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Research Article

Genotoxic and Antigenotoxic Effects of Some Plant Species of Lamiaceae Family[1]

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Abstract

Lamiaceae family is one of the members of important plants, using as a agents for alternative medicine treatments. Our aim was to investigate both antigenotoxic and genotoxic effects three different plants species belonging to Lamiaceae family: *Stachys annua, Scutellaria salviifolia, Nepeta nuda* in Amasya, using SMART (*Drosophila melanogaster* somatic mutation and recombination test) and also plasmid DNA interaction method, respectively. SMART analyses revealed that genotoxic effect of ethyl methanesulfonate (EMS) was eliminated by plant extract of *S. salviifolia*, indicating antigenotoxic effects. Different extract concentrations [(0.1, 0.25, 0.50 and 1 % (v/v)] of other plants species decrased the flecks number on *D. melanogaster* but the results did not show any statistical significance ($p>0.05$). In addition to SMART, interaction with pUC18 plasmid DNA was also analysed by using all concentrations (50 µg/mL-3.125 µg/mL) of plant extracts *N. nuda* and *S. annua.* The extracts did not cause changed in Form I DNA (supercoil) but trasferred from Form II DNA (open circular) to Form III DNA. Moreover, there was no effect of *S. salviifolia* plant extracts on DNA and band density. SMART results were supported by DNA interaction analyses, cocncluding that *S. salviifolia* plants indicated the high-impact antigenotoxic effect, but not genotoxic effects.

Key words: Lamiaceae, *Drosophila melanogaster*, plasmid DNA

1. Introduction

Biologically active extracts and compounds from medicinal and aromatic plants have commonly been studied to discover new sources to treat various animal and human diseases (Essawi and Srour, 2000; Kianbakht and Jahaniani, 2003; Chung et al., 2004; Ünal et al., 2008; Kunduhoglu et al., 2011). Lamiaceae also called as the mint family is a family of flowering plants with more than 3000 species, spreading in the warm and temperate region all over the world. Turkey is one of the important gene centers for Lamiaceae family (Baser, 1993; Sarac and Ugur, 2007). *Stachys annua, Scutellaria salviifolia* and *Nepata nuda* species belonging to Lamiaceae family which is one of the largest families of flowering plants are widely used in folk medicine of many nations (Si et al., 2006; Nemeth and Bernath, 2008).

Different inhibitos and also supressors used as a mutagens and carcinogens have been commonly investigaed in plants (Bhattacharya, 2011) and even plant extracts (Skandrani et al., 2007; Boubaker et al., 2010; Donya and Nancy, 2012). For this purpose, different methods have also been developed. One of them, SMART (somatic mutation and recombination test), uses *Drosophila melanogaster* with experimental accessible model organism to examine toxicity and genotoxicity *in vivo* because of extensive knowledge on its genetics and genomics, high homology to human genes and its short lifespan (Ong et al., 2014).

Another method used in toxicity studies is DNA interaction. In this procedure, the interaction of a compound with DNA might depend on the binding/cleavage properties with the nucleobases of DNA, resulting in changes to the DNA conformation. These changes in DNA conformation can play an important role in the rate of migration of DNA in an electric field (Legin et al., 2014). Agarose gel electrophoresis has been commonly to show the conformational change and damage caused to plasmid DNA. The plasmid DNA is found in three different forms: supercoiled circular form I, singly nicked relaxed circular form II, and doubly nicked linear form III. Untreated plasmid DNA migrates on the gel with two DNA

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bands, which form I migrating faster and form II migrating slower. Form III takes place in the middle when plasmid DNA restricted with the enzyme or damage. Interactions with compounds may cause conformational changes on the plamid DNA, and in mobility of DNA through gel (Mitchell and Teh, 2005; Enjun et al., 2009).

In this study, aim was to screen selected Turkish medicinal plants for their biological activity. Extracts were obtained from the flowers, leaves and stems of three different plant species (*Stachys annua*, *Scutellaria salviifolia*, *Nepeta nuda*) distributed to different ecological conditions in Amasya to analyse biological effects via two different test: SMART and DNA interaction properties using pUC18 plasmid DNA interaction method.

2. MATERIAL and METHOD

2.1. Plants material

The plant species were selected based on their traditional uses reported in available literature (Baytop, 1999). *Stachys annua*, *Scutellaria salviifolia* and *Nepata nuda* of Lamiaceae family were collected (between April and May 2015) from the Amasya region of Turkey and leaves, stems and flowers were used for further studies.

2.2. Plants Extract

200 mg of dried and pulverized each plants were extracted with 30 mL of the extraction solvent mixture. After filtration through a 0.45 µm membrane filter and washing of the residue with 20 mL 80% methanol each extract was evaporated under reduced pressure (Salem et al., 2013). The evaporated extract was dissolved in dimethyl sulfoxide (DMSO) (Sigma, Steinheim, Germany) to a final concentration of 50 mg/mL for each plant extract.

2.3. SMART Analyses

Drosophila somatic mutation and recombination test (SMART) was used for analysing the antagonistic effects. This test is based on the investigations of Drosophila wing spot (Graf et al., 1984). For this purpose, *flr*^{3} virgin females were crossed with *mwh* males to generate transheterozygous larvae. Then, eggs were collected optimally at 8-h periods, storing in culture bottles containing Drosophila Instant Medium (DIM). After 72 + 4 h, they were placed into a glass tube containing 1.5 g dry DIM prepared with 5 mL of the test solutions (1 ppm EMS, EMS + plant extracts) at different concentrations (0.1, 0.25, 0.50 and 1%), distilled water and 1% DMSO for the negative controls. The larvae were fed on this medium for the rest of their development. All the experiments were conducted at a temperature of 25 + 1ºC and at a relative humidity of 60–70%.

The hatching adult flies were collected from vials containing 70% ethanol. The wings were removed and mounted in Faure's solution on microscope slides and inspected under 400X magnification for the presence of spots. On marker-heterozygous wings (*mwh*/*flr*^{3}), it is possible to record three different categories of spots: (i) small single spots (SSSs, 1–two cells in size), (ii) large single spots (LSSs, more than two cells), expressing either the multiple wing hairs (*mwh*) or the flare (*flr*^{3}) phenotype and (iii) twin spots (TSs), consisting of both mwh and *flr*^{3} subclones. On balancer heterozygous wings (mwh/TM3), only mwh single spots can be observed, as the inverted TM3 balancer chromosome does not carry *flr*^{3} or any other suitable marker mutation. In the *mwh*/*flr*^{3} flies, mutant clones can be originated by somatic point mutation, chromosome aberration and/or mitotic recombination, whereas in the mwh/TM3 genotype, these last recombinogenic events are suppressed due to the presence of multiple inversions in the TM3 balancer chromosome (Frei et al., 1992; Amkiss et al., 2013; Uysal et al., 2015). All the experiments have been repeated three times and the averages of the the three data points obtained have been reported.

2.4. DNA interaction analysis

DNA interaction analysis were performed by using pUC18 plasmid DNA and plant extracts. Firstly, plants extracts were dissolved in DMSO (final concentration % 0.1) and then diluted with TE (Tris-EDTA) buffer (50 µg /mL-3.125 µg/mL). Later, samples were immediately mixed with 0.03 µg/µL pUC18 plasmid DNA (Thermo Scientific) and 2 µl loading buffer (0.1 % bromophenol blue and 0.1 % xylene cyanol) in 10 µl total volume. After incubating 37 ºC for 24 hours, samples and 0.05 µg/µL marker DNA were loaded onto the 1 % agarose gel. Untreated and 0.1 % DMSO treated plasmid DNAs were used as control. Electrophoresis was carried out for 1 hours at 100 V with 1 x TBE (Tris-Boric acid-EDTA) buffer. After running the gel, it was stained with 0.5 µg/L ethidium bromide. Gel was viewed using imaging system (Vilber Lourmat Fusion FX5).

3. Results and Discussion

We analysed the genotoxic and antigenotoxic effects of *Stachys annua*, *Scutellaria salviifolia*, *Nepeta nuda* plants by using SMART and DNA interaction methods. When SMART data was investigated with *D. melanogaster*, genotoxic effect of ethyl methanesulfonate (EMS) was largely eliminated especially by plant extract of *S. salviifolia* and indicated antigenotoxic effects. Although different concentration [(0.1, 0.25, 0.50 and 1 % (v/v)] other plants species reduces flecks number on *Drosophila* but no statistical significance was determined ($p>0.05$) (Table 1-3).

SMART methods have been widely used for antigenotoxicity effects. In these studies, they revealed different results, depending on the concentrations, plants, and even plant parts. Furthermore, diffeent plants (*Foeniculum vulgare* and *Echium amoenum*) were also analysed in terms of genotoxic effects via SMART (Amkiss et al., 2013). In addition to plants, antigenotoxicity of different compounds have been also studied (Ayar and Uysal, 2013; Avalos et al., 2015; Orsolin et al., 2015).

In addition to SMART analysis, we also investigated DNA interactions. According to the results of method, the extracts have resulted in the cleavage of the plasmid DNA hence converting the plasmid form I to either form II and/or form III. From this assay, it has been found that at higher concentrations (50 µg/ml-3.125 µg/ml) extracts partially binds to the plasmid DNA. Interaction with pUC18 plasmid DNA was determined with all concentrations (50 µg/mL-3.125 µg/mL) of plant extracts *N. nuda* and *S. annua* (Figure 1). The extracts did not cause changed in Form I DNA (supercoil) but trasferred from Form II DNA (open circular) to Form III DNA. Moreover, there was no effect of *S. salviifolia* plant extracts on DNA and band density (data not shown).

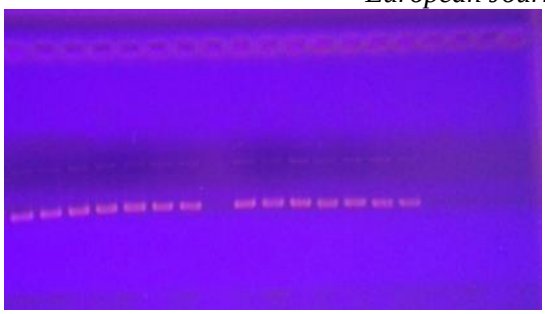

Figure 1. Gel image of extracts of *Nepata nuda* and *Stachys annua*.

1. Lane: DMSO control, 2. Lane: TE control, 3-7. Lane: 1-5: 50 µg/mL-3.125 µg/mL extracts added plasmid, 9. Lane: DMSO control, 10. Lane: TE control, 11-15. Lane: 50 µg/mL-3.125 µg/mL extracts added plasmid, respectively.

Similiar to SMART analyses, genotoxic potential of plants could be different because of *in vivo* and *in vitro* environmental factors. In addition, the concentration of plant extracts might be also an important parameter (Soares et al., 2014). DNA interaction methods has been commonly used for different plant extracts to protect supercoiled plasmid DNA against the deleterious effects of hydroxyl radicals, by using the structural conformation of plasmids (De Mattos et al., 2000; Lee et al., 2002; Kumar et al., 2010). In addition to plants, other compounds have also been tested to investigate genotoxicity via this method (Asmafiliz et al., 2013; Mutlu et al., 2015).

Table 1. SMART results of different concentrations of S.annua plant extracts + EMS application

Treatments	**Number of wings**	**Small single spot (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)***		
		No	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***
Control (water)	80	12	(0.15)		2	(0,03)		1	(0,01)		14	(0,18)		15	(0,19)	
1mM EMS	80	108	(1.35)	+	31	(0,39)	+	17	(0,21)	+	149	(1,86)	+	156	(1,95)	+
***Stachys annua* (%)**																
0,10	80	10	(0.13)	-	2	(0.03)	i	0	(0.00)	i	12	(0.15)	-	12	(0.15)	-
0,25	80	11	(0.14)	-	2	(0.03)	i	2	(0.03)	i	15	(0.19)	i	15	(0.19)	i
0,50	80	14	(0.18)	i	4	(0.05)	i	2	(0.03)	i	18	(0.23)	i	20	(0.25)	i
1	80	14	(0.18)	i	6	(0.08)	i	1	(0.01)	i	20	(0.25)	i	21	(0.26)	i
0,10SA+EMS	80	98	(1.23)	+	26	(0.33)	+	15	(0.19)	+	120	(1.50)	+	129	(1.61)	+
0,25SA+EMS	80	90	(1.13)	+	20	(0.25)	+	11	(0.14)	+	118	(1.48)	+	121	(1.51)	+
0,50SA+EMS	80	50	(0.63)	+	11	(0.14)	i	4	(0.05)	i	60	(0.75)	+	65	(0.81)	+
1SA+EMS	80	45	(0.56)	+	9	(0.11)	i	4	(0.05)	i	50	(0.63)	+	58	(0.72)	+

EMS: Ethyl methanesulfonate; SA: *Stachys annua*, No: number of clones; Fr: frequency; D: statistical diagnosis according to Frei and Wurgler (1995); +: positive; -: negative; i: insignificant difference; m: multiplication factor. *p=0.05*

Table 2. SMART results of different concentrations of S. salviifolia plant extracts + EMS application

Treatments	**Number of wings**	**Small single spot (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)***		
		No	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***
Control (water)	80	12	(0.15)		2	(0.03)		1	(0.01)		14	(0.18)		15	(0.19)	
1mM EMS	80	108	(1.35)	+	31	(0.39)	+	17	(0.21)	+	149	(1.86)	+	156	(1.95)	+
***Scutellaria salviifolia* (%)**																
0,10	80	9	(0.11)	-	1	(0.01)	i	0	(0.00)	i	10	(0.13)	-	10	(0.13)	-
0,25	80	8	(0.10)	-	1	(0.01)	i	1	(0.01)	i	10	(0.13)	-	10	(0.13)	-
0,50	80	12	(0.15)	i	2	(0.03)	i	1	(0.01)	i	15	(0.19)	i	15	(0.19)	i
1	80	11	(0.14)	i	3	(0.04)	i	0	(0.00)	i	14	(0.18)	i	14	(0.18)	i
0,10SS+EMS	80	91	(1.14)	+	18	(0.23)	+	9	(0.11)	i	110	(1.38)	+	118	(1.48)	+
0,25SS+EMS	80	51	(0.64)	+	9	(0.11)	i	3	(0.05)	i	60	(0.75)	+	63	(0.79)	+
0,50SS+EMS	80	47	(0.59)	+	6	(0.08)	i	4	(0.05)	i	50	(0.63)	+	57	(0.71)	+
1SS+EMS	80	16	(0.20)	i	4	(0.05)	i	2	(0.03)	i	22	(0.28)	i	22	(0.28)	i

EMS: Ethyl methanesulfonate; SA: *Stachys annua*, No: number of clones; Fr: frequency; D: statistical diagnosis according to Frei and Wurgler (1995); +: positive; -: negative; i: insignificant difference; m: multiplication factor. *p=0.05*

Table 3. SMART results of different concentrations of Nepeta nuda plant extracts + EMS application

Treatments	Number of wings	Small single spot (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)*		
		No	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***	**No**	**Fr.**	***D***
Control (water)	80	12	(0.15)		2	(0.03)		1	(0.01)		14	(0.18)		15	(0.19)	
1mM EMS	80	108	(1.35)	+	31	(0.39)	+	17	(0.21)	+	149	(1.86)	+	156	(1.95)	+
***Nepeta nuda* (%)**																
0,10	80	9	(0.11)	-	2	(0.03)	i	1	(0.01)	i	12	(0.15)	-	12	(0.15)	-
0,25	80	13	(0.16)	i	0	(0.00)	-	1	(0.01)	i	14	(0.18)	i	14	(0.18)	i
0,50	80	18	(0.23)	i	4	(0.05)	i	2	(0.03)	i	20	(0.25)	i	24	(0.30)	i
1	80	20	(0.25)	i	3	(0.04)	i	2	(0.03)	i	23	(0.29)	i	25	(0.31)	i
0,10NN+EMS	80	100	(1.25)	+	29	(0.36)	+	18	(0.22)	+	140	(1.75)	+	147	(1.84)	+
0,25NN+EMS	80	98	(1.22)	+	27	(0.34)	+	15	(0.19)	+	135	(1.69)	+	140	(1.75)	+
0,50NN+EMS	80	91	(1.14)	+	27	(0.34)	+	17	(0.21)	+	129	(1.61)	+	135	(1.69)	+
1NN+EMS	80	88	(1.10)	+	20	(0.25)	+	10	(0.12)	i	110	(1.38)	+	118	(1.48)	+

EMS: Ethyl methanesulfonate; NN: Nepeta nuda, No: number of clones; Fr: frequency; D: statistical diagnosis according to Frei and Wurgler (1995); +: positive; -: negative; i: insignificant difference; m: multiplication factor. *p=0.05*

4. Conclusions

This study showed that *Scutellaria salviifolia*, *Stachys annua* and *Nepata nuda* species belonging to the Lamiaceae family have *in vitro* biological activities. Especially *S. salviifolia* plants showed the high-impact antigenotoxic effect on *D. melanogaster*. Moreover, only *N. nuda* and *S. annua* had genotoxic effects on plasmid DNA. After additional studies should be performed to analyse toxicological and pharmacological properties.

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