ELSEVIER

Journal of Ethnopharmacology 55 (1996) 69–75

Journal of ETHNO-PHARMACOLOGY

Antiinflammatory activity of extracts from *Aloe vera* gel

Beatriz Vázquez[a,*], Guillermo Avila[a], David Segura[a], Bruno Escalante[b]

[a]*Laboratories of Pharmacology and Natural Products, Division of Investigation, Escuela Nacional de Estudios Profesionales Iztacala (E.N.E.P-I), Universidad Nacional Autónoma de México, Tlalnepantla, México C.P. 54090, Mexico*

[b]*Department of Pharmacology, Centro de Investigación y Estudios Avanzados, Av. Instituto Politécnico Nacional 2508, México D.F. 07300, Mexico*

Received 26 March 1994; revised 19 August 1996; accepted 30 August 1996

Abstract

We studied the effects of aqueous, chloroform, and ethanol extracts of *Aloe vera* gel on carrageenan-induced edema in the rat paw, and neutrophil migration into the peritoneal cavity stimulated by carrageenan. We also studied the capacity of the aqueous extract to inhibit cyclooxygenase activity. The aqueous and chloroform extracts decreased the edema induced in the hind-paw and the number of neutrophils migrating into the peritoneal cavity, whereas the ethanol extract only decreased the number of neutrophils. The antiinflammatory agents indomethacin and dexamethasone also decreased carrageenan-induced edema and neutrophil migration. The aqueous extract inhibited prostaglandin E_2 production from [^{14}C]arachidonic acid. The chemical tests performed in the aqueous extract for anthraglycosides, reductor sugars and cardiotonic glycosides were positive. In the ethanol extract, the chemical tests performed for saponins, carbohydrates naftoquinones, sterols, triterpenoids and anthraquinones were also positive. In the chloroform extract, the chemical tests performed for sterols type Δ^5, and anthraquinones were positive. These results demonstrated that the extracts of *Aloe vera* gel have antiinflammatory activity and suggested its inhibitory action on the arachidonic acid pathway via cyclooxygenase.

Keywords: *Aloe vera*; Indomethacin; Dexamethasone; Inflammation; Cyclooxygenase

1. Introduction

Aloe vera Tourn. ex Linn (syn: *Aloe barbadensis* Miller) (Liliaceae) is used with other species of Aloe in the folk medicine of Mexico and several other countries, for many medical and cosmetic purposes (Morton, 1961; Diez-Martínez, 1981).

* Corresponding author. Laboratorios de Farmacología y Productos Naturales, División de Investigación, E.N.E.P. Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios s/n, Los Reyes Iztacala, Tlalnepantla, Estado de México C.P. 54090, México.

PII S0378-8741(96)01476-6

The fresh leaves of *Aloe vera* are used to obtain two components: (1) a bitter yellow juice (exudate) with high content of 1,8 dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloins), which are used for their cathartic effects (Fairbairn, 1980) and (2) a mucilaginous gel from the parenchymatous tissue, which has been used for topical treatment of skin burns and wounds (Rovatti and Brennan, 1959). Some chemical constituents with immunomodulatory and lectin-like properties have been isolated from the extracts of *Aloe vera* gel (Holdsworth, 1972; Winters et al., 1981). These reports have stimulated research on the chemical, biochemical and pharmacological properties of these compounds as well as on other medical uses (Grindlay and Reynolds, 1986). In spite of these data, there is no clear correlation between the pharmacological properties of the *Aloe vera* gel and its constituents.

The aim of this study was to investigate whether the extracts of *Aloe vera* gel showed antiinflammatory activity and to analyze some of its chemical constituents.

2. Materials and methods

2.1. Preparation of the gel

Fresh *Aloe vera* leaves were collected (14 kg) from the Municipality of Apaxco, State of Mexico, in March, 1990. The plant was identified in the herbarium of the Department of Botany of the Escuela Nacional de Estudios Profesionales Iztacala, U.N.A.M., where a specimen was deposited under accession number RBP 9052. The gel was harvested from the green leaves, minced, homogenized, dried at 35°C and weighed.

2.2. Preparation of extracts

The powder (1 kg) was fractionated successively with n-hexane, benzene, ethyl acetate, chloroform, acetone and 96% ethanol (Merck and Backer) in a Soxhlet extractor; to give the hexane (1.5 g), benzene (2.0 g), chloroform (12.0 g), ethyl acetate (90.0 g), acetone (150.0 g) and ethanol (282.0 g) the solvent was evaporated at low temperature, under reduced pressure, in a rotavapor. The dried extracts were freshly dissolved in propylene glycol or saline solution before administration.

The aqueous extract was prepared with 1 g powder in 20 ml of saline solution (0.45%), mixed for 20 min at 40°C and centrifuged at $100 \times g$ for 10 min; the supernatant was adjusted to pH 7.4, before administration and contained 40 mg/ml of extract material.

2.3. Animals

Male Wistar rats (180–200 g) grown in our animal house and fed on standard chow diet and water ad libitum were used for the experiments (groups of 5–8) at 20–25°C.

2.4. Antiinflammatory activity

2.4.1. Carrageenan-induced edema

The antiinflammatory activity of extracts was studied in groups of 6–8 rats. Edema was induced according to the method described by Van Arman et al. (1965). Briefly, 0.1 ml of 1% carrageenan (type II, Sigma, St Louis, MO) in sterile saline, was injected into one hind paw, under the plantar aponeurosis. A similar volume of saline solution was injected into the other hind paw. The paw volume was measured before injection of carrageenan or saline by the mercury displacement method (Van Arman et al., 1965) and the time course of edema formation was followed over 8 h. In separate groups of animals, either indomethacin (Sigma, St Louis, MO) 10 mg/kg or dexamethasone (Merck-Sharp and Dohme) 0.5 mg/kg were administered subcutaneously (s.c.) as standard drugs, saline solution and propylene glycol as solvent controls. Different amounts of the extracts or solvents were administered intraperitoneally (i.p.) 60 min before carrageenan injection. The volume increase (Δ volume) of the inflamed paw was estimated by subtracting the volume of the contralateral paw. The antiinflammatory effect of the drugs was evaluated as the degree of edema inhibition. Since the aqueous, chloroform and ethanol extracts showed more antiinflammatory effect than the other extracts, the following studies were done with these extracts.

2.4.2. Neutrophil migration into peritoneal cavity

Rats were injected with 3 ml of carrageenan (100 μg/ml prepared in sterile saline solution) into the peritoneal cavity, and 4 h later the abdominal cavity was washed with 10 ml of phosphate buffered saline solution containing 5 U/ml of heparin (Sigma, St Louis, MO) and 5% of bovine serum albumin. Only 5 ml were withdrawn for cell counts; the total cell counts were done in a Neubauer chamber and differential cell counts were performed by the technique reported by Souza and Ferreira (1985). The results are expressed as number of cells/ml of collected fluid. One hour before the carrageenan injection, the experimental groups were treated orally with 200 mg/kg of the chloroform extract, 400 mg/kg of the aqueous extract and 50, 200 and 600 mg/kg of the ethanol extract. The control groups were also given saline or propylene glycol by the oral route and the standard reference groups were treated subcutaneously with indomethacin (10 mg/kg) or dexamethasone (0.5 mg/kg).

2.4.3. Incubation of aqueous extracts with ram seminal vesicles (RSV) microsomes

A RSV microsomal powder was prepared as described previously by Laniado et al. (1989) and kept at −70°C. The incubation was made according to the method described, aqueous extract (1, 10 and 100 μg), 2 μl [^{14}C]arachidonic acid (specific activity 58 mCi/mmol) obtained from Amersham Corp., were incubated with the RSV cyclooxygenase preparation (500 μg of protein), in the presence or absence of glutation (GSH) 0.1 mM for 10 min. (Sigma, St Louis, MO). In control experiments, incubations were done in the presence of indomethacin (1 μg). Arachidonic acid metabolism was terminated by addition of 1 N citric acid to decrease pH to 4–5. Arachidonic acid metabolites were extracted with two volumes of ethyl acetate. The ethyl acetate extract was concentrated to dryness under nitrogen and resuspended in 300 μl of methanol and applied to Silica Gel G plates (Merck). The plates were developed in methanol/chloroform/acetic acid/water, 16:18:2:16 (v/v). For the separation of prostaglandins, the thin layer chromatography plate was examined by the autoradiographic method. Authentic prostaglandin standards were used to identify retention times of prostaglandin E_2. The concentration of the PGE_2 was estimated by densitometry after the autoradiography.

2.5. Phytochemical analysis

Chemical tests were done on the *Aloe vera* gel for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, coumarins, anthraquinones, saponins, sterols and triterpenes (Domínguez, 1973: Harborne, 1984). Thin layer chromatography of the extracts with biological activity was performed on silica gel 60 G plates (Merck), with ethyl acetate/methanol/water, 100:16.5:13.5 (v/v) or benzene/methanol 4:1 (v/v); the chromatograms were observed with an ultraviolet lamp at 254–300 nm, to detect the separated fractions which were marked and eluted from the plate. The chemical tests were performed on the eluted fractions.

2.6. Statistical analysis

Significant differences were assessed by Student's *t*-test for unpaired samples and values of $P < 0.05$ were considered significant.

3. Results

The edema induced by carrageenan in the hind paw lasted for more than 8 h; the maximum effect was seen at the 4th hour (Fig. 1). The aqueous and chloroform extracts showed antiinflammatory activity preventing the formation of edema after administration of carrageenan, i.e. carrageenan increased paw volume by 160 μl whereas carrageenan administered concomitant with aqueous or chloroform extracts increased paw volumes to 75 and 62 μl, respectively (Fig. 1). Moreover, the use of two well known antiinflammatory agents, indomethacin and dexamethasone, also prevented the carrageenan-induced increase in the paw volume of the rat (Fig. 1). The aqueous chloroform and ethanol extracts decreased the number of neutrophils migrating into the peritoneal cavity (Fig. 2). The aqueous extract decreased migration

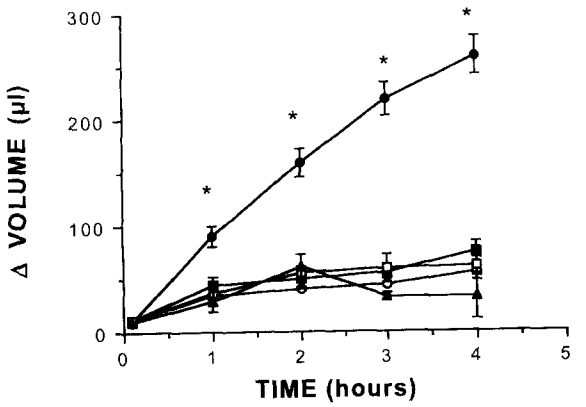

Fig. 1. Effects of aqueous and chloroform extracts of the *Aloe vera* gel on the edema induced by carrageenan (100 μg in 0.1 ml); administered intraplantarly, ●–● control group 0.1 ml (i.p.) propylene glycol; ○–○ 10 mg/kg indomethacin; ▲–▲ 0.5 mg/kg dexamethasone; ■–■ 100 mg/kg (i.p.) aqueous extract of *Aloe vera* gel; □–□ 100 mg/kg (i.p.) chloroform extract. Values are the mean ± SE for the number of animals used ($n = 7$). $P < 0.05$ when compared vs. treated groups.

by 28.6% and the chloroform extract by 42.9%. The ethanol extract decreased the average neutrophil migration in a dose-dependent fashion and the maximum effect was 48% and was obtained with 600 mg/kg (Fig. 3). When we used two well

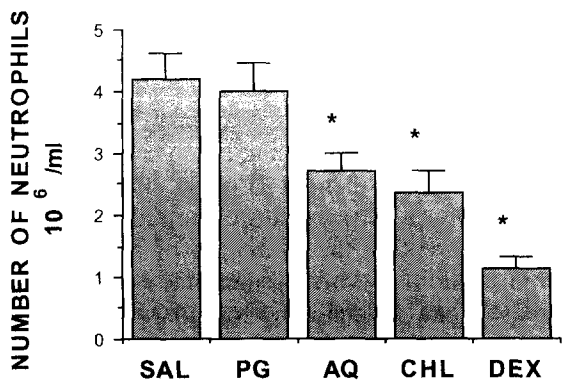

Fig. 2. Effects of 400 mg/kg (AQ) aqueous and 200 mg/kg chloroform extracts (CHL) of the *Aloe vera* gel, orally administered, on neutrophil migration into the peritoneal cavity induced by carrageenan (300 μg). Saline solution (SAL) and propylene glycol (PG) were used as vehicle control whereas dexamethasone (0.5 mg/kg s.c.) was used as standard anti-inflammatory. Values are the mean ± SE for the number of animals used ($n = 8$). $^*P < 0.05$ when compared vs. saline- or propylene glycol-treated groups.

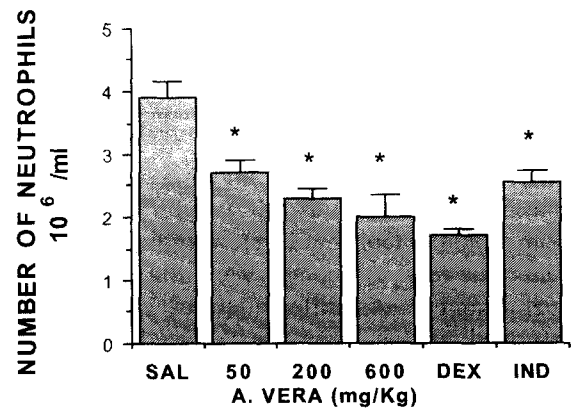

Fig. 3. Dose-dependent effect of ethanol extract of the *Aloe vera* gel on neutrophil migration. Increasing doses of *Aloe vera* (ethanol extract) were administered (v.o.) and compared with vehicle control (SAL), dexamethasone (DEX) (0.5 mg/kg, s.c.) or indomethacin (10 mg/kg, s.c.). Values are the mean ± SE for the number of animals used ($n = 6$). $^*P < 0.05$ when compared vs. saline-treated.

known antiinflammatory agents, dexamethasone and indomethacin, we observed that dexamethasone inhibited migration by 71.4% and indomethacin by 36%.

Incubation of the RSV with ^{14}C-AA, produced several radioactive metabolites. According to the retention time of authentic prostaglandins standard, the main metabolite was PGE_2, and incubation of the microsomes with indomethacin gave a significant decrease in the PGE_2 produced from 6.2% to 2.3% of arachidonic acid converted/mg protein/min. This suggested that induced arachidonic acid metabolism was mediated via cyclooxygenase products. Incubation with 100 mg of the *Aloe vera* aqueous extract also significantly decreased the PGE_2 production from 6.2% to 3.2% (Fig. 4).

The phytochemical analysis of the *Aloe vera* gel and its extracts revealed the presence of anthraquinones, sterols, sterols type Δ^5, saponins and carbohydrates (Table 1). The aqueous and ethanol extracts had a pH of 5. The aqueous extract showed high amounts of mucilagus components and pectins whereas in both extracts the anthraquinones were either free or bound to carbohydrates. The chloroform extract was only soluble in propylene glycol at 30°C.

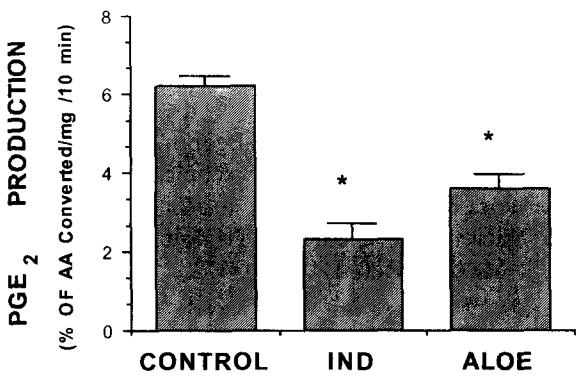

Fig. 4. In vitro inhibitory effect of *Aloe vera* extract on the production of PGE_2. RSV microsomes were incubated with [^{14}C]arachidonic acid and glutation in the absence of inhibitor (control) or in the presence of indomethacin 1 μg/ml (IND) or aqueous *Aloe vera* extract 100 μg/ml (Aloe). Each bar represents the mean ± SE of five experiments. $*P < 0.05$ when compared vs. control.

4. Discussion and conclusion

This study showed that the aqueous and chloroform extracts of the *Aloe vera* gel contain compounds with a potential to reduce carrageenan induced-edema. We found that at the doses used, *Aloe vera* inhibited edema formation, by a percentage close to the inhibition produced by the well established antiinflammatory agents, indomethacin and dexamethasone. Indomethacin, like most of the non-steroidal antiinflammatory compounds, inhibits the biosynthesis of prostaglandins and this effect might explain its antiinflammatory activity in carrageenan-induced rat paw edema (Higgs et al., 1979). On the other hand, dexamethasone is a steroidal antiinflammatory drug that inhibits phospholipase A_2 enzyme which is responsible for arachidonic acid liberation, the substrate for prostaglandin production (Blackwell et al., 1980). Thus, the antiinflammatory action of these agents may be related to the inhibition of prostaglandins and leukotriene synthesis. Further, the dose of dexamethasone used produced a higher inhibition of neutrophil migration than the effect produced by indomethacin, supporting the possible role of leukotrienes in the inflammation model.

Table 1
Chemical groups identified in the extracts with antiinflammatory activity of the *Aloe vera* gel

Extract	Chemical class
Aqueous	Anthraglycosides, reductor sugars, cardiotonic glycosides, mucilagus and pectins
Chloroform	Sterols type Δ^5 and anthraquinones
Ethanol	Carbohydrates, naftoquinones, anthraquinones, saponins, sterols and triterpenoids

The antiedema effect of these two extracts correlated with their ability to decrease the number of neutrophils migrating into the peritoneal cavity. The effect on formation and neutrophil migration has been suggested as some of the characteristics of the antiinflammatory agents whose mechanism of action is related to inhibitory action of the arachidonic acid pathway (Higgs et al., 1979). Our results clearly demonstrate that the aqueous extract inhibited in vitro conversion of arachidonic acid to PGE_2 suggesting the idea that the extract has cyclooxygenase inhibitory properties. Since we are using exogenous arachidonic acid, thereby bypassing the release of arachidonic acid by lipooxygenase activity and because the microsomes have high cyclooxygenase activity and negligible lipooxygenase activity, we could suggest that the antiinflammatory effect of *Aloe vera* is related to cyclooxygenase inhibition, rather than an effect on lipooxygenase activity. However, we demonstrated that dexamethasone in this model is an important antiinflammatory agent, which suggested that probably both arachidonic acid metabolite products, prostaglandins and leukotrienes are involved in the pathology of the inflammation. Thus the need to measure lipooxygenase metabolites on the exudate of the edema and the effect of *Aloe vera* on these products, become necessary to discard inhibition of the lipooxygenase products as one of the *Aloe vera* mechanisms of action. PGE_2 formation inhibition by the extract may account for the antiinflammatory effects since PGE_2 is the main prostaglandin in the inflammatory exudate (Ferreira et al., 1974). The ethanol extract did not show an antiedema effect but reduced the migration of neutrophils. These results suggest the ab-

sence in the ethanol extract of the compounds involved in the antiinflammatory effect.

A number of Japanese workers have found antiinflammatory compounds in *Aloe* species other than *Aloe vera*. Fujita et al. (1979) described in vitro bradykininase and carboxypeptidase activities in *Aloe arborescens* while Yagi et al. (1982) reported in vitro antibradykinin activity in *Aloe saponaria*; the faster breakdown of bradykinin might reduce pain and inflammation. We have also demonstrated this activity of *Aloe vera* gel in the isolated rat ileum (results not shown). Chemical tests on the *Aloe vera* gel and its extracts revealed the presence of components with low molecular weights (Table 1). In the aqueous and ethanol extracts, we identified glycosides of anthraquinones while in the chloroform extract, there was only the genin. We have suggested that the 1-2, 1-4, and 1-8, dihydroxyanthraquinones as well as the exudate from the leaves had no inhibitory effect in carrageenan-induced edema (results not shown). Analysis of the exudate showed 32% barbaloin and these results support our suggestion that anthraquinones do not have a role in the antiinflammatory process. Sterols identified in the ethanol and chloroform extracts had been reported by Suga and Hirata (1983) in *Aloe arborescens*. This suggests that *Aloe vera* sterols might have an antiinflammatory effect due to their structural similarity with the antiinflammatory steroids.

In summary, we have demonstrated that the aqueous, chloroform extracts of *Aloe vera* gel showed an inhibitory effect on carrageenan-induced edema and that the aqueous extract inhibited the production of PGE_2 in vitro. These effects suggest an inhibitory action on the arachidonic acid pathway via cyclooxygenase. Based on the results of this study, we came to the conclusion that the *Aloe vera* gel has potential antiinflammatory activity, and thus provides a scientific basis for the utilization of this plant in folk medicine for the treatment of inflammatory processes. Studies are in progress to elucidate the components responsible for the effects described.

Acknowledgements

The authors wish to thank Drs Francisco Posadas and José L. Reyes for their critical review of this manuscript; to Leonor Zúñiga and Gabriel Martínez for expert technical assistance. This work was supported by a grant from Consejo Nacional de Ciencia y Tecnología (CONACyT), México, Ref. D111-903672.

References

Blackwell, G.J., Carnuccio, R., DiRosa, M., Flower, R.J., Parente, L., and Persico, P. (1980) Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoid. *Nature* 287, 147–149.

Diez-Martínez, S.D. (1981) *La Zábila. Comunicado No. 46 Sobre Recursos Bióticos Potenciales del País*. Instituto Nacional De Investigaciones de Recursos Bióticos (INIREB), México.

Domínguez, X.A. (1973) *Métodos de Investigación Fitoquímica*. Limusa, México, pp. 281.

Fairbairn, J.W. (Ed.) (1980) Natural anthraquinone drugs. *Pharmacology* 20 (Suppl. 1), 2–122.

Ferreira, S.H., Moncada, S. and Vane, R.J. (1974) Prostaglandins and signs and symptoms of inflammation. In: H.J. Robinson and J.R. Vane (Eds.), *Prostaglandins Synthetase Inhibitors*. Raven Press, New York.

Fujita, K., Ito, S., Teradaira, R. and Beppec, H. (1979) Properties of a carboxypeptidase from aloe. *Biochemical Pharmacology* 28, 1261–1262.

Grindlay, D. and Reynolds, T. (1986) The *Aloe vera* phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *Journal of Ethnopharmacology* 16, 117–151.

Harborne, J.B. (1984) *Phytochemical Methods*. Chapman and Hall, New York.

Higgs, A.G., Flower, J.R. and Vane, R.J. (1979) A new approach to antiinflammatory drugs. *Biochemical Pharmacology* 28, 1959–1961.

Holdsworth, D.K. (1972) Chromosomes in *Aloe* species. Part II — Aloesone. *Planta Medica* 22, 54–58.

Laniado, M.S., Falck, J.R., Yadagiri, P. and Escalante, B. (1989) Metabolism of 20-hydroxyeicosatetraenoic acid by cyclooxygenase. *The Journal of Biological Chemistry* 264, 11658–11662.

Morton, J.F. (1961) Folk uses and commercial exploitation of Aloe leaf pulp. *Economic Botany* 15, 311–319.

Rovatti, B. and Brennan, R.J. (1959) Experimental thermal burns. *Industrial Medicine and Surgery* 28, 364–368.

Souza, G.E.P. and Ferreira, S.H. (1985) Blockade by antimacrophage serum of the migration of PMN neutrophils into the inflamed peritoneal cavity. *Agents and Actions* 17, 97–103.

Suga, T. and Hirata, T. (1983) The efficacy of the *Aloe* plant chemical constituents and biological activities. *Cosmetics and Toiletries* 98, 105–108.

Van Arman, C.G., Begany, A.J., Miller, L.M. and Pless, H.H. (1965) Some details of the inflammations caused by Yeast and Carrageenin. *Journal of Pharmacology and Experimental Therapeutics* 150, 328–333.

Winters, W.D., Benabides, R. and Clouse, W.J. (1981) Effects of Aloe extracts on human normal and tumour cells in vitro. *Economic Botany* 35, 89–95.

Yagi, A., Harada, N., Yamada, H., Iwadare, S. and Nishioka, I. (1982) Antibradykinin active material in *Aloe saponaria*. *Journal of Pharmaceutical Sciences* 71, 1172–1174.