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Turk J Biol
34 (2010) 235-246
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doi:10.3906/biy-0812-27

The genotoxic and antigenotoxic effects of *Aloe vera* leaf extract in vivo and in vitro

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Received: 19.12.2008

Abstract: The genotoxic and antigenotoxic effects of *Aloe vera* leaf extract (AV) were investigated using the chromosome aberrations (CAs) test for the bone marrow cells of rats, sister chromatid exchanges (SCEs) and micronucleus (MN) and CAs tests for human lymphocytes, and the Ames Salmonella/microsome test system.

In the bone marrow cells of rats, AV extract significantly induced structural and total CAs at all concentrations and in all treatment periods. In human peripheral lymphocytes, AV did not increase the mean SCE; however, it significantly induced the MN frequency and structural CAs. In addition, AV showed a cytotoxic effect by decreasing the replication index (RI), mitotic index (MI), and nuclear division index (NDI) in human lymphocytes and by decreasing the MI in the bone marrow cells of rats. AV did not decrease the genotoxicity or cytotoxicity of urethane (ethyl carbamate, EC) in the bone marrow cells of rats or in the mitomycin-C (MMC) in human lymphocytes. AV was a weak mutagen in the TA98 strain of *Salmonella typhimurium* in the absence of S9mix; however, AV+NPD (4-nitro-o-phenylenediamine) and AV+SA (sodium azide) exhibited a synergism in increasing the number of revertants for the TA98 and TA100 strains in the absence of S9mix, respectively.

Key words: *Aloe vera*, culture of human lymphocytes in vitro, bone marrow cells, rat, *Salmonella typhimurium*

Aloe vera yaprak ekstraktının in vitro ve in vivo genotoksik ve anti-genotoksik etkisi

Özet: *Aloe vera* (AV) yaprak ekstraktının genotoksik ve anti-genotoksik etkileri sıçan kemik iliği hücrelerinde kromozom aberasyon testi (KA), insan lenfositlerinde kardeş kromatid değişimi, mikronukleus ve KA testleri ve Ames/Salmonella/mikrozom test sistemleri ile araştırılmıştır.

AV ekstraktı sıçan kemik iliği hücrelerinde uygulanan tüm konsantrasyon ve muamele sürelerinde yapısal ve total kromozom anormalliklerini önemli düzeyde uyarmıştır. AV, insan lenfositlerinde ortalama kardeş kromatid değişimi (KKD) sayısını artırmamış, fakat, mikronukleus frekansı ve yapısal kromozom anormalliklerini istatistiksel olarak önemli düzeyde artırmıştır. AV insan lenfositlerinde replikasyon indeksini (RI), mitotik indeksi (MI) ve nukleus bölünme

indeksini (NBI) düşürerek, sıçan kemik iliği hücrelerinde ise sadece mitotik indeksi (MI) düşürerek sitotoksik etki göstermiştir. AV sıçan kemik iliği hücrelerinde üretanın (etil karbamat, EK) insan lenfositlerinde ise mitomisin-C'nin (MMC) genotoksik ve sitotoksik etkisini düşürmemiştir. AV, *Salmonella typhimurium*'un TA98 suşu üzerinde S9mix yokluğunda zayıf mutajenik etki gösterirken, AV+NPD (4-nitro-o-phenylenediamine) ve AV+SA (sodium azide) karışımları TA98 ve TA100 suşlarında S9mix'in yokluğunda revertantların sayısını sinerjistik olarak artırmıştır.

Anahtar sözcükler: *Aloe vera*, in vitro insan lenfosit kültürü, kemik iliği hücreleri, *Salmonella typhimurium*

Introduction

Aloe vera is one of the 432 species of the genus *Aloe* belonging to the family Liliaceae. *A. vera* (L.) Burm.f. (AV) (synonyms: *Aloe barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* var. *vera* L., and *Aloe vulgaris* Lam.) is a native plant of southern and eastern Africa, subsequently introduced into northern Africa, the Arabian peninsula, China, Gibraltar, the Mediterranean countries, and the West Indies. The only medicinal use of AV that is supported by clinical data is the short-term treatment of occasional constipation; however, it is widely used in cosmetics and folk medicine (1-3). According to a WHO report (1), *Aloe vera* gel promotes wound healing by directly stimulating the activity of macrophages and fibroblasts that increase both collagen and proteoglycan synthesis, thereby promoting tissue repair. Furthermore, a complex carbohydrate, acemannan, which can be isolated from *A. vera* leaves, accelerates wound healing and reduces radiation-induced skin reactions. The therapeutic effects of *Aloe vera* gel also include the prevention of progressive dermal ischemia caused by burns, frostbite, electrical injury, and intraarterial drug abuse (1). It was also reported that *Aloe vera* gel acts as an inhibitor of thromboxane A2, a mediator of progressive tissue damage (1). AV, as a cosmetic substance, is available in a large range of skin moisturizers, face and hand creams, cleansers, soaps, suntan lotions, shampoos and hair tonics, shaving preparations, bath aids, makeup and fragrance preparations, and infant lotions and wipes. In folk medicine, AV is used in the treatment of seborrheic dermatitis, peptic ulcers, tuberculosis, and fungal infections, and for reduction of blood sugar (glucose) levels (1-3). Many recent studies have focused on the chemical activities of AV in several test systems. *Aloe vera* gel is reported to be effective in reducing the genotoxicity of EMS in the *Drosophila* sex-linked recessive lethal test (4). The chemopreventive effect of the polysaccharide fraction isolated from *Aloe barbadensis* might be associated with its inhibitor activity on BPDEI (benzo[α]pyrene)-DNA adduct formation in rat hepatocytes, both in vivo and in vitro (5). In addition, aloe emodin, a natural constituent of *A. vera* leaves, significantly inhibits the growth of Merkel carcinoma cell lines (6). However, there is no evidence about the clinical efficacy of topical *A. vera* applications in preventing or reducing radiation-induced skin reactions in cancer patients (7). Furthermore, it has been reported from analysis of survival fractions, bacterial transformation, and agarose gel electrophoresis that *Aloe vera* leaf pulp extract could produce single-strand breaks (SSB) in plasmid DNA in a dose-dependent manner (8). According to recent data, no study in the literature includes the genotoxic and antigenotoxic effects of *Aloe vera* leaf extract together. Thus, the aim of the present study was to evaluate the genotoxic and antigenotoxic effects of *A. vera* extract by the in vivo and in vitro test systems using rat bone marrow cells, human peripheral lymphocytes, and the Salmonella/microsome test system.

Materials and methods

In the present study, *Aloe vera* leaf extract was used as the test substance in in vivo and in vitro tests. The leaves of *Aloe vera* were collected on the campus of Çukurova University in Adana, Turkey. The leaves were cut from the base of the plant and cleaned, and the outer layers were removed. The leaves were homogenized with an Ultra-Turrax homogenizator at 25,000 rpm for 5 min. The pellet was filtered, frozen in liquid nitrogen at -178 °C, lyophilized at -40 °C and 10^{-2} torr for 2 days, and stored at 4 °C. The powder was dissolved in sterile distilled water to obtain the concentrations that were used in the present study.

In vivo assay

The assay design was based on OECD Guideline 475, updated and adopted on 21 July 1997, with minor modifications (9). The in vivo test was performed according to the method of Topaktaş et al. (10). Young adult rats of the Sprague-Dawley strain (*Rattus norvegicus* var. *albinos*) were purchased from the Medical Sciences, Experimental Research and Application Center of Çukurova University. Four rats (2 male and 2 female, 12-16 weeks old, weight 210.678 ± 1.98 g) were used for each treatment and control. Plastic cages (32 × 46 × 18 cm) were used for handling the rats in the Genetics Laboratory. The powdered AV leaf extracts were dissolved in sterile distilled water. The rats were intraperitoneally treated with 3 different concentrations of AV 750, 1000, and 1250 mg/kg b.w. for 12 and 24 h. To investigate the antigenotoxic effect of AV against the mutagenicity induced by urethane (ethyl carbamate, EC), the rats were intraperitoneally treated with 400 mg/kg b.w. EC and with different concentrations of AV for 12 and 24 h treatment times. In the present study, the untreated group (2 male and 2 female rats) was used as the negative control. In order to arrest metaphase, colchicine (3 mg/kg b.w., Sigma C9754) was injected intraperitoneally 2 h before the animals were sacrificed by cervical dislocation. The femurs were stripped proximally, and the bone marrow was aspirated in 4 mL of 0.9% NaCl (37 °C). The suspension was centrifuged for 10 min at 1200 rpm, and the bone marrow pellet was resuspended in 0.4% KCl at 37 °C for 30 min and then fixed in cold methanol-glacial acetic acid (3:1) for 20 min at room temperature. The treatment with fixative was repeated 2 times. Then the cells were spread on glass slides and air-dried. The slides were stained with Giemsa (5% in Sorensen buffer) for 15 min and 100 well-spread metaphases per animal (a total of 400 metaphases per group) were examined at 1000× magnification for the occurrence of structural and numerical CAs. The MI was also determined by scoring 3000 cells from each animal.

In vitro assay

The methods of Evans (11) and Perry and Thompson (12) were followed in preparation of CA and SCE tests, with minor modifications. This study was conducted according to International Programme on Chemical Safety (IPCS) guidelines (13).

Whole blood (0.2 mL) from 4 healthy donors (2 males and 2 females, non-smokers, ages 20-24) was added to 2.5 mL of Chromosome Medium B (Biochrom, F5023) supplemented with 10 μg/mL of bromodeoxyuridine (Sigma, B5002). The cultures were incubated at 37 °C for 72 h. The cells were treated with 1.25, 2.5, and 5.0 mg/mL concentrations of AV that had been dissolved in sterile distilled water for 24 h (AV was added 48 h after initiating the culture) and 48 h (AV was added 24 h after initiating the culture). A negative control (untreated cultures) and a positive control (0.25 μg/mL mitomycin-C (MMC; Kyowa Hakko Chemical Co., Japan)) were also used. The cells were exposed to colchicine (0.06 μg/mL, Sigma C9754) for 2 h before harvesting. The cells were harvested with 0.4% KCl as a hypotonic solution and methanol-glacial acetic acid (3:1) as a fixative. The staining of air-dried slides was performed following the standard method, using 5% Giemsa stain for CAs and a modified fluorescence-plus-Giemsa method for SCEs (14). The slides were irradiated with a 30 W, 254 nm UV lamp at 15 cm distance in Sorensen buffer, then incubated with 1× SSC (standard saline citrate) at 60 °C for 45-60 min, and stained with 5% Giemsa prepared with Sorensen buffer.

The modified methods of Roncada et al. (15) and Mendelsohn (16) were used for evaluating the antigenotoxicity of AV. In the present study, MMC was used as a mutagenic agent. To investigate the antigenotoxic effect of AV against the mutagenicity induced by MMC, the cultures were co-treated with 0.25 μg/mL of MMC and with different concentrations of AV for 24 and 48 h treatment times.

The number of CAs was obtained by calculating the percentage of metaphases from each concentration and treatment period that showed structural and/or numerical alterations. The CAs were classified according to the International System for Human Cytogenetic Nomenclature (17). CAs were evaluated in 100 well-spread metaphases per donor (in total, 400 metaphases per concentration). Gaps were not evaluated as CAs, according to Mace et al. (18). The scoring of SCEs was carried out according to IPCS guidelines (13) and a total of 100 second-division metaphases (25 cells per sample) were analyzed. The results were used to determine the

mean number of SCEs (SCE/cell). In addition, a total of 400 cells (100 cells from each donor) were scored for replication index (RI). The MI was also determined by scoring 3000 cells from each donor. The MI explained the effects of the chemicals on the G2 stage of the cell cycle, and the RI reflected the effects of chemicals on the S and G2 stages of the cycles. The RI was calculated according to the following formula: RI = (M1 × 1) + (M2 × 2) + (M3 × 3)/total scored cells. M1, M2, and M3 are the fraction of cells undergoing the 1st, 2nd, and 3rd mitosis during the 72 h cell culture period.

For the analysis of micronuclei in binucleated lymphocytes, 0.2 mL of fresh blood was used to establish cultures. The cells were treated with 1.25, 2.5, and 5.0 mg/mL concentrations of AV alone and with MMC (0.25 μg/mL) for 24 and 48 h treatment periods.

Cytochalasin B (Sigma, C6762) was added at 44 h of incubation to a final concentration of 6 μg/mL to block cytokinesis. After an additional 24 h of incubation at 37 °C, cells were harvested by centrifugation and processed for a micronucleus test of the peripheral lymphocytes (19,20). In all subjects, 2000 binucleated lymphocytes were scored from each donor (8000 binucleated cells were scored per concentration). For each donor, a total of 1000 viable cells were scored to determine the frequency of cells with 1, 2, 3, or 4 nuclei and to calculate the NDI (nuclear division index) for the cytotoxicity of AV using the following formula: NDI = (1 × M1) + (2 × M2) + (3 × M3) + (4 × M4)/N, where M1-M4 represent the number of cells with 1-4 nuclei and N is the total number of viable cells scored (19).

Ames Salmonella/microsome test

Nicotinamide adenine dinucleotide phosphate (NADP) was purchased from Roche. Glucose-6-phosphate (G-6-P) (G7879), DMSO (D8418), L-histidine (H8125), D-biotin (B4501), ampicillin (A6140), sodium azide (SA) (S2002), and 2-aminofluorene (2-AF) (A9031) were purchased from Sigma. 4-Nitro-o-phenylenediamine (NPD) was purchased from Aldrich. The other chemicals, such as agar (aquamedia, 7178), nutrient broth (NB) (oxoid B241116), and 3-methylcholanthrene (Oekanal, 200-276-4), were also purchased.

The recommended maximum test concentration for soluble noncytotoxic substances is 5 mg/plate (21). Therefore, AV was dissolved in sterile bidistilled water at the concentrations of 1, 2, 3, 4, and 5 mg/plate. In this study, 2-AF (dissolved in DMSO), NPD (dissolved in DMSO), and SA (dissolved in distilled water) were used as the positive controls.

Albino male rats (*Rattus norvegicus* var. *albinos*) weighing 200 g were pretreated with an 80 mg/kg concentration of 3-methylcholanthrene (dissolved in sunflower oil) for 5 days, and the S9 fraction and S9mix were prepared following the procedure of Maron and Ames (22). The freshly prepared S9 fraction was distributed in 1 mL portions into small plastic tubes frozen immediately in crushed dry ice and stored at 80 °C. The S9mix was prepared fresh for each mutagenicity assay. For preparation of S9mix, NADP (4 mM), G-6-P (5 mM), $MgCl_2$ (8 mM), KCl (33 mM), and 6.2 mL of phosphate buffer (0.2 mM) were added to 18 mL of sterile bidistilled water supplemented with 2 mL of microsome fraction (S9); 0.5 mL of S9mix was used for each plate (0.05 mL S9mix/plate).

Histidine-deficient (his^-) tester strains TA98 and TA100 of *Salmonella typhimurium* were kindly provided by Alejandro P. Rooney, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA. The TA98 strain was used for the detection of frameshift mutagens and the TA100 strain was used for the detection of base-pair substitution mutagens. Prior to use in the assay, each strain was checked for the presence of strain-specific markers as described by Maron and Ames (22).

The standard plate incorporation assay was examined with *Salmonella typhimurium* TA98 and TA100 in the presence or absence of S9mix, according to the methods of Maron and Ames (22); 0.5 mL of S9mix per plate was used for the assay. For the mutagenicity test, AV was dissolved in sterile distilled water and used in 1, 2, 3, 4, and 5 mg concentrations per plate. The toxicity of AV to bacteria was observed based on the significantly reduced number of revertants compared to the spontaneous control. On the other hand, NPD (4-nitro-o-phenylenediamine) and SA (sodium azide) were used as positive mutagens for TA98 and TA100, respectively. In the

presence of S9mix, 2-AF (2-aminofluorene) was used as a positive mutagen for both the TA98 and TA100 strains. Each sample was examined with a 3-plate count and all experiments were performed twice.

To investigate the antimutagenic effect of *A. vera*, the tester strains were treated with both AV and NPD or both AV and SA for the TA98 and TA100 strains in the absence of S9mix, respectively. In the presence of S9mix, 2-AF was used as a positive mutagen for both strains because 2-AF required a metabolic activator for the presence of its mutagenic effect.

The t-test was used to determine the statistical significance of all the parameters. Dose-response relationships were determined from the correlation and regression coefficients for the percentage of structural and total CAs, mean SCEs, MN, RI, MI, and NDI for in vivo and in vitro test systems. The significance between control revertants and revertants of treated groups was determined using the t-test. Dose-response relationships were determined using regression and correlation (r) test systems.

Results and discussion

In the bone marrow cells of rats, *Aloe vera* leaf extract (AV) significantly induced structural and total chromosome aberrations (CAs) at all concentrations and in all treatment periods (Table 1). However, it did not induce numerical CAs. AV decreased the mitotic index (MI) at the highest concentration (1250 mg/kg) for a 12 h treatment period when compared with the control, but there was no dose-dependent effect. A similar outcome was noted for the 24 h treatment period, yet it was statistically significant when compared to the control. On the other hand, no other dose-dependent relationships were observed between concentration ranges and chromosomal aberrations (Table 1).

AV and EC as a mixture (AV+EC) significantly induced structural and total CAs at all concentrations in the 12 and 24 h treatment periods when compared with the control. In addition, AV+EC induced structural and total CAs as the positive control means while AV did not decrease the genotoxicity of EC in the bone marrow cells of rats (Table 1). AV+EC significantly decreased the MI in the 12 and 24 h treatment periods when compared with the control. In addition, this decrement in the MI was found to be dose-dependent for the 2 treatment periods (r = -0.99 and r = -0.99, respectively) (Table 1).

AV did not increase the mean sister chromatid exchange (SCEs/cell) for either treatment period when compared with the control in the case of human peripheral lymphocytes (Table 2). Moreover, AV significantly decreased the RI at the highest concentration (5 mg/mL) in the 24 h treatment period and at all concentrations in the 48 h treatment period without dose-dependency, as compared with the control.

The 24 and 48 h cotreatments with AV+MMC (AV and MMC as a mixture) significantly increased the mean SCEs at all concentrations when compared with the control in human peripheral lymphocytes (Table 2). This increment showed dose-dependency (r= 1.0 and r = 0.99, respectively). AV+MMC also increased the mean SCEs of the positive control in the 24 h treatment period, while AV+MMC significantly increased the mean SCEs at all concentrations in the 48 h treatment period when compared with MMC alone. AV+MMC dose-dependency increased the mean SCEs in both the 24 and 48 h treatment periods (r = 1.0 and r = 0.99, respectively). AV+MMC significantly decreased the RI in human peripheral lymphocytes at all concentrations and in all treatment periods when compared with the control and with the positive control, MMC (Table 2).

In human peripheral lymphocytes, AV slightly induced the micronucleus (MN) frequency in the 24 h treatment period; however, it significantly induced the MN frequency at all concentrations in the 48 h treatment period when compared with the control (Table 3). AV decreased the nuclear division index (NDI) in the 24 h treatment period when compared with the control, while it did not decrease the NDI in the 48 h treatment period (Table 3).

AV+MMC significantly induced the MN frequency in human peripheral lymphocytes in the 24 h treatment period when compared with the control, and in the 48 h treatment period as compared with both the control and the MMC positive control. On the other hand, AV+MMC caused a significant reduction in NDI in the 24 and 48 h treatment periods

Table 1. CAs and MI in the bone marrow cells of rats intraperitoneally treated with AV alone or with AV+EC.

Test Substance[+]	Treatment		Chromosome Aberrations[++]			Structural CAs/Cell ± SE	Numerical CAs ± SE (%)	Total CAs/Cell ± SE	MI ± SE
	Period (h)	Concentration (mg/kg)	Structural CAs		Numerical CAs				
			B´ type	B˝ type					
Control	-	-	0	0	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.77 ± 0.40
EC	12	400	32	3	0	8.75 ± 2.13	0.00 ± 0.00	8.75 ± 2.13	3.68 ± 0.31
AV	12	750	13	1	3	3.50 ± 0.86 a1b2	0.75 ± 0.47	4.25 ± 0.47 a2b2	5.36 ± 0.60
	12	1000	17	5	3	5.50 ± 0.50 a2b2	0.75 ± 0.75	6.25 ± 1.03 a2	5.29 ± 0.54
	12	1250	22	3	3	6.25 ± 1.54 a1	0.75 ± 0.47	7.00 ± 1.08 a2	4.59 ± 0.13 a2
AV+EC	12	750 AV+EC*	36	3	0	9.75 ± 2.05 a1	0.00 ± 0.00	9.75 ± 2.05 a1	3.72 ± 0.49 a1
	12	1000AV+EC*	24	1	1	6.25 ± 0.94 a2	0.25 ± 0.25	6.50 ± 0.86 a2	3.48 ± 0.29 a1
	12	1250 AV+EC*	30	4	2	8.50 ± 1.84 a1	0.50 ± 0.28	9.00 ± 1.82 a1	3.17 ± 0.10 a3b1
EC	24	400	27	5	3	8.00 ± 1.00	0.75 ± 0.47	9.00 ± 1.08	3.81 ± 0.19
AV	24	750	23	4	2	6.75 ± 1.10 a2	0.50 ± 0.50	7.25 ± 1.37 a1	3.74 ± 0.64
	24	1000	23	5	1	7.00 ± 1.08 a2	0.25 ± 0.25	7.25 ± 1.25 a2	3.70 ± 0.31 a1
	24	1250	23	4	1	6.75 ± 0.75 a2	0.25 ± 0.25	7.00 ± 0.70 a2	3.63 ± 0.32 a1
AV+EC	24	750 AV+EC*	37	5	1	10.50 ± 1.19 a2	0.25 ± 0.25	10.75 ± 1.10 a2	3.66 ± 0.32 a1
	24	1000AV+EC*	26	4	1	7.50 ± 0.50 a3	0.25 ± 0.25	7.75 ± 0.25 a3	3.40 ± 0.29 a2
	24	1250 AV+EC*	27	8	0	8.75 ± 0.94 a2	0.00 ± 0.00	8.75 ± 0.94 a2	3.20 ± 0.43 a1

*EC (Ethyl carbamate, urethane): 400 mg/kg b.w.
[+]EC: Ethyl carbamate; AV: *Aloe vera* leaf extract; AV+EC: Mixture of *Aloe vera* leaf extract and ethyl carbamate
[++]B' type: Chromatid breakage; B" type: Chromosome breakage
a: Significant from control; b: Significant from positive control, EC; a1b1: $P < 0.05$; a2b2: $P < 0.01$; a3b3: $P < 0.001$.

Table 2. SCEs and RI in human lymphocytes treated with AV alone or with AV and MMC.

Test Substance	Treatment		SCE/Cell ± SE	M1	M2	M3	RI ± SE
	Period (h)	Concentration (mg/mL)					
Control			6.23 ± 0.49	82	118	200	2.29 ± 0.14
MMC	24	0.25 µg/mL	29.44 ± 1.02	105	185	110	2.01 ± 0.15
AV	24	1.25	7.20 ± 0.36 b3	97	138	165	2.17 ± 0.12
		2.5	7.99 ± 0.39 a1b3	104	149	147	2.10 ± 0.15
		5.0	8.31 ± 1.31 b3	144	173	83	1.84 ± 0.07a2
AV+MMC	24	1.25 AV+MMC*	32.20 ± 3.01 a2	184	149	67	1.70 ± 0.03 a3b2
		2.5 AV+MMC*	34.09 ± 3.60 a2	181	173	46	1.66 ± 0.02 a3b3
		5.0 AV+MMC*	37.90 ± 3.34 a2	176	182	42	1.66 ± 0.04 a3b2
MMC	48	0.25 µg/mL	52.00 ± 2.32	129	208	63	1.83 ± 0.07
AV	48	1.25	7.20 ± 0.95 b3	73	131	196	2.30 ± 0.02 b3
		2.5	8.45 ± 0.96 b3	68	130	202	2.33 ± 0.05 b2
		5.0	9.80 ± 1.42 b3	93	150	157	2.16 ± 0.14
AV+MMC	48	1.25 AV+MMC*	73.11 ± 3.68 a3b1	220	168	12	1.48 ± 0.12 a2
		2.5 AV+MMC*	80.86 ± 9.46 a2b1	223	171	6	1.45 ± 0.09 a2b1
		5.0 AV+MMC*	93.87 ± 8.96 a2b1	322	78	0	1.19 ± 0.04 a3b3

*MMC (mitomycin-C): 0.25 µg/mL
a: Significant from control; b: Significant from positive control, MMC; a1b1: $P < 0.05$; a2b2: $P < 0.01$; a3b3: $P < 0.001$.

Table 3. MN and NDI in human lymphocytes treated with AV alone or with AV and MMC.

Test Substance	Treatment		MN ± SE(%)	NDI ± SE
	Period (h)	Concentration (mg/mL)		
Control	-	-	0.57 ± 0.09	1.38 ± 0.03
MMC	24	0.25 μg/mL	1.21 ± 0.27	1.18 ± 0.03
AV	24	1.25	0.86 ± 0.12	1.27 ± 0.03
		2.5	0.96 ± 0.10 a1	1.23 ± 0.03 a1
		5.0	0.66 ± 0.10	1.20 ± 0.02 a2
AV+MMC	24	1.25 AV+MMC*	1.13 ± 0.30 b1	1.19 ± 0.01 a3
		2.5 AV+MMC*	1.03 ± 0.11 a1	1.13 ± 0.03 a2
		5.0 AV+MMC*	1.46 ± 0.16 a1	1.10 ± 0.01 a3b1
MMC	48	0.25 μg/mL	6.08 ± 0.50	1.25 ± 0.06
AV	48	1.25	1.06 ± 0.13 a1b3	1.43 ± 0.09
		2.5	1.11 ± 0.23 a1b3	1.37 ± 0.10
		5.0	1.11 ± 0.08 a2b3	1.21 ± 0.05
AV+MMC	48	1.25 AV+MMC*	11.98 ± 2.32 a1b1	1.20 ± 0.03 a1
		2.5 AV+MMC*	11.78 ± 1.91 a1b1	1.17 ± 0.04 a1
		5.0 AV+MMC*	14.85 ± 3.97 a1b1	1.12 ± 0.03 a2b1

*MMC (mitomycin-C): 0.25 μg/mL
a: Significant from control; b: Significant from positive control, MMC; a1b1: $P < 0.05$; a2b2: $P < 0.01$; a3b3: $P < 0.001$.

when compared with the control. Moreover, this decrement in NDI for the 48 h treatment period was in a dose-dependent manner ($r = -0.99$). In addition, AV+MMC significantly decreased the NDI at the highest concentration (5 mg/mL) for the 2 treatment periods when compared with MMC alone (Table 3).

In human peripheral lymphocytes, AV induced structural CAs at the highest concentration (5 mg/mL) in only the 48 h treatment period when compared with the control (Table 4). AV did not induce numerical CAs (euploidy and aneuploidy) in human peripheral lymphocytes. Similarly, AV significantly decreased the MI at the highest concentration in both the 24 and 48 h treatment periods. This was shown with dose-dependent effects in inducing the structural CAs in the 48 h treatment period ($r = 0.99$) and in decreasing the MI in the 24 h treatment period ($r = -1.0$) (Table 4).

AV+MMC significantly induced structural CAs in the 24 and 48 h treatment periods in a dose-dependent manner when compared with the control ($r = 0.99$ and $r = 0.99$, respectively) (Table 4). On the other hand, AV+MMC significantly induced structural CAs in the 48 h treatment period when compared with the positive control, MMC, which means that AV showed a synergism with MMC on the induction of CAs. In addition, AV+MMC significantly decreased the MI in the 24 and 48 h treatment periods when compared with the control, and decreased the MI at the highest concentration in

Table 4. CAs and MI in human lymphocytes treated with AV alone or with AV and MMC[+].

Test Substance	Treatment		Number of Structural CAs		Structural CAs ± SE(%)**	MI ± SE(%)
	Periods (h)	Concentration (mg/mL)	B' type	B" type		
Control	-	-	8	9	4.25 ± 1.17	4.64 ± 0.75
MMC	24	0.25 µg/mL	43	26	17.25 ± 1.49	3.04 ± 0.45
AV	24	1.25	25	13	9.50 ± 2.32 b1	3.58 ± 0.68
		2.5	22	4	6.50 ± 1.32 b2	3.10 ± 0.57
		5.0	22	6	7.00 ± 2.08 b1	2.01 ± 0.72 a1
AV+MMC	24	1.25 AV+MMC*	47	30	19.25 ± 2.49 a1	2.31 ± 0.54 a1
		2.5 AV+MMC*	55	36	22.75 ± 4.23 a1	1.76 ± 0.40 a2b1
		5.0 AV+MMC*	63***	40	34.33 ± 7.21 a1	1.52 ± 0.50 a2b1
MMC	48	0.25 µg/mL	65	49	28.50 ± 6.19	3.74 ± 0.27
AV	48	1.25	9	9	4.50 ± 1.75 b3	4.70 ± 0.66
		2.5	10	12	5.50 ± 1.55 b3	4.39 ± 0.64
		5.0	27	6	8.25 ± 1.18 a1b3	2.90 ± 0.40 a1
AV+MMC	48	1.25 AV+MMC*	136	89	56.25 ± 8.43 a2b1	2.93 ± 0.35 a1
		2.5 AV+MMC*	160	100	65.00 ± 8.11 a2b1	2.57 ± 0.74 a1
		5.0 AV+MMC*	253	134	97.75 ± 25.57 a1b1	1.62 ± 0.43 a2b1

[+]For abbreviations, see Table 1
*MMC (methyl methanesulfonate): 0.25 µg/mL
**Three polyploid cells were scored and their number was not included in the ratio of structural CAs
***A total of 300 cells were scored due to excessive toxicity
a: Significant from control; b: Significant from positive control, MMC; a1b1: $P < 0.05$; a2b2: $P < 0.01$; a3b3: $P < 0.001$

both treatment periods when compared with MMC. However, a dose-dependent decrement in MI was detected only in the 48 h treatment period (r = -0.99) (Table 4).

AV significantly increased the number of revertants of the TA98 strain of *Salmonella typhimurium* in the absence of S9mix, while AV did not increase the number of revertants of the TA98 strain in the presence of S9mix or of the TA100 strain in the absence or presence of S9mix. Furthermore, no dose-dependent effects were observed (Table 5).

AV+MMC significantly increased the number of revertants of the TA98 strain in the absence of S9mix in a dose-dependent manner (r = 0.90) (Table 6). Similarly, AV+MMC induced the number of revertants of the TA100 strain in the absence of S9mix. This increase was also in a dose-dependent manner (r = 0.98). There was not a significant increase in the number of revertants of *S. typhimurium* TA98 or TA100 strains in the presence of S9mix. AV+MMC exhibited a synergism in increasing the number of revertants for both the TA98 and TA100 strains in the absence of S9mix (Table 6).

In the present experiment, AV, alone or with positive mutagens (EC or MMC), significantly induced structural CAs in both in vivo and in vitro assays. Among these, chromatid-type breaks were the most common abnormalities. AV did not show antigenotoxic effects; in contrast, a synergism was observed between AV and MMC on the induction of SCEs, MN, and CAs in human lymphocytes. AV, alone or as a mixture with NPD, showed mutagenic

Table 5. Mutagenic effects of *Aloe vera* on *S. typhimurium* TA98 and TA100 strains.

Test substances	Concentrat. mg/plate	TA98		TA100	
		-S9	+S9	-S9	+S9
Spontaneous control	-	12.50 ± 1.92	20.66 ± 3.42	103.00 ± 9.56	105.25 ± 7.08
NPD[+]	200 µg/plate	3510.0 ± 391.0	-	-	-
2-AF[+]	20 µg/plate	-	836.5 ± 45.8	-	1347.0 ± 171.4
SA[+]	1 µg/plate	-	-	758.4 ± 42.9	-
Aloe vera leaf extract	1	23.50 ± 2.40 **	21.50 ± 3.12	118.83 ± 5.49 *	84.25 ± 8.61
	2	15.66 ± 0.76 **	22.16 ± 2.41	106.16 ± 7.17	92.00 ± 4.37 *
	3	20.66 ± 2.76 *	20.50 ± 2.82	117.33 ± 4.14 *	107.25 ± 4.78
	4	18.83 ± 2.31 *	29.16 ± 3.93	109.00 ± 7.34	92.25 ± 2.21 **
	5	15.33 ± 1.05 *	22.20 ± 2.81	121.83 ± 10.26	103.75 ± 6.40

Significant from spontaneous control: *P < 0.05; **: P < 0.01; [+]NPD: 4-nitro-o-phenylenediamine; 2-AF: 2-aminofluorene; SA: sodium azide

Table 6. Antimutagenic effects of *A. vera* on *S. typhimurium* TA98 and TA100 strains.

Test substances	Concentrat. mg/plate	TA98		TA100	
		-S9	+S9	-S9	+S9
Spontaneous control	-	15.83 ± 1.13	35.50 ± 5.09	102.66 ± 9.67	141.33 ± 11.05
NPD[+]	200 µg/plate	2586.3 ± 141.7	-	-	-
2-AF[+]	20 µg/plate	-	4285.0 ± 1595.0	-	1943.6 ± 129.4
SA[+]	1 µg/plate	-	-	1465.0 ± 49.9	-
AV+Mutagen[++]	1	4145.3 ± 296.5**	NE[+]	1416.8 ± 20.7	NE[+]
	2	4992.2 ± 199.9***	5780.5 ± 330.9*	1762.3 ± 251.8	1701.0 ± 234.2
	3	6257.1 ± 310.9***	6528.0 ± 448.8*	2221.8 ± 183.6*	1618.5 ± 158.0
	4	6206.3 ± 112.3***	5623.5 ± 752.3	2414.5 ± 291.5*	1859.8 ± 235.4
	5	6332.5 ± 179.1***	5291.0 ± 471.3	3168.1 ± 177.1***	2175.1 ± 177.9

Results were compared with positive controls: *P < 0.05; **:P < 0.01; [+]NE: Non-evaluated

[+]Positive control substances: NPD: 4-nitro-o-phenylenediamine; 2AF: 2-Aminofluorene; SA: sodium azide

[++]To investigate the antimutagenic effect of *A. vera*, NPD and SA were used for TA98 and TA100 in the absence of S9mix, respectively. 2-AF was used for both strains in the presence of S9mix.

activity against the TA98 strain in the absence of S9mix. In addition, AV+SA synergically induced the number of revertants of the TA100 strain in the absence of S9mix.

The most important constituents of the *Aloe* plant were anthraquinones like aloin, barbalion, anthranol, cinnamic acid, aloetic acid, emodin, chrysophanic acid, resistanol, and enzymes (including

cyclooxygenase and bradykininase), together with other compounds such as vitamins, saccharides, and amino acids (2,23).

Paes-Leme et al. (8) reported that *A. vera* leaf extract could produce single-strand breaks (SSB) in the plasmid DNA of *E. coli*. Heidemann et al. (24) reported aloe emodin-induced CAs in CHO cells; however, no mutagenic potential of aloe emodin was observed in an in vitro HGPRT test with V79 cells. It was reported that the other anthraquinones of *Aloe* plants had mutagenic and genotoxic effects in bacterial and mammalian test systems (25,26). In addition, it was reported that emodin was mutagenic in *S. typhimurium* TA1537 and TA98 without S9mix and mutagenic in the TA1538 strain with and without S9mix (24,27,28). In the present study, AV significantly induced the number of revertants of the TA98 strain in the absence of S9mix. On the other hand, several studies have reported that *Aloe* plant constituents were nonmutagenic and nongenotoxic in different tests systems using mice, rats, and bacterial strains (24,26,28,29).

AV caused structural CAs instead of numerical CAs, which means that AV as a clastogen can trigger the formation of CAs by breaking the phosphodiester backbone of DNA. It can be additionally said that AV induced MN because of its clastogenic effects. It was reported that anthraquinones bound noncovalently to DNA and inhibited topoisomerase II activity (30,31). These data support the idea that inhibition of catalytic activity of topoisomerase II contributes to anthraquinone-induced genotoxicity and mutagenicity (31). Furthermore, some chemicals were reported to act in an insidious fashion and kill cells by increasing levels of covalent topoisomerase II-cleaved DNA complexes that are normally fleeting intermediates in the catalytic cycle of the enzyme. All of these compounds induced concentration-dependent increases in the formation of topoisomerase II-stabilized cleavage complexes, providing evidence to support a threshold concept for clastogenicity with topoisomerase II poisons (32,33). It might be concluded that AV promotes chromosomal damage and also acts as a topoisomerase II poison.

AV was not potent enough to decrease the mutagenicity of EC in vivo or of MMC in vitro. Certain studies indicated that emodin caused gland carcinomas in rats and adenocarcinoma in mice (13). In addition, it was reported that powdered or crude extracts of *Aloe* plants caused neoplastic and preneoplastic widespread hemorrhagic lesions in Syrian hamsters, rats, and mice (34,35), while it was reported that *A. vera* extract and anthraquinones like emodin, alonin, and aloctin showed anticancerogenic and antigenotoxic activity against certain cancerogenic substances in animals (4-6,26,36-39). The anticancerogenic effects of the *Aloe* plant might be caused by the cytotoxic properties of these constituents. According to our results, AV had cytotoxic effects, decreasing the RI and NDI in human lymphocytes and the MI in rat bone marrow cells and human lymphocytes. It was reported that *A. vera* extracts emodin and aloe emodin caused cytotoxicity and induced apoptosis in human cell lines CH27 (human lung squamous carcinoma cell), H460 (human non-small-cell lung carcinoma), HepG2 and Hep3B (2 human liver cancer cells), neuroectodermal tumor cells, and mouse lymphoma cells (3,30,40-43). The cytotoxicity of AV might be caused by the decreasing ATP level and the pressure from the functioning of the energy production center (44,45). On the other hand, it was reported that substances are capable of causing cytotoxicity by inducing chromosomal abnormalities and DNA double-strand breaks (46-50). In this study, AV had a cytotoxic effect, most probably by inducing structural CAs. In addition, Madle et al. (51) reported that the mitotic selection of the cells having chromosome abnormalities is capable of decreasing the MI. According to these results, the anticancerogenic effect of the *Aloe* plant was probably caused by the mitotic selection of the cells bearing chromosomal aberrations. It was reported that *A. vera* extract caused external morphological changes, visceral toxicity, hematological changes, sperm abnormality, and abortion in rats and mice (34,52).

Conclusion

In this study, AV alone exerted genotoxic and cytotoxic effects in vivo, in vitro, and in the Salmonella/microsome test systems. AV also exhibited a synergism that is capable of increasing the mutagenic effects of EC and MMC. We therefore

conclude that AV is a potential mutagen in our experimental design and should be further examined in different test systems to better understand its potential genotoxicity before presentation into public usage.

Acknowledgements

This study was supported by Çukurova University Research Fund, FEF2004BAP18.

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