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Effects of Aloe Extracts on Human Normal and Tumor Cells in Vitro[1]

W. D. WINTERS, R. BENAVIDES, AND W. J. CLOUSE[2]

Fractions of leaf extracts from 2 local types, labeled Aloe vera *(subsequently identified as* Aloe barbadensis *Mill. and* A. saponaria *Haw.), were prepared by differential centrifugation and tested by in vitro assays for the presence of lectin-like activities and for effects on the attachment and growth of human normal and tumor cells. Fractions of extracts of fresh leaves and commercially "stabilized"* Aloe vera *gel had high levels of lectin-like substances measured by immunodiffusion and hemagglutination assays. Substances in fluid fractions from both fresh leaf sources were found to markedly promote attachment and growth of human normal, but not tumor, cells and to enhance healing of wounded cell monolayers. In contrast, fractions of "stabilized"* Aloe vera *gel were equally cytotoxic for human normal and tumor cells in vitro. Results from cell assays suggested that the observed growth promotion and wound healing effects of aloe substances in vitro may be analogous to what has been observed in vivo during healing of wounds and burns.*

Fresh leaves and exudates therefrom of aloe plants have been used for many centuries as popular remedies for a variety of unrelated human afflictions. Claims have been made for the curative properties of aloe gel, a juicy mucilaginous extract of leaves from different species of aloe plants, which is used in folk medicine as a topical treatment for skin and other cutaneous wounds, burns, and diseases (Ship, 1977). In the last few decades, reports of biochemical studies of extracts of aloe leaves have described common chemical and physical compositions of gels extracted from various aloe species (Rowe and Parks, 1941; Roboz and Haagen-Smit, 1948; Bouchey and Gjerstad, 1969; Gjerstad, 1971). More recently, biochemically analyzed substances purified from aloe leaves have been found to have immunochemical properties consistent with strong lectins (Suzuki et al., 1979).

Pharmacognosical characteristics of substances extracted from aloe leaves, as reported in clinical research studies using human and experimental animal subjects, suggest that these aloe substances may be effective in reducing inflammation and in promotion of skin wound and burn healing (Crewe, 1939; Rowe et al., 1941; Gjerstad and Riner, 1968; Fujita et al., 1976).

In contrast to the research information described in published reports about in vivo healing effects of aloe substances, there is a paucity of information about similar biological activities of these substances on cells grown in vitro.

In vitro response assays can be designed to exclude a myriad of unknown in vivo factors which could obscure the direct biological actions of aloe substances during in vivo tests. Therefore, the aims of these studies were to see if substances extracted from *Aloe vera* leaves had lectin-like properties and to determine if

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[2] Department of Microbiology, University of Texas Health Science Center, San Antonio, TX 78284.

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these substances markedly promoted healing, enhanced attachment and stimulated growth of human cells in vitro.

MATERIALS AND METHODS

Preparation of aloe extracts

Mature adult plants, labeled *Aloe vera* and *Aloe vera chinensis* by the local nursery where they were purchased, have subsequently been identified as *Aloe barbadensis* Mill. and *Aloe saponaria* Haw., respectively, through the kind efforts of Professor B. L. Turner, Botany Department, University of Texas, Austin, Texas. Both of these plants used in the present studies are commonly grown and used as medicinal aloes in southwestern United States (Gjerstad and Riner, 1968). Voucher materials from *Aloe barbadensis* Mill. (*Winters 1*) and *Aloe saponaria* Haw. (*Winters 2*) are on deposit at the Plant Resources Center Herbarium of the University of Texas at Austin (TEX). Unfortunately, the voucher specimens consist of vegetative materials only, but according to the Director, Professor B. L. Turner, these should be identifiable by specialists in the field.

Fresh clean leaves were first cut from each plant and then they were weighed, minced, and homogenized in a Lourder blender for 1 min at a 50 setting. The homogenate, a mixture of juice and particles approximately 2 mm in size, was centrifuged for 15 min at 2,500 rpm at 4°C and the greenish colored particle-free liquid supernatant was collected (SI fraction). Pelleted materials were recentrifuged for 1 hr at 20,000 rpm at 10°C. The high speed supernatant (SII fraction) and pellet (HP fraction) were individually collected. SI and SII fractions were placed in dialysis bags and then concentrated by immersion in Aquacide II (Calbiochem, CA) for 4 hr at 4°C. *Aloe vera* gel, a commercial preparation claimed by the producer to be "stabilized" for long shelf life without refrigeration, was purchased at a local health food store. (Additional product information will be supplied on request by the authors.) *Aloe vera* gel used in these studies was weighed, minced, homogenized and separated into fractions as described for the fresh aloe leaf specimens. SI and SII fractions from this aloe source were then concentrated and refrigerated at 4°C together with all other aloe fractions.

Hemagglutination titration

Hemagglutination (HA) assays were carried out as described by Suzuki et al. (1979). Human and canine erythrocytes (RBCs) were diluted to a 2.0% final suspension in sterile phosphate buffered saline (PBS; 0.15 M, pH 7.2) following 4 wash cycles in PBS. For HA assays, 50 μl aliquots of 2-fold serial dilutions of individual fractions for each aloe source were mixed with an equal volume of RBCs suspension and left for 3 hr at room temperature. The degree of agglutination was evaluated macroscopically and HA titer was the reciprocal of the highest dilution of an aloe fraction showing complete agglutination.

Immunodiffusion tests

Microimmunodiffusion (ID) tests were performed as previously described (Winters and Snow, 1974; Winters, 1979). ID tests were performed using 0.9% Noble special agar dissolved in PBS layered on microscope slides coated with

0.2% agar. Wells were 5.0 mm distances between central and peripheral wells (center to center). Incubation in a moist chamber at room temperature for 72–96 hr was followed by fixation in a 2.0% NaCl-water solution. Gels were stained in 0.1% thiazine red K dissolved in 1.0% acetic acid and destained in 1.0% acetic acid. ID reactions were evaluated with the aid of an indirect light source.

Cell attachment and growth assays

Human normal fetal lung (HFL) and human cervical carcinoma (ME180) cells were grown in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics, as recently described (Winters and Sykes, 1978; Guest and Winters, 1979). DMEM was used as diluent and as a suspension medium in all assays. Cells cultured in plastic petri plates were incubated at 37°C in an atmosphere of 5% CO_2.

In cell attachment studies, tissue culture tubes in each set received 1×10^5 cells suspended in 2.0 ml DMEM and then either 2.0 ml of diluted aloe fractions or 2.0 ml of DMEM. The untreated control cell suspensions and aloe fraction-cell mixtures were immediately seeded into culture plates. Cell attachment and colony formation were monitored by microscopic observations and by counts of stained cells in cultures fixed at 24-hr intervals (Winters et al., 1974).

In assays of cell growth, 1×10^5 were seeded into tissue culture plates and left undisturbed for 24 hr. At this time medium was decanted and replaced by DMEM alone or by aloe fractions diluted in DMEM as described in the cell attachment assays. Cell cultures were harvested and cell counts were performed at 2-day intervals for 12 days after aloe treatment (Winters et al., 1974).

In vitro wounded cell monolayer assays

Confluent plate cultures of HFL and ME180 cells were used. Three parallel lanes approximately 1.5 mm wide and 2.0 cm long were scraped through the center portion of cell monolayers using a Pasteur pipette with a flattened tip while viewed through a dissecting microscope. Cells within the lanes were completely removed. The cell monolayers were then washed and one-half the total number of wounded plates received DMEM, while the other half received aloe fractions diluted in DMEM. All aloe treated and control wounded cultures were inspected microscopically at daily intervals for 14 days. Cell densities at the edges of the wounds, which reflected the rate of movement of cells into the wound area, were counted as described by Currie (1976).

RESULTS

Immunological activities of aloe extracts

Hemagglutination and immunodiffusion tests were used to monitor the activity levels of lectin-like substances in fraction of each aloe specimen during the separation procedures. Positive HA and ID reactions were detected after the supernatants, i.e., SI and SII, had been concentrated at least 67% (Table 1). Comparable fractions from all 3 aloe sources after separation were found to have approximately the same ratio of recoverable supernatant fluids to high speed pellet materials.

TABLE 1. YIELD OF BIOLOGICALLY ACTIVE FRACTIONS OF *Aloe* LEAF AND GEL SPECIMENS.

Fraction	*Aloe barbadensis* Mill. leaves		Commercial *Aloe vera* gel		*Aloe saponaria* Haw. leaves	
	Weight[a] (g)	Volume[a] (ml)	Weight (g)	Volume (ml)	Weight (g)	Volume (ml)
Homogenized slurry	88	100	118	150	172	150
Low speed supernate	54	64	82	104	96	115
after concentration (SI)	18	21[b]	7	9	15	18
High speed supernate	28	33	27	36	67	56
after concentration (SII)	8	9	4	5	3	2
High speed pellet (HP)	6	4	9	3	9	3

[a] Weight and volume values are actual for one series of aloe specimen preparations. In other preparations of aloe fractions using up to 15% more starting materials the relative weights and volumes of the fractions were in the same ratios as shown above.

[b] Underlined values represent fractions which showed positive reactions in preliminary ID and HA tests.

In ID tests against human, canine and baboon sera collected from 6 donors of each species, concentrated SI fractions at 1:4–1:8 dilutions prepared in PBS from all 3 aloe sources reacted with human and baboon sera. In contrast, none of the aloe fractions reacted in ID tests with canine sera from normal and 2 tumor-bearing adult dogs (Winters, 1979). Concentrated SII and HP fractions from the 3 aloe specimens did not show immunoprecipitation reactions with any sera, although the control lectin Concanavalin A (Con A; Sigma, 25 mg/ml original concentration) at 1:32 dilutions showed precipitin reactions against proteins in all sera.

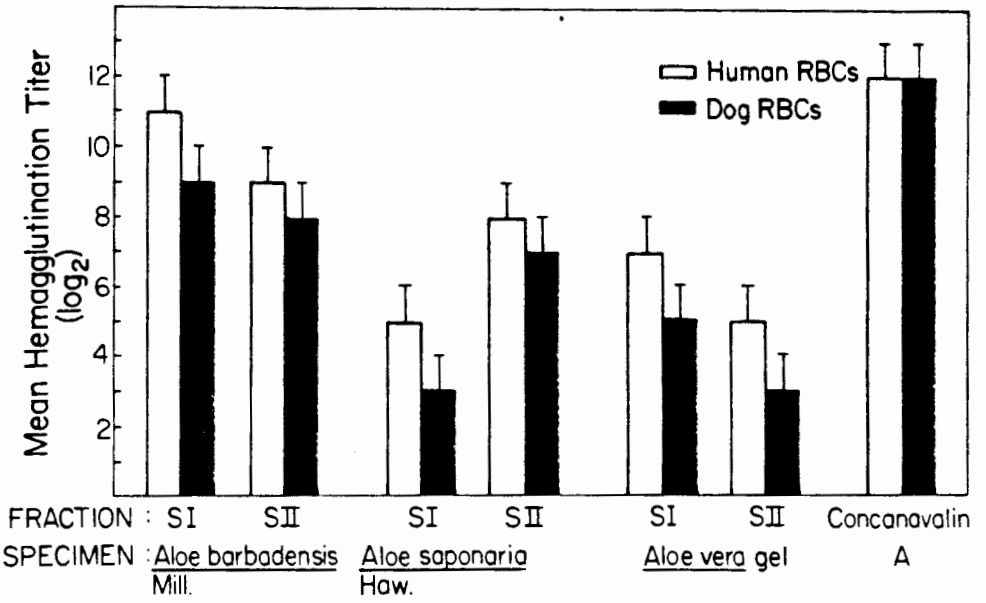
HA titers of Con A and concentrated SI and SII fractions representative of the 3 aloe sources are shown in Fig. 1. Concentrated SI fractions of *Aloe barbadensis* Mill. contained markedly higher amounts of HA reactive substances than comparable fractions from *Aloe saponaria* Haw. or *Aloe vera* gel.

Human RBCs were more sensitive indicators of HA than canine RBCs for tests of aloe fractions, while both human and canine RBCs were equally sensitive in HA tests of the control lectin Con A.

Effects of aloe extracts on cell attachment, growth and wound healing

Neither HFL or ME180 cells in single cell suspensions were aggregated after mixing with dilutions of fractions of *Aloe barbadensis* Mill. which were selected for use in cell studies because of their high lectin-like activities. Observations made during the course of 2 days after seeding cells into culture plates revealed that attachment of HFL cells, but not ME180 tumor cells, was markedly enhanced by 1:10 dilutions of concentrated SI fractions. No differences in cell attachment were observed when the HFL and ME180 cells treated with the other *Aloe barbadensis* Mill. fractions were compared with untreated control cells.

As seen in Fig. 2, marked enhancement of growth of HFL cells treated in suspension and in monolayer cultures with *Aloe barbadensis* Mill. fractions SI and HP was observed. In contrast, ME180 tumor cells treated with aloe fractions in suspension did not grow as well as untreated control cells. The growth of



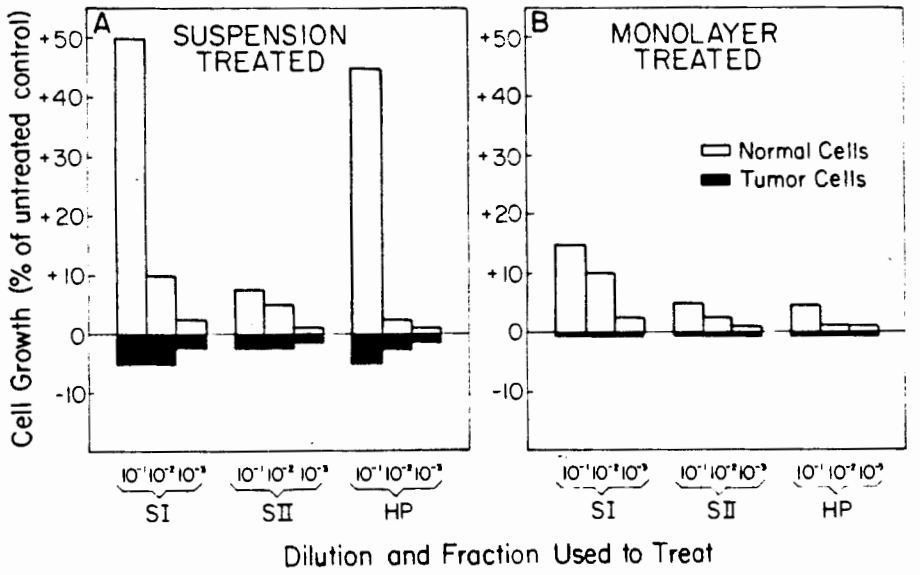


Fig. 1. (*Above*) Hemagglutination of human and canine erythrocytes (RBCs) by concentrated fractions SI and SII prepared from *Aloe barbadensis* Mill. and *Aloe saponaria* Haw. leaves and commercially stabilized *Aloe vera* gel. Each column represents mean titer ± s.e. of 6 assays using RBCs from 3 normal adult dogs and 3 normal human donors.

Fig. 2. (*Below*) Changes in the growth of human normal (HFL) and tumor (ME180) cells treated in suspension (panel A) and in monolayer (panel B) with dilutions of fractions prepared from *Aloe barbadensis* Mill. leaves. Each column represents cell growth results which are mean cell counts of replicate cultures treated with each *Aloe barbadensis* Mill. fraction and are expressed as percent (%) of control values, i.e., counts of cells in corresponding untreated cultures.

ME180 cells in treated monolayer cultures was not different from the growth of untreated cells in control cultures.

Counts of cells at the edges of wounds in monolayer HFL and ME180 cell cultures treated with *Aloe barbadensis* Mill. fraction SI were observed to be higher than cell densities at the wound edges in other cultures treated with SII and HP fractions and in untreated cultures. Moreover, larger numbers of cells were observed to be growing into the wound areas in SI fraction-treated cultures at 7 and 14 days than in other untreated and aloe fraction SII and HP-treated cultures of both HFL and ME180 cells.

Fractions of *Aloe vera* gel, when tested at dilutions up to 1:200, caused marked cellular granularity, inhibition of attachment of cells treated in suspension and cell detachment in monolayer cultures within 2 days after treatment of HFL and ME180 cells. Accordingly, these cytotoxic responses prevented the completion of cell attachment and growth experiments using *Aloe vera* gel fractions.

DISCUSSION

We describe new biological activities of lectin-like substances prepared from leaves of *Aloe barbadensis* Mill. and *Aloe saponaria* Haw. plants and from commercially produced *Aloe vera* gel. Extracts prepared from the 3 aloe sources repeatedly yielded similar ratios of supernatant to high speed pellet materials. Thus, comparisons of lectin-like and other biological activities detected in the experimental fractions revealed that the supernatant fractions of *Aloe barbadensis* Mill. contained the highest levels of reactive substances in all in vitro tests. The concentrated supernatant fractions from the 3 aloe sources closely resembled the AS-0-40 fractions described by Suzuki et al. (1979) in possessing almost all of the HA activities. In the present HA studies, canine RBCs, not used in previously reported HA tests (Suzuki et al., 1979), were agglutinated by the lectin-like substances in supernatant fractions from all aloe sources. Based on the results of HA tests, it appeared that fractions from commercially prepared *Aloe vera* gel were least reactive. Immunodiffusion tests showed that baboon sera, not tested in previous studies (Fujita et al., 1978; Suzuki et al., 1979), and human sera contained serum proteins reactive with supernatant fractions derived from all 3 aloe sources. These baboon and human sera ID reactions were similar to those described by Fujita et al. (1978) and Suzuki et al. (1979), who tested sera from a number of species against crude and purified extracts of *Aloe aborescens* Mill. In contrast to results reported in these 2 previous studies, aloe fractions in our studies did not react with serum proteins in the canine sera tested.

In order to mimic topical treatment of skin wounds in vivo with aloe preparations, monolayers of human normal and tumor cells growing in individual cultures in vitro were artificially wounded and then treated with fractions of *Aloe vera* extracts. The rate of movement of cells into wounded areas was greatest in both HFL and ME180 cell cultures treated with only 1:10 or lower dilutions of SI fractions of *Aloe barbadensis* Mill. Substances promoting cell attachment and growth were found at highest levels of *Aloe barbadensis* Mill. fractions SI and HP when these fractions were used to treat HFL cells in suspension. All aloe fractions tested had similar levels of activity in attachment and growth assays of human tumor cells treated in suspension and in monolayer cultures. The in vitro

cell assays used here were designed to reduce or eliminate the possible influences of factors, such as cells of immune systems and their products, which may play active roles during aloe treatments of wounds in vivo.

Substances in aloe fractions shown here to have lectin-like activities may also prove, in studies currently underway, to have enzyme, mitogenic and other biological activities which may be involved in the resolution of inflammation, wounds and burns (Fujita et al., 1976; Fujita et al., 1978, Suzuki et al., 1979).

The cytotoxic effects of commercially prepared *Aloe vera* gel fractions on human normal and tumor cells in culture suggest that these commercial preparations contain substances introduced during commercial processing which can alter the levels of lectin-like activities and can markedly disrupt the in vitro attachment and growth of human cells.

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