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Antimutagenic and Antileukemic Activities of *Aloe vera* L.

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Abstract – We investigated that the extract of *Aloe vera* L. and its fractions exert antimutagenic activity against *Salmonella typhimurium* TA98 and TA100, and antileukemic effect against K562 human leukemia cell line. The aqueous ethanolic extract of *A. vera* L. was revealed to have antimutagenic effect on the AF-2 (2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide) in Salmonella mutation assay. Among the three fractions (fractions A, B and C) separated by silica gel chromatography, fraction C (50 μg/plate) exhibited the greatest antimutagenic effect on the AF-2 with inhibition rate of 84 and 90% in *Salmonella typhimurium* TA98 and TA100, respectively. The fraction C (500 μg/ml) inhibited the growth of K562 human leukemia cell line by 93% in MTT assay. However, the components of *A. vera* L. did not exhibit cytotoxic effect against MDBK bovine normal kidney in MTT assay.
Key words – Antimutagenic activity, antileukemic effect, *Aloe vera* L.

Introduction

Although 600 species of the aloe genus are known, only 5 species (*Aloe vera* L., *A. ferox* Miller, *A. barbadensis* Miller, *A. africana* and *A. saponaria* Hawaii) have been most extensively investigated for their biological activities (Klein and Penneys, 1988). Among these aloe species, *A. vera* is, perhaps, the best-known plant that has been used in medical field. This plant shows many biological activities such as healing capacity against skin burns (Schmidt and Greenspoon, 1991), antiulcer activity (Saito *et al.*, 1989; Wang *et al.*, 1989), antiinflammatory effect (Davis *et al.*, 1989; Davis *et al.*, 1991; Vázquez *et al.*, 1996), inhibition of the AIDS virus (Montaner *et al.*, 1996; Vlietinck *et al.*, 1998), antitumor effect (Imanishi *et al.*, 1981; Wintera *et al.*, 1981; Yoshimoto *et al.*, 1987; Zhang and Tizard, 1996) and chemopreventive effect (Kim and Lee, 1997).

In these papers, polysaccharides and glycoprotein (aloctin A, acemannan, ATF1011) were suggested to act as antitumor agents. Aloe-emodin in trace anthraquinone compounds was found to show significant antileukemic activity against P388 leukemia in mice (Kupchan and Karim, 1996). In addition, aloe-emodin showed mutagenicity against *Salmonella typhimurium* TA1537 and V79 cells (Westendrof *et al.*, 1990).

In view of new pharmacological potential of *A. vera*, the present study was conducted to evaluate the antileukemic and antimutagenic activities of the extract of *A. vera* L. against K562 leukemia cell line and *Salmonella typhimurium* TA98 and TA100.

Experimental

Chemicals and media – AF-2 (2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, CAS 3688-53-7) was from Wako Pure Chemical Industries Co. Ltd., in Japan. The solvent used was dimethylsulfoxide (DMSO) supplied by Merck in USA. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and S9 were from Sigma Chemical Co. in USA. Nutrient broth No. 2 was obtained from Oxoid in USA, biotin and histidine from Sigma Chemical Co. in USA.

Preparation of test samples – Freeze-dried powder of *A. vera* L. was obtained from Kim Jung Moon Aloe Co., Ltd. (Seoul, Korea). 50g of the *A. vera* powder was extracted with 2L of 75% ethanol (ethanol:water, 75/25, v/v) at room temperature for 3

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hours and then evaporated to yield 75% ethanol extract. The EtOH extract was dissolved in water and then partitioned with $CHCl_3$ in a separatory funnel. $CHCl_3$ fraction was separated in a silica gel column (50×550 mm) eluting with *n*-hexane:ethyl acetate (3:1, v/v). Chromatography was accomplished on open column system with UV detector (254 nm, 2238 UVICORD SII, LKB) and linear chart recorder (2210 recorder, LKB). Test samples were dissolved in DMSO for antimutagenicity assay, in 5% DMSO for cell growth inhibition assay.

Assay for antimutagenicity – The tester strains were *Salmonella typhimurium* TA98 and TA100. The cultures were grown in 20 ml of Oxoid nutrient broth No. 2, and incubated for 16 hours at 37 in a rotary incubator in order to ensure adequate aeration. Testing for antimutagenic activity was carried out by the preincubation method (Mastsushima *et al.*, 1980). The positive mutagen used was 0.2 μg of AF-2. In brief, 100 μl (2×10^9 cells/ml) of tester strain, 100 μl of AF-2 plus test sample and 500 μl of PBS were preincubated for 30 minutes at 37°C. After preincubation, the mixture with 2 ml of top agar, containing 0.5 mM histidine and 0.5 mM biotin, was poured immediately onto a minimal glucose agar plate with a Voge-Bonner E medium. All the experiments were performed with duplicate plates and repeated five times. The number of revertant colonies on the plates was counted after being incubated at 37°C for 48 hours. The positive controls were 0.2 μg/plate of AF-2 for *S. typhimurium* TA98 and TA100. The evaluation of antimutagenic activity was performed in the absence of rat liver microsomal fractions (S9 mix), since AF-2 is a direct mutagen. The inhibition rate (%) was calculated as [1-(treated revertants-spontaneous revertants)/(positive mutagen revertants-spontaneous revertants)]×100.

Assay for growth inhibition in K562 and MDBK – K562 (ATCC CCL 243, human chronic myelogenous leukemia cell line) was maintained in RPMI1640 culture medium supplemented with 10% fetal bovine serum (GIBCO USA). MDBK (ATCC CCL 22, bovine kidney normal cell line) was maintained in Ham's F12 medium with 10% fetal bovine serum (GIBCO USA). K562 and MDBK were plated in 96 well culture plate at 1×10^4 cells/well in their respective medium. The methodology described below represents a modification of the original MTT assay (Monks *et al.*, 1991). The MTT assay is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). In brief, the cells were harvested during the exponential phase of maintenance cultures, counted by trypan blue exclusion and dispensed into triplicate 96-well culture plates in quantities of 100 μl. Following 24 hours of incubation at 37°C in a 5% CO_2 incubator, 100 μl of sample solution was dispensed into the appropriate wells and then incubated for 48 hours. A 50 μl aliquot of MTT solution (0.5 mg/ml) was added directly to all the appropriate 96-well microtiter plates and was incubated to form formazan at 37°C for an additional four hours. After this time, the supernatant was aspirated and 100 μl of DMSO was added to dissolve the formazan. The plates were agitated on a plate shaker to ensure a homogeneous solution, and the optical densities were read on an automated spectrophotometric plate reader at the single wavelength of 570 nm.

The means of data from five experiments were calculated. The inhibition rate of growth was calculated as [1-(OD of treated cells/OD of control cells)] ×100. *In vitro* response was classified at each concentration level as either sensitive, if the inhibition rate was more than 50%, or resistant, if the inhibition rate was less than 50%.

Results and Discussion

Before the testing of the antimutagenicity of the aqueous EtOH extract of *A. vera* L., the mutagenicity of the extract was assayed by the Ames method. It was not mutagenic for *Salmonella typhimurium* strains TA98 and TA100 with and without S9mix up to 1mg extract (Data not shown). This indicated that the extract of *A. vera* was non-toxic at 1 mg/plate.

It was found that *A. vera* extract exerted significant antimutagenic and antileukemic effects in the Salmonella mutation assay and MTT assay. The number of mutant colonies of *S. typhimurium* strains TA98 and TA100 upon exposure to AF-2 was decreased on concentration-dependent manner. At the concentration of 1 mg of extract per plate, the number of revertants in *S. typhimurium* strains TA98 and TA100 was reduced to 63 and 66%, respectively (Table 1).

The extract yielded 200 fractions (15 ml/fraction) by silica gel chromatography. Fractions A (fraction No. 60-67), B (fraction No. 94-104) and C (fraction No. 174-192) were examined for inhibitory effect against mutagenicity induced by AF-2 mutagen.

Table 1. Inhibitory effects of *Aloe vera* extract on mutagenicity induced by 0.2 μg of AF-2 in *Salmonella typhimurium* strains TA98 and TA100

Amount of *Aloe vera* extract	TA98	TA100
	Revertants/plate(% inhibition)	Revertants/plate(% inhibition)
AF-2 with *Aloe vera* extract 0.01mg	695±19 (6)[a]	1813±17 (6)
AF-2 with *Aloe vera* extract 0.1mg	425±13(45)	1094±15(46)
AF-2 with *Aloe vera* extract 1mg	300± 8(63)	741± 8(66)

[a]Data are means±SD from each of five separated experiments
In *Salmonella typhimurium* strains TA98, spontaneous revertants was 38±2, positive mutagenic revertants was 738±14, In *Salmonella typhimurium* strains TA100, spontaneous revertants was 147±11, positive mutagenic revertants was 1915±12.

Fraction C showed the strongest inhibition. At the concentration of 50, 5 and 0.5 μg/plate, fraction C reduced AF-2-induced mutagenicity by 84, 67 and 50% in *S. typhimurium* strain TA98, respectively. In case of *S. typhimurium* strain TA100, fraction C reduced AF-2-induced mutagenicity by 90, 74 and 44%, respectively. No inhibitory effect of fractions A and B were observed on mutagenicity of AF-2 (Table 2). The overall yield of fraction C which showed the antimutagenicity was approximately 300 mg/50 g *A. vera* powder.

Table 3 shows the inhibitory effect of the extract of *A. vera* in K562 human leukemia cell line and MDBK bovine normal kidney cell line. At the high dose of 10 mg/ml, *A. vera* extract was examined to show inhibitory effect by 74% in K562. The cytotoxicity of aloe extract was not examined in MDBK yet.

Table 4 shows the growth inhibition of fractions in K562 and DMBK. At the concentration of 500 to 50 μg/ml, fraction C showed the growth inhibition by 93 and 50% in K562, respectively. But, the fractions A and B did not show inhibitory effect against

Table 2. Inhibitory effects of the fractions separated by chromatography on mutagenicity induced by 0.2 μg of AF-2 in *Salmonella typhimurium* strains TA98 and TA100

Amount of fractionation	TA98	TA100
	Revertants/plate (% inhibition)	Revertants/plate (% inhibition)
AF-2 with fractionation A(μg)		
0.5	676±17 (9)[a]	1903± 4(1)
5.0	684±18 (8)	1903±27(1)
50.0	670±15(10)	1808±14(6)
AF-2 with fractionation B(μg)		
0.5	665± 7(12)	1913±18 (1)
5.0	641± 4(14)	1900±15 (1)
50.0	630±11(15)	1888±12 (2)
AF-2 with fractionation C(μg)		
0.5	390± 7(50)	920±13(44)
5.0	267±10(67)	610± 9(74)
50.0	150± 6(84)	331± 8(90)

[a]Data are means±SD from each of five separated experiments.
In *Salmonella typhimurium* strains TA98, spontaneous revertants was 38±2, positive mutagenic revertants was 738±14, In *Salmonella typhimurium* strains TA100, spontaneous revertants was 147±11, positive mutagenic revertants was 1915±12.

Table 3. Comparative growth inhibition of the extract of *Aloe vera* L. against K562 human leukemia cell line and MDBK bovine kidney normal cell line using MTT assay

Extract (Conc. mg/ml)	K562	MDBK
	% inhibition	% inhibition
1	10±0.7	0±0.0
5	35±4.4	0±0.0
10	74±0.9[a]	12±1.1

[a]Sensitive i.e., ≥ 50% inhibition, Data are mean±SD of five separate experiments.

Table 4. Comparative growth inhibition of fractions separated by chromatography against K562 human leukemia cell line and MDBK bovine kidney normal cell line using MTT assay

Fractions	Conc. μg/ml	K562	MDBK
		% inhibition	% inhibition
A (60-67)*	5	0±0.0	0±0.0
	50	3±1.0	0±0.0
	500	3±2.0	0±0.0
B (94-104)	5	14±3.8	0±0.0
	50	19±2.6	3±0.2
	500	29±2.1	12±0.5
C (174 -192)	5	9±2.1	0±0.0
	50	50±2.1[a]	9±1.0
	500	93±3.8[a]	10±0.8

[a]Sensitive i.e., ≥ 50% inhibition, Data are mean±SD of separate experiments.
*Fraction number

K562.

Anthraquinone derivatives in trace components of aloe include aloin, barbaloin, aloe-emodin. Among these anthraquinone derivatives, aloe-emodin has been investigated for the antimutagenicity on *S. typhimurium* TA1537 and V79 cells (Westendrof *et al.*, 1990). But, in these anthraquinone derivatives including aloe-emodin, antimutagenicity of *A. vera* have not been examined yet.

The gel of aloe has been studied for antitumor activity by acemannan, aloctin A, alomicin, ATF1011 and emodin (Darzyndiewicz *et al.*, 1989; Koyama *et al.*, 1989, Imanishi *et al.*, 1981; Soeda, 1969; Zhang and Tizard, 1996), but antimutagenicity has not been examined yet.

Thus, we carried out the screening for antimutagenic and antileukemic activity from *A. vera* L., and evaluated antimutagenicity on the fractions isolated from *A. vera* L. against *S. typhimurium* strains TA98 and TA100, and then antileukemic effect against K562. The present finding clearly demonstrates that *A. vera* L. decreases the mutation in *S. typhimurium* TA98 and TA100 which are induced by AF-2. The results suggest that active compounds in *A. vera* L. may prevent mutations by AF-2 and have antileukemic activity. This research is a preliminary study on the antimutagenicity and antileukemic effect of *A. vera* L. commonly used in diet and medicine. The results from our studies seem to have some of its advantages as health food and pharmacological agents as well. There are still many compounds whose biological activities have not been revealed in *A. vera* L. Further studies on their biological effects of the components of *A. vera* L. are expected to be performed in the future to find their use in health foods, food additives and drugs.

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