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Anti-leukaemic and Anti-mutagenic Effects of Di(2-ethylhexyl)phthalate Isolated from *Aloe vera* Linne

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Abstract

Extracts of *Aloe vera* Linne have been found to exhibit cytotoxicity against human tumour cell lines. This study examines the anti-tumour effects of di(2-ethylhexyl)phthalate (DEHP) isolated from *Aloe vera* Linne, in human and animal cell lines. Its anti-mutagenic effects were examined using *Salmonella typhimurium* TA98 and TA100 strains.

Growth inhibition was specifically exerted by DEHP against three leukaemic cell lines at concentrations below $100\,\mu g\,mL^{-1}$. At $100\,\mu g\,mL^{-1}$ DEHP, K562, HL60 and U937 leukaemic cell lines showed growth inhibition of 95, 97 and 95%, respectively. DEHP exhibited an inhibitory activity of 74, 83 and 81%, respectively, in K562, HL60 and U937 cell lines at a concentration of $10\,\mu g\,mL^{-1}$. At a concentration of $1\,\mu g\,mL^{-1}$, DEHP exerted an inhibitory activity of 50, 51 and 52%, respectively, in K562, HL60 and U937. In a normal cell line, MDBK, DEHP exerted 30% growth inhibition at a concentration of $100\,\mu g\,mL^{-1}$, and showed no inhibitory activity at concentrations below $50\,\mu g\,mL^{-1}$.

It was found that DEHP exerted anti-mutagenic activity in the *Salmonella* mutation assay. The number of mutant colonies of *Salmonella typhimurium* strain TA98 upon exposure to AF-2 ($0{\cdot}2\,\mu g$/plate) decreased in a concentration-dependent manner in the presence of different DEHP concentrations (decreasing to 90·4, 83·9, 75·4, 69·6 and 46·9%, respectively, for DEHP concentrations of 100, 50, 10, 5 and $1\,\mu g$/plate). In the case of *Salmonella typhimurium* strain TA100, DEHP reduced AF-2-induced mutagenicity at 1, 5, 10, 50 and $100\,\mu g$/plate to 57·4, 77·5, 80·0, 89·0 and 91·5%, respectively.

The isolated compound from *Aloe vera* Linne, DEHP, was considered to be the active principle responsible for anti-leukaemic and anti-mutagenic effects in-vitro.

Aloe, a common constituent of laxative preparations, is widely used for manufacturing food products, beverages, pharmaceuticals and cosmetics because of its aromatic properties, bitter taste, cathartic activity of anthraquinones and other pharmacological activity. This plant shows many biological activities such as healing capacity against skin burns (Schmidt & Greenspoon 1991), anti-ulcer activity (Saito et al 1989; Wang et al 1989), anti-inflammatory activity (Vázquez et al 1996), anti-tumour activity (Imanishi et al 1981; Wintera et al 1981; Yoshimoto et al 1987; Zang & Tizard 1996) and chemopreventive activity (Kim & Lee 1997). The different species of aloe have different chemical compositions. Chemical components of *Aloe vera*, which have been described by many investigators, include polysaccharides, lectin-like substances (aloctin A, acemannan, etc.), inorganic components (calcium, magnesium, etc.), vitamins (vitamin B_1, vitamin B_2, etc.), amino acids (histidine, glutamic acid, etc.), enzymes (oxidase, catalase, etc.), anthraquinones (aloin, emodin, etc.) and other chemicals (β-sitosterol, salicylic acid, etc.) (Shelton 1991). Anti-tumour compounds contained in aloe are known to be chrysophanol, emodin, aloctin A and acemannan. Chrysophanol and emodin are reported to have anti-leukaemic activity in-vivo (Koyama et al 1988, 1989) and aloctin A and acemannan have a tumour-killing effect mediated by immunomodulatory activity (Imanishi & Suzuki 1984; Peng et al 1991). The chemistry of the species shows that acetylglucomannan and acetylmannan are characteristic for the genus of *Aloe*. In trace compounds, anthraquinone glycoside (aloin) and anthraquinone

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derivatives (aloe-emodin, emodin and chrysophanic acid) show cathartic and other pharmacological activity (Capasso et al 1983; Ishii et al 1990; Kupchan & Karim 1996; Westendrof et al 1990).

In view of the new pharmacological potential of *Aloe vera*, this study was conducted to evaluate the anti-tumour and anti-mutagenic activity of *Aloe vera* Linne against various tumour cell lines and *Salmonella typhimurium* TA98 and TA100. We isolated and identified the active principle responsible for anti-tumour and anti-mutagenic activity from the leaves of *Aloe vera* Linne.

Materials and Methods

Plant material

The freeze-dried powder (5 kg) of *Aloe vera* Linne leaves used for this study was supplied by Kim Jeong Moon Aloe Co. Ltd (Seoul, Korea).

Culture of cell lines

A panel of human and animal cell lines were employed. The human tumour cell lines for this study were A549, Calu-3, Sk-mes-1 and Sk-lu-1 derived from lung carcinoma, SNU-1 derived from stomach carcinoma, SF-188 derived from brain carcinoma, WiDr derived from colon carcinoma, Farrow derived from melanoma, Caki-2 derived from kidney carcinoma, HEp-2 derived from larynx carcinoma, KB derived from oral epidermoid carcinoma, HEC-1B derived from endometrial adenocarcinoma, Raji derived from Burkitt's lymphoma, Hs-578T derived from breast carcinoma and K562, HL60, U-937 and THP-1 derived from leukaemias. Animal tumour cell lines were 3LL derived from mouse lung carcinoma, S180 derived from mouse sarcoma and P388 derived from mouse leukaemia. The MDBK normal cell line for cytotoxicity was derived from a bovine kidney.

All cell lines were adapted to a standard culture medium. The cell lines A549 and MDBK were maintained in Ham F12 (Gibco, BRL) supplemented with 10% foetal bovine serum (FBS) (JRH, Bioscience). The cell lines Calu-3, Sk-mes-1 and Sk-lu-1 were maintained in minimum essential medium (MEM) supplemented with 10% FBS, 1% penicillin (10 000 U mL^{-1})/streptomycin (10 000 μg mL^{-1}), 1% L-glutamine (200 mM), 1% non-essential amino acid (Gibco, BRL) and 1% MEM vitamin (Gibco, BRL). The cell lines Farrow, SNU-1, Raji, K562, HL60, U-937 and THP-1 were maintained in RPMI 1640 supplemented with 10% FBS. The cell lines SF-188, HEp-2, HEC-1B and S180 were maintained in Eagle's MEM supplemented with 10% FBS. The cell line Hs-578T was maintained in Dulbecco's modified Eagle's medium containing 10% FBS. The cell line Caki-2 was maintained in MaCoy's 5A medium supplemented with 10% FBS.

Culture of bacterial tester strains

The Screening and Toxicology Center, Korea Research Institute (Daejon, Korea), supplied the tester *Salmonella typhimurium* strains TA98 and TA100. To confirm the subculture and genotype of the tester strains before anti-mutagenic assay, the strains were grown on a nutrient agar plate, and tests for histidine requirement, *rfa* mutation, R-factor and *uvr*B mutation were carried out before anti-mutagenic assay (Maron & Ames 1983). The His- character of the tester strains was confirmed by demonstrating the histidine requirement for growth on minimal glucose agar containing 0·1 mL of 0·1 M histidine.

In brief, the strains were streaked on the minimal glucose agar plate either with or without histidine. The plates were incubated overnight at 37°C and examined for growth. For *rfa* mutation, the nutrient agar plates were seeded with a 0·1-mL culture of the strains and placed on a paper disc soaked in 10 μL of a solution of crystal violet (1 mg mL^{-1}). The plates were incubated overnight at 37°C and examined for a clear zone of inhibition. To examine ampicillin resistance (R factor), the strains were streaked on an ampicillin plate using the procedure described above for the histidine requirement. For *uvr*B mutation, tester strains grown on a nutrient agar plate were irradiated for 6 s with a 15-W germicidal lamp at a distance of 30 cm, incubated overnight at 37°C and the growth was estimated. The tester strains prepared from a nutrient broth containing a final dimethylsulphoxide (DMSO) concentration of 5% (v/v) were stored at −80°C.

Extraction and identification

Pure freeze-dried powder (50 g) of *Aloe vera* Linne leaves was vigorously agitated with chloroform (2 L) at room temperature for two days, followed by filtration through a Whatman No. 4 paper. After dryness and vacuum concentration, the chloroform extract (3% of aloe powder weight) was dissolved with a 1 : 1 mixture of *n*-hexane/ethylacetate, and loaded on a 50 × 5·5-cm silica gel column that had been eluted with a 3 : 1 mixture of *n*-hexane/ethylacetate (2 L) to obtain active fractions.

Fractions were analysed with a UV detector (254 nm, 2238 UVICORD SII, LKB) and linear chart recorder (2210 recorder, LKB). Active fractions (fraction no. 175–182) were obtained by vacuum evaporation, and were further purified by preparative TLC (silica gel 60 GF254, Merck). The purity of the compound was checked by HPLC with diode-array detection on a Hewlett-Packard 1100 system fitted with a μ-bondapak C18 (10 μm, 150 mm × 3·9 mm, Waters), that had been eluted with 50% methanol solution for 40 min. The purified compound was identified by GC-MS, ^{1}H NMR, UV and FT-IR data.

Anti-tumour activity

A modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Monks et al 1991) was used to investigate the cytotoxic effect of a compound from *Aloe vera* Linne against various tumour cell lines and a normal cell line. The MTT assay is based on its metabolic reduction. 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. After incubation for 24 h at 37°C in a 5% CO_2 incubator, 100 μL of the sample solution was dispensed into appropriate wells and then incubated for 48 h. A 50-μL sample of MTT solution (0·5 mg mL^{-1}) was added directly to the appropriate 96-well microtiter plates and was incubated to form formazan at 37°C for an additional 4 h.

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 rate of growth inhibition 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%.

Anti-mutagenic activity

The cultures were grown in 20 mL of Oxoid nutrient broth No. 2, and incubated for 16 h at 37°C in a rotary incubator to ensure adequate aeration. Testing for anti-mutagenic activity was carried out by the pre-incubation method (Mastsushima et al 1980). The positive mutagen used was 0·2 μg of 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2, CAS No. 3688-53-7, Wako Pure Chemical Ind.). In brief, 100 μL (2×10^9 cells mL^{-1}) of tester strain, 100 μL of AF-2 plus test sample and 500 μL of PBS were pre-incubated for 30 min at 37°C. After pre-incubation, 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 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 incubation at 37°C for 48 h. The positive controls were 0·2 μg/plate of AF-2 for *Salmonella typhimurium* TA98 and TA100. The anti-mutagenic activity assessment was performed in the absence of rat-liver microsomal fractions (S9 mix), since AF-2 is a direct mutagen. The inhibitory rate (%) was calculated as [1−(treated revertants−spontaneous revertants)/(positive mutagen revertants−spontaneous revertants)] × 100.

Results

The active anti-leukaemic and anti-mutagenic compound was isolated from pure powder concentrates of *Aloe vera* Linne using solvent extraction and chromatography. The obtained compound had a poor yield (2·9 mg (kg aloe powder)$^{-1}$) with a purity of 98% by HPLC. The properties of the compound, purified by GC-MS, ^{1}H NMR, UV and FT-IR data, suggested the active compound to be DEHP. The identity was confirmed using an authentic sample (DEHP, CAS No. 117-81-7, Sigma).

The inhibitory rates in the various cell lines exposed to DEHP are shown in Table 1. At a concentration of 500 μg mL^{-1} DEHP, sensitive inhibitory activity was observed in eight tumour cell lines of the 21 tested cell lines. In Sk-mes-1, Farrow and SF-188 cell lines, a mild response was achieved at a DEHP concentration of 500 μg mL^{-1}, with inhibitory rates of 50, 54 and 51%, respectively. In the leukaemic K562, HL60, U937, THP-1 and P388 cell lines, DEHP 500 μg mL^{-1} exerted significant inhibitory rates, with 98, 100, 98, 72 and 67%, respectively. At concentrations below 100 μg mL^{-1} DEHP, inhibitory activity was considerable in three leukaemic cell lines – K562, HL60 and U937 – which were inhibited at 1 μg mL^{-1} DEHP by 50, 51 and 52%, respectively. In the normal cell line, MDBK, an inhibitory rate of 48 and 30% was achieved with 500 μg mL^{-1} and 100 μg mL^{-1} DEHP, respectively, but no inhibitory activity was seen with concentrations below

Table 1. Cytotoxicity (% growth inhibition) of the DEHP isolated from *Aloe vera* Linne against various tumour cell lines and a normal cell line using MTT assay.

Cell lines	Cell type	Concn of DEHP ($\mu g\,mL^{-1}$)					
		500	100	50	10	5	1
A549	Human lung	32	12	4	0	0	0
Calu-3	Human lung	42	30	0	0	0	0
Sk-mes-1	Human lung	50*	34	2	0	0	0
Sk-lu-1	Human lung	11	0	0	0	0	0
Farrow	Human melanoma	54*	41	20	0	0	0
SNU-1	Human stomach	14	0	0	0	0	0
SF-188	Human brain	51*	48	10	0	0	0
WiDr	Human colon	0	0	0	0	0	0
Caki-2	Human kidney	32	13	0	0	0	0
HEp-2	Human larynx	31	24	7	0	0	0
KB	Human mouth epidermoid	18	11	3	0	0	0
HEC-1B	Human uterus	0	0	0	0	0	0
Raji	Human lymphoma	41	26	20	8	0	0
Hs-578T	Human breast	27	15	4	0	0	0
K562	Human leukaemia	98*	95*	94*	74*	56*	50*
HL60	Human leukaemia	100*	97*	90*	83*	60*	51*
U937	Human leukaemia	98*	95*	83*	81*	60*	52*
THP-1	Human leukaemia	72*	40	35	10	0	0
3LL	Mouse lung	38	25	11	0	0	0
S180	Mouse sarcoma	21	0	0	0	0	0
P388	Mouse leukaemia	67*	41	20	10	0	0
MDBK	Bovine kidney	48	30	2	0	0	0

*Sensitive (i.e., % inhibition $\geq$ 50). Data are means from five separate experiments.

50 $\mu g\,mL^{-1}$. The results of the MTT assay in-vitro show that DEHP exerted strong anti-tumour activity specifically against leukaemic cell lines.

Before testing for anti-mutagenic activity, the mutagenicity of the DEHP isolated from *Aloe vera* Linne was tested. This indicated that the DEHP was non-toxic at concentrations up to 10 mg/plate (data not shown). Table 2 shows the anti-mutagenic activity of the DEHP isolated from *Aloe vera* Linne against *Salmonella typhimurium* TA98 and TA100 activated with a known mutagen, AF-2 (0·2 μg/plate). It was found that the DEHP exerted surprising anti-mutagenic activity in the *Salmonella* mutation assay. The number of mutant colonies of *Salmonella typhimurium* strain TA98 and TA100 exhibited upon exposure to AF-2 (0·2 μg/plate) was decreased in a concentration-dependent manner. With *Salmonella typhimurium* strain TA98, in the presence of DEHP at concentrations of 1, 5, 10, 50 and 100 μg/plate a decrease to 46·9, 69·6, 75·4, 83·9 and 90·4% was seen, respectively. With *Salmonella typhimurium* strain TA100, the same concentrations of DEHP effected a decrease to 57.4, 77.5, 80.0, 89.0, 91.5% respectively.

Table 2. Anti-mutagenicity of the DEHP isolated from *Aloe vera* Linne on *Salmonella typhimurium* TA98 and TA100 activated with AF-2 (0·2 μg/plate).

Amount of DEHP (μg/plate)	TA98 Revertants/ plate (% inhibition)	TA100 Revertants/ plate (% inhibition)
100	105 (90·4)	298 (91·5)
50	151 (83·9)	342 (89·0)
10	210 (75·4)	500 (80·0)
5	251 (69·6)	544 (77·5)
1	410 (46·9)	900 (57·4)

Data are means from five separate experiments. In *Salmonella typhimurium* TA98, spontaneous revertants were 38 per plate, and positive mutagenic revertants were 738. In *Salmonella typhimurium* TA100, spontaneous revertants were 147 per plate, and positive mutagenic revertants were 1915. Inhibition rate (%) was calculated from [1−(treated revertants−spontaneous revertants)/(positive mutagen revertants−spontaneous revertants)] × 100.

Discussion

DEHP is one of the phthalate esters, a group which also includes monoethylhexylphthalate (MEHP), dimethylphthalate (DMP), butylbenzylphthalate (BBP), dibutylphthalate (DBP) and dioctylphthalate (DOP). Phthalate esters, particularly DBP and DEHP, are found in food packing, blood packs and other environmentals, and some phthalate esters are reported to be toxic compounds (Jobling et al 1995; Sharpe et al 1995; Soto et al 1995).

Recently, however, DBP has been proved to have a new pharmacological activity, namely a purging

effect on leukaemic cells associated with apoptosis, and has been employed as an effective ex-vivo purging agent in autologous bone-marrow transplantation for the treatment of acute myeloid leukaemia (Wu et al 1993, 1995; Wang et al 1996a, b). It was found that DEHP could increase the incidence of benign and malignant liver cell tumour in mice and rats, and may cause carcinogenicity by peroxisome proliferation (Kluwe et al 1982; Reddy & Rao 1988). There has been no evidence, however, of carcinogenicity in humans (Morita et al 1997). When tested in 89 different in-vitro genotoxicity assays, DEHP was negative in 73 and showed effects in only 16, detecting mainly cell-transforming and aneuploidy-inducing properties. From these results, it is not clear whether DEHP is a mutagen (Ashby et al 1985). The phthalate esters found in compounds isolated from *Aloe vera* Linne were reported to be DBP, diheptylphthalate, monooctylphthalate and dioctylphthalate (Yamaguchi et al 1993).

In this study, the DEHP isolated from *Aloe vera* Linne exerted significant anti-mutagenic activity. At the low DEHP concentration of 1 μg/plate, on the *Salmonella typhimurium* TA98 and TA100, inhibitory rates of 46 and 51% were found upon exposure to AF-2 0·2 μg/plate and the mutation test was negative at a high concentration of 10 mg/plate (data not shown). In an in-vivo experiment using the spot test with C57BL mice, DEHP reduced ethylnitrosourea (30 mg kg^{-1})-induced gene mutation from 14% to a mean of 5% when the ethylnitosourea was combined with 5 mL kg^{-1} DEHP (Fahring & Stenikamp-Zucht 1996). Taking the results of this in-vitro test together with Fahring's spot test, it is concluded that DEHP can serve as an anti-mutagenic agent.

In summary, the DEHP specifically exerted growth inhibition on three particular human leukaemic cell lines at a low concentration, and exhibited a new pharmacological activity as an anti-mutagenic agent. Therefore, it seems that *Aloe vera* Linne, having anti-tumour and anti-mutagenic characteristics, is suitable for the development of healthy food and chemopreventive agents. There are still many compounds whose biological activity has not been revealed in *Aloe vera*. Further study of their biological effects and of the active compounds from aloe can be expected in the near future.

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References

Ashby, J., de Serres, F. J., Draper, M., Ishidate, M., Margolin, B. H., Matter, B. E., Shelby, M. D. (1985) In: Evaluation of Short-term Tests for Carcinogens. Elsevier Science Publishers, Amsterdam, pp 1–752

Capasso, F., Mascolo, N., Autore, G., Duraccio, M. R. (1983) Effect of indomethacin on aloin and 1, 8 dioxianthraquinone-induced production of prostaglandins in rat isolated colon. Prostaglandins 26: 557–562

Fahrig, R., Steinkamp-Zucht, A. (1996) Co-recombiogenic and anti-mutagenic effects of diethylhexylphthalate, inactiveness of pentachlorophenol in the spot test with mice. Mutation Res. 354: 59–67

Imanishi, K., Suzuki, I. (1984) Augmentation of natural cell-mediated cytotoxic reactivity of mouse lymphoid cells by Aloctin A. Int. J. Immunopharmacol. 6: 539–543

Imanishi, K., Ishiguro, T., Saito, H., Suzuki, I. (1981) Pharmacological studies on a plant lectin, Aloctin A. I. Growth inhibition of mouse methylcholanthrene induced fibrosarcoma(Meth A) in ascites from by Aloctin A. Experientia 37: 1186–1187

Ishii, Y., Tanizawa, H., Takino, Y. (1990) Studies of aloe. III. Mechanism of cathartic effect (2). Chem. Pharm. Bull. 38: 197–200

Jobling, S., Reynolds, T., White, R., Parker, M. G., Sumpter, J. P. (1995) A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ. Health Persp. 103: 582–587

Kim, H. S., Lee, B. M. (1997) Inhibition of benzo[a]pyrene-DNA adduct formation by *Aloe barbadensis* Miller. Carcinogenesis 18: 771–776

Kluwe, W. M., Haseman, J. K., Douglas, J. F., Huff, J. E. (1982) The carcinogenicity of dietary di-(2-ethylhexyl) phthalate (DEHP) in Fischer 344 rats and $B6C3F_1$ mice. J. Toxicol. Environ. Health 10: 797–815

Koyama, M., Kelly, T. R., Watanabe, K. A. (1988) Novel type of potential anticancer agents derived from chrysophanol and emodin. Some structures-activity relationship studies. J. Med. Chem. 31: 283–284

Koyama, M., Takahashi, K., Chou, T. C., Darzynkiewicz, Z., Kapuscinski, J., Kelly, T. R., Watanabe, K. A. (1989) Intercalating agents with covalent bond forming capability. A novel type of potential anticancer agents. 2. Derivatives of chrysophanol and emodin. J. Med. Chem. 32: 1594–1599

Kupchan, S. M., Karim, A. (1996) Tumor inhibition 114, Aloe emodin: antileukemic principle isolated from *Rhamnus frangula* L. Lloydia 39: 223–224

Maron, D. M., Ames, B. N. (1983) Revised methods for the Salmonella mutagenicity test. Mutation Res. 113: 173–215

Matsushima, M., Sugimura, T., Nagao, T., Shirai, A., Sawamur, M. (1980) Factors modulating mutagenicity in microbial test. In: Norpoth, K. H., Garner, R. C. (eds) Short-term Test System for Directing Mutagens and Carcinogens. Springer, Berlin, pp 273–285

Monks, A., Scudiero, D., Skehen, P., Robert, S., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-goodrich, M., Campbell, H., Mayo, J., Boyd, M. (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J. Natl Cancer Inst. 83: 757–766

Morita, T., Asano, N., Awogi, T., Sasaki, Y. F., Sato, S. I., Shimada, H., Sutou, S., Suzuki, T., Wakata, A., Sofuni, T., Hayashi, M. (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Group 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS.MMS. Mutation Res. 389: 3–122

Peng, S. Y., Norman, J., Curtin, G., Corrier, D., McDaniel, H. R., Bubee, D. (1991) Decreased morality of Norman murine sarcoma in mice treated with the immunomodulator, acemannan. Mol. Biother. 3: 79–87

Reddy, J. K., Rao, M. S. (1988) Oxidative DNA damage caused by persistent peroxisome proliferation: its role in hepatocarcinogensis. Mutation Res. 214: 63–68

Saito, H., Imanishi, K., Okabe, S. (1989) Effects of aloe extracts, aloctin A, on gastric secretion and on experimental gastric lesions in rats. Yadugaku Zasshi 109: 335–339

Schmidt, J. M., Greenspoon, J. S. (1991) *Aloe vera* dermal wound gel is associated with a delay in wound healing. Obstet. Gyneol. 78: 115–117

Sharpe, R. M., Fisher, J. S., Miller, M. M., Jobling, S., Sumpter, J. P. (1995) Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular and sperm production. Environ. Health Persp. 103: 1136–1143

Shelton, R. M. (1991) Aloe vera, its chemical and therapeutic properties. Int. J. Dermatol. 30: 679–683

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., Serrano, F. O. (1995) The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ. Health Persp. 103: 113–122

Vázquez, B., Avila, G., Sequra, D., Escalante, B. (1996) Antiinflammatory activity of extracts from *Aloe vera* gel. J. Ethnopharmacol. 55: 69–75

Wang, H. H., Chung, J. G., Ho, C. C., Wu, L. T., Chang, S. H. (1989) Aloe-emodin effects on arylamine N-acetyl transferase activity in the bacterium Helicobacter pylori. Planta. Med. 64: 176–178

Wang, L. S., Wu, C. T., Pei, X. T., Xu, L., Cao, J. R., Xue, H. H. Yang, K., Teng, A. P., Ji, S. Q. (1996a) Dibutylphthalate purged antologous bone marrow transplant in the treatment of leukemia. Leukemia Res. 20: 343–348

Wang, L. S., Wu, C. T., Pei, X. T., Xu, L., Cao, J. R., He, F. C. (1996b) Purging effect of dibutylphthalate on leukemic cells associated with apoptosis. Leukemia Res. 20: 989–992

Westendrof, J., Marzuardt, H., Poginsky, B., Dominiak, M., Schmidt, J. (1990) Genotoxicity of naturally occurring hydroxy-anthraquinones. Mutation Res. 240: 1–12

Wintera, W. D., Benavides, R., Clause, W. J. (1981) Effects of Aloe extracts on human normal and tumor cells in vitro. Economic Botany 35: 89–95

Wu, C. T., Pei, X. T., Cao, J. R., Xue. H. H. (1993) A new pharmacological activity of dibutylphthalate (DBP) on selective elimination of tumor cells from bone marrow. Leukemia Res. 17: 557–560

Wu, C. T., Yang, K., Pei, X. T., Tang, A., Wang, F. S., Wang, L. S. (1995) Bone marrow purging with dibutyphthalate experimental basis and a preliminary clinical application. Leukemia Res. 19: 557–560

Yamaguchi, I., Mega, N., Sanada, H. (1993) Components of the gel of Aloe vera (L.) Burm. f. Biosci. Biotech. Biochem. 57: 1350–1352

Yoshimoto, R., Kondoh, N., Isawa, M., Hamuro, J. (1987) Plant lectin, ATF 1011, on the tumor cell surface augments tumor-specific immunity through activation of T cells specific for the lectin. Cancer Immunol. Immunother. 25: 25–30

Zhang, L., Tizard, I. R. (1996) Activation of a mouse macrophage cell line by acemannan. The major carbohydrate fraction from *Aloe vera* gel. Immunopharmacology 35: 119–128