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 (Tyler, 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 (Carli 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 (Vazquez et al., 1996; Yagi and Takeo, 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 (Pecere et al., 2003). Scientific studies exist that support an antibacterial and antifungal effect for substances in aloe vera (Klein et al., 1988). Antimutagenic and cancer preventive activities of sage (*Salvia officinalis* L.) have also been reported (Simic et al., 2000, Knezevic et al., 2001).

This study provides evidence showing that aloe exhibits anti-genotoxic effects against mutagenicity induced by by alkylation 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 recessive stocks of *Drosophila melanogaster* (obtained from the Umea Stock Center, Sweden). One is Canton-S, whose individuals have the normal phenotype (wild type), while Base 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 appears in 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 (wa) 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 (Lee 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 (Lee et al., 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 F₁ 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 F₂, 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 nutritious 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 – Petz, 1985).

**RESULTS**

The results obtained regarding the mutagenic effect of EMS and antimutagenic effect 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
ed 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 Collins 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 medicinal, and in the 1960s it started to be used in treatment of gastric ulcer patients. Waller (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. McCann (1985) separated a polysaccharide (carrysin) containing a molecule of acemannan, which 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 (Yagi 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% (Yoo 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 spermatogonia) and prereproductive (spermatocytes) germ cell lines. It can be presumed that aloe reduces mutation induction by chemicals in different ways: 1) it inhibits formation of

<table>
<thead>
<tr>
<th>Brood</th>
<th>No of lethals (negative control)</th>
<th>EMS (positive control)</th>
<th>EMS + ALOE (co-treatment)</th>
<th>t寂寞</th>
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<tbody>
<tr>
<td>Brood I Δ</td>
<td></td>
<td>5</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>% of lethals</td>
<td>1.67</td>
<td>5.84</td>
<td>4.02</td>
<td></td>
</tr>
<tr>
<td>Brood II Δ</td>
<td></td>
<td>5</td>
<td>86</td>
<td>4</td>
</tr>
<tr>
<td>% of lethals</td>
<td>1.86</td>
<td>61.43</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Brood II Δ</td>
<td></td>
<td>252</td>
<td>59</td>
<td>185</td>
</tr>
<tr>
<td>% of lethals</td>
<td>2.58</td>
<td>69.49</td>
<td>4.52</td>
<td></td>
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<tr>
<td>1+II-III Δ</td>
<td></td>
<td>821</td>
<td>276</td>
<td>693</td>
</tr>
<tr>
<td>% of lethals</td>
<td>1.95</td>
<td>61.59</td>
<td>3.17</td>
<td></td>
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</tbody>
</table>

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***).
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 sugarcane that it can be used in prevention of DNA damage caused by chemical agents.

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REFERENCES


АНТИГЕНТОКСИЧНО ДЕЈСТВО ALOE VERA-GEL® 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 поло везаних рецесивних леталних мутација нижа у односу на позитивну контролу како у предмезоотичкој, тако и у постмезоотичким герминативним линијама.