipid peroxidation and improve anti-oxidant enzyme status in the liver, lungs, and kidneys (154). Aloe vera was also shown to be effective in minimizing the radiation-induced increase in plasma glucose levels without affecting insulin levels, suggesting that the hypoglycemic effects of Aloe vera may function via decreased hepatic gluconeogenesis. Ethanol, chloroform, and aqueous extracts of Aloe vera were also used on carrageenan-induced edema in the rat paw and examined for neutrophil migration and inhibition of cyclooxygenase activity (155). The aqueous and chloroform extracts of Aloe vera decreased the carrageenan-induced edema in the hind-paw and decreased neutrophil migration; the aqueous extract also inhibited prostaglandin E_2 production from arachidonic acid. Similarly, a commercial preparation of Aloe vera gel was shown to inhibit reactive oxygen metabolites and the production of prostaglandins in human colorectal mucosa cells and colorectal biopsies (156). Aloe vera gel, at 1:50 dilution in culture medium, inhibited prostaglandin E_2 production by 30% in inflamed colorectal biopsies, but had no effect at higher or lower concentrations, and thromboxane B_2 release was not affected at any dose. The reduced inhibition of prostaglandin E_2 at higher concentrations of Aloe vera gel suggests that one or multiple components in the gel may actually

stimulate prostaglandin production and outweigh the inhibitory effects by other components.

The ability of the Aloe vera gel and polysaccharides to reduce the severity of acute inflammatory responses has been evaluated in several animal models. Unfortunately, many of these studies were conducted using either complex commercial products or poorly characterized plant components. A commercial topical Aloe vera solution was used to evaluate leukocyte adhesion and cytokine production in a second-degree burn wound rat model (157). Burn wounds were untreated or treated with either saline or Aloe vera. The daily treatment with Aloe vera significantly reduced leukocyte adhesion and serum levels of TNF-α and IL-6. Saline treatment was also shown to lower the levels of these parameters, but significance was only attained in lowered levels of IL-6. In contrast, Peng et al. (158) reported that intraperitoneal administration of acemannan to mice stimulated the macrophage production of cytokines, including IL-1 and TNF-α.

The ability of Aloe vera gel extract to influence lymphocyte function and prevent the suppression of delayed-type hypersensitivity and contact hypersensitivity by ultraviolet (UV) irradiation has been examined by several investigators. Strickland *et al.* (159) investigated the ability of Aloe vera gel to ameliorate the UV irradiation-induced immune suppression in mice. UV-B irradiation and fluorescein isothiocyannate (FITC) sensitization was used to induce local immune suppression. A plant source of Aloe vera gel was used and incorporated into a cream base, consisting of petrolatum, mineral oil, and lanolin. The topical application of the Aloe vera cream to irradiated skin ameliorated UV-B suppression of contact hypersensitivity and delayed-type hypersensitivity responses. Aloe vera gel did not prevent the formation of UV-induced pyrimidine dimmers in the DNA or accelerate the repair of these lesions in UV-irradiated mouse skin; however, the Aloe vera gel partially protected the number and morphology of accessory cells, such as Langerhan and dendritic epidermal cells. In an effort to identify and characterize the Aloe vera gel components that offered protection against UVB-impairment of accessory cell function, Lee *et al.* (160) conducted *in vitro* studies and isolated at least two small-molecular-weight ($<$1 kDa) immunomodulators that were capable of restoring UV-B suppressed activity *in vivo*. The components offered 50% and 81% recovery of accessory cells at low UV-B doses ($<$180 J/m^2), but were ineffective at higher doses.

Similarly, Aloe vera gel was investigated to determine whether protection from UV-induced immunosuppression was afforded by a single or multiple agents, and whether protection decreased upon storage of the gel (161). For these studies, mice were administered UV irradiation in combination with topical application of a crude Aloe vera gel extract or a highly purified oligosaccharide fraction. The results indicated that maximum protection of contact hypersensitivity was afforded by the gel extract. However, the activity of the gel was shown to decay with time, despite storage of the material as lyophilized powder.

Within three to nine months of storage, none of the gels prevented UV-induced suppression of contact hypersensitivity, and the commercial preparations of gel were uniformly inactive even when tested within a month after being prepared. In contrast, time and storage of the gel extracts had little effect on the protection afforded for delayed-type hypersensitivity, even after 12 month of storage, suggesting that protection from UV-induced contact and delayed-type hypersensitivity is mediated by at least two separate factors in crude Aloe vera gel. In addition, a purified oligosaccharide fraction offered *in vivo* protection against UV-induced suppression of delayed-type hypersensitivity. Furthermore, when mice were injected with spent culture media of keratinocytes that were exposed to the oligosaccharide and UV-B irradiation, the media reduced IL-10 levels and blocked the immunosuppression of UV irradiation, suggesting that oligosaccharides may prevent UV-induced suppression of delayed-type hypersensitivity by suppressing keratinocyte-derived cytokines.

Inhibitors of cyclooxygenase, such as indomethacin, decrease sunburn redness and increase tissue perfusion when applied to sunburn skin (162). The effects of Aloe vera gel on UV-B induced erythema and increased blood flow was examined in twelve male and female volunteers (163). The men and women received UV-B irradiation to two sites on each forearm followed by hourly applications of plant-derived Aloe vera gel. Doppler blood flow measurements and clinical assessment of erythema at six and twenty-four hours indicated there were no significant alterations in blood or qualitative differences in erythema in Aloe-treated areas compared with untreated control sites.

In a randomized, double-blind, placebo-controlled trial of the efficacy of Aloe vera gel for the treatment of mildly to moderately active ulcerative colitis, patients were administered Aloe vera gel in a drink twice daily for four weeks. Clinical remission, sigmoidoscopic remission, and histological remission were the primary endpoints measured (164). The drink was a commercial product of Aloe vera gel and the placebo was a flavored liquid, identical in taste and appearance to the Aloe vera preparation. The physician's global assessment showed no change during the treatment period, and none of primary end-points of the study was met in terms of clinical, endoscopic, or histologic remission. The Simple Clinical Colitis Activity Index and histological scores decreased significantly; however, the sigmoidoscopic scores and laboratory values showed no significant differences from placebo controls.

Anti-Bacterial/Anti-Viral effects

Macrophage and neutrophil cells are primarily responsible for phagocytosis and intracellular killing of viruses and other pathogens. Interest in acemannan has grown since demonstration of its anti-viral properties *in vivo*. Womble and Helderman (165) published the first study suggesting that acemannan had an anti-viral effect on human cells. Human peripheral mononuclear cells served as stimulator cells in a mixed lymphocyte assay, and commercial

acemannan was shown to enhance significantly the alloantigenic response in a dose-dependent fashion. Additionally, the function and proliferation of cytotoxic T cells increased in a dose-related response when human monocytes were pre-treated with acemannan and co-cultured with phytohemagluttinin-stimulated T-lymphocyte cells (T-cells). Acemannan-induced IL-1 release by monocytes was suggested as the mechanism of action since IL-1 is a known stimulator of T-cells.

Studies have shown that certain mannosylated substances activate macrophage cells and enhance the killing of pathogens, such as *Candida albicans* (166). Stuart *et al.* (167) investigated whether acemannan would also enhance macrophage function. Exposure of macrophage cells to acemannan resulted in significantly enhanced killing of C. *albicans* compared with untreated controls. Acemannan was shown to initiate the production of reactive oxygen species, the secretion of pro-inflammatory cytokines, and the generation of other cytotoxic factors such as NO by stimulated macrophages. Stimulation of macrophages often results in the generation of these cytotoxic factors, and, while beneficial under certain circumstances, these agents are known to cause inflammation and lead to subsequent pathology.

Retroviral infections are associated with a number of pathologic abnormalities, including a variety of cancers, immunologic diseases, and neurologic disorders (168), and the influence of acemannan to alter the *in vivo* course of retroviral infections has been examined in several studies. In each case evaluated, the acemannan was provided by the supplier who funded the studies. The administration of acemannan to cats with clinically symptomatic feline leukemia virus was examined by Sheets *et al.* (78). Acemannan administration by intraperitoneal injection significantly improved both the quality of life and the survival rate of feline leukemia virus-infected cats. Similarly, Yates *et al.* (169) demonstrated the anti-viral activity of acemannan in a pilot study of clinically-symptomatic immunodeficiency virus-infected cats. Acemannan was administered by weekly intravenous or subcutaneous injections or by daily oral administration. There were no control groups. After 0, 6, or 12 weeks, there were no significant differences in clinical scores, laboratory values, or long-term survival among groups administered acemannan by the different routes; however, the survival rates for the acemannan-treated animals exceeded those observed in limited number of historical feline immunodeficiency virus (FIV)-infected controls. Significantly increased lymphocyte and decreased neutrophil counts were also observed.

Acemannan in combination with suboptimal non-cytotoxic concentrations of the anti-viral agents azidothymidine (AZT) and acyclovir (ACY) was shown to inhibit synergistically the replication of human immunodeficiency virus (HIV-1) and herpes simplex virus type 1 in human peripheral mononuclear (170). Acemannan in combination with AZT protected the cells from rapid (HIV)-1 replication, which is known to cause premature cell death. In a subsequent study, the anti-viral effects of acemannan was evaluated in a variety of cell

lines, and the inhibition of glycosylation of viral glycoproteins was found to be the mechanism of action for its anti-viral effects. It was suggested that, while acemannan might not be considered potent enough for anti-viral therapy, acemannan may be able to act synergistically with more toxic agents to reduce their toxicity and enhance anti-viral efficacy.

Aloe vera gel was shown to inhibit the growth of gram-positive bacteria, *Shigella flexneri* and *Streptococcus pyrogenes* (171). Effective growth inhibition for up to 24 hours was achieved with concentrations of 100 mg of Aloe vera gel per ml. The activities of Aloe vera gel were low by comparison with the activities ampicillin and nalidixic; however, it was suggested that use of Aloe vera as an ingredient in anti-microbial products may be beneficial given the direct effect of the gel on accessible areas of the body. However, the incorporation of Aloe vera gel in various soft-soaps did not exhibit any significant effect on the anti-microbial activities against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida , Aergillus clavaatus, or Tychophyton* (172).

Anti-Tumor Effects

Polysaccharides isolated from Aloe vera have been reported to have anti-tumor activity, and the anti-tumor activity of acemannan has been examined in several animal species. A modified Aloe vera polysaccharide, G2E1DS2, isolated from cellulose-treated Aloe vera gel was shown to activate macrophages and exhibit potent anti-tumor activity when injected into the peritoneum of mice implanted with sarcoma cells (143). Similarly, intraperitoneal administration of both enriched and commercial forms acemannan to mice implanted with murine sarcoma cells significantly reduced the tumor burden and increased the survival rate (158). *In vivo* treatment of peritoneal macrophages with acemannan stimulated the production of monkines, including TNF-α and IL-1, and resulted in activation of the host defense system and regression of the implanted tumors. Evidence of an immunological attack, as demonstrated by marked increases in TNF-α and lymphocyte infiltration, was also observed in the spontaneous tumors of dogs and cats treated with intraperitoneal and intralesion administration of acemannan (173). Clinical improvement, as assessed by tumor shrinkage, tumor necrosis, or prolonged survival, was observed in approximately 50% of the animals. In addition, tumor shrinkage was observed following acemannan treatment in conjunction with surgery and radiation therapy in dogs and cats with recurring spontaneous fibrosarcomas (174).

Aloe vera was also shown to have chemopreventive and anti-genotoxic effects on benzo[*a*]pyrene-DNA adducts. Treatment of primary rat hepatocyte culture media with a polysaccharide fraction isolated from Aloe vera showed a time- and dose-dependent inhibition of benzo[*a*]pyrene-DNA adduct formation (175). Additionally, incubating rat hepatocytes simultaneously with Aloe vera and benzo[*a*]pyrene significantly reduced the formation of DNA adducts

and the uptake of benzo[*a*]pyrene by the cells. When benzo[*a*]pyrene was administered orally to mice followed by daily administration of Aloe vera, DNA adduct formation in various organs, including liver, kidney, and lung, was significantly inhibited. Aloe vera did not affect the content of liver cytochrome P450, and liver glutathione S-transferase activity was only slightly increased, suggesting that the chemoprotective effects of Aloe vera is by the inhibition of benzo[*a*]pyrene absorption. In a follow up screening of several plant polysaccharides for their anti-tumor promoting effects, Aloe vera was shown to inhibit significantly phorbol myristic acetate-induced ornithine decarboxylase, tyrosine kinase, and superoxide formation (36).

The effects of ingestion of a diet supplemented with Aloe vera gel and vitamin C on hepatocarcinogenesis induced by intraperitoneal injections of diethylnitrosamine and 2-acetylaminofluorene was studied in rats. Parameters assessed included histochemical determination of γ-glutamyl transferase and glutathione-*S*-transferase, determination of marker enzymes in the plasma, and liver microsomal and cytosolic fractions. Supplementation of the cancer-induced rats with vitamin C or Aloe vera gel extract significantly inhibited the development and severity of carcinogenesis, as reflected in the reduction of the percentage surface area of enzyme-positive foci (176).

The concomitant administration of Aloe vera tincture and melatonin hormone was studied in fifty patients with advanced solid tumors, including lung cancer, gastrointestinal tract tumors, breast cancer, or brain glioblastoma, for whom no effective standard anti-cancer therapies were available (177). The percentage of non-progressing patients, which included a partial response and stable disease, was significantly higher in the group treated with the Aloe vera tincture combined with melatonin than the melatonin alone group. In addition, the percentage of individuals surviving 1 year was significantly higher with melatonin plus Aloe vera compared with melatonin treatment alone. The Aloe vera tincture consisted of 10% Aloe vera and 90% alcohol.

Adverse or Toxic Effects

Several studies have attempted to determine whether or not Aloe vera causes toxicity in animals or humans. Various preparations were studied including plant-derived Aloe vera gel, commercial forms of the gel, and isolated components, either commercial or native. Single or repeated doses of a commercial preparation of acemannan were administrated at four-day intervals by intravenous or intraperitoneal injections to mice, rats, and dogs (178). No signs of treatment-related toxicities were apparent after a single injection of acemannan in mice or rats; however, emesis and diarrhea were observed in dogs following intraperitoneal or intravenous injections. Repeated administration of acemannan was associated with an increased accumulation of macrophages and monocytes in the lungs of intravenously-treated animals and in the liver and

spleen of intraperitoneally-treated animals; however, there were no subsequent inflammatory reactions detected after a six-day recovery period. Clinical signs of intoxication included decreased activity, abnormal gait and stance, flaccid body tone, piloerection, and tremors in mice, and emesis, abdominal discomfort, decreased activity, and diarrhea in dogs. Early deaths occurred in 30% of the high (80 mg/kg/dose) dose and 15% of the middle (40 mg/kg/dose) dose mice treated intravenously, and in 25% of the mice dosed intraperitoneally at 100–200 mg/kg.

Foggleman *et al.* (179) also examined the effect of oral administration of a commercial preparation of acemannan in acute and subchronic studies in rats and dogs. Technical acemannan was mixed in the basal diet for the rats or in a canine meal for the dogs. The acemannan was administered to the rats for 14 days at 5% of the diet (approximately 4,000 mg/kg/day) and for 6 months at up to 2,000 mg/kg/day, and acemannan was administered to dogs for 90 days at up to 1,500 mg/kg/day. There were no significant treatment-related effects or mortality in the fourteen-day study in rats or in the ninety-day sub-chronic study in dogs. In the sub-chronic rat study, bleeding, enlarged kidneys, and pyelonephritis were observed at necropsy. The technical acemannan used in this and the previous study was lyophilized and contained an average of 78–84% acemannan, less than 10% water, and the balance as calcium, magnesium, and other salts.

Mice administered an ethanol extract of Aloe vera leaf pulp by oral gavage at three doses (500 mg/kg, 1 g/kg, and 3 g/kg) showed no acute signs of toxicity at 500 mg/kg during the 24 hour observation period. However, at higher doses, a decrease in central nervous activity was noticed. During a chronic ninety-day study, the Aloe vera leaf pulp at a dose of 100 mg/kg in the drinking water caused decreased body and vital organ weights, decreased red cell counts, a significant spermatogenic dysfunction, and a 30% lethality compared with control animals (180).

The ingestion of crude and charcoal-processed Aloe vera leaf pulp on growth, dietary intake, and a variety of metabolic parameters in rats was examined in sub-chronic studies (181, 182). Ingestion of the crude Aloe vera gel at concentrations of 3%, 5%, and 10% of the diet (approximately equivalent to 330, 550, and 1,100 mg/kg/day) produced diarrhea, a slower growth, polydipsia, and polyuria in rats compared with control animals. At a dietary concentration of 1% (approximately 110 mg/kg/day), neither the crude nor the charcoal-processed leaf pulp elicited adverse effects on growth or pathology. Longer-term studies showed that ingestion of the crude or charcoal-processed Aloe vera gel by rats resulted in marked changes in serum parathroid hormone and calcitonin concentrations, suggesting that Aloe vera gel may alter calcium metabolism (182).

The effects of lifetime administration of dietary-supplemented Aloe vera leaf fillet or charcoal-processed Aloe vera gel or Aloe vera charcoal-processed whole-leaf dosed drinking water was examined in rats (183). Commercial

preparations of Aloe vera crude pulp and charcoal-processed gel were incorporated into a semi-purified diet at 1% (wt/wt) and administered *ad libitum*. The charcoal-processed whole leaf was administered at 0.02% (wt/vol) in the drinking water of rats. In general, the life-long ingestion of Aloe vera exerted no apparent harmful effects or changes in physiological parameters in the rat. Lim *et al.* (184) used an almost identical protocol of administration to examine supplementation of rats with Aloe vera on anti-oxidant protection and cholesterol-lowering effects. A life-long intake of Aloe vera reduced hepatic phosphatidylcholine hydroperoxide levels. Dietary administration significantly enhanced catalase and superoxide dismutase levels, but the supplementation of Aloe vera in the drinking water had no effect. Total cholesterol levels were not different from control levels at four months but were significantly lower after sixteen months of Aloe vera administration.

A case report was presented where a female patient had begun using tablets containing 500 mg of an extract of *Aloe barbadensis* Miller about four-weeks before admission with a one-week history of progressive jaundice, pruitus, acoholic bowel movements, and abdominal discomfort (185). Liver biopsy revealed severe acute hepatitis with portal and acinar infiltrates of lymphocytes, plasma cells, granulocytes along with bridging necrosis and bilirubinostasis. The hepatitis was linked to the ingestion of Aloe vera tablets, and symptoms resolved upon discontinuance with one week.

There is potential for herb-drug interactions with Aloe vera components in patients using prescribed mediations. Compounds in Aloe vera may cause a reduction in prostaglandin synthesis, which may inhibit secondary aggregation of platelets. Vasquez *et al.* (155) showed that Aloe vera gel caused a 48% reduction in prostaglandin synthesis compared with a 63% reduction by indomethacin. A case was presented in which a female patient lost five liters of blood during surgery as a result of a possible herb-drug interaction between oral consumption of Aloe vera tablets and sevoflurane, an inhibitor of thromboxane A_2 (185). Interactions of Aloe vera gel have also been reported for hydrocortisone, antidiabetic agents, and UV radiation (186).

Despite its reported wound-healing and emollient properties, the topical application of Aloe vera gel has been reported to cause contact dermatitis, erythema, and photodermatitis (27, 187, 188). A case report of a male patient presenting pruritic eczematous eruptions that were present on various parts of the body for a period of three or more months indicated that he had take ingested one teaspoon of a powdered form of Aloe vera gel three times a day for approximately three years, and he had applied the jelly-like material to his face and neck area after shaving for one year (189). Hives had developed only at the sites of application; however, examination indicated nummular, eczematous lesions on the arms, trunk, and legs. Biopsy specimens showed epidermal spongiosis with microvesicles of chronic inflammatory cells.

Aloe Latex

Metabolism

Aloe vera latex contains a mixture of anthracene compounds including O- and C-glycosides of anthrones and anthraquinones, as well as free anthrones and dianthrones and a small amount of free anthraquinones (100). Orally ingested anthranoid glycosides pass through the upper part of the gastrointestinal tract without chemical modification. The sugar moiety confers hydrophilic characteristics to the anthraquinone glycoside, which prohibits absorption by intestinal epithelial cells and results in their passage to the lower gastrointestinal tract and colon unmodified, where resident microflora of the *Bifidobacterium* sp. catabolize the *O*-glycosidic anthranoids, while bacterium of the *Eubacterium* sp. act upon the C-glycoside anthranoids releasing glucose and the free anthraquinone aglycone (190, 191). The laxative activity of the Aloe vera latex is not due to the ingested form of the anthraquinone, but rather to a common metabolite, Aloe-emodin-9-anthrone (Figure 2), which is the active ingredient that results by activity of the *Eubacterium* BAR (94, 190, 192). The *Eubacterium* sp. is expressed differentially across species; for example, rats but not guinea pigs are able to generate the Aloe-emodin-9-anthrone (Figure 2) (193). Subsequent systemic metabolism of the free anthranoids depends upon their absorption and ring constituents (194). Free anthraquinone aglycones undergo oxidation to form anthrones and anthraquinones that are absorbed through the small intestine, where they are transported to the liver and glucuronidated (195). The glucuronidated compounds are partly excreted in the urine and partly returned to the intestine through the bile (194). The glucuronidated anthraquinones are transported to the colon and released as free anthraquinones after metabolism by gut bacterial enzymes (193, 196). Most of the free anthranoids absorbed systemically in humans are excreted in the urine as rhein (Figure 2) or as conjugates (93, 193, 196). The ability of free anthraquinones to be absorbed in the small intestine appears to determine their toxic potential (194). Pharmacokinetic studies after oral administration of ^{14}C-aloe-emodin to male and female rats showed that 20–30% of the dose was excreted in the urine and the rest was excreted in the feces as rhein and conjugates. Ten percent of the radioactivity was identified as free aloe-emodin in the plasma, with maximum concentrations peaking at 1.5–3.0 hours post administration. Maximum plasma levels were about three and ten times higher than the concentrations in the ovaries and testes, respectively. Only the liver, kidney, and intestinal tract showed higher concentrations than the plasma. The terminal half-life of the radioactivity in the blood was 50 hours (197).

The kinetic dynamics of aloe-emodin and rhein were determined after orally administering therapeutic doses of senna laxatives to ten healthy volunteers in a two-way cross-over design. Blood samples were collected up to 96 h after the

first dose, and plasma levels of total aloe-emodin and rhein were determined by fluorometric HPLC. Aloe-emodin was not detectable in any plasma sample of any subject. The concentration of rhein showed the highest level at 3–5 hr and another peak maxima at 10–11 h after dosing, which were probably dependent upon the absorption of free rhein and rhein released from the pro-drugs (e.g., sennosides) by bacterial metabolism, respectively (198).

Endocrine System Effects

There is some evidence that aloe may have a blood glucose lowering effect. The oral administration of aloe was shown to lower plasma glucose levels in non-insulin dependent diabetic patients and in alloxan-induced diabetic mice (47). Significant reductions in fasting plasma glucose and glycated hemoglobin levels were observed; although, in chemically-induced diabetic mice, levels of these remained four-fold higher than control levels. An anti-oxidant mechanism was the proposed to account for these effects.

Cathartic Effects

Aloe vera latex possesses laxative properties, and use of the latex to relieve constipation dates back to classic Greece with first recordings of its use in the first century A.D. (199). In general, diarrhea is induced by an increase in water content and/or peristalsis in the large intestine. The major C-glycosides of Aloe vera latex, barbaloin and isobarbaloin (Figure 2) are the principal agents responsible for the cathartic activities of Aloe vera in humans and animals, although considerable variation exists in purgative potency among animal species; for example, barbaloin is potent in humans, but shows reduced activity in the mouse and rat (92, 94, 95). In addition, there are inter-individual differences in sensitivity to the laxative activity of barbaloin (200). Both barbaloin and isobarbaloin are inactive as laxatives themselves but undergo decomposition to form aloe-emodin-9-anthrone (Figure 2) and aloe-emodin and other metabolites by human and animal intestinal flora (95, 201, 202). The human intestinal anaerobe, *Eubacterium* BAR, was shown to metabolize barbaloin and induce severe diarrhea in gnotobiote rats (94, 190, 192). Diet and nutrition were also shown to play important roles in the laxative action of Aloe vera latex. The metabolism of barbaloin to aloe-emodin-9-anthrone was promoted by a diet containing iron salts and iron-rich meat and was decreased by cereals and complex carbohydrates (203). In addition, individual anthrones exhibit less purgative activity than mixtures of anthrones or of mixtures of anthrones and anthraquinones, suggesting that metabolites of barbaloin synergistically exert purgative effects (204).

Confirmation of aloe-emodin-9-anthrone as the purgative principle of Aloe vera latex was demonstrated by the intracecal administration of barbaloin and subsequent detection of aloe-emodin-9-anthrone in the large intestine,

with accompanying diarrhea (205). The aloe-emodin-9-anthrone and anthraquinones of barbaloin and isobarbaloin are thought to utilize multiple mechanisms in producing their cathartic effects. *In vitro* and *in vivo* studies in rats demonstrated that aloe-emodin-9-anthrone disturbs the equilibrium between the absorption of water from the intestinal lumen via inhibition of active sodium/potassium-adenosine triphosphatase and increases the paracellular permeability across the colonic mucosa (202), stimulates peristaltic activity in the large intestine, stimulates mucus secretion (205), and secretes water into the lumen by a prostaglandin-dependent mechanism (206). The result is a net reduction in water absorption and more frequent stools with softer consistency. Aloe-emodin-9-anthrone was shown to enhance the membrane permeability of water-soluble and poorly permeable compounds in the rat colon (207). The permeation-enhancing activity was estimated by changes in the permeability coefficient of 5(6)-carboxyfluorescein, and aloe-emodin-9-anthrone was shown to increase significantly its permeation in a dose-dependent manner. The enhancing effects were inhibited by an inhibitor of protein kinase C and significantly suppressed by a histamine H_1 receptor antagonist and a mast cell stabilizer. The results suggest that aloe-emodin-9-anthrone stimulates colonic mast cells to release histamine, which activates the protein kinase C pathway and opens tight junctions in colonic membranes.

Although there is no doubt that Aloe vera latex exerts its action on the colonic mucosa, its mechanism of action is still not fully understood. Under physiological conditions, endogenous NO appears to function as a pro-absorptive molecule, based on findings that NO synthetase inhibitors reverse net fluid absorption to net secretion in rodents, dogs, and rabbits (208). When rats were treated with several laxatives, including castor oil and anthraquinones of senna and cascara, NO was elevated in their colon, and L-NAME, a NO synthetase inhibitor, reduced their diarrhea response (208). L-NAME was also shown to prevent the diarrhea and fecal water excretion in rats administered aloe or barbaloin; however, in contrast with castor and senna laxatives, aloe and barbaloin produced a dose-dependent inhibition of calcium-dependent NO synthase activity in the rat colon, suggesting that the inhibition of NO synthetase by aloe or barbaloin may be a mechanism to reduce the cathartic activity of aloe (209). Aloe-emodin was also shown to inhibit the autotoxic release of NO in a dose-dependent manner in murine L929 fibrosarcoma cells that were stimulated with interferon-gamma and interleukin-1 (210).

Anti-Bacterial/Anti-Viral Activity

The phenolics and aloins of Aloe vera were found to have dose-dependent non-competitive inhibitory effects on *Clostridium histolyticum* metalloproteinases and collagenases (211). Structure active relationships drawn between the aloins and tetracyclines suggest that the inhibitory effects of aloins are via

a destabilizing effect on the structure of the granulocyte metalloproteinases and diminishing intracellular calcium availability (212). Barbaloin was also shown to disrupt membranes by weakening hydrophobic interactions between hydrocarbon chains in the phospholipids bilayers. Moreover, barbaloin showed specificity for two major phospholipids (phosphatidylethanolamine and phosphatidylglycerol) present in bacterial membranes (213). In screenings of Aloe vera for anti-viral effects, aloe-emodin purified from barbaloin, was also shown to inactivate a variety of viruses, including herpes simplex virus type I and type II, varicella-zoster, and the influenza virus (214). In tests of barbaloin to inhibit the infectivity of the viral hemorrhagic septicemia rhabdovirus or the growth of *Escherichia coli*, barbaloin exhibited anti-viral but not virucidal activity (213). Others reported differing results (215). The mechanism proposed for the anti-bacterial and anti-viral effects of aloe-emodin is the inhibition of nucleic acid biosynthesis after which protein syntheses is also inhibited (216). The tetracyclins are also able to inhibit protein synthesis at the ribosome level, probably by interference with the ribosome messenger and RNA, and perhaps aloe-emodin acts similarly (217).

Anti-Oxidant / Pro-Oxidant Activity

The anti-oxidant activities of anthraquinone and anthrones of Aloe vera have been evaluated using different model systems (11, 53, 218). An aloesin derivative from Aloe vera was found to exhibit potent anti-oxidant activity and inhibit cyclooxygenase-2 and thromboxane A_2 synthase. Aloe-emodin was also shown to have some protective effects against carbon tetrachloride-induced lipid peroxidation in rat liver (219). Aloe-emodin not only protected against hepatocyte death but also protected against the inflammatory response subsequent to lipid peroxidation.

Anthraquinone and anthrones of Aloe vera absorb UV light in the UV-B range. *In vitro* studies on the photobiological and photochemical properties of barbaloin and aloe-emodin were conducted in human skin fibroblasts (220). Cells were incubated with barbaloin or aloe-emodin and exposed to UV or visible light. Cells pretreated with aloe-emodin showed increased sensitivity to both UV-A and visible light. Significant photo-oxidative damage to both RNA and DNA was associated with the phototoxicity induced by aloe-emodin. Oxidative damage was observed even at low levels of phototoxicity, which suggested that photo-oxidative damage may cause rather than result from cellular death induced by aloe-emodin. The phototoxicity mechanism for aloe-emodin appears to involve the generation of reactive oxygen species and stable phototoproducts with cellular components (221). Aloe-emodin was found to generate singlet oxygen efficiently when irradiated with UV light, and the survival of human skin fibroblast in the presence of aloe-emodin was found to decrease when irradiated (222).

Cytotoxicity/Anti-Tumoral Effects

Aloe vera, in general, and aloe-emodin, in specific, have been reported to have *in vitro* cytotoxic effects against tumor and not normal cells. Aloe-emodin was shown to have specific dose-dependent cytotoxic effects on non-epithelial tumors, in particular neuroblastoma cells; however, human epithelial tumors, blood-derived tumors, and normal fibroblasts were almost refractory to the aloe-emodin treatments (44). In addition, of five purified anthraquinone compounds isolated from Aloe vera, only aloe-emodin produced cytotoxic effects against the multi-drug resistant human leukemia cells, although the effective dose range was in the micromolar concentration range (223). The aloin glycosides, aloesin and aloeresin, were devoid of anti-tumor cell activity, implying that only aloe-emodin exerted cytotoxic responses. Treatment of human leukemia cells with aloe-emodin was shown to induce cell cycle arrest, with the subsequent accumulation of cells in the S and G_2-M phases of the cell cycle, and at increased doses, aloe-emodin was also shown to induce apoptosis in human lung squamous carcinoma cells (224). Subsequently, it was demonstrated that the mechanism of aloe-emodin induced apoptosis involved the modulation of the expression of Bcl-2 family proteins, activation of caspases, and decreased the expression of certain isozymes of protein kinase C suggesting that aloe-emodin induced apoptosis occurred via activation of the Bax and Fas pathway (225, 226). The expression of p38 may also be an important determinant of apoptotic death induced by aloe-emodin (227). The exposure of aloe-emodin to two liver cancer cell lines that differed in p53 expression, however, suggested alternative mechanisms for the differing anti-proliferative activities of aloe-emodin. In human liver cancer cells that express p53, aloe-emodin induced a p53-dependent pathway that was accompanied with enhanced expression of p21 and resulted in cell cycle arrest. In human liver cancer cells that were p-53 deficient, aloe-emodin was shown to induce a p21-dependent pathway that did not cause cell cycle arrest, but rather promoted apoptosis (228). In cell-based ELISA and Western blot analysis, aloe-emodin was shown to abolish cisplatin-triggered activation of extracellular signal-regulated kinase (ERK) in rat glioma and murine fibroscarma cells (229).

Adverse or Toxic Effects

Aloe vera latex contains many biologically active compounds, but it is usually taken as a purgative (96). Tumor-promoting, as well as anti-mutagenic activities, have been ascribed to the latex of Aloe vera. Mutagenic and genotoxic activities in bacteria and eukaryotic cells have been shown for some, but not all anthraquinones. Westendorf and coworkers (230) investigated naturally occurring hydroxyanthraquinones for mutagenicity and cell-transforming activity. Aloe-emodin, which is present in Aloe vera-anthraquinoid laxatives, exhibited dose-related effects in mutation assays, in rat hepatocyte DNA-repair

induction assays, and in assays to determine malignant transformation of C3H/M mouse fibroblasts. Mueller and colleagues (231) investigated the genotoxicities of several anthraquinone derivatives found as natural constituents in plants, and showed that some of the 1,8-dihydroxyanthraquinone derivatives, including aloe-emodin, are intercalating agents that inhibit the interaction between topoisomerase II and DNA. The compounds induced a moderate increase in *Tk*-mutations and a dose-dependent induction of micronuclei. A micronuclei test indicated that danthron was more potent than aloe-emodin, which was more potent than emodin. Kodama and associates (232) observed DNA strand breaks and the generation of free radical and hydrogen peroxide by some anthraquinone derivatives from plant sources; and, subsequently, Mueller *et al.* (233–235) showed that some anthraquinone derivatives are biotransformed by cytochrome P450 1A2 *in vitro*, and that this may be relevant for the disposition of anthraquinone derivatives *in vivo*.

Aloe-emodin and other dihydroxyanthraquinones were examined for activities associated with tumor promotion, such as stimulation of cell proliferation and enhancement of malignant transformation (236). The *In vivo* treatment of primary rat hepatocytes with danthron, aloe-emodin, crysophanol, and rhein resulted in a 2-3-fold increase of DNA synthesis, whereas emodin was inactive. This marked stimulation of DNA synthesis was in the range with other known *in vitro* tumor promoters, such as phenobarbital and hexachlorocyclohexane. The results suggested that anthraquinones that possess hydroxyl groups in two positions may have tumor promoting activities.

Muller *et al.* (231, 235) investigated the dihydroxyanthraquinones of emodin, danthron, and aloe-emodin for genotoxicity in a number of *in vitro* assays, including mutation and micronucleus assays in mouse L5178Y cells, kinetochore analysis, topoisomerase II assay, and comet assays. Emodin, danthron and aloe-emodin reduced the amount of monomer DNA generated by topoisomerase II, indicating that all three compounds were capable of inhibiting the topoisomerase II-mediated decatenation. Furthermore, a modified comet assay showed that pretreatment of the cells with the test compounds reduced the effects of etoposide, an inhibitor of topoisomerase II. Danthron and aloe-emodin, and not emodin, increased the fraction of DNA moving into comet tails at concentrations of 50 μM in single-cell gel-electrophoresis assays. Results of these assays indicate that danthron and aloe-emodin are genotoxic.

SW480 colorectal tumor cells, VACO235 adenoma cells, and normal colonic epithelial cells were exposed to the dihydroxyanthraquinone compounds (0.2–5 mg/ml) of laxatives to determine if these compounds stimulated growth and the secretion of urokinase (237). Concentrations of 5 μg/ml caused between 50–70% cell loss in colorectal carcinoma SW480 cells; however, DNA synthesis was not similarly reduced. Dihydroxyanthraquinone treatment caused an approximate

doubling in the number of premalignant VACO235 cells; whereas, the growth of normal rat colonic epithelial cells was not affected. Urokinase secretion was increased by all dihydroxyanthraquinones in a dose-dependent manner, and this was the predominant effect of the dihydroxyanthraquinones in the SW480 carcinoma cells. Urokinase facilitates metastasis by matrix degradation and digestion of normal cells, and it was suggested that the release of urokinase caused the loss of cells observed in the SW480 carcinoma line.

Four *in vivo* studies were conducted to investigate the genotoxicity of aloe-emodin and emodin (100). The studies were conducted in rats or mice orally administered aloe-emodin or emodin for 4 h to 9 days. Analyses were conducted on bone marrow cells by micronucleus testing or in mouse fetal melanoblasts with the mouse spot test. The results showed no evidence of compound-induced increases of micronuclei or evidence of mutation induction or clastogenicity, although blood concentrations of aloe-emodin in the animals reached levels in the range of genetically active concentrations *in vitro*. One area of testing that was not addressed is the potential for effects in the gastrointestinal tract where the concentrations would be higher and where the microflora environment may actively participate in the metabolism of these compounds.

Adverse effects resulting from ingestion of the latex have been reported. Prolonged use is associated with watery diarrhea leading to electrolyte imbalance, and the increased loss of potassium can lead to hypokalemia (238). The amount of potassium lost can vary between 25% and 50% of the lean body mass (239). The increased loss of potassium is largely the result of compensatory reaction to the excessive loss of sodium, which induces a compensatory production of aldosterone that can exacerbate the hypokalemic condition and increase rennin production (186). Ishii *et al.* (202) demonstrated that aloe-emodin-9-anthrone inhibited rat colonic sodium, potassium adenosine triphosphatase. Persistent hypokalemia will bring about renal tubular nephropathy and an increased risk to pyelonephritis (240). In a case report, a male patient, who ten days prior to clinical admission had consumed juice extracted from four to five leafs of Aloe vera, presented with severe arthralgias, palpable purpura, and abdominal pain (241). The patient had consumed the same remedy two months prior without incidence. Within twenty-four hours of the last consumption, a rash on his legs and a mild arthralgia was noted on his ankle. His symptoms worsened in the following days with symmetrical arthralgias involving his knees, elbows, wrists, and ankles. Urinalysis showed hematuria, leukocytes, and moderate proteinuria. A diagnosis of Henoch-Schonlein, which is a systemic vasculitis, was confirmed by skin biopsy. Renal function deteriorated, and a renal biopsy demonstrated segmental necrosis. The immunomodulatory therapy response was poor, and the patient succumbed to renal failure. The renal dysfunction, nephritis, and chronic renal failure have been associated with Aloe consumption (242).

The increased loss of potassium may potentiate the actions of conventional drugs, such as cardiac glycosides and cortisteroids. Such interactions may result in cardiac arrhythmias and hypertension (186, 243). In addition, possible antagonism may also occur for anti-diarrhea agents and for non-steroidal anti-inflammatory agents, whereas synergism or exacerbation may result from interactions with glucoresins and diuretics. A decreased gastrointestinal transit time may also reduce the absorption of essential nutrients and many other drugs taken orally.

In recent years, the risk of development of colon cancer has been correlated with constipation and the use of laxatives. Apart from the physical changes, such as increased motility and the secretion of fluid and electrolytes within the lumen, morphological changes induced by laxative use is decidedly of greater importance (238). Siegers *et al.* (244) evaluated the incidence of colorectal cancer and anthranoid laxative abuse in humans, using the presence of pseudomelanosis coli as an indicator of anthranoid abuse. In a retrospective study of 3049 patients who underwent diagnostic colorectal endoscopy, the incidence of pseudo-melanosis coli in patients without pathological changes was 3.1%; the incidence increased significantly to 8.6% in those diagnosed with adenomas, and was 3.3% in patients diagnosed with colorectal carcinomas. In a prospective study of 1095 patients, the incidence was 6.9% for patients with normal diagnoses. The incidence of pseudo-melanosis coli increased to 9.8% for patients with adenomas and 18.6% for patients with carcinomas, suggesting an increased relative for colorectal cancer. Although the intestinal absorption and expected concentrations of 1, 8-dihydroxyanthraquinones in human tissues by food intake or medications is low, local accumulation is possible and the relative increased risk of colorectal cancer among frequent laxative users suggests that further research is warranted.

The onset of colonic lesions was examined in a patient who underwent liver transplantation and was also known to suffer from ulcerative colitis (245). A medical history of the patient revealed a ten-month use of an Aloe-containing anthranoid laxative. Colonoscopy showed marked brownish pigmentation of the mucosa of the entire colon, compatible with melanosis coli, whereas previous colonoscopies revealed no abnormalities. A year later, a large sessile polypoid lesion was found in the traverse colon, and histological examination revealed tubulovillous adenoma with extensive low-grade dysplasia.

Strickland *et al.* (246) found that painting aloe-emodin in an ethyl alcohol vehicle on the skin of mice in conjunction with exposure to UV-B irradiation resulted in the development of melanin-containing skin tumors. In addition, the application of ethanol and aloe-emodin combined with the exposure of mice to UV-B irradiation for thirty-three weeks was shown to cause mutations in the p53 gene, whereas, in the absence of UV irradiation, mice failed to develop tumors or p53 gene mutations (247).

SUMMARY

Aloe vera has enjoyed a long history of lay acceptance as an herbal remedy and is perhaps the most popular herbal remedy in use today (8, 9). Two products are obtained from the inner and outer fleshy leaf pulp of the Aloe vera plant—Aloe vera gel and Aloe vera latex. From these products, three distinct preparations of the Aloe vera plant are used as topical or oral therapeutic agents. Aloe vera latex is a laxative regulated as a drug by the FDA and is also used as a bitter flavoring additive by the food industry; Aloe vera gel is primarily a topical agent for skin wounds and irritations but is also taken internally for the treatment of gastric ulcers and diabetes; and the whole leaf extract, which combines both the gel and latex, is popular as a dietary supplement for various systemic ailments and is promoted as a potential anti-cancer, anti-AIDS, and anti-diabetic agent. A major obstacle in evaluating results of experimental studies and clinical trials of the effects of Aloe vera is differentiating clearly the part of the plant used and which species is under investigation. For example, Aloe vera juice, which is used to describe the latex from the bundle sheath cells, is also the liquid obtained from the maceration of the whole leaf. In addition, the use of the word "Aloe" on its own when Aloe vera gel is meant is misleading, since "Aloe" is used by the pharmaceutical industry as the drug derived from the leaf latex. Furthermore, not all products are equivalent. The climate, season of the year, harvesting, processing, and storage conditions affect the composition and potential biological activity of the plant components.

Both classes of leaf products, the gel and latex, are reported to possess a wide range of pharmacological activities; however, these claims are not supported by well-controlled studies. Dosed water studies in mice revealed no acute toxicity of the leaf pulp at 500 mg/kg (180). At higher doses, however, a decrease of central nervous system activity was observed. During sub-chronic ninety-day studies, increased mortality, decreased red blood cell count, and significant sperm damage were noted, in addition to decreased central nervous system activity (180). Ingestion of crude Aloe vera gel at doses greater than 110 mg/kg/day produced diarrhea, slower growth, polydipsia and polyuria in rats (181). Longer-term studies showed that ingestion of Aloe vera gel altered calcium metabolism (182). In a controlled toxicological evaluation of acemannan, an acetylated mannan, there was an increase in circulating leukocyte counts and an increase in the concentration of macrophages in the liver, lungs and spleens of animals; in addition, emesis and diarrhea were observed in dogs (178).

Colonic fermentation is the anaerobic process in which carbohydrates and proteins are metabolized by intestinal microflora (248). Fermentation of carbohydrates by bacteria mainly leads to the production of gases and short-chain fatty acids, such as acetate, butyrate, and propionate. Short chain fatty acids, especially butyrate, nourish the colonic epithelium and may protect against colon cancer, infection, and ulcerative colitis through their ability to promote

differentiation and select cells with damaged DNA for apoptosis (249, 250). Studies indicated that the production of IL-6 may play an important role in the pathogenesis of colorectal cancers, and that butyrate may exert a protective effect by specifically blocking IL-6-induced signaling events (251). Certain carbohydrates are preferred substrates for selected bacteria species; therefore, they have the potential to alter bacteria populations and their production of short-chain fatty acids in the intestine (252). Senna, a plant with similar characteristics as Aloe vera, was shown to inhibit the growth of *Bacteroides* sp. (253). *Bacteroides* sp. are responsible for the production of most of the butyrate required to nourish the colonic epithelium. Aloe vera may act similarly to alter the bacterial populations in the intestine. Since the mucosal epithelial cells in the intestine rely on the production of small organic acids produced by resident bacteria, an alteration in the microflora population may predispose the colon to chemical insult.

Aloe vera gel is most popularly recognized as a topical agent in the treatment of burns and wounds; however, topical application of Aloe vera gel is not as innocuous as reported. Episodes of contact dermatitis and conventional drug interactions have been observed (155, 185, 189).

Many people believe that cathartics sold in the form of teas, drinks, or herbs are safe, but the continued use of cathartic agents can cause the so-called lazy bowel syndrome (254). Furthermore, self-medication may delay the diagnosis of a more serious but treatable disease. The use of herbal laxatives during pregnancy may also present potential teratogenic and toxicological effects upon the embryo and fetus (255). Aloe vera consumption is associated with an irritating cathartic activity, which produces gripping and pelvic congestion. Consumption may also result in kidney damage in the mother and fetus, and increased intestinal peristalsis in the fetus may result in meconium release into the amniotic fluid. Similar effects may also result from consumption of the whole leaf products since the latex is a component of these. Consumption of Aloe vera latex is also associated with watery diarrhea leading to electrolyte imbalance and hypokalemia (238). Other side effects include weight loss, central nervous system disturbances, and abnormalities and kidney dysfunction. Compounds in Aloe vera latex are also considered genotoxic and may be mutagenic (231). The abuse of Aloe vera latex-containing laxatives is associated with melanosis coli, which may play a role in the development of colorectal cancer (244). Intestinal tumors were induced in rats that consumed a diet containing chrysazin, a synthetic anthraquinone with dihydroxy groups like other natural anthraquinones (256). In addition, compounds in Aloe vera latex are suspected to interact with certain oral conventional drugs, in particular corticosteroids and cardiac glycosides (186, 243).

Aloe vera is not an approved drug except in the form of the dried latex. In this form it is a potent cathartic agent. Reports are conflicting as to the efficacy of Aloe vera as a topical agent in burn and wound management. Too often

there is confusion over the test material used in the studies, which is complicated by the addition of other active ingredients, such as anti-microbials. Topical application of Aloe vera does not appear to be an effective preventative for radiation-induced injuries, and whether it promotes wound healing is unclear. Oral administration of Aloe vera does appear to be a useful adjunct for lowering blood glucose levels in diabetic patients as well as for reducing blood lipid levels in patients with hyperlipidemia (257). In view of the complexities inherent in Aloe vera pharmacology and the inconsistencies reported in literature, the effectiveness and safety of Aloe vera as a topical or oral herbal remedy is insufficiently defined at present.

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