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Assessment of anti-cytotoxic, anti-genotoxic and antioxidant potentials of Bulgarian *Rosa alba* L. essential oil

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Abstract. Bulgarian *Rosa alba* L. essential oil is widely used in perfumery, cosmetics and pharmacy. The scarce data about its cytotoxic/genotoxic effect and anti-cytotoxic/anti-genotoxic potential gave us a reason to set our aim: i) to study its cytotoxic/genotoxic activities, iii) to explore its cytoprotective/genoprotective potential against the experimental mutagen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in two experimental test-systems - barley and human lymphocytes using appropriate endpoints and iii) to assess its antioxidant properties. Findings about chemical composition of rose essential oil would help us to explain its activities. Chromatogaphic profile of rose essential oil was obtained by Gas Chromatography-Mass Spectrometry and quantificaton of particular constituents was done with a Gas Chromatography-FID system. Superoxide anion radical scavenging, DPPH inhibition and iron ion chelating activity were used to study a possible antioxidant potential of the rose oil. Its defense potential was investigated by induction of chromosome aberrations and micronuclei in both test-systems. Cytogenetic analysis showed a low cytotoxic effect in both test-systems and no high genotoxic effect in human lymphocytes *in vitro* in a dose-dependent manner. Rose oil possessed a well-expressed anti-cytotoxic/anti-genotoxic potential against MNNG manifested by decreasing both of chromosome aberrations and micronuclei regardless of the experimental schemes used. A well-expressed concentration-depended free radical scavenging activity of the essential oil was obtained. Current data suggest a promising ethnopharmacological potential of Bulgarian white rose essential oil.

Keywords: *Rosa alba* L. essential oil, rose oil components, genotoxicity, anti-cytotoxicity, anti-genotoxicity, antioxidant effect.

INTRODUCTION

As a general rule, living organisms exist in conditions of continuous attack by various environmental pollutants such as alkylating agents, oxidative stress inducers, etc. As a result serious alteration in the main hereditary molecule DNA could be induced. There is a constant need to obtain products, which could decrease or eliminate the harmful effects of the genotoxins. Plants are known as a rich source of various bioactive natural compounds, which are widely used in various areas of human life. A cursory look at the literature cited in relation to plants' essential oils in recent years indicates that there is a growing interest in evaluation of the biological activities of various extracts of essential plants, their antimutagenic and antigenotoxic potential (Blasiak *et al.* 2002; Mezzoug *et al.* 2007; Bakkali *et al.* 2008; Vicuña *et al.* 2010; Siddique *et al.* 2010; Arumugam *et al.* 2010; Leffa *et al.* 2012; Gokbulut *et al.* 2013; Madrigal-Santillán *et al.* 2013; Oyeyemia and Bakare 2013; Reddy and Devi 2014; Shohayeb *et al.* 2014; Laribi *et al.* 2015), and presuming their role in the prevention of degenerative diseases and other human ailments including cancer (Hajhashemi *et al.* 2002; Raut and Karuppayil 2014; Horváth and Ács 2015). The genus Rosa is one of the largest and most important aromatic and medicinal genera of the Rosaceae family. Numerous rose phytocomplexes, including essential oils isolated from *Rosa damascena* Mill., *Rosa x centifolia* L., *Rosa gallica* L., *Rosa alba* L. and *Rosa rugosa* Thunb. have been identified and used for therapeutic purposes as well (Rangaha 2001; Degraf 2003; Moein *et al.* 2010). The rose extracts help in the reduction of thirst, healing of old cough, special complaints of women, abdominal and chest pain, digestive problems and show skin health effects (Tabrizi *et al.* 2003; Boskabady *et al.* 2006; EMA/HMPC 2013). The rose essential oils and by-products of *Rosa damascena*, from Shafaa, Taif, Saudi Arabia (Shohayeb *et al.* 2014) and from Amman and Ajloun areas, Jordan (Talib and Mahasneh 2010) have antimicrobial activity against various Gram-positive, Gram-negative, acid-fast bacteria and fungi. Absolute oil of *Rosa damascena trigintipetala* Dieck has an antimutagenic activity against mitomycin C in normal human blood lymphocytes (Hagag *et al.* 2014). Methanolic and aqueous extracts of *Rosa damascena* white variety from Iran show better anti-radical activity than some synthetic antioxidants (Kashani *et al.* 2011). Unfortunately, little is known about single- and repeat-dose cytotoxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, local tolerance or other special studies of preparations from rosae flos in animals and humans according to current state-of-the-art standards (Hagag *et al.* 2014).

Rosa alba L. is the second most important plant for Bulgarian rose production. Kovatcheva *et al.* (2011) and Dobreva (2010) demonstrated that Bulgarian *Rosa alba* L. has a similar oil composition but some of compounds are with lower content to that of *Rosa damascena* Mill. collected from Bulgaria. *Rosa alba* L. essential oil, known as Bulgarian rose oil of white rose, has been defined as "original, exclusively fine, only suitable for the highest perfumery" (Degraf 2003). Fukada *et al.* (2011) found that in an experimental model of acute stress in rats, inhalation with *Rosa alba* L. essential oil (supplied by Kanebo Cosmetics) lowered corticosterone levels almost twice. Water extract of calyces of *Rosa alba* from India might be a useful memory restorative agent in the treatment of cognitive disorders (Naikwade *et al.* 2009). Our previous study indicated well-expressed antimicrobial activities of Bulgarian *Rosa alba* L. essential oil (Mileva *et al.* 2014). Insufficient data exist about cytotoxic/genotoxic effect and anti-cytotoxic/anti-genotoxic potential of essential oil from Bulgarian *Rosa alba* L. This gave us a reason to set our aim in the present paper: i) to study cytotoxic and genotoxic activities of Bulgarian *Rosa alba* L. essential oil, ii) to explore its cytoprotective and genoprotective potential against well known experimental mutagen – alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in two types of widely used experimental test-systems (higher plant and human lymphocytes *in vitro*) using appropriate for this purpose endpoints, and iii) to assess its antioxidant properties. Phytochemical analysis of rose essential oil would help us to explain its cytotoxic/genotoxic and anti-cytotoxic/anti-genotoxic effects.

MATERIAL AND METHODS

Chemicals Used

All chemicals, standards and solvents used for analysis of rose essential oil (GC-MS, GC-FID), methods for testing of the antioxidant activity and cytogenetic analysis were of high purity (>99%). Tetracosane [646-31-1] GA14075, EC 2114745, free puriss. p.a. > 99.5 % (GC) used as a reference compound in Predicted Relative Response Factors calculations was purchased from Fluka, USA. Nitroblue tetrazolium (NBT), xanthine, α-tocopherol, butylated hydroxytoluene (BHT), ascorbic acid, ferrous chloride, ferrozine, EDTA, dimetylsulfoxyde (DMSO) used for examination of antioxidant properties and the chemicals used for cell cultivation and cytogenetical analysis (RPMI 1640, bovine serum, phytohaemagglutinin PHA, Giemsa) were purchased from Sigma-Aldrich, Germany. α-bromonaphthaline,

colchicine, KCL were purchased from Merck, Germany, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) from Fluka – AG, Switzerland and Schiff's reagent from Riedel-De Haen AG, Germany.

Plant material and distillation of Rosa alba L. essential oil

Fresh flowers of *Rosa alba* L., from the experimental field of the Institute of Rose and Essential Oil Plants (IREOP), in Kazanlak, Bulgaria were used as raw material in the study. The flowers were picked up in the mornings in May/June 2009, from 8 to 10 am, in a phase of semi-blooming – blooming. Roses were distilled immediately by water-steam distillation using IREOP's semi-industrial processing line. Process parameters of the distillation were as follows: a raw material for charge – 10 kg; hydro module 1:4, rate of 8-10% and duration – 150 min. The aromatic water was re-distilled in the same apparatus. The essential oil, obtained of each charge is the sum of primary and secondary oil in their natural ratio. Total oil is a mixture of distillates collected over 15 days – the time of the collection campaign of *Rosa alba* L. for 2009. Finally, it was dried with sodium sulfate (Merck, Germany), filtered and stored at 4 °C for further use.

The concentration of the main compounds as well as the physicochemical parameters and characteristics of the rose essential oil are controlled through implementation of national (BDS ISO9842:2006) and international Standards (ISO 9842:2003, www.iso.org) only for oil obtained from *Rosa damascena* Mill. So, in our study we used the essential oil from *Rosa damascena* Mill. cultivated in Bulgaria, Kazanlak as one of the controls in the tests for antioxidant activities.

Gas Chromatography-Mass Spectrometry (GC-MS)

Chromatogaphic profile of rose essential oil was obtained by Gas Chromatography-Mass Spectrometry. GC-MS analysis was carried out on a HP 6890 "PLUS" gas chromatograph interfaced with a 5975 mass selective detector. Separations were performed using a HP-5MS silica-fused capillary column – 30 m × 0.25 mm coated with 0.25 μm film of (5%-phenyl)- methylpolysiloxane as the stationary phase (Agilent Technologies, USA). The flow rate of carrier gas (helium) was maintained at 0.8 ml/min. The injector and the transfer line temperature were kept at 250 and 300 °C respectively. The oven temperature program used was 60 °C for 2 min then 3 °C/min to 300 °C for 8 min, total run time - 90 min. The injections were carried out in a split mode with a split ratio of 25:1. The mass spectrometer was scanned from 30 to 550 m/z. The injection volume was 1 μl.

The quantitative analysis was carried out on a HP 5890 "SERIES II" gas chromatograph equipped with a FID detection system. We analysed the same sample and the separation was performed at the same chromatographic conditions, column, carrier gas and temperature program. GC-FID – eluted constituents were identified on the basis of a Kovats Retention Index (RI), determined with reference to a homologous series of *n*-alkanes (C10-C28), under identical experimental conditions. GC-MS – eluted constituents were identified using MS Library search (NIST version 2.1), as well as by comparison with the fragmentation pattern of the mass spectra with data published in the literature (Adams 2007). For each identified constituent, from GC-MS analysis was obtained its Kovats RI. Every particular value for these indices was confirmed by the literature. The differences between the measured and published Kovats RI exceeding 10 units were not reported, except these for *n*-alcanes and a few unsaturated hydrocarbons. The percentage compositions of *Rosa alba* L. essential oil were determined from the GC-FID peak's areas corrected with Predicted Relative Response Factors for the constituents calcutated by formula given by Tissot *et al.* (2012).

Examination of antioxidant properties

Superoxide scavenging properties

The generation of superoxide anion radical $O_2^{\bullet -}$ in the model system xanthine-xanthine oxidase (XO) and the changes occurring upon the *Rosa alba* L. oil effect were investigated by the nitroblue tetrazolium (NBT) test. The detailed procedure was described elsewhere (Mileva *et al.* 2000). Briefly, the spectrophotometric registration of superoxide was carried out measuring the amount of formazan generated by $O_2^{\bullet -}$ induced reduction of NBT. The investigated samples of a volume 1 ml PBS contained: 1 mmol/l xanthine, 2.10^{-3} IU XO, 0.04 mmol/1 NBT, as well as oil at concentrations from 0 to 100 μg/ml. Samples were incubated at 37 °C and the amount of the formed formazan was measured by absorption at 560 nm. The time of incubation was selected so that the absorption for the controls was 0.2. The decrease of absorbance in the presence of oil indicated the consumption of superoxide anion in the reaction mixture. Data were calculated in percentage as spectrophotometric scavenger index (SpSI) - the ratio of the absorption at 560 nm for the sample with oil, and the same absorption for the controls (without oil). The scavenger activity of oil was compared with that of α-tocopherol – commonly used as superoxide radical scavenger.

DPPH Test

Hydrogen atoms and electron-donating potential of essential oils were measured from the bleaching of the purple-colored ethanol solution of DPPH (Sigma-Aldrich, Germany). All compounds were dissolved in ethanol to a concentration of 100 mg/ml stock solutions for the follow dilutions. DPPH assay was performed as follows: freshly prepared ethanolic solution of DPPH (100 mM) was incubated with tested substances in the concentration of 10 to 100 μg/ml, and the absorbance (A) monitored spectrophotometrically at 517 nm after 30 min incubation in dark at room temperature. Inhibition of DPPH in percentage (DPPH inhibition, %) was calculated as given below:

DPPH inhibition (%) = [(A control - A sample)/(A control)] X 100

BHT and ascorbic acid served as positive controls. Each experiment was carried out in triplicate and data were presented as a mean of the three values (Singh *et al.* 2008).

Iron binding capacity of essential oil

Metal chelating activity of *Rosa alba* L. essential oil on ferrous ions was determined according to the method of Decker and Welch (1990). The percentage of inhibition of ferrozine - Fe^{2+} complex formation was calculated using the formula:

Chelating activity (%) = [(A control – A sample)/A control] × 100

Where A control is the absorbance of the ferrozine - Fe^{2+} complex, and A sample is the absorbance of essential oil. As a positive control EDTA was used.

All results for antioxidant properties are presented as mean ± SD, and compared against routinely used reference positive controls. The data were collected from three independent experiments with three parallel measurements for each experiment.

Analysis of cytotoxic/anti-cytotoxic and genotoxic/anti-genotoxic effects of Rosa alba L. essential oil

Rose essential oil and chemicals preparation

Rose essential oil was dissolved in 1% dimetylsulfoxyde (DMSO). A standard well-known experimental mutagen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) 50 μg/ml was used as DNA damage agent in the chromosome aberrations and micronucleus assays. MNNG was dissolved in bidistilled water.

Test-systems

Two types of experimental test-systems were used – *Hordeum vulgare* (barley) and human lymphocytes *in vitro*. Barley seed meristems and human lymphocyte cultures preparation as well as the schemes of treatment are given below.

Hordeum vulgare (barley) meristem cells as a test-system. Seeds of reconstructed karyotype of *Hordeum vulgare* MK14/2034, 2n = 14 (Künzel and Nicoloff 1979) presoaked for 1 hour in tap water were germinated for 18h in Petri dishes on moist filter paper at 24°C. Well-synchronized seeds with a root meristem size of 1-2 mm were selected for further treatment.

Experimental designs used for cytogenetic analysis. Three types of experimental schemes were applied. First to assess cytotoxic/genotoxic effects of rose essential oil, whole germinated seeds of barley with root meristems were treated with essential oil in concentrations from 250 to 1000 μg/ml. To assess the protective potential of rose oil germinated seeds were conditioning treated with 250 and/or 500 μg/ml for 60 min followed by 4h inter-treatment time and subsequent challenge treatment with 50 μg/ml MNNG (60 min). Third part of germinated seeds was pretreated with 250 and/or 500 μg/ml of rose oil (60 min), followed immediately by 50 μg/ml MNNG (60 min) without any inter-treatment time. For chromosome aberrations evaluation the germinated seeds were treated at 24 °C for 2 hours with 0.025% colchicine in a saturated solution of α-bromonaphthaline after the treatment and recovery times for 18, 21, 24, 27 and 30 hours. The extracted embryos were fixed in a mixture of ethanol and acetic acid (3:1), hydrolyzed in 1N HCl at 60°C for 9 min and stained with Schiff's reagent at room temperature for 1h. The root tips were macerated in a 4% pectinase solution for 12 min and squashed onto slides for scoring of metaphases with chromatid aberrations. For scoring of micronuclei, the root tips were fixed after 30h recovery time without colchicine treatment.

Human lymphocytes in vitro as a test-system. Lymphocyte cultures ($1x10^6$ mol/l) were prepared from venous blood of three healthy nonsmoking/nondrinking donors (men and women) aged between 33 to 50 years according to the standard method of Evans (1984). Each

culture contained 3.5 ml RPMI 1640 medium, heat-inactivated fetal bovine serum (20%), phytohemagglutinin PHA (0.1%) and 40 mg/ml gentamycin (Pharmacia, Bulgaria). The study complies with the Declaration of Helsinki. Voluntary written informed consent was taken from all study participants.

Experimental designs used for cytogenetic analysis. Lymphocyte cultures were treated with rose oil in a range of concentrations from 50 to 500 μg/ml to assess cytotoxic and genotoxic effect of *Rosa alba* L. essential oil. To study its protective potential, non/or low cytotoxic concentrations of rose essential oil - 50 and/or 200 μg/ml were applied as a conditioning treatment (60 min) followed by 4 hours inter-treatment time and a challenge treatment (60 min) with 50 μg/ml MNNG. Another part of the lymphocyte cultures was pretreated with 50 and/or 200 μg/ml of rose oil (60 min), followed immediately by MNNG 50 μg/ml (60 min) without any inter-treatment time. After each treatment the lymphocyte cells were washed with a fresh RPMI medium. Untreated cells were used as a negative control. At the 72nd hour of cultivation to each culture was added 0.02% colchicine, then the cells were hypotonized in 0.56 % KCl; afterwards fixed in a mixture of methanol: glacial acetic acid (3:1, v/v) and stained in 2% Giemsa for assessment of chromosome aberrations. Lymphocytes were directly fixed without colchicine for assessment of micronuclei.

*Cytogenetic analysis***.** Cytotoxicity of Bulgarian *Rosa alba* L. essential oil for both test-systems mentioned above was assessed by mitotic index (MI) using a formula: MI‰=A/1000, where A is a number of dividing cells.

Genotoxic effect was evaluated by chromosome aberrations (CA) and micronuclei (MN) induction. Percentage of metaphases with chromosome aberrations (MwA % ± SD) was calculated. 1000 well spread metaphases (in M1 mitosis) of each treatment variant in both test-systems were assessed. Chromatid breaks (B'), isochromatid breaks (B"), translocations (T), intercalary deletions (D), duplication-deletions (DD) and dicentrics (DC) were determined.

"Aberration hot spots" in the plant chromosomes were determined to obtain information about the DNA segments with higher susceptibility to DNA damage. For analyzing the locus specificity of aberration induction the metaphase chromosomes of *H. vulgare* were subdivided into 48 segments of approximately equal sizes. The segments are numbered with respect to their position in the standard karyotype as described earlier by Künzel and Nicoloff (1979).

Percentage of micronuclei was calculated (MN % ± SD) where 4000 nuclei per experiment and treatment variant were assessed.

Data analysis

The results were calculated statistically by Student's t-test and chi-square method. All experiments were repeated three times. An adapted formula was used for comparison of the upper limit of the confidence interval of the expected and observed chromatid aberrations in individual loci and evaluation of aberration „hot spots" in barley (Rieger *et al.* 1975; Künzel and Nicoloff 1979; Jovtchev *et al.* 2010). For multiple comparisons, a one-way analysis of variance (ANOVA) was employed, followed by Bonferroni`s correction.

RESULTS

Chromatogaphic profile of Rosa alba L. essential oil

The chromatographic composition of the essential oil of Bulgarian *Rosa alba* L. is presented in Table 1. The values were compared with literature data. The main groups are aliphatic hydrocarbons (AH) – 40.92% with high molecular weight (C15 – C27) as heneicosane (12.75%), tricosane (2.69%), eicosane (1.46%), pentacosane (1.0%), heptacosane – 0.86%, (Z)-3-heneicosene-0.64%, etc., followed by oxygenated monoterpenes (OM) – 40.35% as citronellol – 17.69 %, geraniol –16.64 %, trans-citral – 2.12%, linalool – 1.37%, β-citral – 0.68%, etc., sesquiterpenes hydrocarbons (SH) – 5.68% as caryophyllene – 1.52%, α-muurolene – 0.41%, b-cubebene – 0.14%, etc., and oxygenated sesquiterpenes (OS) – 1.72%.

The remaining 11.27% of the constituents were not reported due to their low content, less than 0.05% by mass, and/or not sufficiently reliably identification. After squalene some constituents leaving the column were in fact overlay with the column bleed and were also discarded from the final results.

Superoxide scavenging analysis of Rosa alba L. essential oil

The decrease of absorbance at 560 nm in semples with antioxidant supplements indicates the consumption of superoxide anion radical in the reaction mixture. As can be seen from Figure 1, *Rosa alba* L. essential oil exhibits moderate activity in superoxide scavenging assay in concentration range of 0–500 μg/ml. It is sig-

Table 1. Volatile oil constituents of Bulgarian *Rosa alba* L. essential oil

№	Name	Class	*R. alba* oil, relative percetage	Kovats RI (measured)	Kovats RI (literature)	Reference
1	Linalool	OM	1.37	1092	1099	Babushok *et al.* 2011
2	cis Rose oxide	HM	0.06	1124	1128	Babushok *et al.* 2011
3	a-Terpineol	OM	0.27	1186	1190	Babushok *et al.* 2011
4	Citronellol	OM	17.69	1227	1228	Babushok *et al.* 2011
5	β-Citral	OM	0.68	1244	1249	Jalali-Heravi *et al.* 2006
6	Geraniol	OM	16.64	1256	1255	Babushok *et al.* 2011
7	trans-Citral	OM	2.12	1269	1276	Bertuzzi *et al.* 2013
8	Citronellyl format	OM	0.23	1277	1277	Babushok *et al.* 2011
9	Geranyl formate	OM	0.31	1289	1303	Babushok *et al.* 2011
10	Citronellol acetate	OM	0.12	1346	1352	Babushok *et al.* 2011
11	3,7-dimethyl-2,6-Octadien-1-ol acetate	OM	0.92	1380	1385	Wannes *et al.* 2009
12	Tetradecane	AH	0.05	1400	1400	
13	Caryophyllene	SH	1.51	1419	1420	Babushok *et al.* 2011
14	b-Cubebene	SH	0.14	1430	1434	Facey *et al.* 2005
15	Humulene	SH	0.16	1453	1453	Babushok *et al.* 2011
16	Naphthalene, 1,2,3,4,4a,5,6,8a-oct	SH	0.33	1479	1480	Marongiu *et al.* 2006
17	α-Muurolene	SH	0.41	1504	1498	Babushok *et al.* 2011
18	g-Cadinene	SH	0.19	1528	1523	Babushok *et al.* 2011
19	Salvial-4(14)-en-1-one	SH	0.19	1557	1563	Pavlovic *et al.* 2006
20	Caryophyllene oxide	SH	2.75	1580	1580	Babushok *et al.* 2011
21	3-Octadecine	AH	0.15	1615		
22	Heptadecane	AH	0.54	1700	1700	
23	(2Z,6E) Farnesol	OS	1.72	1722	1722	Babushok *et al.* 2011
24	Z-5-Nonadecene	AH	5.20	1880	1885	Tigrine-Kordiani *et al.* 2006
25	Nonadecane	AH	12.18	1900		
26	3-Nonadecyne	AH	0.57	1911		
27	3-Eicosene, (E)-	AH	0.28	1953		
28	Eicosane	AH	1.46	2000		
29	10-Heneicosene (c,t)	AH	0.34	2070		
30	(Z)-3-Heneicosene	AH	0.64	2085		
31	1-Heneicosene	AH	0.55	2091		
32	Heneicosane	AH	12.75	2100		
33	Docosene	AH	0.41	2200		
34	9-Tricosene, (Z)-	AH	0.74	2290		
35	Tricosane	AH	2.69	2300	2300	
36	1-Pentacosene	AH	0.19	2490		
37	Pentacosane	AH	1.00	2500	2500	
38	Hexacosane	AH	0.09	2600	2600	
39	1-Heptacosene	AH	0.08	2697		
40	Heptacosane	AH	0.86	2700	2700	
41	Octacosane	AH	0.15	2800	2800	

Heterocyclic monoterpenes (HM) – 0.06 %
Oxygenated monoterpenes (OM) – 40.35%
Sesquiterpenes hydrocarbons (SH) – 5.68%
Oxygenated sesquiterpenes (OS) – 1.72%
Aliphatic hydrocarbons (AH) – 40.92%
Total identified - 88.73%

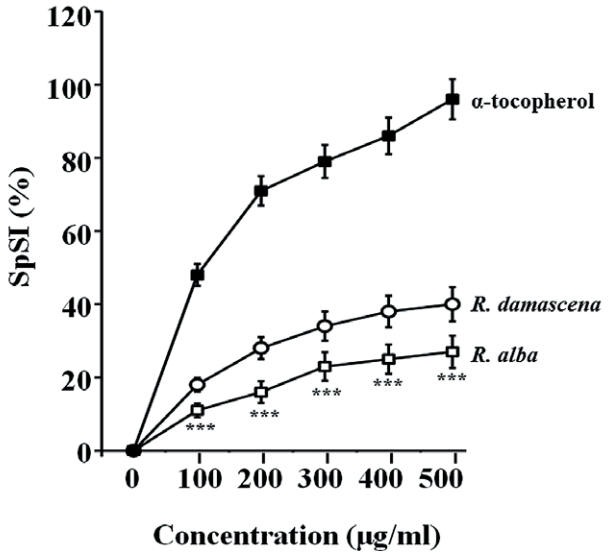


Figure 1. Superoxide scavenging activity of *Rosa alba* L. essential oil. Data were calculated in percentage as spectrophotometric scavenger index (SpSI) – the ratio of the absorption at 560 nm for the sample with oil, and the same absorption for the controls (without oil). The essential oil from Rosa damascena Mill. was used as a control. Data are expressed as mean ± SD of three independent experiments. ***p<0.001 compared with alpha -tocopherol.

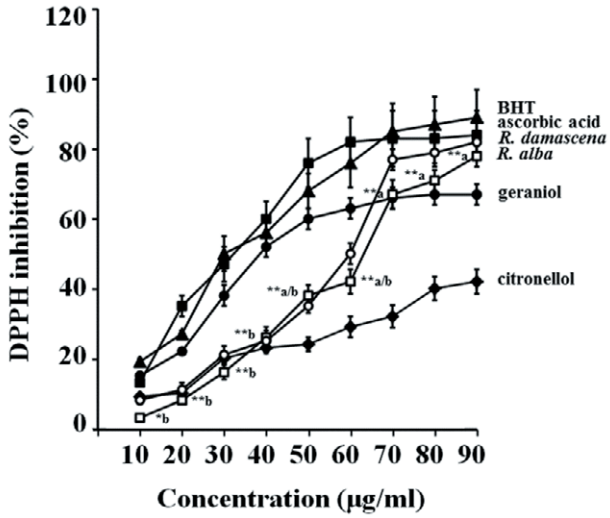


Figure 2. DPPH scavenging activity (%) at various concentrations (µg/ml) of Bulgarian *Rosa alba* L. essential oil and its main ingredients geraniol and citronellol. The essential oil from *Rosa damascena* Mill. was used as a control. Data are expressed as mean ± SD of three independent experiments. ***p<0.001 compared with citronellol (a), compared with BHT and ascorbic acid (b).

nificantly lower than that of alpha tocopherol, but close to that of the *Rosa damascena* Mill. *Our* previous works have shown that essential oils of rose species from different origins in principal does not have high potential as a scavenger of superoxide anion radical (Mileva *et al.* 2014).

DPPH – Radical scavenging assay

Rosa alba L. oil, as well as citronellol and geraniol as main aromatic components of the essential oil were tested in terms of their DPPH – radical scavenging activity. The DPPH assay usually involves a hydrogen atom transfer reaction (Li *et al.* 2009). DPPH radical scavenging test is a sensitive antioxidant assay and depends on substrate polarity. As can be seen from the chromatographic composition of the essential oil, it is rich of components which possess ideal structural chemistry for DPPH radical scavenging activity (Table 1). The presence of multiple hydroxyl functions could be considered as an option for hydrogen donation and/or radical scavenging activity. As can be seen in Figure 2, we found that Bulgarian *Rosa alba* L. essential oil exhibits higher potency in scavenging DPPH radicals than geraniol and citronellol in the applied system, but significantly lower than the benchmark substances (BHT and ascorbic acid). In the concentrations in range of up to 50 µg/ml, *Rosa alba* L. and *Rosa damascena* Mill. rose oils have close activity, but in higher concentracions, over than 50 µg/ml, *Rosa damascena* Mill. oil exhibits about 10% higher activity.

Iron binding capacity of Rosa alba L. essential oil

One of the possible mechanisms of the antioxidant activity of essential oils is the chelation of transition metals. Among the transition metals, iron is known as the most active pro-oxidant, due to its high reactivity. The ferrous form of iron accelerates lipid peroxidation by breaking down hydrogen peroxide and lipid peroxides to reactive free radicals by Fenton's reaction (Li *et al.* 2010). The products of these reactions are able to oxidise cell lipid membranes, modify proteins, as well as to damage DNA. Chelating agents may inactivate metal ions and potentially inhibit the metal-dependent processes (Li *et al.* 2010). Ferrous ion chelating activities of the oil, citronellol and EDTA are shown in Figure 3. The chelating test provided that *Rosa alba* L. essential oil inhibits lipid peroxidation up to 75%. The antioxidant activity obtained in this test is significantly lower than the activity of EDTA but similar to this of citronellol. In the concentration in range of up to 50 µg/ml the two rose oils have close activity, but in higher than 50 µg/ml concentrations *Rosa alba* L. exhibits increasing chelating activity.

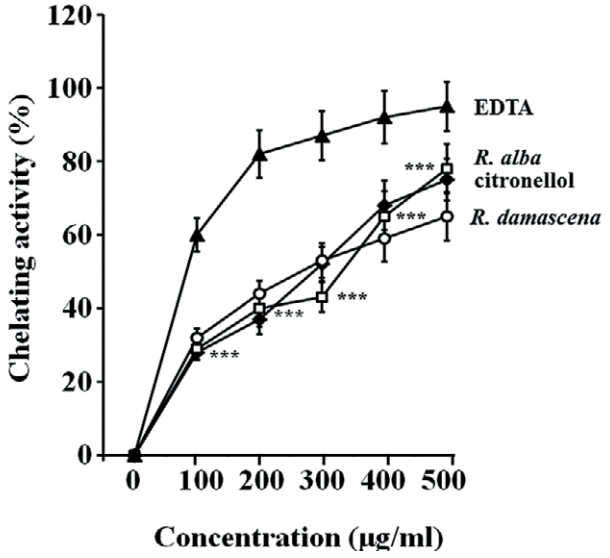


Figure 3. Chelating activity of Bulgarian *Rosa alba* L. essential oil and its main constituent citronellol. The essential oil from *Rosa damascena* Mill. was used as a control. Data are expressed as mean ± SD of three independent experiments. ***p<0.001 compared with EDTA.

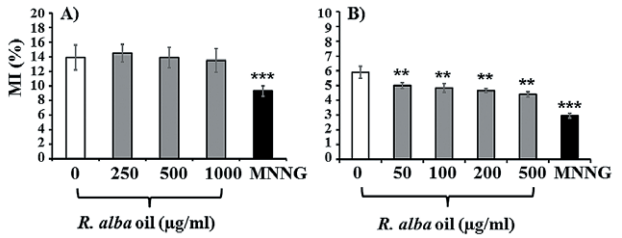


Figure 4. Value of mitotic activity (MI) observed after *Rosa alba* L. essential oil treatment in *Hordeum vulgare* (A) and in human lymphocyte cultures (B). Mitotic activity is calculated as a percent of the control. Data are expressed as mean ± SD of three independent experiments. **p<0.01; ***p<0.001 compared with the negative control.

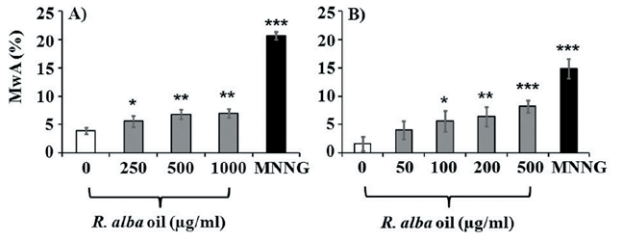


Figure 5. Frequency of chromosome aberrations (MwA) calculated after *Rosa alba* L. essential oil treatment in *Hordeum vulgare* (A) and human lymphocyte cultures (B). Data are expressed as mean ± SD of three independent experiments.*p<0.05; **p<0.01 and ***p<0.001 compared with the negative control.

Cytotoxic and genotoxic effects of Rosa alba L. essential oil

Rosa alba L. essential oil didn't show any cytotoxic effect in barley (Figure 4A). Here, human lymphocytes were found to be more susceptible than barley to rose oil in a concentration range (50-500 µg/ml) used in the present study. The rose oil decreased in a low extent the value of mitotic activity (Figure 4B) compared with the negative control in the lymphocyte cells ($p < 0.01$), whereas after treatment with MNNG (50 µg/ml) a well-expressed cytotoxic effect in both test-systems was observed ($p < 0.001$) (Figure 4A, 4B).

Rosa alba L. essential oil treatment enhanced the induction of chromosome aberrations (MwA) compared to non-treated cells in both test – systems. These results showed its genotoxic effect ($p < 0.05$; $p < 0.01$, $p < 0.001$). The effect is clearly depended on the concentration applied (Figure 5A, 5B). Human lymphocyte cultures were more sensitive to *Rosa alba* L. oil than *Hordeum vulgare*. Genotoxic effect of rose oil was much lower ($p < 0.001$) than the alkylating agent MNNG (50 µg/ml) in both test-systems (Figure 5A, 5B).

Analysis of the chromosome aberrations distribution showed that in barley, rose essential oil induced only isochromatide breaks (data not shown). In human lymphocytes the observed chromosome aberrations were preferably B" 92.0 %, followed by B' 8.0 %. In comparison with the rose oil, MNNG treatment (50 µg/ml) induced more diverse spectrum of chromosome disturbances in both test-systems. In *Hordeum vulgare* were obtained predominantly B"97.0 %, followed by B' 3.0%, whereas in lymphocyte cultures B" were 88.6%, B'- 10.4%, DC-1%, respectively (data not shown) (Figure 6A, 6B).

Treatment with rose oil enhanced the frequency of micronuclei (MN) clearly depending on the test-system and the concentration applied (Figure 6A, 6B; Figure 7A, 7B). No increase of the frequencies of this endpoint were observed in barley meristem cells (Figure 7A). The formation of micronuclei increased ($p < 0.001$) above two-fold (1.1%±0.3 for 50 µg/ml to 1.7%±0.2 for 500 µg/ml) compared to the negative control (0.5%±0.2) in lymphocyte cultures. The genotoxic effect of rose oil assessed as micronuclei is much lower than MNNG in the concentration applied in our study ($p < 0.001$) (Figure 7B).

Anti-cytotoxic and anti-genotoxic potentials of Rosa alba L. essential oil

Anti-cytotoxic potential

Two types of experimental schemes were applied: i) conditioning treatment with non- toxic or low toxic

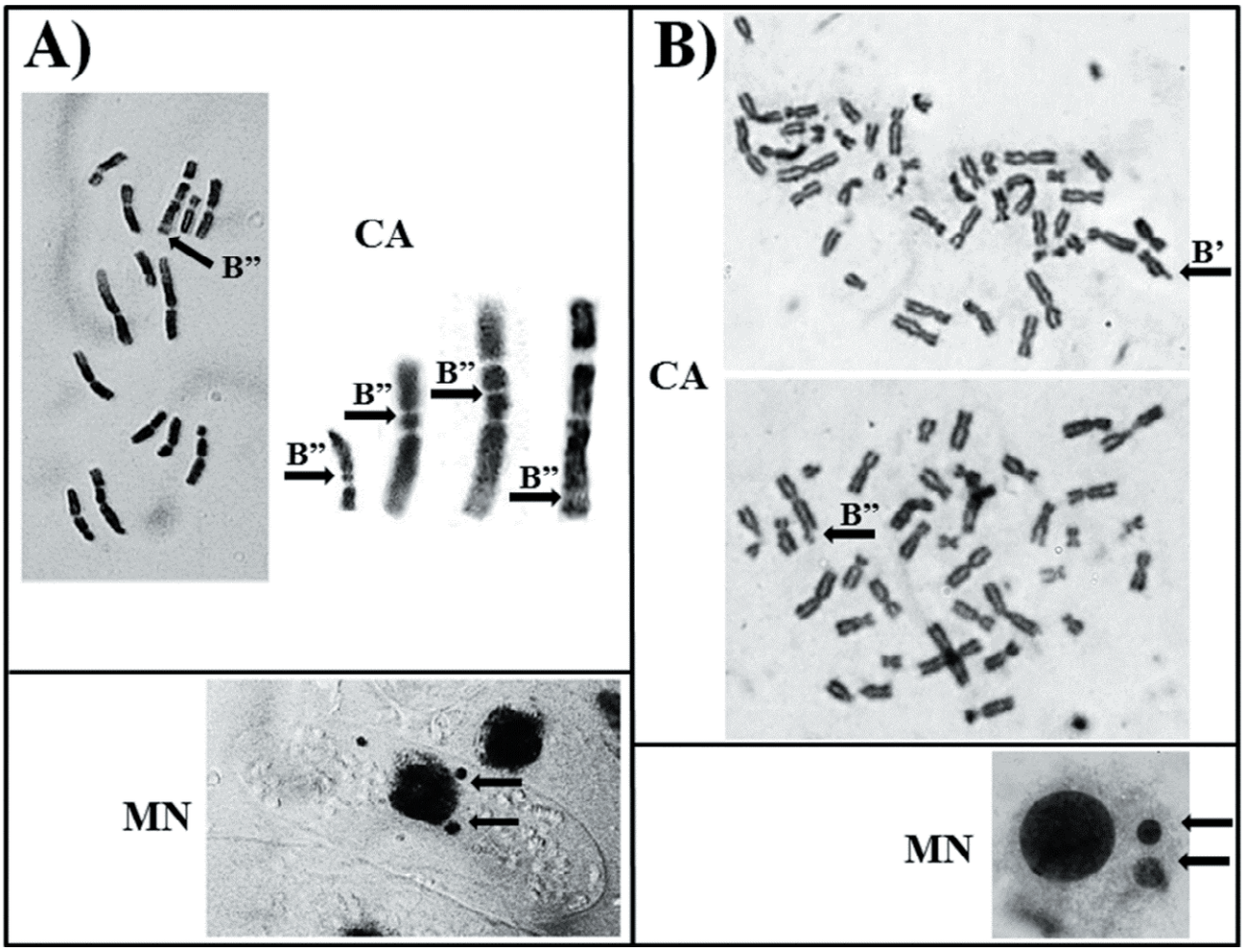


Figure 6. Types of chromosome aberrations (CA) and micronuclei (MN) observed after treatment with *Rosa alba* L. essential oil in *Hordeum vulgare* (A) and in human lymphocyte cultures (B).

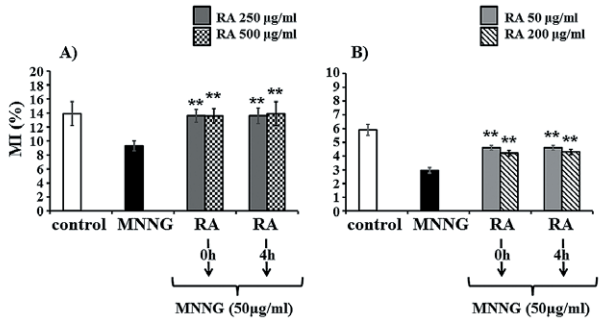


Figure 7. Induction of MN observed after *Rosa alba* L. essential oil treatment in *Hordeum vulgare* (A) and in human lymphocyte cultures (B). Data are expressed as mean ± SD of three independent experiments. **p<0.01 and ***p<0.001 compared with the negative control.

concentrations of rose essential oil followed by challenge treatment with alkylating agent MNNG (50 µg/ml) and 4 hours inter-treatment time, ii) treatment without any inter-treatment time between treatments (Figure 8A, 8B).

Mitotic activity (MI) observed after treatment showed clear dependence on the experimental design and test-systems (Figure 8A, 8B). The value of mitotic index was significantly increased ($p < 0.01$) after treatment applying both schemes of experimental design, compared to those obtained after MNNG (50 µg/ml) treatment alone in both test-systems (Figure 8A, 8B). A lack of any difference was obtained between the values of mitotic activity after rose essential oil conditioning treatment (250, 500 µg/ml for barley and 50, 200 µg/ml for human lymphocytes, respectively) followed by challenge treatment with MNNG (50 µg/ml) with 4 hours

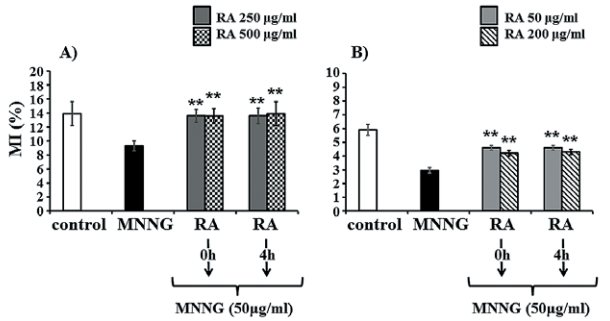


Figure 8. Anti-cytotoxic potential of rose essential oil assessed by mitotic index (MI) applying experimental schemes with: - *Rosa alba* L. essential oil conditioning treatment prior to MNNG challenge (50 µg/ml) with 4 hours inter-treatment time and, - without any inter-treatment time in *Hordeum vulgare* (A) and in human lymphocyte cultures (B). Mitotic activity is calculated as a percent of the control. Data are expressed as mean ± SD of three independent experiments. **p<0.01 compared with MNNG.

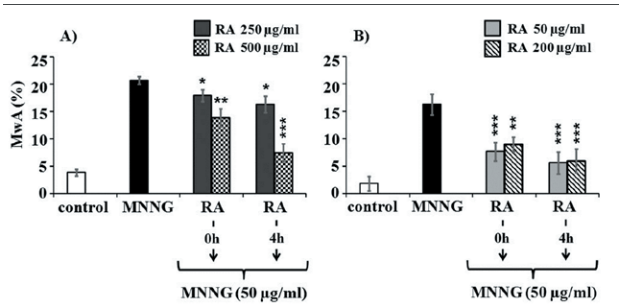


Figure 9. Anti-genotoxic potential of rose essential oil assessed by induction of chromosome aberrations (MwA) applying experimental schemes with: - *Rosa alba* L. essential oil conditioning treatment prior to MNNG challenge (50 µg/ml) with 4 hours inter-treatment time and, - without any inter-treatment time in *Hordeum vulgare* (A) and in human lymphocyte cultures (B). Data are expressed as mean ± SD of three independent experiments. *p<0.05, **p<0.01 and ***p<0.001 compared with MNNG.

inter-treatment time and treatment without any inter-treatment time.

Anti-genotoxic potential

Significantly ($p < 0.05$ till $p < 0.001$) lower frequency of chromosome aberrations was observed in *Hordeum vulgare* after conditioning treatment with rose essential oil (250, 500 µg/ml) prior to MNNG challenge (50 µg/ml) with 4 hours inter-treatment time, compared to that induced after treatment with alkylating agent only (Figure 9A). The reduction of MNNG induced chromosome aberrations was nearly three times lower in samples after rose oil 500 µg/ml conditioning (7.5%±1.6) compared to MNNG single treatment (20.7%±2.0).

Similar anti-genotoxic effect ($p < 0.001$) was observed in human lymphocytes after applying the same experimental scheme of treatment – conditioning with rose essential oil (50, 200 µg/ml) followed by challenge with MNNG (50 µg/ml) with 4 hour inter-treatment time (Figure 9B). Chromosome injuries were decreased approximately three times in samples conditioned with rose oil 50 µg/ml (5.6%±1.7) and with 200 µg/ml (6.0%±1.3) compared to MNNG treatment alone (16.3%±1.9). Lower structural chromosome disturbances were also calculated after treatment with rose essential oil and alkylating agent without any inter-treatment time (Figure 9A). The frequencies of chromosome aberrations were decreased between 2.2-times for samples conditioned with 50 µg/ml rose oil to 1.8–times for samples conditioned with 200 µg/ml. Reduction of the frequencies of chromosome aberrations was observed in barley cells but to a lower extent ($p < 0.01$) also after treatment following the scheme without any inter-treatment time compared to those after MNNG treatment alone (Figure 9A).

The spectrum of chromosome disturbances induced after applying experimental schemes for assessing anti-genotoxic potential of rose essential oil (250, 500 µg/ml) in *H. vulgare* showed predominantly B" 93%, followed by T 4% and B' 3% in samples with 4 hours inter-treatment time, and B" 93%, T-5%, D-1%, B'-1% in samples without any inter-treatment time (data not shown). In human lymphocytes conditioning treated with rose oil (50, 200 µg/ml) followed by MNNG and inter-treatment time of 4 hours were obtained B" 84.4%, B'-11.8%, T-1.9%, RC-1% respectively, whereas in samples treated with rose oil and MNNG without any inter-treatment tine were observed mainly B" 85.5%, B' 12.1% and T 2.4% (data not shown).

By calculating the frequency of micronuclei as another endpoint for genotoxicity, it was observed that rose essential oil showed similar anti-genotoxic effect ($p < 0.01$, $p < 0.001$) in both test-systems (Figure 10A, 10B). The effect was obtained applying both schemes of experimental design. In *Hordeum vulgare* conditioning treatment with rose essential oil decreased MN approximately two-times (0.97%±0.12 for 250 µg/ml and 1.02%±0.10 for 500 µg/ml) compared to that induced after treatment with the alkylating agent (1.95%±0.18). Similarly, lower MN frequency was obtained after treatment of barley cells without any inter-treatment time (Figure 10A). Frequency of micronuclei was decreased roughly 2 times – 1.0%±0.11 for 250 µg/ml and 1.04%±0.14 for 500 µg/ml. In human lymphocytes conditioning treatment with rose

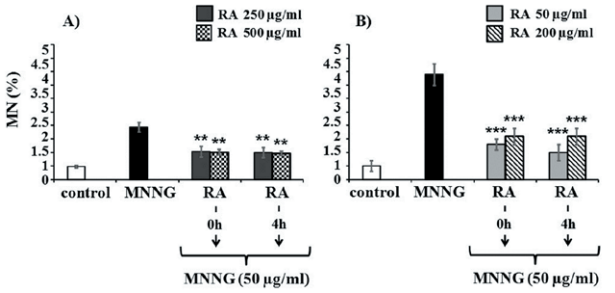


Figure 10. Anti-genotoxic potential of rose essential oil assessed by induction of micronuclei (MN) applying experimental schemes with: - *Rosa alba* L. essential oil conditioning treatment prior to MNNG challenge (50 µg/ml) with 4 hours inter-treatment time and, - without any inter-treatment time in *Hordeum vulgare* (A) and in human lymphocyte cultures (B). Data are expressed as mean ± SD of three independent experiments. **p<0.01; ***p<0.001 compared with MNNG.

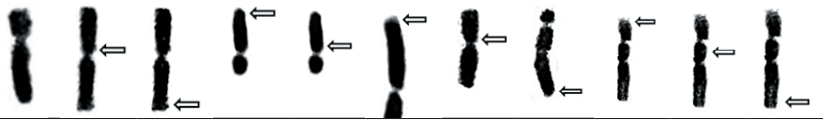
oil decreased MN approximately four-times 1.0%±0.2 for 50 µg/ml and 1.6%±0.3 for 200 µg/ml compared with those induced by MNNG alone (3.9%±0.4). The frequency of micronuclei was from 3-fold (for 50µg/ml) to 2.4-fold (200 µg/ml) lower than that of MNNG after treatment without any inter-treatment time (Figure 10B).

"Aberration Hot Spots" in Barley. "Aberration hot spots" are a good expression tool to investigate genotoxic activity as well as anti-genotoxic potential of *Rosa alba* L. essential oil. They give additional information about the effect of the essential oil on *Hordeum vulgare* root meristem cells.

The potential of MNNG to induce "aberration hot spots" in plant chromosomes was analyzed in the reconstructed barley karyotype MK14/2034. Seven out of the 48 inspected segments showed significant deviation from a random distribution of isochromatid breaks. Aberration clustering of isochromatid breaks (Table 2) was found in segment 10 and segment 14 of chromosome 2 (4.5% and 5.8%, respectively), in segment 17 of chromosome 3^4 (6.4%), in segment 21 of chromosome 4^3 (4.2%), in segment 30 chromosome 5 (9.1%), and in segments 44 and 48 of chromosome 7^1 (7.3%, resp. 6.7%).

All of these segments are located directly adjacent to the centromeres in the heterochromatin rich regions and are not connected with the regions of chromosome reconstruction. After *R. alba* L. essential oil treatment alone concentration dependent aberration hot spots (2 or 3, resp.) were observed (see Table 2), namely: after treatment with *R. alba* L. oil 250 µg/ml – segment 30 of chromosome 5 (8.2%) and segments 41 of chromosome 6 (14.2%); after treatment with *R. alba* oil 500 µg/ml – segment 14 of chromosome 2 (6.2%), segment 21 of chromosome 4^3 (6.2%) and segment 30 of chromosome 5 (10.0%)and after treatment with *R. alba* L. essential oil 1000 µg/ml – segment 17 of chromosome 3^4 (7.2%),

Table 2. Observed aberration "hot spots" in chromosomes of barley root tip meristem cells of the reconstructed karyotype MK14/2034 after different treatment procedures

Treatment variants		Non-Spot segments	Hot spot segments									
												
			Chr.1^7	Chr.2 seg. 10	Chr.2 seg. 14	Chr.3^4 seg. 15	Chr.3^4 seg. 17	Chr.4^3 seg. 21	Chr.5 seg. 30	Chr.6 seg. 41	Chr.7^1 seg. 1	Chr.7^1 seg. 44	Chr.7^1 seg. 48
1.	control	100%											
2.	MNNG (50 µg/ml)	56.0%		4.5%	5.8%		6.4%	4.2%	9.1%			7.3%	6.7%
3.	*Rosa alba* oil (250 µg/ml)	77.6%							8.2%	14.2%			
4.	*Rosa alba* oil (500 µg/ml)	77.6%			6.2%			6.2%	10.0%				
5.	*Rosa alba* oil (1000 µg/ml)	72.2%					7.2%		10.3%			10.3%	
6.	*Rosa alba* oil (250 µg/ml) → MNNG (50 µg/ml)	60.3%				5.6%		6.3%	6.3%		6.9%		14.6%
7.	*Rosa alba* oil (250 µg/ml) → 4h IT → MNNG (50 µg/ml)	60.6%			10.5%	11.4%				7.9%			9.6%
8.	*Rosa alba* oil (500 µg/ml) → MNNG (50 µg/ml)	73.0%		8.5%	8.5%							10.0%	
9.	*Rosa alba* oil (500 µg/ml) → 4h IT → MNNG (50 µg/ml)	81.5%							7.6%			10.9%	

segment 30 of chromosome 5 (10.3%) and segment 41 of chromosome 6 (10.3%).

Conditioning treatment with *R. alba* L. essential oil in concentrations of 250 μg/ml and 500 μg/ml, without any inter-treatment time prior to MNNG challenge decreased the aberration "hot spots" to five or three out of the 48 inspected segments (Table 2): *R. alba* L. essential oil 250 μg/ml – segment 15 chromosome 3^4 (5.6%), segment 21 of chromosome 4^3 (6.3%), segment 30 of chromosome 5 (6.3%), segments 1 and 48 of chromosome 7^1 (6.9% and 14.6%, resp.); *R. alba* L. oil 500 μg/ml – segments 10 and 14 chromosome 2 (both 8.5%) and segment 48 of chromosome 7^1 (10.0%). After inter-treatment time of 4 hours between conditioning and challenge treatment only 4 aberrations "hot spots" for conditioning with *R. alba* L. oil 250 μg/ml showed a significant deviation from a random distribution of isochromatid breaks – segment 14 of chromosome 2 (10.5%), segment 15 of chromosome 3^4 (11.4%), segment 41 of chromosome 6 (7.9%) and segment 48 of chromosome 71 (9.6%). Two aberrations "hot spots" were found after conditioning with *R. alba* L. essential oil 500 μg/ml, namely segment 30 of chromosome 5 (7.7%) and segment 44 of chromosome 7^1 (10.9%) (Table 2).

"Aberration hot spots" were found in all treated variants in barley chromosomes – karyotype MK 14/2034, with exception of chromosome 1^7 (Table 2).

In summary, applying both experimental schemes for assessing anti-genotoxic effect of *R. alba* L. essential oil the frequency of aberration "hot spots" was statistically significant decreased compared to MNNG 50 μg/ml (7 aberration "hot spots") (see Table 2). After consecutive treatment *R. alba* L. oil 500 μg/ml – 4 h inter-treatment time – MNNG the best result was achieved – reduction of aberration "hot spots" to 2 (Table 2).

DISCUSSION

In the current study valuable information was obtained about cytoprotective/genoprotective and antioxidant potentials of *Rosa alba* L. essential oil. The use of two types of test-systems – barley and human lymphocytes *in vitro,* which are widely applied in the genotoxic studies makes evaluation more representative.

Our results showed that rose essential oil possessed good expressed DPPH radical scavenging activity; the effect increased with the increasing of the concentration. It confirmed the data of Hatano *et al.* (1989) who proposed that rose's extract radical scavenging ability is due to the polar-bounded hydrogen. The compounds, which are able to donate hydrogen, are able to break the chain reaction of lipid peroxidation at the first initiation step, and/or to produce redox-silent compounds. Gordon (1990) reported that the chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion. The data shown in Figure 3 reveal that the Bulgarian *Rosa alba* L. oil demonstrates an effective capacity as iron binding agent, depending on the concentration applied, so its behavior as liposomal membrane peroxidation protector could be due to its iron binding capacity (Mileva *et al.* 2014). Moreover, in concentrations higher than 400μg/ml *Rosa alba* L. oil has a higher activity than *Rosa damascena* Mill.

In the present study Bulgarian *Rosa alba* L. essential oil didn't show significant cytotoxic effect in barley but it has relatively low cytotoxicity in human lymphocytes *in vitro* depending on the concentration. Sinha *et al.* (2014) demonstrate that essential oils may be safe at low concentrations, but show toxicity to humans at high concentrations represented as lethal doses. As typical lipophiles, essential oils can pass through the cell membrane and cytoplasmic membrane. They disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids and permeabilize through them (Bakkali *et al.* 2008). Their mode of action affects several targets at the same time. The cytotoxic activity of some essential oils for example of *Ocotea quixos* and others is mostly due to the presence of phenols, aldehydes and alcohols (Bruni *et al.* 2003; Sacchetti *et al.* 2005). In our study aliphatic hydrocarbons (AH) are the major compounds of *Rosa alba* L. essential oil. Here heneicosane (12.75%) and tricosane (2.69%) are representatives of the main components of this group (Table 1). Similar oil composition but in higher concentrations for some ingredients was detected for *Rosa damascena* Mill. by Kovacheva *et al.* (2010; 2011) and Mileva *et al.* (2014). The essential oils of plants such as *Ceratonia siliqua* (Hsouna *et al.* 2011), *Ailanthus altissima* (Albouchi *et al.* 2013) and *Viscum album* leaves (Cebovic *et al.* 2008), contain the same compounds in similar concentrations as in the white rose oil. They showed obvious cytotoxic effects, high antioxidant and phytotoxic activities. Shokrzadeh *et al.* (2017) reported a sensitivity of cancer cell line (A549) to high concentrations of rose oil obtained from *Rosa damascene* Mill. from Kashan. Probably these substances play a role in the cytotoxicicity of rose essential oil that we obtained for human lymphocytes. The higher resistance of *Hordeum vulgare* cells compared to the human lymphocytes is probably due to their different cell permeability and a cell wall existence compared to lymphocyte cells.

The current investigation detects genotoxic activity of Bulgarian *Rosa alba* L. essential oil depending on

the concentrations in both test-systems applied. Shokrzadeh *et al.* (2017) also reported that at concentrations of 50-200 μg/ml *Rosa damascena* Mill. oil significantly increased the frequency of micronuclei in human lymphocytes. According to Bakkali *et al.* (2008) in the case of cytotoxicity and genotoxicity, essential oils can damage the cellular and organelle membranes and can act as pro-oxidants on proteins and DNA *via* production of reactive oxygen species (ROS).

As it is known numerous plant extracts or phytochemicals have dual aspects, showing both genotoxicity and anti-genotoxicity against mutagens and carcinogens *in vivo* and *in vitro* test-systems (Kopaskova *et al.* 2012; Reddy *et al.* 2014; Gateva *et al.* 2015). Here we made an attempt to study the defense potential of Bulgarian *Rosa alba* L. essential oil and to determine the experimental conditions under which it can occur against alkylating agent MNNG. This is a typical mutagenic agent, which damages DNA. As a result it induced intra-strand, inter-strand crosslinks and double-strand breaks as well as base methylations, respectively (Kinzella and Radman 1980; Black *et al.* 1989). Extracts and essential oils of various plants were used to decrease cytotoxicity and genotoxicity induced by numerous genotoxins including alkylating agents (Vicuña *et al.* 2010; Mezzoug *et al.* 2007; Leffa *et al.* 2012; Madrigal-Santillán *et al.* 2013; Agabeyli 2012; Kuzilet *et al.* 2013; Matić *et al.* 2015).

The current results clearly show anti-genotoxic potential of Bulgarian *Rosa alba* L. essential oil manifested by decreasing of the frequencies of chromosome aberrations and micronuclei after conditioning treatment with rose oil before MNNG challenge and 4 hours inter-treatment time in both experimental test-systems used by us. This is in agreement with our study (Gateva *et al.* 2019) where the preventive effect of acyclic monoterpenoid geraniol (which is one of the major rose essential oil compounds) was obtained against MNNG in human lymphocytes *in vitro* and *Hordeum vulgare*. Geraniol was found to be effective in limiting the genotoxic effect of MNNG applied as conditioning treatment (4h inter-treatment time) prior challenge with MNNG compared to the samples treated with MNNG alone. Our current study also showed an increased resistance to the damaging effect of MNNG both in barley and in human lymphocytes after treatment with rose essential oil and MNNG without any inter-treatment time. Hence the defence potential of Bulgarian *Rosa alba* L. essential oil is manifested regardless of the experimental conditions. The results about anti-cytotoxic and anti-genotoxic effect of the rose oil corresponded with those for antioxidant activity of rose essential oil obtained by DPPH test and iron chelating analysis. As a rule, the anti-cytotoxic, anti-genotoxic, and antioxidant properties of the plant extracts cannot be attributed of activities to single constituents. Their biological activity could be explained with the combination of effects to one another. As can be seen on chromatographic profil of *Rosa alba* L. oil (Table 1), geraniol and citronellol are in major amounts in oil, so they could have an over additive participation in total antioxidant and iron chelating properties. Ruberto and Baratta (1999) demonstrated that most radical scavenging activities of essential oils are mainly due to the cumulative effect of ingredients nerol, citronellol and geraniol, within whose structure polar bounded hydrogen has been observed. Undoubtedly, DPPH radicals have little relevance as presence in biological systems, but the results are indicative of the capacity of the Bulgarian white rose oil to scavenge free radicals which relate to hydrogen atom or electron donation ability.

There are data indicating that not only geraniol and citronellol, but citral (which belongs to the bioactive compounds of the Bulgarian *Rosa alba* L. essential oil) also possess antioxidant activity (Raut and Karuppayil 2014). Madankumar *et al.* (2013) showed that geraniol has a potent antioxidant effect by scavenging oxygen-free radicals and increasing the level of total glutathione content (GSH) in murine skin. It could modulate the activity of enzymatic and non-enzymatic antioxidants to exert its chemopreventive activity against 4-Nitroquinoline 1-oxide induced oral cancer in rats. Manoharan and Selvan (2012) proposed that geraniol inhibits abnormal cell proliferation occurring in skin carcinogenesis by modulating the activities of Phase II detoxification agents and through free radical scavenging potential. Geraniol and camphene were found to significantly decrease lipid peroxidation, inhibit Nitric oxide release (83.84% and 64.61%) and ROS generation in the pre-treated cells as compared to stressed cells (Tiwari and Kakkar 2009). Kashani *et al.* (2011) described a significant correlation between the phenolic content and DPPH scavenging capacity of white rose extracts. Our results indicate good iron chelating and DPPH radical-scavenging activities, medium superoxide scavenging ability of Bulgarian *R. alba* L. essential oil, which is probably due to its chemical composition. Some authors reported that **α**-terpineol (which is one of the white rose essential oil compounds obtained by us) has remarkable ferrous ions chelating agent and possees antimutagenic activity against 2-aminoanthracene in *S. typhimurium* TA100 (Di Sotto *et al.* 2013).

According to Bakkali *et al.* (2008) the mechanism of the decrease of mutagenicity did not depend only of the type of essential oil but on the type of mutagen, thus on the type of lesions and consequently on the DNA repair

or lesion avoidance system involved. The protective effect obtained by us for Bulgarian *Rosa alba* L. essential oil against alkylating agent MNNG suggests an activation of repair pathways in addition to antioxidant and scavenging activity of rose essential oil. It is well known that N^7-alkylG is responsible for about 90% of the total frequency of alkylation events among the alkylation damages induced by alkylating agents. Quantity of O^6-alkylG (DNA adduct formed by the alkylating agent) is less, but if not repaired could led to DNA damage such as cross-linking, strand breaks and modification of bases (Kondo *et al.* 2009). However N^7-alkylG is considered to be as mutagenic as O^6-alkylG because it is efficiently repaired by base excision repair (BER) pathway (Drablos *et al.* 2004). O^6-methylG lesions are repaired by direct damage reversal repair carried out by the enzyme O^6-methylguanine-DNA methyltransferase (MGMT) also referred to as alkylguanine transferase (AGT). MGMT efficiently repairs O^6-methylG before replication, through direct transfer of the adducted methyl group from the oxygen in the guanine to a cysteine residue in the catalytic site of MGMT (Ramos *et al.* 2011). Niture *et al.* (2006; 2007) showed that both the ethanolic and aqueous extracts and compounds of some medical plants increased MGMT expression and its activity in lymphocytes and cancer cell lines. To understand the real mechanism of protective potential of rose essential oil when applied in combination with alkylating agents more studies are needed.

CONCLUSION

Bulgarian *Rosa alba* L. essential oil has low cytotoxicity in barley and human lymphocytes *in vitro* in a dose-dependent manner, as well a good cytoprotective/genoprotective effect against DNA damaging agent MNNG when applied both with 4 hours between treatments and without any inter-treatment time. Anti-genotoxic potential of rose essential oil was manifested by the decrease of the frequency of chromosome aberrations and the micronuclei in both test-systems. Something more, white rose's oil demonstrated well-pronounced anti-oxidant potential and very good metal chelating activity. The results show that rose oil contained protective compounds that can decrease DNA damage. Data suggest a promising ethnopharmacological potential of Bulgarian white rose essential oil. It could serve in medical cosmet as a prophylactic agent, and as an adjuvant in cancer prevention and therapy.

GEOLOCATION

Fresh flowers of *Rosa alba* L., from the experimental field of the Institute of Rose and Essential Oil Plants (IREOP), in Kazanlak, Bulgaria were used. All studies were conducted in the laboratories of the Republic of Bulgaria.

ACKNOWLEDGMENTS

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