Arch. Biol. Sci., Belgrade, 59 (3), 223-226, 2007. DOI:10.2298/ABS0703223S

ANTI-GENOTOXIC EFFECT OF *ALOE VERA GEL*[R] ON THE MUTAGENIC ACTION OF ETHYL METHANESULFONATE

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Abstract – The antimutagenic effect of aloe vera gel[R] was investigated using the *Drosophila* sex-linked recessive lethal test (or SLRL test). In this assay, 3-day-old adults were treated with a direct-acting mutagen – ethyl methanesulfonate (EMS), which was the positive control. The other group of individuals of the same age was firstly treated with EMS, and then with aloe vera gel[R] (co-treatment). When co-treatment experiments with aloe were carried out, it was effective in reducing genotoxicity of the direct-acting mutagen.

Key words: *Drosophila melanogaster*, SLRL test, genotoxicity, aloe vera gel[R]

UDC 595.773.4 : 57.088.7

INTRODUCTION

Aloe barbadensis Miller is the type of aloe used in most commercial products with aloe content available today. Commonly known as aloe vera, the plant can be separated into two basic products: gel and latex. Aloe vera gel is the leaf pulp of mucilage, a thin clear jelly-like substance obtained from the parenchymal tissue that makes up the inner portion of the leaves (T y l e r, 1993). Aloe is a well-known natural dietary supplement and chemopreventive agent. It is a source of polysaccharides and has been found to have anti-inflammation, wound healing, antihepatitis, antigastric ulcer, and antineoplastic (stops the transformation of normal cells into tumor cells) activities (C a r l i et al., 2003).

Aloe gel, made from the central part of the aloe leaf, is a common household remedy for minor cuts and burns as well as sunburns. Aloe gel contains active substances known as glycoproteins and polysaccharides. Glycoproteins are protein-carbohydrate compounds that speed the healing process by stopping pain and inflammation (V a z q u e y et al., 1996; Y a g i and T a k e o, 2003). Polysaccharides are a type of carbohydrate that stimulates skin growth and repair. Studies in test tubes and animals suggest that active substances in aloe leaf extracts (which contain aloe gel) may have immunostimulant and anti-cancer effects (P e c e r e et al., 2003). Scientific studies exist that support an antibacterial and antifungal effect for substances in aloe vera (K l e i n et al., 1988). Antimutagenic and cancer preventive activities of sage (*Salvia officinalis* L.) have also been reported (S i m i ć et al., 2000, K n e ž e v i ć - V u k č e v i ć et al., 2001).

This study provides evidence showing that aloe exhibits anti-genotoxic effects against mutagenicity induced by by alkylating agent ethyl methanesulfonate (EMS).

MATERIAL AND METHODS

Chemicals

The mutagen used in this study was EMS – ethyl methanesulfonate. Aloe vera gel (Forewer Living, USA) was used in a co-treatment experiment and 1% sucrose was used as a negative control.

Strains

The sex-linked recessive lethal test (SLRL test) was done with laboratory stocks of *Drosophila melanogaster* (obtained from the Umea Stock Center, Sweden). One is Canton-S, whose individuals have the normal phenotype (wild type), while Basc line flies are characterized by individuals homozygous for a balancer X-chromosome that carries two genetic markers: Bar (B), which produces a narrow eye shape in homo- and hemizygous conditions

and a kidney-shaped eye when heterozygous in females. An eye restricted to a narrow vertical bar of 80± facets appears in males, and one restricted to 70± facets appearsin homozygous females. Heterozygous females have a number of facets (360±) intermediate between homozygous females (70±) and the wild type (780±). The given character can be regarded as partially dominant, while white-apricot (w^a) changes the red eye color to light orange and is expressed only in homozygous females and hemizygous males; and *scute (sc)* is a recessive mutation that reduces the number of thoracic bristles. This mutation is linked with a long inversion on the X-chromosome, which is necessary for suppression of crossing-over that could change the existing gene combinations on the treated chromosome (L e e et al., 1983).

Test procedure

Three-day-old Canton-S males were starved in empty bottles for 5 h prior to treatment, then transferred and fed in bottles with a filter paper soaked with solution of 2 ppm ethyl methanesulfonate for 24 h (positive control). After another 24 h of recovery on the standard medium, each male was mated individually to three Basc females, in 30 bottles, which made brood I. After two days males were transferred to new vials with three virgins of the Basc line (brood II), and after three days males were transferred again to the fresh vials with three Basc virgins (brood III). These males stayed with females for three days and were removed afterwards. Females were left for five days to lay eggs, and then they were removed. The solvent 1% sucrose served as a negative control (L e w i s and B a c h e r, 1968), while 2 ppm ethyl methanesulfonate (EMS) for 24 h + aloe vera gel in the relaxation period (the next 24 h) was the test group for anti-genotoxicity (co-treatment).

After the F_1 emerged, brother-sister matings were allowed for several days and 10 females from each vial were put individually into new vials. Each vial would give the progeny of one treated X-chromosome.

In the F_2, phenotypes were scored according to eye color and shape. The absence of wild type males indicated the presence of a recessive lethal induced by the test substance.

The stocks were maintained and all experiments were done under optimal conditions (t = 25°C, relative humidity = 60%, 12/12 h of a light/dark regime) on a nutritive medium standard for *Drosophila* (corn flour, yeast, agar, sugar, and nipagin to prevent mold and infection).

The total number of treated X-chromosomes is equal to the sum of lethal and nonlethal cultures, and the frequency of sex-linked recessive lethals was calculated by the ratio of the number of lethal to the totals number of treated X-chromosomes. Testing of the significance of differences in the percentage of lethals was done by the test for large independent samples (testing of differences between proportions – P e t z, 1985).

RESULTS

The results obtained regarding the mutagenic effect of EMS and antimutagenic effect of of aloe are shown in Table 1. In our experiment, a 2 ppm concentration of EMS was shown to be clearly genotoxic, inducing significant increases in the frequency of mutants in all three broods: in both premeiotic (spermatocytes) and postmeiotic (spermatids and spermatozoids) germ cell lines. Results of our co-treatment experiment show that aloe vera gel drastically reduced the genotoxicity of EMS in all germ cell lines and at the same time significantly increased fertility of the tested individuals (compared to the positive control).

DISCUSSION

Antitoxic, anticancer, and antimutagenic activities of aloe have been studied using both *in vivo* and *in vitro* systems (for example, in the *Salmonella typhimurium* bacterial mutation assay and the chromosome aberration assay using Chinese hamster ovary cells), but there are not enough data on its antimutagenic effects in *in vivo* system with *Drosophila melanogaster*. The present study was designed to determine the effect of aloe on the mutagenicity of a direct-acting alkylating agent-ethyl methanesulfonate (EMS), which is mutagenic both *in vivo* and *in vitro* - using the SLRL test of *Drosophila melanogaster*.

The studies carried out so far have shown that alkylating agents belong to the group of chemical mutagens that cause direct changes in DNA. They react directly with certain bases in DNA. Active DNA synthesis is not necessary for this reaction to take place, but it is necessary for it to be fixed.

These studies show that the most reactive places are nitrogen atoms in purine rings, and especially N-7 of the base guanine, followed by N-3 and N-7 in the adenine

Table 1. Frequencies of SLRL mutations after treatment of *Drosophila melanogaster* males with EMS and co-treatment with aloe (Statistically significant difference: $p < 0.001$***).

	SUCROSE (negative control)	EMS (positive control)	EMS + ALOE (co-treatment)	$t_{ems/aloe}$
Brood I Σ	300	77	249	8.98
N_o of lethals	5	43	10	p<0.001***
% of lethals	1.67	55.84	4.02	
Brood II Σ	269	140	259	14.20
N_o of lethals	5	86	4	p<0.001***
% of lethals	1.86	61.43	1.54	
Brood III Σ	252	59	185	10.53
N_o of lethals	6	41	8	p<0.001***
% of lethals	2.38	69.49	4.32	
I+II+III Σ	821	276	693	18.67
No of lethals	16	170	22	p<0.001***
% of lethals	1.95	61.59	3.17	

ring. Formation of alkylated derivatives, ethyl- or methylpyrimidine and purine, results in incorrect pairing in the replication processes because the changed bases show slightly different chemical affinities that deviate from the classical principle of complementarity. Furthermore, certain products of covalent interactions of purine and pyrimidine with mutagens are unstable, which causes the appearance of apurine and apyrimidine sites in polynucleotide DNA chains. For that reason, the alkylating agents are very powerful mutagens that lead to various types of mutations: transition, transversion and outphase mutations (W h e e l e r, 1962).

It has been found that ethyl methane-sulfonate changes guanine into ethyl-guanine (adds the ethyl-CH_3-CH_2-group), causing a substitution type of mutations. Due to this reaction, instead of a G-C pair, an A-T base pair appears in DNA. Besides guanine, EMS can also affect thymine, causing the same kind of reaction. These changes in DNA, caused by the influence of chemical agents, represent an adequate stimulus for activation of molecular mechanisms of DNA repair whose basic aim is to remove the local changes. If the reparation mechanisms do not establish the previous state, the first replication to occur will lead to fixation of the mutation. Genetic mutations induced by these agents can be detected by SLRL tests based on their phenotypic effect (eye color of the F_2 generation brood).

Although aloe has been used in traditional medicine since ancient times (in 1500 B.C., the Egyptians recorded use of the herbal plant in treating burns, infections, and parasites; and the ancient Greeks, Arabs, and Spaniards have used the plant throughout the millennia), the first modern medical study on aloe was reported by C o l l i n s et al. (1935). The author described the application of this plant, from the family of Liliaceae, in treating radiation dermatitis. In the 1940s it was concluded that aloe heals burns much faster than any known medicament, and in the 1960s it started to be used in treatment of gastric ulcer patients. W a l l e r (1978) stated that aloe contains a wide range of free amino acids, polysaccharides, sterols (mostly B-sitosterol), and lupeol. It has been shown that B-sitosterol is effective in treatment of excess cholesterol and has anti-inflammatory effects, while lupeol has analgesic and antibacterial activities. M c A n a l l y (1985) separated a polysaccharide (carrysin) containing a molecule of acemannan, wich has an antiviral effect. Studies have shown that acemannan boosts T-lymphocyte cells (which aid the immune system). Researchers from Japan showed that aloe contains at least three antitumor agents – emodin, mannose, and lecithin. The glycoprotein aloe lecithin has been shown to stimulate DNA synthesis in baby hamster kidney (BHK) cells and to have the properties of a lecithin that reacts with sheep blood cells (Y a g i et al., 1985, 2003).

In the November 2005 issue of Journal of Toxicology and Environmental Health, the authors study the chemopreventive effects of aloe. This study provides evidence showing that aloe exhibits chemopreventive activity against the mutagenicity of BaP (benzopyrene is formed as a result of smoking cigarettes and other tobacco products). The authors found that aloe inhibited chromosomal damage in a dose-dependent manner. This means the more aloe used, the fewer the mutations. They found that the highest concentration of aloe compared with the control inhibited mutagenicity by 47% (Y o o et al., 2005).

The results shown in Table 1 suggest that the components of aloe (probably a combination of polysaccharides) that promote anti-genotoxic activity in an *in vivo* system of *Drosophila melanogaster* can be considered as potential agents for chemoprevention, as well as for improvement of the fertility of individuals. Treatment of adults with mutagenic doses of EMS combined with aloe supplementation clearly reduced the recessive mutation frequencies in both postmeiotic (spermatids and spermatozoids) and premeiotic (spermatocytes) germ cell lines. It can be presumed that aloe reduces mutation induction by chemicals in different ways: 1) it inhibits formation of

alkylated derivatives of ethyl-pyrimidine and purine that cause incorrect pairing in the replication processes and occurrence of genetic mutations; or 2) it activates molecular mechanisms of DNA repair that effectively and permanently remove the damage.

Chemoprevention or co-treatments that decrease damaging effects of chemical agents are very important for preservation of the genetic system of the species because all metabolic processes that influence both physical and mental health depend on DNA function. It is essential to maintain the germ cell line because damage in this cell line is transferred to the offspring, and it can also decrease the reproductive ability of its bearer. These results show obvious antigenotoxic potentials of aloe and suggest that it can be used in prevention of DNA damage caused by chemical agents.

Acknowledgment – This study was financially supported by a grant (No. 143008) from the Serbian Ministry of Science and Environment Protection.

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АНТИГЕНОТОКСИЧНО ДЕЈСТВО *ALOE VERA-GEL*^{R} НА МУТАГЕНУ ДОЗУ ЕТИЛМЕТАНСУЛФОНАТА

СНЕЖАНА СТАНИЋ

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Испитиван је антигенотоксични ефекат *Aloe vera*-гела на *Drosophila melanogaster* применом SLRL теста. Резултати показују значајан антимутагени ефекат тестираног агенса јер је фреквенција полно везаних рецесивних леталних мутација нижа у односу на позитивну контролу како у премејотичкој, тако и у постмејотичким герминативним линијама.