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The Influence of Long-term Aloe Supplement on Anti-oxidative Defenses and Cholesterol Content in Brain and Kidney of Aged Rats

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ABSTRACT : **The present study was investigated the anti-oxidative effects of aloe vera ingestion on brain and kidney in aged rats by monitoring several oxidative-related parameters. Male specific pathogen-free Fischer 344 rats were randomly divided into four groups of five rat each: Group A was fed test chow without aloe supplementation; Group B was fed a diet containing a 1% freeze-dried aloe filet; Group C was fed a diet containing a 1% charcoal-processed, freeze-dried aloe filet; and Group D was fed a diet containing a charcoal-processed, freeze-dried, whole leaf aloe in drinking water. Analyses of tissues were done at 4 months and 16 months of age. Results showed that a long-term intake of aloe, regardless of the preparation used, enhanced antioxidant defenses against lipid peroxidation, as indicated by reduced phosphatidylcholine hydroperoxide levels in both brain and kidney. The additional benefit of aloe intake on the anti-oxidative action was evidenced by enhanced superoxide dismutase and catalase activity in all aloe-ingested groups. Another beneficial effect of aloe shown in this study, although not an anti-oxidative parameter, was its cholesterol-lowering effect as detected in brain and kidney with significant decreases at age16 months of aloe-fed rats. Based on these findings, we conclude that a long-term dietary aloe supplementation modulated the anti-oxidative defense systems and cholesterol level.**

Key words : Aging; aloe vera, oxidative stress, brain, kidney, cholesterol

INTRODUCTION

Aloe Vera has been used for health, medical and cosmetic purposes for centuries (Haller, 1990). In addition, aloe has become more popular as a skin emollient and as an aid in the treatment of skin disorders and in burn therapy (Davis, 1997). Scientific research also shows that the ingestion of aloe seems to have beneficial efficacy for the amelioration of age-related diabetes (Ghannam *et al.*, 1986) and hypertension (Agarwal, 1985). Herlihy *et al.* (1998a) reported in their comprehensive study that the life-long ingestion of several aloe propagations at moderated levels (1%) causes no apparent adverse effects in rats. Ikeno *et al.* (2002) reported pathological data showing that the life-long dietary supplementation of aloe suppresses many age-related disease processes in rats.

Another a well-known effect of aloe is its anti-inflammatory property related to skin and wound healing (Davis *et al.*, 1989). In addition to its anti-inflammation action, aloe vera also has been tested for its protection against free radical damage and other reactive oxidative species (ROS) (Hutter *et al.*, 1996). 't Hart *et al.* (1988; 1989; 1990) reported in several papers that aloe inhibits the release of ROS from polymorphonuclear leukocytes and that it suppresses the oxidation of arachidonic acid (Penneys, 1982). Additional evidence supporting the anti-oxidative action of ingested aloe came from data obtained on decreased lipid peroxide levels in rats (Herlihy *et al.* 1998b). Evidence of aloe's anti-oxidative property may related to an effective phenolic antioxidant that was isolated from aloe leaves (Lee *et al.* 2000).

Aging processes are gradual and progressive time-dependent changes that compromise an organism's ability to meet both internal and external stresses. A good example of such challenges is the age-related oxidative stress caused by the both endogenous and exogenous generation of free radicals that exacerbate tissue injury (Yu, 1994). 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

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(CAT), which hydrolyzes hydrogen peroxide formed from the dismutation reaction.

In the present study, we studied the anti-oxidative effects of long-term aloe supplementation in its ability to protect lipid damage in brain and kidney of aging rats. To assess age-related lipid peroxidation, we determined phosphatidylcholine hydroperoxide (PCOOH) as a sensitive, key indicator of oxidative lipid injury using CL-HPLC, which was developed to monitor active oxygen-derived radical activity (Choi *et al.* 1998; 2000). 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 (Ikeno *et al.*, 2002; Herlihy *et al.* 1998a).

MATERIAL AND METHODS

Animal treatment

The specific-pathogen free (SPF) male Fischer 344 rats used this study were purchased as weanlings from Charles River Laboratories (Kingston, NY, USA). The standards operation procedures for the barrier and rat maintenance were described previously (Yu *et al.*, 1982). At six weeks of age, rats were randomly assigned to one of four groups of five rat each: 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 the drinking water. The food intake of each rat in every group was measured as described previously (Yu *et al.*, 1982). A recent publication gives a complete pathological analysis of these rats (Ikeno *et al.*, 2002).

Total lipids and anti-oxidant activity measurement

Total lipids were extracted from the brain and kidney by the method of Folch *et al* (1957). The CL-HPLC procedure for the quantification of phosphatidylcholine hydroperoxide (PCOOH) concentration was done by the method of Miyazawa *et al* (1992). Standards for PCOOH were prepared by oxidation of phosphatidylcholine using a method of Terao *et al.* (1994). Total superoxide dismutase (SOD) activity was determined by the method previously described (Lee *et al.* 1990). Brain and kidney catalase activity was determined spectrophotometrically by measuring the decomposition of hydrogen peroxide at 240 nm (UVIKON XS, Secomam) (Aebi, 1984). Brain and kidney total lipids were extracted and purified by the Folch method (1957), and cholesterol was measured as described previously (Gu *et al.* 1995).

Table 1. Effect of aloe vera on the amount of phosphatydilcholine hydroperoxide (PCOOH), catalase, and superoxide dismutase (SOD) in the brain and kidney of rat at ages 4 and 16 months

Group/Oxidative level	A	B	C	D
Brain				
PCOOH (pmol/mg protein)				
4 mo	6.0 ± 0.7^{ab}	4.2 ± 0.5^{ab}	2.8 ± 0.5^{a}	1.4 ± 0.2^{a}
16 Mo	16.5 ± 3.7^{c}	8.9 ± 2.1^{b}	9.2 ± 1.0^{b}	6.2 ± 1.3^{ab}
Catalase (IU/mg protein)				
4 mo	34.4 ± 2.6^{ab}	54.4 ± 2.9^{c}	53.8 ± 4.3^{c}	41.1 ± 4.1^{b}
16 Mo	27.6 ± 2.8^{a}	40.1 ± 3.0^{b}	41.6 ± 3.1^{b}	38.4 ± 4.3^{b}
SOD (IU/mg protein)				
4 mo	0.7 ± 0.1^{a}	1.5 ± 0.1^{b}	1.4 ± 0.1^{b}	1.0 ± 0.1^{b}
16 Mo	0.7 ± 0.1^{a}	1.9 ± 0.1^{b}	1.5 ± 0.1^{b}	1.4 ± 0.1^{b}
Kidney				
PCOOH (pmol/mg protein)				
4 mo	8.0 ± 0.6^{cd}	7.4 ± 0.8^{c}	7.2 ± 0.2^{bc}	6.8 ± 0.1^{bc}
16 Mo	8.9 ± 0.5^{d}	6.7 ± 0.3^{bc}	5.9 ± 0.5^{b}	3.5 ± 0.3^{a}
Catalase(IU/mg protein)				
4 mo	4.7 ± 0.4^{a}	7.1 ± 1.0^{b}	7.7 ± 0.9^{bc}	7.0 ± 0.1^{b}
16 Mo	4.4 ± 0.5^{a}	7.2 ± 0.9^{bc}	7.9 ± 1.0^{bc}	6.3 ± 1.0^{b}
SOD (IU/mg protein)				
4 mo	0.8 ± 0.1^{a}	1.9 ± 0.1^{b}	1.7 ± 0.1^{b}	2.0 ± 0.1^{b}
16 Mo	0.4 ± 0.1^{a}	2.0 ± 0.1^{b}	2.2 ± 0.1^{b}	1.6 ± 0.1^{b}

Each value represents the mean ± SEM of five rats. $^{a\text{-}c}$ Means 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 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 the drinking water.

Statistical analysis

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 (Duncan, 1955).

RESULTS AND DISCUSSION

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 months were measured. PCOOH levels in brain were about 2 times higher in the 16 month-old rats than in the 4-month-old rats, indicating an age-related change (Table 1). The aloe-fed rats in Groups B, C, and D showed mostly reduced PCOOH amounts at 4 months and 16 months as compared to the control rats, Group A.

The anti-oxidative effect of aloe feeding was manifested by 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, and C showed increased levels with age (Table 1). In particular, SOD levels were significantly higher in Group C rats at 16 months of age. In the case of catalase activity, Group C rats showed the highest increase among the aloe-fed groups at 4 months of age, and this trend continued to 16 months of age.

In brain MDA activity, aloe vera supplementation was shown to significantly lower MDA levels at age 4 and 16 months (Table 2). This finding indicates that aloe vera supplementation has an effective suppressive action against age-related lipid peroxidation. However, the suppressive effect of aloe on kidney MDA activity was not observed at the age of 4 months.

Ingestion of aloe vera has long been thought to exert its beneficial effects on a wide variety of human diseases (Haller, 1990; Agarwal, 1985; Klein, 1988). In the current study, we examined the anti-oxidative effects of life-long feeding of aole vera on age-related lipid peroxidation. Our rationale to study aloe is based on several reasons: 1) our long interest in the anti-oxidative compounds of natural products that protect against membrane phospholipid peroxidation as we found in our previous studies (Lim *et al.* 1998a; 1998b; 1999); 2) previous studies by others showing the potential anti-oxidative property of aloe (Herlihy *et al.* 1998a; Lee *et al.*, 2000); and 3) a search for a natural product with the putative cholesterol-lowering efficacy of aloe ingestion in humans with heart disease (Agarwal, 1985). Our current data clearly demonstrated that dietary aloe supplementation decreases age-related oxidative stress, as evidenced by the reduced PCOOH levels in both age groups (Table 1). This observation is significant in view of the putative anti-inflammatory property of aloe when administered *in vitro* (Pennys, 1982), when topically applied (Hutter, 1996) and when ingested (Saito, 1982). Because inflammation processes involve the mediation of free radicals and pro-inflammatory prostaglandins, the anti-oxidative action we observed in the current study may well be the underlying mechanism of 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.(Yu, 1996) Our current results showed that aloe feeding enhances catalase in Groups B, C and D in 16-month-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, shown in Table 2.

The cholesterol concentration was shown to increase with

Table 2. Effect of aloe vera feeding on the amount of MDA in the brain and kidney of rat at ages 4 and 16 months

Group / MDA level	A	B	C	D
Brain				
TC (nmol/mg protein)				
4 mo	1.8 ± 0.2^{a}	0.9 ± 0.1^{bc}	1.3 ± 0.2^{b}	1.4 ± 0.1^{b}
16 Mo	1.8 ± 0.3^{a}	1.4 ± 0.2^{b}	1.3 ± 0.3^{ab}	1.4 ± 0.2^{ab}
Kidney				
TC (nmol/mg protein)				
4 mo	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.2^{a}	0.9 ± 0.2^{ab}
16 Mo	1.6 ± 0.2^{a}	1.3 ± 0.1^{b}	1.3 ± 0.1^{b}	1.2 ± 0.1^{b}

Each value represents the mean ± SEM of five rats. $^{a\text{-}c}$ Means 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 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 the drinking water.

Table 3. Effect of aloe vera feeding on the amount of total cholesterol in the brain and kidney of rat at ages 4 and 16 months

Group / TC level	A	B	C	D
Brain				
TC (nmol/mg protein)				
4 mo	128.1 ± 4.4 [a]	121.8 ± 5.3 [a]	131.9 ± 10.8 [ab]	123.9 ± 6.2 [a]
16 Mo	164.6 ± 4.5 [a]	125.4 ± 6.7 [b]	130.2 ± 11.3 [b]	128.4 ± 8.8 [b]
Kidney				
TC (nmol/mg protein)				
4 mo	56.5 ± 7.2 [a]	49.5 ± 4.2 [ab]	54.5 ± 4.8 [a]	53.1 ± 1.7 [a]
16 Mo	70.3 ± 5.2 [a]	56.0 ± 3.1 [b]	65.1 ± 1.5 [bc]	65.9 ± 2.8 [bc]

Each value represents the mean ± SEM of five rats. a-c Means 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 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 the drinking water.

age in rat brain and kidney (Table 3). At 16 months of age, the concentration was significantly lower in the aloe-supplemented groups compared to the control group. The decrease in liver cholesterol observed in this current study is consistent with previous findings of our laboratory (Herlihy, 1998), where reduced serum cholesterol was found in rats fed aloe for 7 months. Our current results from 16-month-long feeding data are much clearer and more convincing.

This research demonstrated that the life-long ingestion of aloe exerts substantial benefits by attenuating the lipid peroxidation of phospholipids and MDA, lowering brain and kidney cholesterol, and enhancing antioxidant enzyme activity during aging. We suggested that aloe vera protects against age-related increases in oxidative stress *in vivo*. Some active antioxidants in aloe are considered responsible for its anti-oxidative action, as reported by Lee *et al.* (2000)

We further suggest the possibility that potentially aloe vera can be beneficially protective against oxidative injury to the brain and kidney. Future investigations are warranted for the exploration of the anti-oxidative effects of aloe ingestion during aging and for its modulation of anti-inflammatory processes and wound healing at cellular and molecular levels.

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LITERATURE CITED

Aebi H (1984) Catalase in Vitro. In: Colowick, S.P., and Kaplan, N.O. (Eds.), Methods in Enzymology. p. 121-126.

Agarwal OP (1985) Prevention of atheromatous heart disease. *Angiology*, 36:485-492.

Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH, Park DK (2000) Application of flow injection-chemiluminescene to the study of radical scavenging activity in plants. Phytotheraphy Res 14:250-253.

Choi HY, Song JH, Park DK (1998) A combined flow injection-chemiluminescent method for the measurement of radical scavenging activity. Anal. Biochem 264:291-293.

Davis RH (1997) Aloe Vera: Scientific Approach. Vantage Press, New York.

Davis RH, Leitner MG, Russo JM, Byrne ME (1989) Wound healing. Oral and topical activity of aloe vera. J. Am. Podiatry Med. Assoc 79:559-562.

Duncan DB (1955) Multiple range and multiple F test. Biometrics 11:1-42.

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem 226:497-509.

Ghannam N, Kingston M, Al-Meshaal IA, Tariq M, Parman NS, Woodhouse N. (1986) The antidiabetic activity of aloes: preliminary clinical and experimental observations. Hormone Res 24:288-294.

Gu JY, Wakizono Y, Tsujita A, Lim BO, Nonaka M, Yamada K, Sugano M (1995) Effects of sesamin and α-tocopherol, individually or in combination, on the polyunsaturated fatty acid metabolism, chemical mediator production, and immunoglobulin levels in Sprague-Dawley rats. Biosci. Biotech. Biochem 59:2198-2202.

Haller Jr JS (1990) A drug for all seasons. Medical and Pharmacological history of aloe. Bull. N. Y. Acad. Med 66:647-659.

Herlihy JT, Bertrand HH, Kim JD, Ikeno Y, Yu BP (1998) Effects of aloe vera ingestion in the rat. I. Growth, food and fluid intake and serum chemistry. Phytotheraphy Res 12:183-188.

Herlihy JT, Kim JD, Kalu DN, Nelson JF, Ward WF, Ikeno Y, Yu BP (1998) Effects of aloe vera ingestion in the rat. II. Hormonal and metabolic characteristics. Phytotheraphy Res 12:355-360.

Hutter JA, Salman M, Stavinoha WB, Satsangi N, Willims RF, Streeper RT, Weintrub ST (1996) Antiinflammatory C-glucosyl chromone from Aloe barbardensis. J. Nat. Prod

59:541-543.

Ikeno Y, Hubbard GB, Lee MS, Yu BP, Herlihy JT (2002) The influence of long term aloe ingestion on age-related disease in male Fischer 344 rats. Phytotherapy Res 16:712-718.

Klein AD, Pennys NS (1988) Aloe vera. J. Am. Acad. Dermatol 18:714-720.

Lee DW, Yu BP (1990) Modulation of free radicals and superoxide dismutase by age and dietary restriction. Aging Clin. Exp. Res 2:357-362.

Lee KY, Weintrub S, Yu BP (2000) Isolation and identification of a phenolic antioxidant from Aloe barbadensis. Free Rad. Biol. Med 28:261-265.

Lim BO, Yu BP, Cho SI, Her E, Park DK (1998) The inhibition by quercetin and Ganhuangenin on oxidatively modified low-density lipoprotein. Phytotherapy Res 12:340-345.

Lim BO, Yu BP, Kim SC, Park DK (1999) The antioxidative effect of ganhuangenin against lipid peroxdiation. Phytotherapy Res 13:479-483.

Lim BO, Yu BP, Oh JH, Park DK (1988) The inhibitory effects of ginsenoside and quercetin on oxidatively damage by puromycin aminonucleoside in rat. Phytotherapy Res 12:375-377

Miyazawa T, Suzuki T, Fujimoto K, Yasuda K (1992) Chemiluminesecent simultaneous determination of phosphatidylcholine hydroperoxide and phosphatidylethanolamine hydroperoxide in the liver and brain of the rat. J. Lipid Res 33:1051-1059.

Penneys NS (1982) Inhibition of arachidonic acid oxidation in vitro by vehicle components. Acta Dermato-Venerelogica 62:59-61.

Saito H, Ishiguro T, Imanishi K, Suzuki I (1982) Pharmacological studies on a plant lectin aloctin A. II. Inhibitory effect of aloctin A on experimental models of inflammation in rats. Jpn J Pharmacol 32:139-142.

Terao J, Piskula M, Yao Q (1994) Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch. Biochem. Biophys 308:278-284.

't Hart LA, Nibbering PH, Labadie RP (1990) Effects of low molecular constituents from Aloe vera gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. Int. J. Immunopharmacol 12:427-434.

't Hart LA, van den Berg AJ, Kuis L, van Dijk H, Labadie RP (1989) An anti-complementary polysaccharide with immunological adjuvant activity from the leaf parenchyma gel of aloe vera. Planta Med 55:509-512.

't Hart LA, van Enckevort PH, van Dijk H, Zaat R, de Silvia, KT, Labadie RP (1988) Two functionally and chemically distinct immunomodulatory compounds in the gel of aloe vera. J. Ethnopharmacol 23:61-71.

Yu BP (1994) Cellular defenses against damage from reactive oxygen species. Physiol. Rev 74:139-162.

Yu BP (1996) Aging and oxidative stress: modulation by dietary restriction. Free Radic. Biol. Med 21:651-668.

Yu BP, Masoro EJ, Murata I, Bertrand BA, Lynd FT (1982) Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and diseases. J. Gerontol 37:130-141.