Note

Efficacy of Dietary Aloe vera Supplementation on Hepatic Cholesterol and Oxidative Status in Aged Rats

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Summary In the current study, we show the anti-oxidative and hypocholesterol effects of aloe vera in the liver. Male specific pathogen-free (SPF) Fischer 344 rats were randomly assigned to one of four groups: Group A (control) was fed test chow without aloe supplementation; Group B was fed a diet containing a 1% (per weight basis) freeze-dried aloe filet; Group C was fed a diet containing a 1% (per weight basis) charcoal-processed, freeze-dried aloe filet; and Group D was fed a diet containing a charcoal-processed freeze-dried, whole leaf aloe (0.02% per weight basis) in the drinking water. Our results show that a life-long intake of aloe had superior anti-oxidative action against lipid peroxidation *in vivo*, as indicated by reduced levels of hepatic phosphatidylcholine hydroperoxide. Additional anti-oxidative action was evidenced by enhanced superoxide dismutase (SOD) and catalase activity in groups B and C. Furthermore, our study revealed that hepatic cholesterol significantly increased in the control group during aging in contrast to the aloe-supplemented groups, which showed approximately 30% lower cholesterol levels, thereby an effective hypocholesteremic efficacy. In this report, we suggest that life-long dietary aloe supplementation suppresses free radical-induced oxidative damage and age-related increases in hepatic cholesterol.

Key Words Aging, aloe vera, oxidative stress, phospholipid hydroperoxide, cholesterol

Aloe vera has been used for health, medical, and cosmetic purposes for many centuries (1–3). Currently, aloe vera is a popular skin emollient and is used as an aid in the treatment of skin injuries and burns (4). Literature also shows that the ingestion of aloe was implicated in the amelioration of several age-related diseases, e.g., diabetes (5, 6) and hypertension (6). Herlihy et al. (7) reported in their comprehensive study that the life-long ingestion of aloe at moderated levels (1%) causes no apparent adverse effects in the rat. More recently, Ikeno et al. (8) showed that the life-long dietary supplementation of aloe suppresses many age-related disease processes in rats.

Aloe's anti-inflammatory property has been documented in various types of inflammation (9). Aloe vera has been tested for its anti-inflammation action and its suppression of damaging free radicals and other reactive oxidative species (ROS) (10). Aloe was found to inhibit the release of ROS from polymorphonuclear leukocytes (11–13) and suppress the oxidation of arachidonic acid (14). Additional evidence supporting the

anti-oxidative action of ingested aloe came from data obtained on decreased lipid peroxide concentrations in young rats (15). These findings of the antioxidative action of aloe were recently documented by the isolation and characterization of a potent antioxidant from aloe leaves (16).

Aging processes are gradual and progressive time-dependent changes that compromise an organism's ability to meet both internal and external disruptions. A good example of such disruptions is the age-related oxidative stress caused by both the endogenous and exogenous generation of free radicals to exacerbate tissue injury (17). To prevent such damage, endogenous primary defense scavenging enzymes exert a protective action as exemplified by superoxide dismutase (SOD), which catalyzes the dismutation of superoxide, and catalase (CAT) that hydrolyzes hydrogen peroxide formed from the dismutation reaction.

In the present study, we studied the effects of long-term aloe supplementation on the protective action against lipid damage and age-related increases in the hepatic cholesterol of aging rats. To assess age-related lipid peroxidation, we determined phosphatidylcholine

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hydroperoxide (PCOOH) as a sensitive, reliable indicator of oxidative lipid injury using CL-HPLC, which is developed to monitor active ROS activity (18, 19).

The aloe vera additions we used in this study were prepared under the careful supervision of Aloecorp (Harlingen, TX, USA). Detailed preparation procedures of the aloe vera samples were previously described (8, 15). Briefly, aloe vera filets were obtained by skinning the leaf, with care taken to avoid contamination of the gel from the outer layers. The filets were dried, blended, and immediately freeze-dried. The product was then finely ground and stored at -20°C until used. For the charcoal-processed preparations, the whole leaf was subjected to routine procedures for commercial preparation, which included charcoal-filtration. The filtrate was freeze-dried, and care was taken to avoid the development of rancidity and possible breakdown of the aloe vera ingredients. Frozen aliquots of the freeze-dried products were shipped to Purina Test Diet Mills (St. Louis, MO, USA) for mixing and preparation of the test diets. The test diets were mixed every two months and air-shipped to the University of Texas Health Science Center. With the exception of air shipment, the diets were kept at -20°C at all times until fed to the rats.

The specific pathogen-free (SPF) male Fischer 344 rats used in this study were purchased as weanlings from Charles River Laboratories (Kingston, NY, USA). Upon arrival by air, the rats were housed in a SPF barrier facility where they were individually housed in plastic cages with wire mesh floors. The SPF status of the rats was maintained throughout their life. The standard operation procedures for the barrier and rat maintenance were described previously (20).

At six weeks of age, rats were randomly assigned to one of four groups: Group A (control) was fed test chow without aloe supplementation; Group B was fed a diet containing a 1% (per weight basis) freeze-dried aloe filet; Group C was fed a diet containing a 1% (per weight basis) charcoal-processed, freeze-dried aloe filet; and Group D was fed a diet containing charcoal-processed freeze-dried, whole leaf aloe (0.02% per weight basis) in drinking water.

The food intake of each rat in every group was measured as described previously (20). For Group D, the drinking water was changed every three days, and replaced with freshly acidified water to which charcoal-processed aloe vera was added. At 4 and 16 mo of age, rats from each group were anaesthetized using methoxyflurane. Major organs were excised, weighed, and prepared for biochemical analyses. A recent publication gives a complete pathological analysis of these rats (8).

Total lipids were extracted from the liver by the method of Folch et al. (21). The CL-HPLC procedure for the quantification of phosphatidylcholine hydroperoxide (PCOOH) concentration was done by the method of Miyazawa et al. (22). Standards for PCOOH were prepared by oxidation of phosphatidylcholine using a method of Terao et al. (23).

Total superoxide dismutase (SOD) activity was determined by a method previously described (24) (i.e., mon-

itoring the inhibition of cytochrome c reduction at 550 nm using the xantine and xantine oxidase system). One SOD unit was defined as the amount of enzyme that inhibited reduction of cytochrome c by 50%. Liver catalase activity was determined spectrophotometrically by measuring the decomposition of hydrogen peroxide at 240 nm. One catalase unit is defined as the amount of enzyme required to decompose $1.0\text{ }\mu\text{mol}$ of H_2O_2 per min, pH 7.0, at 25°C (25, 26).

Liver total lipids were extracted and purified by the Folch method (21), and cholesterol was measured as described previously (27, 28). Briefly, total lipid was extracted using acetone-absolute ethanol (1:1), and the alkali was neutralized with 10% acetic acid. To 2 mL of the digitonin solution were added. After overnight standing at room temperature, the amount of isolated cholesterol was estimated using the Sperry and Merrill method (29).

Differences among the mean of the individual groups were assessed by one-way ANOVA with Duncan's multiple range test (SPSS 7.5, SPSS Institute, USA). Differences of $p < 0.05$ were considered significant (30).

We determined that PCOOH is a sensitive, key indicator for oxidative injury because phospholipids are important structural and functional components of the biological system and are commonly recognized as a major target of lipid peroxidation. Thus, to determine the inhibitory effect of aloe on lipid peroxidation, the amounts of PCOOH formed in rats at 4 and 16 mo were examined. PCOOH levels were 2 times higher in the 16-mo-old rats than in the 4-mo-old rats, indicating an age-related change (Table 1). The aloe-fed rats in groups B, C, and D showed significantly reduced PCOOH amounts at 4 mo as compared to the control rats, Group A.

The anti-oxidative effect of aloe feeding was manifested in two major scavenging enzymes, namely, SOD and catalase. In the case of SOD activity, while rats in Group A showed reduced SOD activity levels with age, the aloe-fed rats in groups B, C, and D showed increased levels with age (Table 1). In particular, SOD levels were significantly higher in Group C rats at 4 mo of age.

In the case of catalase activity, Group C rats showed the highest increase among the aloe-fed groups at 4 mo of age, and this trend continued to 16 mo of age.

The suppressive effect of aloe on hepatic cholesterol was not observed at the age of 4 mo, indicating that 2.5 mo-long aloe feeding exerts little effect. However, aloe vera supplementation was shown to significantly lower cholesterol levels at the age of 16 mo (Fig. 1). This finding indicates that aloe vera supplementation has an effective suppressive action against age-related hypercholesterolemia.

Aloe vera dietary supplementation has long been claimed to exert beneficial effects on a wide variety of human diseases (1, 2, 6, 31). In the current study, we examined the anti-oxidative effects of the life-long feeding of aloe vera on age-related lipid peroxidation. Our rationale to study aloe is based on several criteria: 1) our continuous interest in the anti-oxidative compounds of

Table 1. Effects of aloe vera on the amount of phosphatidylcholine hydroperoxide (PCOOH), catalase, and superoxide dismutase (SOD) in the liver of rat between ages 4 and 16 mo.

Groups/Oxidative level	A	B	C	D
PCOOH (pmol/mg protein)				
4 mo	14.4±1.3 ^c	9.8±0.9 ^b	9.3±2.1 ^b	3.2±0.5 ^a
16 mo	30.3±3.8 ^c	19.9±2.8 ^b	3.2±0.5 ^a	17.6±2.4 ^b
Catalase (IU/mg protein)				
4 mo	78.1±4.3 ^a	81.5±5.6 ^a	109.4±4.4 ^b	81.9±6.3 ^a
16 mo	54.6±3.0 ^a	71.4±4.8 ^b	84.0±5.2 ^b	53.9±5.2 ^a
SOD (IU/mg protein)				
4 mo	1.6±0.1 ^a	1.9±0.1 ^a	2.3±0.1 ^b	1.6±0.1 ^a
16 mo	1.2±0.1 ^a	1.8±0.1 ^b	1.9±0.2 ^b	1.6±0.1 ^b

Each value represents the mean±SEM of five rats. ^{a-c}Mean values with different superscripts are significantly different in the same age group (*p*<0.05). Group A (control) was fed test chow without aloe supplementation; Group B was fed a diet containing 1% (on a dry weight basis) freeze-dried aloe fillet; Group C was fed a diet containing a 1% (per weight basis) charcoal-processed, freeze-dried aloe fillet; and Group D was given charcoal-processed freeze-dried, whole leaf Aloe vera (0.02% per weight basis) in drinking water.

Fig. 1. Effects of aloe feeding on total cholesterol. Each bar represents the mean±SEM of five rats. a, b: Mean values with different superscripts are significantly different in the same age group (*p*<0.05). Group A (control) was fed test chow without aloe supplementation; Group B was fed a diet containing a 1% (per weight basis) freeze-dried aloe fillet; Group C was fed a diet containing a 1% (per weight basis) charcoal-processed, freeze-dried aloe fillet; and Group D was fed a diet containing charcoal-processed freeze-dried, whole leaf aloe (0.02% per weight basis) in drinking water.

natural products, for example, our previous findings of their protective value against membrane phospholipid peroxidation (18, 32–36); 2) previous studies showing the potential anti-oxidative property of aloe (7, 15, 16); and 3) evidence of the putative cholesterol-lowering effect by aloe ingestion in humans with heart disease (6). Our current study attempted to document the anti-oxidative effect of life-long aloe ingestion on a biological system. In addition, we examined the anti-oxidative activities of various aloe preparations processed by different methods.

Our current data clearly demonstrated that dietary aloe supplementation decreases age-related oxidative stress, as evidenced by the reduced phosphatidylcholine hydroperoxide levels in both age groups (Table 1). Our findings are in agreement with two previous reports showing the suppression action of aloe against age-

related oxidative stress. This observation is significant in view of the putative anti-inflammatory property of aloe when administered in vitro (14, 31–33), when topically applied (10), and when ingested (37). Because inflammation processes involve the mediation of ROS and pro-inflammatory prostaglandins, the anti-oxidative action we observed in the current study may be the underlying mechanism for aloe’s claimed anti-inflammatory actions.

Additional evidence showing the anti-oxidative action of aloe was in its ability to enhance two major free-radical scavenger enzymes, namely SOD and catalase. Oxidative stress is known to weaken defense systems during aging, including these two enzymes (38). Our current results show that aloe feeding enhanced catalase in groups B and C in 16-mo-old rats (Table 1), suggesting the possible maintenance of an anti-stress strategy against age-related oxidative insults. Such a possibility was shown by the reduced amount of lipid hydroperoxides (Table 1).

The cholesterol concentration was shown to increase with age in rat liver (Fig. 1). At 16 mo of age, the concentration was significantly lower in the aloe-supplemented groups as compared to the control group. The decrease in liver cholesterol observed in this study is consistent with the previous findings of our laboratory (7, 15), where reduced serum cholesterol was found in rats fed aloe for 7 mo. Our current results from 16-mo-long feeding data are much clearer and more convincing.

Our research demonstrated that the life-long ingestion of aloe exerts substantial benefits by attenuating the lipid peroxidation of phospholipids, lowering liver cholesterol, and enhancing antioxidant enzyme activity during aging. We concluded that aloe vera protects against age-related increases in oxidative stress in vivo. It is speculated that, at present, some active antioxidants in aloe as reported by Lee et al. (16), are responsible for its anti-oxidative action. We further suggest the possibility that aloe vera can be potentially beneficial

against oxidative injury to the liver. Future investigations on the exploration of the anti-oxidative effects of aloe ingestion on aging and the modulation of anti-inflammatory processes and wound healing at the cellular and molecular levels is warranted.

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