Cumhuriyet Üniversitesi Fen Fakültesi
Fen Bilimleri Dergisi (CFD), Cilt 37, No. 3 (2016)
ISSN: 1300-1949

Cumhuriyet University Faculty of Science
Science Journal (CSJ), Vol. 37, No. 3 (2016)
ISSN: 1300-1949

*http://dx.doi.org/*10.17776/csj.18487

Genoprotective Effects of Aqueous Extracts of *Rosa Canina* L. Fruits on Ethyl Methanesulfonate-Induced DNA Damage in *Drosophila Melanogaster*

Caner KASIMOĞLU, Handan UYSAL*

Department of Biology, Faculty of Science, Atatürk University, Erzurum, Turkey

Received: 28.04.2016; Accepted: 16.05.2016

Abstract. In this study, the possible genotoxic effects of ethyl methanesulfonate (EMS) which are one of alkylating agent and genoprotective effects of *Rosa canina* L. fruit water extract (RC_{wtr}) was studied with *Drosophila* wing somatic mutation and recombination test (SMART). Five different application groups (distilled water, 1 mM EMS, 1% RC_{wtr}, 3% RC_{wtr} and 5% RC_{wtr}) were formed with preliminary studies. 3-day-old transheterozygous larvae of *mwh/flr*^{3} genotype of *Drosophila melanogaster* were fed chronically on the Drosophila instant medium (DIM) including the application groups. The wing slides of normal wing (*mwh*/*flr*^{3}) phenotype individuals were prepared and examined under light microscope (400X).

As a result of experiments, the total clone frequencies of distilled water control group, 1 mM EMS, 1% RC_{wtr}, 3% RC_{wtr} and 5% RC_{wtr} application groups were determined as 0.15, 3.55, 2.58, 2.78 and 2.20, respectively. The difference between distilled water control group and 1 mM EMS application group is statistically significant ($P<0.05$). According to the results obtained from RC_{wtr} application groups, each group's total clon frequencies decreased compared with 1 mM EMS application group. It was found that the differences between the 1mM EMS and $RC_{wtr.}$ application groups were statistically important too ($P<0.05$). The findings demonstrate that the constituents of *Rosa canina* L. have great potential as a natural genoprotective product.

Keywords: *Rosa canina*, SMART, *Drosophila melanogaster*, Ethyl methanesulfonate, genoprotective

Drosophila Melanogaster'de Etil Metansülfonat ile Uyarılan DNA Hasarı Üzerine *Rosa Canina* L. Meyvelerine Ait Su Ekstraktinin Genomik Koruyucu Etkisi

Özet. Bu çalışmada, alkilleyici ajanlardan birisi olan etil metansülfonatın olası genotoksik etkileri ve *Rosa canina* L. meyvelerinin su ekstraktının (RC_{su}) genomik koruyucu etkisi Drosophila kanat somatik mutasyon ve rekombinasyon testi (SMART) ile araştırılmıştır. Yapılan ön çalışmalar ile beş farklı uygulama grubu (saf su, 1 mM EMS, 1% RC_{su}, 3% RC_{su} ve 5% RC_{su}) hazırlanmıştır. *Drosophila melanogaster*'in *mwh/flr*^{3} genotipli 3. evre trans-heterozigot larvaları, uygulama gruplarını içeren hazır Drosophila besiyerinde kronik olarak beslenmiştir. Normal kanat fenotipli (*mwh*/*flr*^{3}) bireylerin kanat preparatları hazırlanmış ve ışık mikroskobunda incelenmiştir (400X).

Deneylerin sonucunda saf su kontrol grubu, 1 mM EMS, %1 RC_{su}, %3 RC_{su} ve %5 RC_{su} uygulama gruplarının toplam klon frekansları sırasıyla 0.15, 3.55, 2.58, 2.78 ve 2.20 olarak belirlenmiştir. Saf su kontrol grubu ve 1 mM EMS uygulama grupları arasındaki fark istatistiksel olarak önemlidir ($P<0.05$). RC_{su} uygulama gruplarından elde edilen sonuçlara göre ise, 1 mM EMS uygulama grubu ile karşılaştırıldığında her grubun toplam klon frekansı azalmıştır. 1mM EMS ve RC_{su} uygulama grupları arasındaki farkların istatistiksel olarak oldukça önemli olduğu bulunmuştur ($P<0.05$). Bulgular, *Rosa canina* L. bileşenlerinin doğal bir genomik koruyucu ürün olarak büyük bir potansiyele sahip olduğunu göstermektedir.

Anahtar kelimeler: Paraben, *Rosa canina*, SMART, *Drosophila melanogaster*, Etil metansülfonat, Genomik koruyucu

* Corresponding author. *Email address:* hauysal@atauni.edu.tr

1. INTRODUCTION

Alkylating agents appear in the same way as mutagenic and carcinogenic agents. The alkyllating agents are very powerful mutagens that lead to various types of mutations such as transition and transversion. Ethyl methanesulfonate (EMS), metyl 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 [1]. EMS leads to both point mutations and chromosomal damage [2]. It has been used as a positive control group in genotoxicity tests due to the mutagenic and carcinogenic effects.

Various natural resources have been used for medicinal purposes since the ancient times. Medical plants have always played an important role in public health [3]. Extacts of several plants are used against different effects of chemicals at the present. *Rosa canina* L. (Rosaceae) is one of the plants that is used for alternative treatment purposes. The rosehip fruits (*R. canina*) are rich in vitamin C and phenolic compound [4, 5, 6]. There are also a lot of mineral such as potassium, sodium, calcium, magnesium, iron, phosphorus, copper, zinc in the rosehip berries [7]. Therefore the rosehip plant (*R.canina*) has antioxidant properties [8, 9] and protective effects against to DNA damage [10]. Studies which used different plants as curative are available. In a study by Uysal *et al*. [11], it was observed that the methanol extract of *Echium amoenum* Fisch. and Mey (Boraginaceae) decreased the genotoxic effects of EMS. In another study, extract of *Bauhinia variegata* L. (Fabaceae) reduced the MN frequency in mice and showed an anti-mutagenic effect [12]. Furthermore, extracts of plants such as *Salvia lavandulifolia* Vahl. (Lamiaceae)[13] and *Urtica dioica* L. (Urticaceae) [14] have removed the harmful effects of free radicals.

In our study, antigenotoxicity of water extract of the rosehip fruits have been investigated on genotoxic effects of EMS using Drosophila SMART assay.

2. MATERIALS and METHODS

2. 1. Chemicals

Ethyl methanesulfonate (EMS, CAS no. 62-50-0) was purchased from Sigma (St. Louis, MO). Drosophila instand medium was obtained from Carolina Biological Supply Company (Burlington, NC).

2. 2. Plant extract

Berries of the rosehip plants were collected from natural areas at an altitude of 2000–2200m in the highlands of Erzurum, Turkey, in September 2012, during the maturation period. The plant was identified by Meryem Sengul Koseoglu (Atatürk University, Turkey). Voucher specimens are deposited in the Herbarium of Atatürk University's Faculty of Science (Erzurum, Turkey). Rosehip berries were dried in indirect light and a clean environment. Then, both the berries and the seeds in the fruit were milled with the help of a blender. The water extract of rosehips was prepared on the basis of the method applied by Halici *et al*. [15]. According to this, 100 g of the milled rosehips was placed in 200 ml of distilled water. Rosehip–water mixture was afflicted in the water-bath which was adjusted 50°C for 2 h and subsequently filtered. After the released solution was passed lyophilizator, the water extract of rosehip plant (RC_{wtr}) was obtained.

2. 3. Preparation of Application Groups

Application groups were determined according to the larval mortality. Five application groups were formed with preliminary studies (Distilled water, 1 mM EMS, 1% RC_{wtr}, 3% RC_{wtr} and 5% RC_{wtr}). Rosehip extracts were applied together with 1 mM EMS.

2. 4. Somatic Mutation and Recombination Test (SMART)

The principles and basic procedures for the *Drosophila* wing spot test have been described by Graf *et al*. [16]. In order to generate trans-heterozygous larvae, flr^3 virgin females were crossed with *mwh* males. When the larvae were 72±4h, they were placed into *Drosophila* Instant Medium containing application groups. The larvae were fed on this medium for the rest of their development. The normal phenotype wings of hatching adult flies were inspected under 400 X magnification for the presence of spots The data were evaluated according to the multiple-decision procedure of Frei and Würgler [17]. Statistical comparisons of survival rates were made by using Chi-square test for ratios for independent samples.

3. RESULTS

For SMART, it was determined 0.14 small single spots frequency, 0.01 large single spots frequency, 0.00 twin spots frequency, 0.15 total *mwh* and total spots frequencies in the distilled water negative control group. The CIF value is 0.61. In the 1 mM EMS application group, these values were found as 2.07, 1.18, 0.30, 3.25 and 3.55, respectively. The CIF value was calculated 14.55. 1 mM EMS application caused to a statistically significant increase in the frequency of all clones according to the negative control group ($P<0.05$). Small single spots, large single

spots, twin spots, total *mwh* spots and total spots values were calculated as 1.83, 0.53, 0.23, 2.35, 2.58 in EMS 1+ 1% RC_{wtr} application group, 1.80, 0.70, 0.28, 2.50, 2.78 in EMS 1+ 3% RC_{wtr} application group and 1.45, 0.58, 0.18, 2.03, 2.20 in EMS 1+ 5% RC_{wtr} application group, respectively. The CIF values were found 10.55 in EMS 1+ 1% RC_{wtr} application group, 11.37 in EMS 1+ 3% RC_{wtr} application group and 9.02 in EMS 1+ 5% RC_{wtr} application group (Table 1).

According to these results, when 1 mM EMS application group compared with RC_{wtr} application groups, reductions in the all clone frequencies were observed. The Clone frequencies with the application of rosehip extract decreased from 2.07 to 1.45 in the small single spots, from 1.18 to 0.58 in the large single spots, from 0.30 to 0.18 in the twin spots, from 3.25 to 2.03 in the total *mwh* spots, from 3.55 to 2.20 in the total spots (Table 1). All these reductions were statistically negative effect ($P<0.05$).

Table 1. SMART data obtained after EMS and EMS + RC_{wtr} treatments.

Application groups	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	80	11	(0,14)		1	(0,01)		0	(0,00)		12	(0,15)		12	(0,15)		0,61
EMS 1 mM	80	166	(2,07)	+	94	(1,18)	+	24	(0,30)	+	260	(3,25)	+	284	(3,55)	+	14,55
EMS 1 mM	80	166	(2,07)	+	94	(1,18)	+	24	(0,30)	+	260	(3,25)	+	284	(3,55)	+	14,55
EMS 1 + 1% RC_{wtr}	80	146	(1,83)	-	42	(0,53)	-	18	(0,23)	-	188	(2,35)	-	206	(2,58)	-	10,55
EMS 1 + 3% RC_{wtr}	80	144	(1,80)	-	56	(0,70)	-	22	(0,28)	-	200	(2,50)	-	222	(2,78)	-	11,37
EMS 1 + 5% RC_{wtr}	80	116	(1,45)	-	46	(0,58)	-	14	(0,18)	-	162	(2,03)	-	176	(2,20)	-	9,02

EMS:ethyl methanesulfonate; No: number of clones; Fr:frequency; D:statistical diagnosis according to Frei and Würgler (1988); +:positive; -: negative; m: multiplication factor; P=0.05

4. DISCUSSION

EMS caused to increases in the frequency of all clones in our study. These increases have confirmed that the EMS is a clearly mutagen. EMS is preferred as a positive control group in genotoxicity testing. Alkylating agents such as EMS cause both gene mutations and chromosomal damage [2]. EMS 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 [18]. Alkylating agents such as methyl

metansulfonate (MMS) and N-methyl-N-nitrosourea directly alkylate nitrogen and oxygen atoms of DNA bases [19]. Additionally, MMS forms covalent bonds with DNA and causes methylation of guanine [20]. EMS induced DNA damage in different organs on Syrian hamsters [21] and rats [22]. It was observed that EMS increased the micronucleus frequency in the mice [23], sister chromatid exchanges (SCE) and chromosomal aberrations (CA) in fish [24], rat [25] and human peripheral lymphocytes [26]. In a study by Madrigal-Bujaidar *et al*. [27], mutagen effect of MMS has been detected as *in vivo* and *in vitro* studies.

When EMS and the rosehip extract applied together, the frequency values of all clones decreased. These reductions were statistically significant ($P<0.05$). As a result of the obtained data, we can say that the rosehip water extract has reduced mutagenic effects of EMS. Several previous studies show that the antigenotoxic effects of rosehip are available. Kasımoğlu and Uysal [28] expressed that both water and ethanol extract of the rosehip removed mutagenic effects of cypermethrin and fenvalerate insecticides in the human peripheral lymphocytes. Kızılet *et al*. [29] reported that the ethanol extract of the rosehip reduced genotoxic effects of EMS in *Drosophila melanogaster*. Ascorbic acid which was abundantly found in *R. canina* decreased the genotoxic effects of mutagen compounds such as EMS, MMS, and ENU was identified [30]. In a study conducted by using the Ames test by Westhuizen *et al*. [31], *Rosa roxburghi* Tratt (chestnut rose) plant located in the same family with *R. canina* significantly reduced mutagenic effects of aflatoxin B1. We think that the antigenotoxic effects of rosehip may be associated with antioxidant capacity. Many researchers have stated that the rosehip has antioxidant activity. Tumbas *et al*. [32] have determined that there is a positive correlation between antioxidant capacity with vitamin C and phenolic compounds in the rosehip tea. 25 mg / ml of the dried rosehip extract inhibited 83.7 % lipid peroxidation *in vitro* [8]. Extracts of the rosehip reduced the formation of free radicals in the polynuclear neutrophil cells [9]. Consequently, the rosehip is an effective radical scavenging and has the healing effects. Therefore, *Rosa canina* could be used as curative in alternative medicine.

REFERENCES

1. Gocke E., Burgin H., Muller L., Pfister T., 2009. Literature review on the genotoxicity, reproductive toxicity, and carcinogenicity of ethyl methanesulfonate. Toxicol. Lett. 190, 254.
2. Doak S.H., Jenkins G.J.S., Johnson G.E., Quick E., Parry E.M., Parry J.M., 2007. Mechanistic influences for mutation induction curves following exposure to DNA-reactive carcinogens. Cancer Res. 67, 3904.

3. Anyinam C., 1995. Ecology and ethnomedicine: exploring links between current environmental crisis and indigenous medical practices. Soc. Sci. Med. 40, 321.
4. Cemeroglu B., 1992. Basic analysis methods of fruit and vegetable processing industry. Biltav Press: Ankara, 381.
5. Ozcan M., 2000. Antioxidant activity of seafennel (*Crithmum maritimum* L.) essential oil and rose (*Rosa canina*) extract on natural olive oil. Acta Aliment. Hung. 29, (4), 377.
6. Serteser A., Kargıoglu M., Gok V., Bagcı Y., Ozcan M.M., Arslan D., 2008. Determination of antioxidant effects of some plant species wild growing in Turkey. Int. J. Food Sci. Nutr. 59, (7-8), 643.
7. Hvattum E., 2002. Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. Rapid Commun. Mass Sp. 16, 655.
8. Gao X., Björk L., Trajkovski V., Uggla M., 2000. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J. Sci. Food Agr., 80, 2021–2027.
9. Daels-Rakotoarison D.A., Gressier B., Trotin F., Brunet C., Luyckx M., Dine T., Bailleul F., Cazin M., Cazin J.C., 2002. Effects of *Rosa canina* fruit extract on neutrophil respiratory burst. Phytother Res, 16, 157–161.
10. Kılıcgun H., Altıner D. 2009. *In vitro* antioxidant effect of *Rosa canina* in different antioxidant test systems. Phcog. Res. 1, 417.
11. Uysal H., Kizilet H., Ayar A. and Taheri A., 2015. The use of endemic Iranian plant, *Echium amoenum* against the ethyl methanesulfonate and the recovery of mutagenic effects, Toxicol. Ind. Health., 31, 44–51.
12. Agrawal R.C., Pandey, S., 2009. Evaluation of anticarcinogenic and antimutagenic potential of *Bauhinia variegata* extract in Swiss albino mice. Asian Pac. J. Cancer P., 10, 913-916.
13. Ünver S., Uysal H., 2014. Neonikotinoid İnsektisitlere Bağlı Olarak *Drosophila melanogaster*'in AChE Aktivitesinde Meydana Gelen Değişikliklerin Bitkisel Ekstraktlar ile Giderilmesi Üzerine Araştırmalar. Cumhuriyet Üniversitesi Fen Fakültesi Fen Bilimleri Dergisi, Cilt 35, No 4.
14. Kan Y., Orhan İ., Koca U., 2009. Fatty acid profile and antimicrobial effect of the seed oils of *Urticadioica* and *U. Pilulifera*. Turk. J. Pharm. Sci., 6, 21–30.
15. Halici M., Odabasoglu F., Suleyman H., Cakir A., Aslan A., Bayir Y., 2005. Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine, 12, 656-662.
16. Graf U., Würgler F.E., Katz A.J., Frei H., Juon H., Hall C.B., Kale P.G., 1984. Somatic mutation test in *Drosophila melanogaster*. Environ. Mol. Mutagen., 6, 153-188.
17. Frei H., Würgler F.E. 1988. Statistical methods to decide whether mutagenicity test data from Drosophila assays indicate a positive, negative, or inconclusive result. Mutat. Res. Environ. Mutagen. Relat. Subj. 203, 297.
18. Lai Y.P., Huang J., Wang L.F., Li J., Wu Z.R. 2004. A New Approach to Random Mutagenesis *in vitro*. Biotechnol. Bioeng. 86, 622.
19. Fatur T., Lah T.T., Filipi M., 2003. Cadmium inhibits repair of UV-, methyl methanesulfonate- and N-methyl-N- itrosourea-induced DNA damage in Chinese hamster ovary cells. Mutat. Res., 529 109–116.

20. Hernandez-Ceruelos A, Madrigal-Bujaidar E, Cruz C., 2002. Inhibitory effect of chamomile essential oil on the sister chromatid exchanges induced by daunorubicin and methyl methanesulfonate in mouse bone marrow. Toxicol. Lett., 135, 103–110.
21. Donovan P., Smith G., 2010. Mutagenicity of N-ethyl-Nnitrosourea, N-methyl-N-nitrosourea, methyl methanesulfonate and ethyl methanesulfonate in the developing Syrian hamster fetus. Mutat. Res-Gen. Tox. En., 699, 55.
22. Smith C.C., Adkins D.J., Martin E.A., O'donovan M.R., 2008. Recommendations for design of the rat comet assay. Mutagenesis. 23, 233.
23. Kondo K., Suzuki H., Hoshi K., Yasui H., 1989. Micronucleus test with ethyl methanesulfonate administered by intraperitoneal injection and oral gavage. Mutat. Res-Gen Tox. En. 223, 373.
24. Maddock M.L., Northrup H., Ellingham T.J., 1986. Induction of sister chromatid exchange and chromosomal aberrations in hematopoietic tissue of a marine fish following *in vivo* exposure to genotoxic carcinogens. Mutat. Res., 29, 145–147.
25. Adhikari N., Grover I.S., 1988. Genotoxic effects of same systemic pesticides: *in vivo* chromosomal aberrations in bone marrow cells in rats. Environ. Mol. Mutagen., 12, 235–242.
26. Topaktas M., Speit G., 1990. Sister chromatid Exchange (SCE) test make use of determine in the mutagen and carcinogen. Cumhuriyet Medical Journal, 5, 73–84.
27. Madrigal-Bujaidar E., Velazquez N., Morales-Ramirez P., Mendiola M.T., Lagunas A., Chamorro G., 1999. Sister-chromatid exchanges induced by disulfiram in bone marrow and spermatogonial cells of mice treated *in vivo*. Food Chem. Toxicol., 37, 757–763.
28. Kasimoglu C., Uysal H., 2015. Mutagenic biomonitoring of pirethroid insecticides in human lymphocyte cultures: Use of micronuclei as biomarkers and recovery by *Rosa canina* extracts of mutagenic effects. Pharm. Biol., 53, 625–629.
29. Kızılet, H., Kasimoğlu, C., Uysal, H., 2013. Can the *Rosa canina* plant be used against alkylating agents as a radical scavenger? Pol. J. Environ. Stud., 22, 1263-1267.
30. Kaya, B., 2003. Anti-genotoxic effect of ascorbic acid on mutagenic dose of three alkylating agents. Turk J. Biol., 27, 241-246.
31. Westhuizen F.H., Rensburg C.S., Rautenbach G.S., Marnewick J.L., Loots T., Huysamen C., Louw R., Pretorius P.J., Erasmus E., 2008. *In vitro* antioksidant, antimutagenic and genoprotective activity of *Rosa roxburghii* fruit extract. Phytother Res, 22, 376-383.
32. Tumbas V.T., Čanadanovic-Brunet J.M., Cetojevic-Simin D.D., Cetkovic G.S., Dilas S.M., Gille L., 2012. Effect of rosehip (*Rosa canina* L.) phytochemicals on stable free radicals and human cancer cells. J. Sci. Food. Agric., 92, 1273–1281.