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Short Communication

Can the *Rosa canina* Plant be Used Against Alkylating Agents as a Radical Scavenger?

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Abstract

In this study we used the somatic mutation and recombination test (SMART) in *Drosophila melanogaster* to evaluate the genotoxicity of ethyl methanesulfonate (EMS) and the effect of *Rosa canina* L. ethanol extract (RC_{eta}) on the genotoxicity of EMS. Application groups were prepared as *Drosophila* instant medium (DIM), including only 1ppm EMS and different concentrations (1, 2, and 4ppm) of RC_{eta} were added to DIM, including 1 ppm EMS. As a result of our study, statistically significant genotoxic effects of EMS ($P<0.05$) were removed by RC_{eta}. A particularly positive genotoxic effect of EMS has become inconclusive/negative efficacy in both wing phenotypes on 2 and 4 ppm EMS+RC_{eta} application groups.

Keywords: *Drosophila melanogaster*, *Rosa canina*, EMS, SMART, antigenotoxicity

Introduction

Since ancient times, man has used various natural resources for medicinal purposes. Plants have always played an important role in both medicine and public health [1]. Various types of inhibitors and suppressors against mutagens and carcinogens are found in several organisms, especially plants [2]. Alkylating agents appear in the same way as mutagenic and carcinogenic agents. The alkylating agents are very powerful mutagens that lead to various types of mutations: transition and transversion [3]. Ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), and N-Ethyl-N-nitrosourea (ENU) are some of the most well-known alkylating agents. EMS causes transition by ethylating with thymine or guanine directly, and incorrect base pairing between nucleotides during replication [3]. Extracts of various plants can be used against genotoxicity. According to Kılıçgün and Altıner [4], one of these plants is *R. canina*, which has protective properties against DNA damage.

The fruit of *R. canina* (100 g) has the highest antioxidant activity due to the high content of vitamin C and phenolic compounds, including 250-1,500 mg of ascorbic acid [5, 6]. Özcan [7] stated that the methanol extract of *R. canina* contained antioxidant properties, and a concentration of 0.4% reached the highest value of antioxidant activity. According to Serteser et al. [8], rosehip is a potent free radical hunter and a natural antioxidant source. Gao et al. [9], observed that *Rosa* species antioxidant activity is related to the amount of carotenoid, phenolic compound, and ascorbic acid in its content and 25µg/ml rosehip extract inhibited 83% of lipid peroxidation due to its iron ions.

The aim of this study is to evaluate the antigenotoxicty of ethanol extract of *R. canina* vs. EMS-mediated genotoxicity using *Drosophila* SMART assay.

Methods

The chemicals used in this study were ethyl methanesulfonate (EMS), ethanol, and dimethyl sulfoxide (DMSO) supplied by Sigma Chemical Co. (St. Louis, USA). *Drosophila* instant medium was obtained from Carolina Biological Supply Company (Burlington, NC).

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R. canina fruits were collected from the Aşkale district of Ezurum, Turkey, ranging from approximately 1,500 to 2,000 m. The plant was identified by Meryem Şengül Köseoğlu (Atatürk University, Turkey). *R. canina* fruits were extracted with ethanol. The dried extracts were later dissolved in 1% DMSO followed by a culture medium and prepared in different concentrations.

The principles and basic procedures for the *Drosophila* wing spot test have been described by Graf et al. [10]. In order to generate trans-heterozygous larvae, *flr*^{3} (*flr*^{3}/*In (3LR)TM3*, *ri p*^{p} *sep l(3)89Aa bx*^{34e} *e Bd*^{s}) virgin females were crossed with *mwh* (*mwh/mwh*) males. When the larvae were 72±4 h, they were placed into a glass tube containing 1g dry *Drosophila* Instant Medium prepared with 4 ml of the test solutions (1 ppm EMS+RC_{eta}) at different concentrations (1, 2, and 4 ppm) and distilled water, 1% DMSO for the negative controls. The larvae were fed on this medium for the rest of their development. The wings of hatching adult flies were inspected under 400X magnification for the presence of spots. The wing spot data were evaluated according to the multiple-decision procedure of Frei and Würgler [11].

Results

The findings obtained from EMS and EMS+RC_{eta} application groups for the normal wings (*mwh/flr*^{3}) and serrate wing (*mwh/TM3*) phenotypes are presented in Table 1. As is shown in Table 1, there were no significant differences between the values that were obtained with distilled water and 1ppm DMSO applications for both normal and serrated wing phenotypes. When the 1ppm EMS application group was compared with the DMSO application group even though positive results were obtained for small single spot, total *mwh* spots, and total spots despite the increase in mutation frequency in *mwh/flr*^{3} genotype. However, inconclusive/negative results for large single spots and twin spots were observed (Table 1).

While the CIF value for *mwh/flr*^{3} genotype in the application of 1ppm EMS was 2.00, this value for the DMSO application group was calculated as 0.92. Conversely, no significant differences were observed for all spots in *mwh/TM3* genotype of the 1 ppm EMS application group. CIF values in *mwh/TM3* genotype were calculated as 1.49 and 0.92 for EMS and DMSO, respectively. As seen in Table 1, EMS increased mutation frequencies in each genotype in comparison with the DMSO application group.

In the second part of our study three different doses (1, 2, and 4 ppm) of ethanol extracts of *R. canina* were applied to 72±4 hours trans-heterozygous larvae along with 1 ppm EMS (EMS+1 RC_{eta}, EMS+2 RC_{eta}, EMS+4 RC_{eta}). EMS+RC_{eta} (Fig. 1) applications decreased numbers of clones in all spots of both *mwh/flr*^{3} and *mwh/TM3* genotypes when compared with EMS applications. The results were statistically transformed from positive efficient to inconclusive/negative efficient. In the application groups of RC_{eta}, CIF value for *mwh/flr*^{3} declined from 2.00 to 1.23. This value for *mwh/TM3* has been determined to decrease from 1.49 to 0.56.

The present results demonstrate that the highest antigenotoxic effect was obtained with the dose of 4 RC_{eta} caused among the other RC_{eta} doses tested (Table 1). In this dose application, the total spot frequency has been found to be decreased from 0.54 to 0.30 in *mwh/flr*^{3} from 0.36 to 0.14 in *mwh/TM3*.

Our results in the co-treatment experiments show that RC extracts reduced the genotoxicity of EMS in all types of mutant clones. Under the effect of 4 RC_{eth}, the mutation rate decreased to 17%, 100%, 100%, 39%, and 44% in *mwh/flr*^{3} for small single spots, large single spots, twin spots, mwh spots, and total spots, respectively. These rates were determined 25%, 100%, 61%, and 61% in *mwh/TM3* for small single spots, large single spots, mwh spots, and total spots, respectively (Fig. 1).

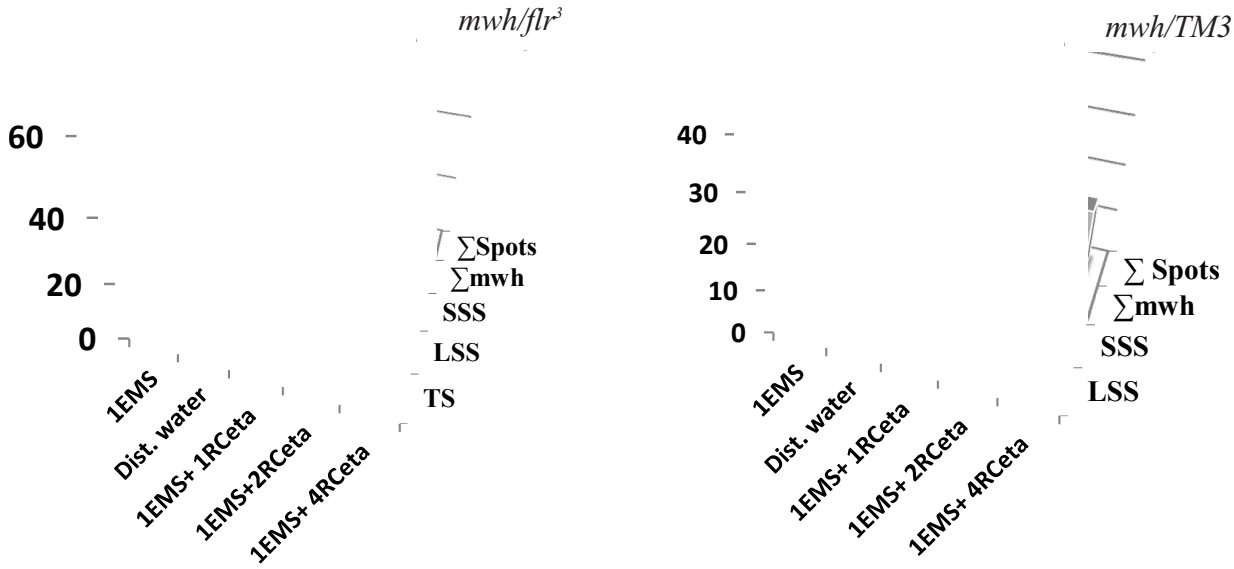

Fig. 1. The antimutagenic effect of RC_{eta} against EMS.
SSS – small single spots, LSS – large single spots, TS – twin spots, EMS – ethyl methanesulfonate, RC_{eta} – ethanol extract of *Rosa canina*.

Table 1. Wing spot test data obtained after EMS and EMS+RC_{eta} treatments.

Compound concentration (ppm)	Number of wings (N)	Small single spots (1-2 cells) (*m*=2)			Large single spots (>2 cells) (*m*=5)			Twin spots (*m*=5)			Total *mwh* spots (*m*=2)			Total spots (*m*=2)			Frequency of clone formation per 10^5 cells (CIF)
		No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	
Normal wings (*mwh/flr*^{3})																	
Distilled water	120	18	0.15		1	0.01		1	0.01		19	0.16		20	0.17		0.65
DMSO	80	14	0.18	–	4	0.05	i	2	0.03	i	18	0.23	i	20	0.25	i	0.92
EMS	80	29	0.36	+	11	0.14	i	3	0.04	i	39	0.49	+	43	0.54	+	2.00
EMS + 1x RC_{eta}	80	21	0.26	i	11	0.14	i	0	0.00	–	33	0.41	+	33	0.41	+	1.69
EMS + 2x RC_{eta}	80	21	0.26	i	8	0.1	i	0	0.00	–	29	0.36	i	29	0.36	i	1.49
EMS + 4x RC_{eta}	80	24	0.3	i	0	0.00	–	0	0.00	–	24	0.3	i	24	0.3	i	1.23
Serrate wings (*mwh/TM3*)																	
Distilled water	120	20	0.17		5	0.04		Balancer chromosome *TM3* does not carry the *flr*^{3} mutation			25	0.21		25	0.21		0.85
DMSO	80	12	0.15	–	6	0.08	i				18	0.23	–	18	0.23	–	0.92
EMS	80	19	0.24	i	10	0.13	–				29	0.36	i	29	0.36	i	1.49
EMS + 1x RC_{eta}	80	21	0.26	i	0	0,00	–				21	0.26	i	21	0.26	i	1.08
EMS + 2x RC_{eta}	80	13	0.16	i	3	0.04	–				16	0.2	–	16	0.2	–	0.82
EMS + 4x RC_{eta}	80	11	0.14	–	0	0.00	–				11	0.14	–	11	0.14	–	0.56

No – number of clones, Fr – frequency, D – statistical diagnosis according to Frei and Würgler [11], "+" – positive, "–" – negative, i – inconclusive, m – multiplication factor, probability levels = 0.05.

Discussion

EMS, which is highly mutagenic and carcinogenic, is preferred as a positive control group in genotoxicity testing. It has been observed that *in vivo* and *vitro* studies, such as alkylating agents, EMS, and MMS cause both gene mutations and chromosomal damage [12], EMS induces DNA damage by a direct mechanism [3]. EMS is an aklylating agent that acts as a powerful alkyl donor, which provides an alkyl residue to the N7-glycodidic bond of guanine or thymine, resulting in G-T mismatch and introducing AT→GC and GC→AT transition mutations [13]. In studies conducted by various researchers on Syrian hamsters [14] and rats [15], EMS induced DNA damage in different organs, and the micronucleus rate increased oral intake in the mice observed [16]. These results of EMS also correlate with our results of EMS.

Many studies conducted in the last 30 years have focused on the evaluation of anticarcinogenic and antimutagenic activity in plants. Medical herbs are a potential source of antioxidants and ROS (reactive oxygen species) scavenging molecules, and they have the highest levels and a wide variety of vitamins and minerals. One such plant was the rosehip fruit, which is rich in minerals, vitamins, sugars, phenolic compounds, carotenoids, tocopherol, bioflavonoids, tannins, organic acids, fruit acids, aminoacids, volatile oils, and pectin [17]. The structure of rosehip fruits, which are rich in minerals and flavonoids, contains cations such as potassium, sodium, calcium, magnesium, iron, manganese, phosphorus, copper and zinc, and anions such as sulphate, chloride, and nitrate [18]. In addition, rosehip does not contain pesticides and heavy metals (arsenic, cadmium, lead, mercury, etc.) that are harmful to human health. This property is a safe source for baby food production [19]. Between the antioxidant activity and phenolic compounds in *R. canina,* many plant species have strong relations [20] and these phenolic compounds were determined as antimutagenic and anticarcinocenic [21]. *R. canina* was reported to have a metal ion chelating activity [22] and demonstrate a strong radical scavenging effect [9]. *In vivo* and *in vitro* studies show that the formation mechanism of the antioxidant effect was the cleaning capacity of H_2O_2 and O_2^- [6]. Therefore, the antioxidant and antimicrobial effects of the rose fruit are significantly strong [23]. In a study conducted by Kılıçgün and Altıner [24], it was observed that the *R. canina* inhibited liver damage in rats, even at low concentrations. According to Gao et. al [9]; *R. canina* inhibited lipid oxidation in humans *in vitro*. Ascorbic acid and beta-carotene are abundant in *R. canina*. Ascorbic acid reduced the genotoxic effects of EMS, MMS, and ENU [25], if beta-carotene decreased the genotoxic effects of doxorubicin [26].

In vivo experiments of *D. melanogaster* using medicinal herbs support our study. It was observed in a study by Uysal et al. [27] that methanol extract of *E. amoenum* decreased the genotoxic effect of EMS. According to Pereira [28] extract of *P. ginseng* has an antirecombinogenic effect.

According to the data obtained in our study, we can state that the combatting effects on the EMS of *R. canina* are due to the antioxidative properties of the phenolic compound as well as the vitamins and minerals it contains.

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