Antiarthritic Activity Of Anthraquinones Found In Aloe Vera For Podiatric Medicine

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Rheumatoid arthritis is a painful and crippling systemic disease for which there is no cure. The best experimental model for studying rheumatoid arthritis in humans is the adjuvant-induced arthritis in rats. One of the group of compounds found in Aloe is the anthraquinones. These substances have been recognized for their use in veterinary medicine against inflammation. The authors evaluate the anti-inflammatory and antiarthritic activity of anthraquinone, anthracene, cinnamic acid, and anthranilic acid found in the Aloe vera plant, and show what contribution each ingredient makes toward the total activity found in Aloe.

In previous studies, the authors have shown that Aloe gel extract has anti-inflammatory and antiarthritic activity.[1] Combinations of Aloe with ascorbic acid, thymus extract, and RNA significantly improved the activity.[2] The chemical makeup of Aloe holds a valuable key to antiarthritic activity that could be used by podiatrists to treat patients.[3] Elements in Aloe gel include lignin, saponins, anthraquinones, inorganic ingredients / minerals, vitamins, enzymes, and amino acids. Anthraquinones such as anthracene have been recognized for their use in veterinary medicine against inflammation. They possess anti-inflammatory, analgesic, and tissue repair properties.

No doubt anthraquinones have a bearing on the healing and pain-killing effectiveness of the fresh leaf gel. Few people understand the meaning of the anthraquinone complex in Aloe. Many studies verify the successful treatment of burns, ulcers, and dermatitis, but no one knows why Aloe has these healing qualities.[5] The authors propose to test the antiarthritic and anti-inflammatory activity of anthraquinone, anthracene, cinnamic acid, and anthranilic acid in an adjuvant arthritis model in order to determine if there are possible ingredients that can be used to treat rheumatoid arthritis. This approach will help us understand the antiarthritic activity of Aloe. The purpose of this study is to determine, in part, the active elements in Aloe so as to unlock the mystery of the gel. Many medicines in common use today, such as digitalis and quinidine, were derived in a similar way from barks and leaves.

Materials And Methods

Adult male Sprague-Dawley rats (175 to 200 gm, 12/group) were injected with heat-killed *Mycobacterium butyricum*. The bacteria were suspended in light mineral oil at 5 mg/ml. Under ether anesthesia, two groups of rats were injected in their right hind paw with 0.1 ml of oil. All the other groups were injected with the suspended bacteria. Six hours after the injections, the day 0 measurements were taken. Two experiments were conducted together. One study investigated the effect of anthraquinones on the prevention of adjuvant arthritis. The other study determined their effect on established adjuvant arthritis. Three control groups were used. The animals injected with oil alone were used to be sure that the oil, itself, did not cause swelling. One of the groups injected with adjuvant was also injected with distilled water at the same times and amounts as those groups being treated. This was done to determine whether the volume and frequency of injections affected the amount of swelling. A third group was injected with the adjuvant.

In the first experiment, prevention of adjuvant arthritis by anthraquinones was studied. The right hind paws of two control groups were injected with the adjuvant, and day 0 paw volume measurements were taken. One of the control groups was injected with 10 ml of water subcutaneously daily for 13 days beginning on day 0. Either anthraquinone, anthracene, cinnamic acid, or anthranilic acid was injected into rats in four other groups that had received *M. butyricum*. Each day the anthraquinones were injected subcutaneously at 150 mg/kg aqueous suspensions.

In the regression study, the rats were injected with *M. butyricum* suspension. The symptoms of adjuvant arthritis usually take from 14 to 21 days to develop. After 21 days, treatment was initiated with 150 mg/kg suspensions of anthraquinones subcutaneously daily from day 21 through day 33.

Adjuvant arthritis in rats manifests itself by swelling in all paws, especially the hind paws, gonads, and ears. A water plethysmograph was used to monitor the hind paw volume. The plethysmograph was at zero prior to each reading while maintaining constant sensitivity to obtain consistency. Edema in the injected (right) hind paw was considered to be inflammation caused by trauma. Prolonged inflammation in this paw was maintained by the slow release of the *M. butyricum*. Any edema in the left paw was considered to be an immunological phenomenon.[6,7]

The rats were anesthetized with ether on paw-measuring days. Their hind paw volumes were determined by dipping them into a fluid-filled cell up to the anatomical hair line. Day 0 measurements were taken 6 hours after the paws were injected with *M. butyricum* adjuvant or oil alone. This initial measurement was used as a reference from which units of edema were calculated in the prevention study. These units of edema were calculated by

subtracting the day 0 volumes from those measured on days 7, 14, and 21. The base-line values for calculating units of edema in the regression study were measured on day 21. Day 21 values were viewed as 0 units of edema. The hind paws were measured on days 0, 21, 28, 35, and 38 for this study.

The animal's body weight was measured on days 0 and 2l in both studies and day 38 in the regression study. The change in weight during the experiments was calculated by subtracting from day-38 weights in the regression experiment. The change in edema was divided by the change in weight to obtain a relative change in edema. This served to rule out any gain in paw volume caused by weight gain. A CU-5 Medical Land Camera was used to photograph representative rats on days 21 (prevention study) and 38 (regression study). This was done to demonstrate the difference in paw edema between the rats receiving anthraquinones and the control rats. Mean paw volumes and body weights were recorded for all animals. Standard errors were determined by using the formula SE = E $d^2/N(N - 1)$. The deviation of individual values from the mean is Ed^2, and $N - 1$ represents the degrees of freedom.

Results And Discussion

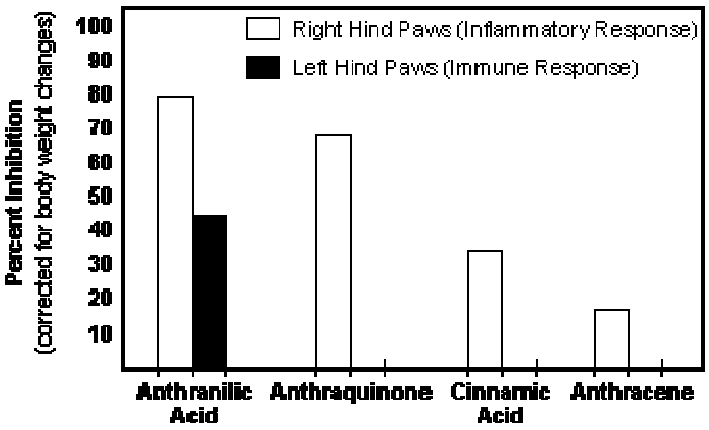
Prevention of Arthritis. Aloe has antipyretic and anti-inflammatory activity that has been used to treat burns and skin conditions over the years. Splitting the leaves of Aloe and applying the gel directly to burns produces beneficial effects.[8] The curative principle occurs within the pulp and rind of the leaf.[9] The authors have administered fresh Aloe leaf extract to prevent or regress adjuvant arthritis. Aloe extract produced a 48% inhibition of inflammation and a 72% inhibition of the immune response (the arthritis) when 150 mg/kg of Aloe extract was administered subcutaneously, daily, over 13 days as preventive measure.[1]

The anti-inflammatory and antiarthritic activity of the anthraquinones were tested to determine if they could prevent the formation of arthritis. An increase in volume of the right hind paw is an anti-inflammatory response to the presence of the adjuvant, whereas the swelling of the left hind paw is an autoimmune response, in part, against the animal's own cartilage. *Mycobacterium butyricum* is similar to cartilage so that the immune response attacks both. Swelling occurs in both hind paws injected with adjuvant. This swelling continues to increase over the entire experimental period. Aqueous controls received equal amounts of water injections as those receiving injections of anthraquinone. Since these animals swelled somewhat more than those receiving the adjuvant, the volume or the trauma of daily injections contributed to the disease process. In order to more accurately estimate the effect of the compounds under investigation, the response of anthraquinones was

compared to the aqueous injected adjuvant controls.

Forst and Davis[10] have shown that anthranilic acid has antiarthritic activity. This compound is present in Aloe and was considered as an activity internal control so that the authors could be certain that the responses recorded in the study were real and not phantoms. Edema in the right hind paw (inflammation) was 40% less than the aqueous adjuvant control animals on day 21 *(Table 1; Fig. 1)*. The left hind paw (immune) was 35.5% less than the controls. The percentage of inhibition by anthranilic acid corrected for body weight was 79.7% in the inflammatory paw and 42.4% in the immune paws. Tryptophane, an active anti-inflammatory compound, is produced from anthranilic acid. Previous work showed that tryptophane inhibited arthritis 75% in the immune paw.[10] Anthranilic acid may work through tryptophane.

Figure 1



Prevention of adjuvant arthritis with anthraquinones.

Table 1

Prevention of Adjuvant Arthritis with Anthraquinones in Rats[a,b]

	Protocol	Aqueous Adjuvant Control[c]	Anthraquinone	Anthracene	Cinnamic Acid	Anthranilic Acid
	Treatment (mg/kg x 13)					
	Days 0 to 12		150	150	150	150
	Number of rats	12	12	12	12	12
	Final body weight (g)	254 +/- 12	249 +/- 12	271 +/- 13	261 +/- 9	275 +/- 5
	Edema of hind paws (volume units +/- SE)					
Day 7	Left	1.59 +/- 0.05	1.59 +/- 0.08	1.49 +/- 0.04	1.50 +/- 0.04	1.40 +/- 0.04
	Right	2.59 +/- 0.10	2.28 +/- 0.16	2.41 +/- 0.12	2.41 +/- 0.08	2.41 +/- 0.09
Day 14	Left	1.62 +/- 0.19	1.54 +/- 0.06	1.61 +/- 0.11	1.72 +/- 0.09	1.52 +/- 0.05
	Right	2.38 +/- 0.21	2.53 +/- 0.22	2.43 +/- 0.18	2.41 +/- 0.09	2.22 +/- 0.08
Day 21	Left	0.53 +/- 0.12	1.11 +/- 0.16	0.80 +/- 0.06	0.66 +/- 0.08	0.34 +/- 0.11
	Right	1.56 +/- 0.32	1.42 +/- 0.19	1.82 +/- 0.19	1.26 +/- 0.13	0.93 +/- 0.12
	Percent inhibition[d]					
Day 21	Left		-10.90	-50.90	-23.80	35.50
	Right		9.00	-16.70	19.20	40.20
	Relative weight ratio of hind paws					

Relative change in volume[e]					
Left	0.700	-0.214	-0.178	-0.146	0.297
Right	1.142	0.802	0.021	0.381	0.950
Percent inhibition[d]					
Left		-30.6	-25.4	-20.9	42.4
Right		67.3	17.6	32.0	79.7

[a] Initial body weight, 170-185 g.

[b] Symbols: +/-, standard error; and the negative value (-, minus) means swelling.

[c] Adjuvant arthritis with 0.1 mg/kg H_2O x 13, day 0-12.

[d] Percent difference from aqueous adjuvant controls.

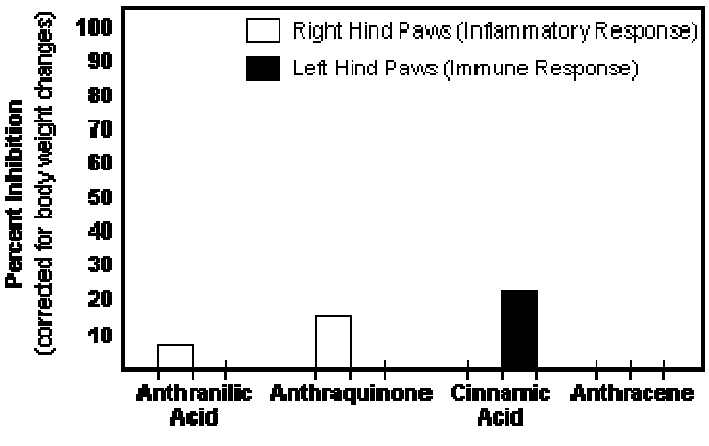
[e] $\frac{\text{Change in hind paw vol. aqueous adjuvant}}{\text{Change in body weight}} - \frac{\text{Change in test paw vol.}}{\text{Change in body weight}}$ = Relative change in volume.

Anthraquinone had the most preventive antiarthritic activity recorded of the three Aloe compounds tested. Anthraquinone inhibited inflammation 67.3%, which was the largest response next to anthranilic acid. Anthracene had no antiarthritic activity, but a 17.6% inhibition of inflammation was obtained in the inflammatory paws. This is about one third the effect seen with anthraquinone and anthranilic acid. A good positive anti-inflammatory response was also obtained with cinnamic acid (32.0%).

Regression of Arthritis.

Anthranilic acid had no antiarthritic activity in the regressive phase, unlike its ability to prevent the onset of the disease *(Table 2; Fig. 2)*. On the other hand, anthraquinone showed anti-inflammatory activity, but exhibited no anti-immune response in the regression phase. Cinnamic acid exhibited no anti-inflammatory effect, but a 17.3% regression of the immune response was recorded. This is somewhat opposite to the effect measured against prevention of arthritis. Since the anthraquinones show activity that helps to explain the overall response

Figure 2



Regression of adjuvant arthritis with anthraquinones.

seen with Aloe, future studies should evaluate other ingredients such as amino acids, enzymes, vitamins, and saponins.

Table 2

Regression of Adjuvant Arthritis with Anthraquinones in Rats[a,b]

	Protocol	Aqueous Adjuvant Control[c]	Anthraquinone	Anthracene	Cinnamic Acid	Anthranilic Acid
Treatment (mg/kg x 13)						
	Days 0 to 12		150	150	150	150
	Number of rats	12	12	12	12	12
	Final body weight (g)	333 +/- 12	315 +/- 8	320 +/- 14	329 +/- 16	336 +/- 9
Edema of hind paws (volume units +/- SE)						
Day 28	Left	1.67 +/- 0.13	1.71 +/- 0.14	1.80 +/- 0.12	1.62 +/- 0.09	1.67 +/- 0.05
	Right	3.07 +/- 0.78	2.61 +/- 0.25	3.38 +/- 0.26	3.13 +/- 0.30	3.00 +/- 0.29
Day 35	Left	1.76 +/- 0.14	1.84 +/- 0.18	1.89 +/- 0.12	1.74 +/- 0.11	1.77 +/- 0.11
	Right	3.41 +/- 0.27	3.00 +/- 0.25	3.69 +/- 0.29	3.91 +/- 0.44	3.52 +/- 0.32
Day 38	Left	0.65 +/- 0.09	0.80 +/- 0.10	0.87 +/- 0.36	0.52 +/- 0.06	1.53 +/- 0.12
	Right	1.63 +/- 0.15	1.26 +/- 0.13	1.96 +/- 0.16	1.82 +/- 0.17	1.65 +/- 0.19
Percent inhibition[d]						
Day 21	Left		-22.9	-34.4	-20.0	-135.0
	Right		22.7	-22.2	-11.7	-1.2
Relative weight ratio of hind paws Relative change in volume[e]						
	Left	-0.157	-0.193	0.072	-0.530	0.297
	Right	1.038	0.138	-0.323	-0.159	0.020
Percent inhibition[d]						
	Left		-37.0	-46.5	17.3	-127.7
	Right		13.3	-31.1	-15.3	1.9

[a] Initial body weight, 170-185 g.

[b] Symbols: +/-, standard error; and the negative value (-, minus) means swelling.

[c] Adjuvant arthritis with 0.1 mg/kg H_2O x 13, day 0-12.

[d] Percent difference from aqueous adjuvant controls.

[e] $\frac{\text{Change in hind paw vol. aqueous adjuvant}}{\text{Change in body weight}} - \frac{\text{Change in test paw vol.}}{\text{Change in body weight}}$ = Relative change in volume.

Summary

Anthraquinones found in Aloe may be responsible for the healing properties and anti-inflammatory activity recorded. This study has shown both anti-inflammatory and antiarthritic activity that can be improved by combining Aloe with ascorbic acid, thymus extract, and RNA. Since the chemical composition of Aloe holds a valuable key to its activity, the antiarthritic and anti-inflammatory activity of the anthraquinone complex in the adjuvant arthritis rat were tested. Anthraquinone and cinnamic acid exhibited anti-inflammatory activity in the prevention study. Anthranilic acid prevented inflammation as well as arthritis. Both anthraquinone and cinnamic acid exhibited activity in the regression phase. This work proves that the anthraquinone complex contributes to the healing properties of Aloe.