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Processed *Aloe vera* Administered Topically Inhibits Inflammation

ROBERT H. DAVIS, PhD*
KENNETH Y. ROSENTHAL†
LINDA R. CESARIO†
GREGORY A. ROUW†

Aloe vera preparations were evaluated for topical anti-inflammatory activity using the croton oil-induced edema assay. The results show that small amounts of *A. vera* given topically will inhibit inflammation induced by a moderate amount of irritant. In general, the decolorized Aloe was more effective than the colorized Aloe (with anthraquinone). A 47.1% inhibition of inflammation was obtained by 5% decolorized irradiated Aloe. These results may be used as a baseline to assess the biologic activity of *A. vera* in the treatment of inflammation by podiatric physicians.

Aloe vera has unique biologic properties. It reduces inflammation, but also improves wound healing. Topically administered Aloe inhibits edema over a dose range of 0.25 to 1%.[1] In the absence of anthraquinones, orally administered *A. vera* has virtually no effect upon croton oil-induced edema. Conversely, when anthraquinones are present, *A. vera* given orally reduces inflammation 54%.[2]

It is hypothesized that anthraquinones aid in the biochemical transport of the active components in Aloe to the site of inflammation. Unlike steroids, Aloe has its optimal action in the local inflammatory phase, as opposed to the chronic fibrosis phase. The possibility of a synergistic relationship between glucocorticoids and *A. vera* must be evaluated in future studies.

Over the past 20 years, an intense search for the active ingredient in Aloe has been carried out. A good deal of the biologic acitvity may be found in the carbohydrate or glycoside fraction.[3] The major component could be a phosphomannose, or a uronic acid, or even gibberellin. However, the authors believe that a strong synergistic relationship exists between the carbohydrate compounds and other active substances in Aloe, such as vitamins and amino acids. Certain vitamins and amino acids show strong anti-inflammatory activity in their own right.[4] These substances may have a triggering effect on enzyme and carbohydrate activity needed for anti-inflammation.[5] Thus, the authors have confined their study to the "entire team" of *A. vera*, rather than individual extracts of the plant gel.

The purpose of this study is to compare the anti-inflammatory activity of processed (irradiated) *A. vera* with the fresh Aloe in the presence (colorized) and absence (decolorized) of anthraquinones. The croton oil-induced edema swelling model provides an effective way of measuring the topical activity of the various preparations of *A. vera* used in podiatric medicine.

Materials and Methods

Adult female ICR mice (20–30 GM; 13 animals/group) were given 25 μg/μl croton oil, applied topically on both surfaces of the right ear.[6] The concentration of croton oil in acetone was 2.5 mg/ml. The irritant was applied by means of a Hamilton®[1] microsyringe. The left ear remained untreated and served as a control. Acetone alone did not induce any changes in ear weight. The peak swelling oc-

* Professor of Physiology, Pennsylvania College of Podiatric Medicine, Eighth at Race St, Philadelphia, PA 19107.

† Submitted during second year, Pennsylvania College of Podiatric Medicine, Philadelphia.

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curred 6 hr later, at which time the ear swelling was measured by obtaining a 6-mm punch biopsy specimen from the inflamed and the control ears. The ear tissue was weighed to the nearest 0.01 mg. The difference in weight between the inflamed and control ear represented the degree of swelling for each group. Each group of animals had its own internal control. Topical applications of 5% fresh, processed, and irradiated processed *A. vera* were applied 30 min after the croton oil to minimize any nonspecific interaction between the irritant and *A. vera*. Colorized (with anthraquinones) and decolorized (without anthraquinones) Aloe were evaluated for each preparation. Ear weight differences were recorded, and the percentage of inhibition of swelling was obtained for each Aloe preparation. The Student's t-test was used to obtain the significant difference of each test group in reference to the irritant control.[7]

Three sets of *A. vera* preparations were evaluated for anti-inflammatory activity. The fresh *A. vera* was obtained by removing the gel from the leaves. The pulp was removed from the gel. The anthraquinones were either left in (colorized) or removed (decolorized), and the preparation was freeze-dried to a powder and sealed in airtight containers. The processed, irradiated Aloe was simply the freeze-dried processed Aloe irradiated by gamma cobalt irradiation at 1 Mrads.

Results and Discussion

Advances have been made over the last few years in the development of Aloe concentrates for medical and cosmetic formulations. Since Aloe gel in its natural state is only 0.5% solids, water must be removed without damaging the active biologic ingredients. Also, the *A. vera* as a liquid must be preserved, and bacteria must be prevented from attacking the Aloe. Since *A. vera* is a composite of many components, a reliable biologic assay must be found to assess the biologic activity of the Aloe gel concentrates for use in podiatric medicine. A quality *A. vera* must be obtained and used at the correct concentration to forecast with accuracy the medical and therapeutic effectiveness in treating inflammation, wounds, and arthritis.

Topical administration of 25 $\mu g/\mu l$ croton oil produced a 68% increase in punch biopsy ear weight over 6 hr. Topical administration of 5% decolorized fresh *A. vera* significantly inhibited inflammation from croton oil 38.2 ± 3.6% ($p < 0.01$). The 5% colorized fresh *A. vera* inhibited ear swelling only 7.3 ± 1.1% ($p > 0.5$) (Table 1 and Fig. 1). The presence of anthraquinones on topical administration did not favor anti-inflammatory activity, possibly because of their slight irritating properties to the skin.

Experience has demonstrated, however, that anthraquinones are required for oral activity.[2] Their presence may be needed to absorb and carry biologic

Table 1. Topical 5% *A. vera* Inhibits Croton Oil-Induced Inflammation

TREATMENT	EAR EDEMA* MG	% INHIBITION
CROTON OIL (25 μg / μl)	6.8 ± 0.8	–
COLOR	6.3 ± 1.0	7.3 ± 1.1
+ FRESH ALOE		
DECOLOR	4.2 ± 0.4	38.2 ± 3.6**
COLOR	6.0 ± 0.8	11.8 ± 1.6
+ PROCESSED ALOE		
DECOLOR	6.2 ± 1.0	8.8 ± 1.4
COLOR	4.8 ± 0.6	29.4 ± 3.7
+ IRRADIATED PROCESSED ALOE		
DECOLOR	3.6 ± 0.5	47.1 ± 6.5**

*EDEMA AFTER 6 HOURS. 13 ANIMALS / GROUP. **P < 0.02.

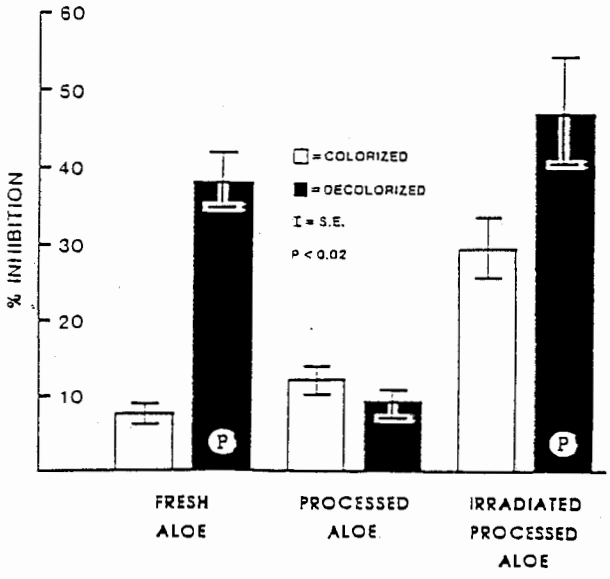

Figure 1. Percentage of inhibition of croton oil-induced edema by topical 5% *A. vera*.

[92] Key Pharmaceutical, Miami, FL.

complexes in Aloe to the site of inflammation. Colorized and decolorized processed *A.vera* inhibited inflammation 11.8 ± 1.6% and 8.8 ± 1.4%, respectively. These values were not significant at the probability level of >0.1. However, it is likely that increasing the topical dose above 5% would yield significant biologic activity. When the processed *A. vera* was irradiated, the decolorized Aloe reduced croton oil-induced edema 47.1 ± 6.5% ($p < 0.02$). The irradiation of Aloe eliminated the influence of bacteria and had a significant influence on the biologic activity.

Even the colorized Aloe showed a 29.4 ± 3.7% inhibition even though the value was not significant at the 95% confidence limit. The small amount of gamma irradiation used (1 Mrads) did not destroy the active biologic compounds in Aloe. However, irradiation did appear to eliminate the bacterial influence, since processed Aloe without irradiation was less active. Evaluation of *A. vera* preparations at low doses allows Aloe gel to be better understood and reliably assayed so that it can be used in treatment.

Summary

The topical influence of *A. vera* on inhibiting croton oil-induced ear swelling provides a valuable tool for evaluating the biologic activity of Aloe concentrates, extracts, and constituents in small amounts for podiatric medical use. Using the coefficient of standard error variation around the mean, the authors calculated a rough index of experimental error for the study to be 13.3 ± 0.8%. Thus, this reliable assay can be used to correlate the chemistry of Aloe, with its biologic activity, to obtain beneficial treatment.

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References

1. DAVIS AH, LEITNER M, RUSSO J: Topical anti-inflammatory activity of *Aloe vera* as measured by ear swelling. JAPMA 77: 610, 1987.
2. DAVIS AH, LEITNER M, RUSSO J, ET AL: Anti-inflammatory activity of *Aloe vera*: an investigation of the spectrum of activity using the irritant-induced edema assay. JAPMA 79: 263, 1989.
3. SKOUSEN, MB: *Aloe Vera: New Scientific Discoveries*, The Aloe Vera Research Institute, Cypress, CA, 1982.
4. HANLEY D, SOLOMON W, SAFFRAN B, ET AL: The evaluation of natural substances in the treatment of adjuvant arthritis. JAPMA 72: 275, 1982.
5. COATS B: *The Silent Healer*, Garland, TX, 1979.
6. GLENN E, BOWMAN B, RHOLOFF N: Simple laboratory procedures for the evaluation of topically active anti-inflammatory drugs. Agents Actions 8: 497, 1978.
7. SNEDECOR G, COCHRAN W: *Statistical Method*, 6th Ed, Iowa State University Press, Ames, IA 1974.