# Salvia Extract Can Decrease DNA Damage Induced by Zeocin

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***Abstract*—Use of natural plant extracts to prevent the damage potency of various mutagens is on the rise. The aims of our study were: i) to evaluate the biological activity of *Salvia officinalis* (sage) water extract and its ability to reduce the zeocin-induced cytotoxic/genotoxic effects in *Hordeum vulgare* (barley) and human lymphocytes, ii) to obtain information about the susceptibility of plant chromosomes on the basis of "aberration hot spots" (AHS) under various experimental conditions of treatment. Sage extract had no or low cytotoxic effect and its clastogenic effects were clearly dependent on the concentration and test-systems. Lymphocytes were more susceptible to sage extract than barley. Sage extract applied in a higher but low-toxic concentration showed well expressed protective potential against zeocin. It decreased the chromosome aberrations and micronuclei induced by zeocin in both test-systems. The protective effect was manifested irrespective of the experimental design and test-systems. All AHS were found to be within heterochromatin-containing or terminal segments when barley meristems were treated with the radiomimetic. AHS were reduced and no longer located in heterochromatin-rich regions after sage treatment following different experimental designs. Our results might be useful in future health research programs and prophylactic therapy based on plant extracts.**

***Index Terms*—*Salvia officinalis*, plant extract, chromosome aberrations, micronuclei, anti-cytototoxicity/anti-genotoxicity, test-systems**

## I. Introduction

In the last decades natural plant extracts as a form of medical treatment have become a subject of intensive research. There is growing interest in the search for and investigation of natural substances of plant origin that possess anti-mutagenic, anti-genotoxic and anti-oxidant activities against inducers of reactive oxygen species (ROS). A variety of medical plants have biological and pharmacological activities that may protect cells against oxidative-induced damage (Mierlici *et al*., 2009, Ramu *et al*., 2012; Agabeyli, 2012, Al-Awaida and Akash, 2014). The wide application of natural plant extracts and their biologically active compounds in human nutrition and pharmacy require studies first on their safety at doses in the pharmacological range and second, on their anti-cytotoxic and/or anti-clastogenic activities. As a general rule crude therapeutic products of plant origin are less toxic than their synthetic counterparts because they contain the whole array of medicinal compounds (known and unknown) just as they are found in their natural source and thus, pose less risk of side effects (Celik, 2012). However, it must be taken into account that the non-prescription use of medicinal plants is an important health problem due to their particular toxicity (Mendonça-Filho, 2006).

*Salvia officinalis* (sage), which belongs to Lamiaceae, is one of the important herbs native to the Mediterranean region and also cultivated to some extent in different European countries. It is used in the food processing industry and also in the area of human health. Extracts of different Salvia species have been examined for a number of biological activities, antimicrobial, anti-inflammatory, antioxidant, anti-spasmolytic, anti-diarrheal, neuroprotective, hepatoprotective and cholinergic binding properties (Capasso *et al*., 2004, Ren *et al*., 2004; Lima *et al*., 2007; Behboud *et al*., 2011; Khan *et al*., 2011; Osman and El –Azime, 2013; Fischedick, 2013, Shahrzad *et al*., 2014, Hamidpour *et al*., 2014). *S. officinalis* is considered to have the highest amount of essential oil compared to the other Salvia species (Rami and Li, 2011). Oil extract of *S. officinalis* shows good antifungal activity (Badiee *et al*., 2012). Al-Ezzy *et al*., (2011) have demonstrated anti-mutagenic effect of sage leaf aqueous extract against cytostar in cultured blood cells of ten acute lymphocytic leukaemia patients. The leaves of *S. offcinalis* are known for their antioxidative properties (Wang *et al*., 1998, Farhoudi *et al*., 2011). Sage can express protective effect against $H_2O_2$ in DNA fragmentation tests (Ewadh *et al*., 2013). Salvia extract can significantly decrease the DNA damage induced by flavomycin in a linear, dose-dependent manner in partridges (Yurtseven *et al*., 2008). *S. officinalis* leaves possesssome therapeutic effects due

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to the presence of biochemicals like tannin, triterpenoids, flavonoids, estrogenic substances, saponins, and volatile oil, together with vitamins C and A (Patenkovic *et al*., 2009).

Various genetic biomarkers and test-systems are used to estimate the cytotoxic/genotoxic effects and anti-cytotoxic/anti-genotoxic potential of medicinal herbs and phytochemicals in order to regulate their consumption (Gadano 2006, Surh and Ferguson, 2003, Liman *et al*., 2012, Gateva *et al*., 2014, Angelova *et al*., 2014, Todorova *et al*., 2014). The genoprotective effects of *S. officinalis* extract was studied using micronuclei and comet assay in Swiss albino mice (Mathew and Thoppil 2012 a). The anti-proliferative and pro-apoptotic effects of water extracts of *S. fruticosa* and *S. officinalis* and their main phenolic compound rosmarinic acid were evaluated in two human colon carcinoma-derived cell lines (Xavier *et al*., 2009). Diterpene manool isolated from *S. officinalis* has been shown to exhibit a protective effect against methyl methanesulphonate in HepG2 cells evaluated based on the level of induced chromosome damage (Nicolella *et al*., 2014).

Valuable and representative information about the biological activity and anti-genotoxic potential of medical plant extracts could be provided by combined studies with *in vitro* and *in vivo* test-systems. This provoked our interest is studying the biological effect and anti-cytotoxic/anti-genotoxic potential of *S. officinalis* water extract against ROS inducer – zeocin, in two different phylogenetically distant experimental test-systems: higher plant (*Hordeum vulgare*) and human lymphocytes *in vitro.* Endpoints for cytotoxicity and genotoxicity were used. Detailed information about the susceptibility of chromosomes based on "aberration hot spots" (AHS) in the reconstructed karyotype of barley chromosomes was also obtained. The use of reconstructed karyotypes allows identification of individual chromosomes and the detection of AHS as a result of clastogenic effects of different mutagenic factors and chemical substances in plant test-systems (Schubert and Rieger, 1977; Nicoloff *et al*., 1987; Künzel *et al*., 2001). The susceptibility to genotoxic impact depends on the type of karyotype reconstruction, particularly with regard to the number and kind of the reconstructed chromosome segments. Here we used the structurally reconstructed barley karyotype MK 14/2034. This translocation line is characterized by seven easily distinguishable chromosome pairs which allow both the investigation of mutagen-specific features of aberration distribution patterns and possess nearly the same sensitivity as the barley standard karyotype.

In the present study zeocin was used as a standard mutagen. It is a radiomimetic which is able to directly induce double strand breaks (DSBs) in DNA similarly to ionizing radiation. It belongs to Bleomycin/Phleomycin antibiotic family. Its effect has been previously investigated and described (Chankova *et al*., 2007, Dimova *et al*., 2009).

The aims of our present study were: i) to evaluate the biological activity of *Salvia officinales* water extract and its ability to reduce the zeocin-induced cytotoxic and genotoxic effects in *H. vulgate* and human lymphocytes *in vitro* as test-systems based on cytotoxic and genotoxic endpoints, ii) to obtain information about the susceptibilities of plant chromosomes on the basis of "aberration hot spots" under various experimental conditions of treatment. Cytotoxic effect was assessed based on mitotic index, DNA damage by induction of chromosome aberrations and micronuclei formation.

## II. Material and Methods

### A. Plant Extract from Salvia Officinalis (Sage)

Plants *Salvia officinalis* were collected in the region of Banska Bystrica, Slovakia. The collection of such herbs from natural medicinal plants is carried out according to the requirements of Bulgarian Medicinal Plants Act, amend., Offic. Gaz. No 66 of 26 July 2013, Chapter 3/Art. 21 (1). The sage extract was prepared as follows:

Haulms and leaves of *Salvia officinalis* were air dried for 20 days at 25℃. The plant material (24 g) was cut into small pieces (1 – 2 cm) and was extracted three times with hot methanol (200, 150 and 100 ml) at 60℃. After evaporation of the methanol, the remaining water was removed by azeotropic distillation with toluene. The solid end-product yield of 2.7 g (11.3 %), yellow-green in color, was stored at 4 ℃. The plant extract of *S. officinalis* was dissolved in 5 % dimethyl sulphoxide (DMSO) to prepare working concentrations.

### B. Standard Mutagen

The radiomimetic zeocin (Zeo) (Cas No.: 11006-33-0) belongs to the bleomycin/phleomycin family of antibiotics. The concentrations of zeocin were selected based on their effectiveness in inducing DNA damage in both test-systems.

### C. Test-Systems and Culture Conditions

Two types of experimental test systems at different levels of hierarchy were used: plant test-system – *Hordeum vulgare*, and human lymphocytes *in vitro*

### D. Hordeum Vulgare (Barley)

Root tip meristems of *Hordeum vulgare* (reconstructed karyotype MK14/2034) were presoaked for 1 h in tap water and germinated for 18 h at 24$^{o}$C. The meristems were treated as described further.

### E. Human Lymphocytes in Vitro

Lymphocyte cultures ($1\times10^{6}$ cells/ml) were prepared from peripheral heparinized venous blood of healthy non-smoking donors, aged 35–48 years, by applying the standard method of Evans (1984). Each culture contained RPMI 1640 medium (Sigma, Germany), 12 % calf serum (Sigma, Germany), 40 μg/ml gentamycin (Pharmacia, Bulgaria) and 0.1% phytohemagglutinin (PHA) (Sigma, Germany). The lymphocyte cultures were treated as described below.

The experiments were in compliance with the Declaration of Helsinki. Voluntary written informed consent was obtained from all participants.

*F. Experimental Design*

*1) Cytotoxic and/or genotoxic effect of Salvia officinalis water extract*

*Hordeum vulgare* meristems were treated with sage water extract for 60 min in concentrations of 10–100 µg/ml; human lymphocyte cultures were treated for 60 min in concentrations of 5–100 µg/ml. Barley cells were treated for 60 min with 300 µg/ml of zeocin and human lymphocyte cultures, for 15 min with 150 µg/ml.

Untreated cells were used as a negative control in both test systems.

*2) Anti-cytotoxic and anti-genotoxic potential of sage*

To study the anti-cytotoxic and anti-genotoxic potential of *S. officinalis*, the most appropriate concentrations were chosen based on our preliminary experiments. Two experimental designs were applied:

*3) Hordeum vulgare:*

- Conditioning treatment (for 60 min) with *Salvia officinalis* water extract (100 µg/ml) followed by 4 h inter-treatment time and challenge treatment (for 60 min) with zeocin (300 µg/ml).
- Treatment (for 60 min) with *Salvia officinalis* water extract followed immediately by zeocin treatment (60 min) without any inter-treatment time.

After the treatment and recovery times (18, 21, 24, 27 and 30 h) at 24°C, each sample was added 0.025% colchicine (2 h) in a saturated solution of α-bromonaphthaline. The meristems were fixed in a mixture of ethanol and acetic acid (3:1), hydrolyzed in 1 N HCl at 60°C for 9 min and stained with Schiff's reagent at room temperature for 1 h. The root tips were macerated in a 4% pectinase solution for 12 min and squashed onto slides for scoring of metaphases with chromosome aberrations.

*4) Human lymphocytes in vitro:*

- Conditioning treatment (for 60 min) with *Salvia officinali* water extract (50 µg/ml) followed by 4 h inter-treatment time and challenge treatment with zeocin (150 µg/ml).
- Treatment (for 60 min) with *Salvia officinalis* water extract followed immediately by zeocin treatment (15 min) without any inter-treatment time.

After each treatment the cultures were washed in a serum-free RPMI medium and the cells were incubated in a fresh medium at 37 ℃. At the 72nd hour of cultivation 0.02% colchicine was added to each culture and the cells were hypotonized in 0.56 % KCl. The cells were fixed in a mixture of methanol: glacial acetic acid (3:1). After centrifugation each cell culture was dropped on clean slides and was stained in 2% Giemsa.

*5) Endpoints*

The marker for cytotoxicity was mitotic index (MI). It was calculated according to the formula: MI = A/1000, where A is the number of dividing cells.

To evaluate the chromosome aberrations (CA) at least 800 well-spread metaphases of each sample were scored for both test-systems. Chromatid breaks (B'), chromosome breaks (B"), chromatid translocations (T), intercalary deletions (D), duplication-deletions (DD), dicentrics (DC) and ring chromosomes (RC) were determined. The yield of "aberration hot spots" was evaluated in barley, using an adapted formula for comparison of the upper limit of confidence interval from the expected and observed chromatid aberrations in individual loci and evaluation of "aberration hot spots" (Jovtchev *et al*., 2010, Rieger *et al*., 1975).

To calculate the micronuclei, 3000 cells per sample were scored. Colchicine was omitted and the cells were directly fixed at 30 h after treatment for *Hordeum vulgare* and at 72 h after PHA stimulation for human lymphocyte cultures.

*6) Statistical analysis*

The metaphases were analyzed for the presence of chromosome aberrations (MwA ± SD). The percentage of MwA in $M_1$ mitosis was determined. Induced micronuclei (MN % ± SD) were evaluated.

An adapted formula was used for comparison of the upper limit of confidence interval of the expected and observed chromatid aberrations in individual loci and evaluation of "aberration hot spots" in barley (Rieger *et al*., 1975; Jovtchev *et al*., 2010: Gateva *et al*., 2011; Gateva *et al*., 2014).

Data are mean values from three independent experiments for both test-systems. The results were analyzed statistically by the Fisher exact-test.

## III. Results

The cytotoxic effect of *Salvia officinalis* water extract evaluated based on the value of the mitotic index (MI) in both test systems is shown in Fig. 1. The sage extract had no or only a weak cytotoxic effect ($p < 0.05$) in *Hordeum vulgare* when applied in a concentration range of 10 µg/ml – 100 µg/ml as compared to the untreated cells (Fig. 1a). Sage water extract was shown to possess a low cytotoxic effect in human lymphocytes in the concentration range of 5 µg/ml – 100 µg/ml compared with the negative control (Fig. 1b). The most cytotoxic concentrations ($p < 0.01$) were 50 and 100 mg/ml, decreasing the MI to 30 % from the value of the untreated control (100%).

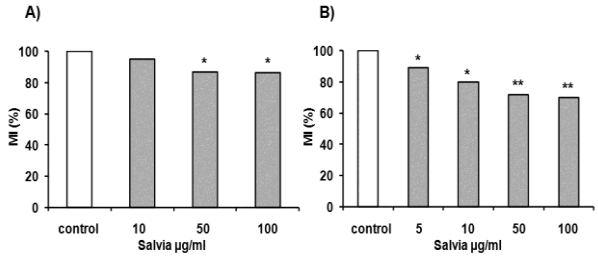


Figure 1. Mitotic activity evaluated based on the value of the mitotic index observed after treatment with *Salvia officinalis* water extract: in *Hordeum vulgare* (a) and in human lymphocytes *in vitro* (b). Data are mean values from three independent experiments. The mitotic activity in all treated variants was calculated as a percentage of the untreated control. *$p < 0.05$, **$p < 0.01$.

No effect of DMSO on the investigated parameter was observed in both test systems (data not shown).

The genotoxic/clastogenic activity of sage water extract was calculated based on induction of chromosome aberrations and micronuclei. Based on the yield of chromosome aberrations, a well expressed clastogenic effect ($p < 0.05$, $p < 0.001$) was observed in both test systems, with a clear dependence on the applied concentrations (Fig. 2). Lymphocyte cultures were more sensitive to *Salvia officinales* water extract than *Hordeum vulgare.* The yield of chromosome aberrations increased from 2.4- to 5.3-fold in human lymphocytes and from 2.0- to 3.8-fold in barley compared with the negative control.

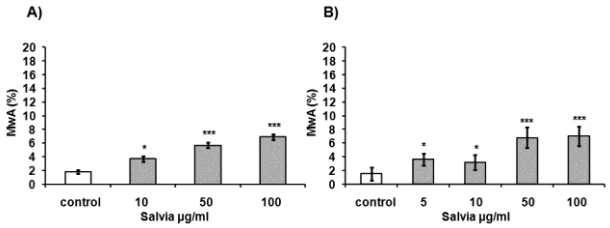


Figure 2. Chromosome aberrations induced after treatment with *Salvia officinalis* water extract: in *Hordeum vulgare* (a) and in human lymphocytes *in vitro* (b). Data are mean values from three independent experiments. *$p < 0.05$; ***$p < 0.001$.

No effect of DMSO on the investigated parameter was observed in both test-systems (data not shown).

There was a 3-fold increase in the percentage of induced micronuclei ($p < 0.001$) in human lymphocytes with increasing the concentrations of extract. compared with the control and 2.3-fold in *H. vulgare*, respectively (Fig. 3 ).

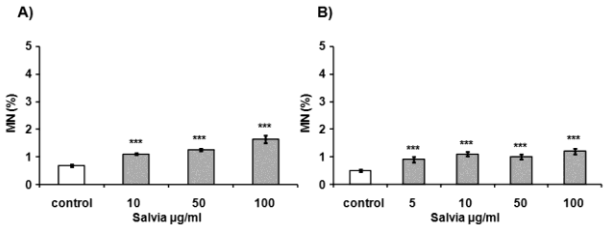


Figure 3. Micronuclei induced after treatment with *Salvia officinalis* water extract: in *Hordeum vulgare* (a) and human lymphocytes *in vitro* (b) test-systems. Data are mean values from three independent experiments. ***$p < 0.001$.

A narrow spectrum of chromosome aberrations after sage extract treatment was observed in both test systems. Mainly chromosome breaks (B"), followed by a low percent of chromatid breaks (B') were observed.

### *A. Anti-Cytotoxic and Anti-Genotoxic Potential of Salvia Officinalis Water Extract*

The anti-cytotoxic potential of sage water extract in both test systems is demonstrated in Fig. 4. Our results showed an increased ($p < 0.001$) value of mitotic activity after conditioning treatment with Salvia water extract in low-toxic concentrations prior to zeocin treatment with a high harmful concentration and 4-hour inter-treatment time compared to that in cells treated with zeocin only in both types of test-systems (Fig. 4(a), Fig. (b)). The cytotoxic effect of zeocin also decreased when Salvia extract was applied in a low-toxic concentration immediately before the radiomimetic without any inter-treatment time in human lymphocytes (Fig. 4 (b)).

The anti-genotoxic/anti-clastogenic potential of *Salvia officinalis* water extract evaluated as induction of chromosome aberrations and micronuclei in both test-systems is presented in Fig. 5 and Fig. 6. The clastogenic effect of zeocin calculated based on chromosome aberrations decreased 2.6-fold in barley and 1.5-fold in human lymphocytes after conditioning treatment with a low-toxic concentration of Salvia extract followed by 4-hour inter-treatment time and a toxic high concentration zeocin (Fig. 5). The yield of chromosome aberrations of zeocin was also reduced ($p < 0.001$) when the radiomimetic was given immediately after the treatment with Salvia extract in both test-systems (Fig. 5).

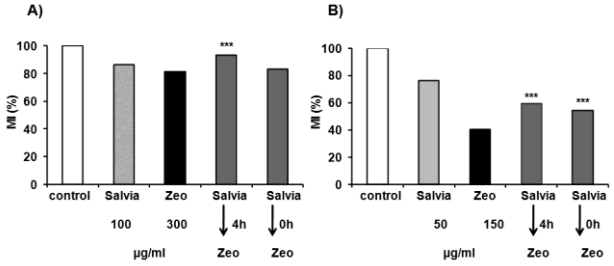


Figure 4. Anti-cytotoxic potential of *Salvia officinalis* water extract evaluated based on the value of the mitotic index after treatment with sage and zeocin in two experimental designs in: (a) *Hordeum vulgare,* (b) human lymphocytes *in vitro.* The mitotic activity in all treated variants was calculated as a percentage of the untreated control. Data are mean values from three independent experiments. ***$p < 0.001$.

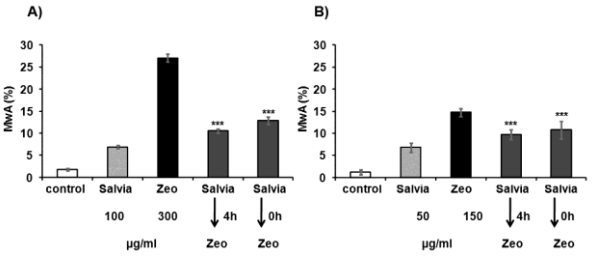


Figure 5. Anti-clastogenic potential of *Salvia officinalis* water extract evaluated based on the yield of chromosome aberrations after treatment with sage and zeocin in two experimental designs in: (a) *Hordeum vulgare,* (b) human lymphocytes *in vitro.* Data are mean values from three independent experiments. ***$p < 0.001$.

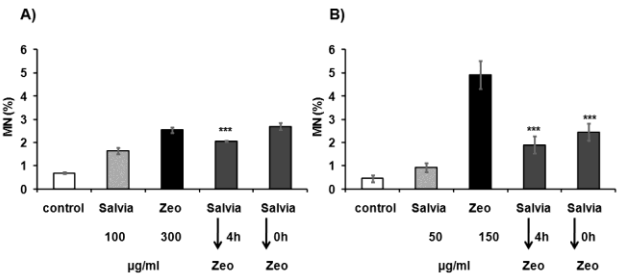


Figure 6. Anti-clastogenic potential of *Salvia officinalis* water extract evaluated based on the yield of micronuclei after treatment with sage and zeocin in two experimental designs in: (a) *Hordeum vulgare,* (b) human lymphocytes *in vitro.* Data are mean values from three independent experiments. ***$p < 0.001$.

The yield of micronuclei depends on the test system and the experimental design. The yield of micronuclei

was reduced approximately 1.5-fold in barley and 2.4-fold in human lymphocytes ($p < 0.001$) after conditioning treatment with low-toxic concentration of Salvia water extract prior to a harmful concentration of zeocin and 4-hour inter-treatment time compared with zeocin treatment only (Fig. 6 (b)). A lower yield of micronuclei compared with zeocin treatment was calculated ($p < 0.001$) after treatment with Salvia extract without any inter-treatment time prior to zeocin only in human lymphocytes. In *Hordeum vulgare* the yield of micronuclei was comparable to that after treatment with zeocin only (Fig. 6 (a)).

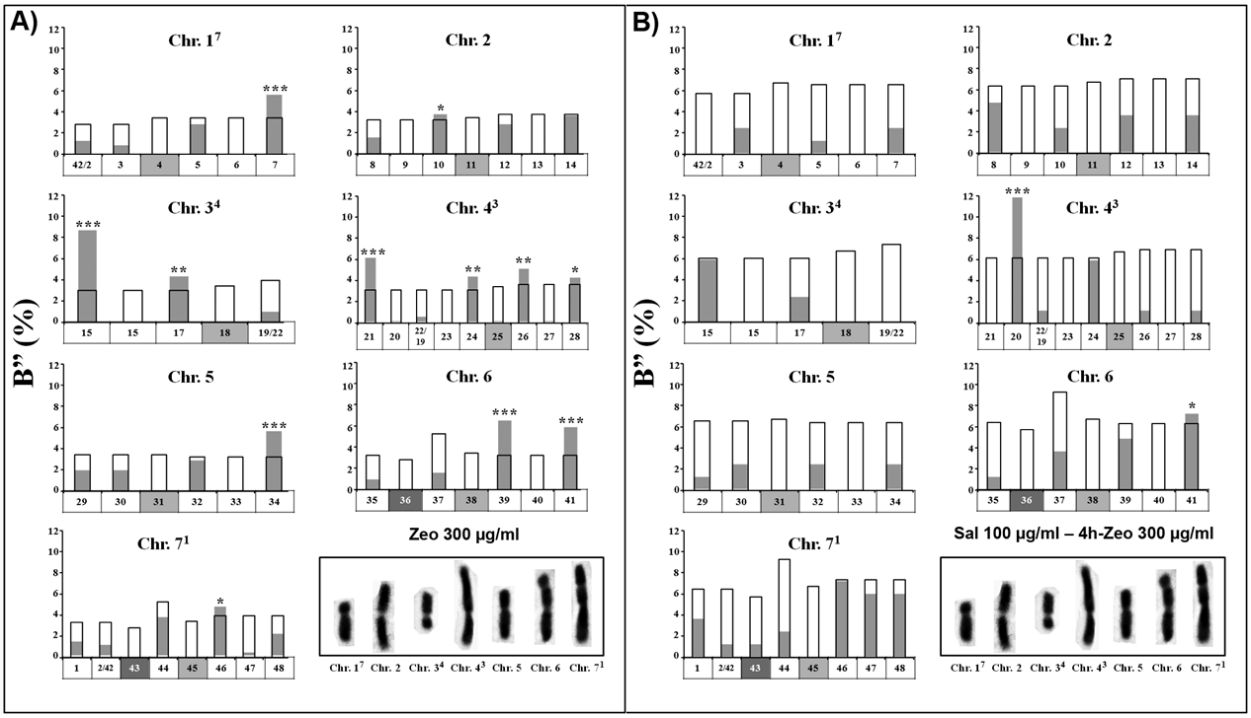


Figure 7. "Aberration hot spots" evaluated after treatment with: (a) zeocin (300 µg/ml) and (b) conditioning treatment with a low-toxic concentration of *Salvia officinalis* extract (100 µg/ml) 4 h inter-treatment time and a challenge treatment with a high toxic concentration (300 µg/ml) of zeocin in the reconstructed barley karyotype MK14/2034. Centromers are labeled in dark grey and NORs of chromosome 6 and 7, in light grey. White columns represent the observed isochromatid breaks; grey columns indicate the calculated breaks based on the segments length. The asterisks denote "aberration hot spots". Data are mean values from three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE I. OBSERVED "ABERRATION HOT SPOTS" AFTER DIFFERENT TREATMENT DESIGNS IN HORDEUM VULGARE ROOT TIP MERISTEN CELLS OF THE RECONSTRUCTED KARYOTYPE MK 14/2034

| Treatment variants | Number of observed cells versus Number of observed breaks | Non-hot spot segments (%) | Observed "hot spot segments" (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7 Chr.$1^7$ | 10 Chr.2 | 15 Chr.$3^4$ | 17 Chr.$3^4$ | 20 Chr.$4^3$ | 21 Chr.$4^3$ | 24 Chr.$4^3$ | 26 Chr.$4^3$ | 28 Chr.$4^3$ | 34 Chr.5 | 39 Chr.6 | 41 Chr.6 | 43 Chr.$7^1$ | 46 Chr.$7^1$ | 48 Chr.$7^1$ |
| Control | 2000 cells 87 breaks | 100 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Sal 10 µg/ml | 2000 cells 169 breaks | 100 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Sal 50 µg/ml | 2000 cells 207 breaks | 88.5 | - | - | - | - | - | - | - | - | - | - | - | - | 11.5 | - | - |
| Sal 100 µg/ml | 2000 cells 281 breaks | 74.8 | - | - | - | - | - | 8.0 | - | 8.0 | - | - | - | - | - | 9.2 | - |
| Zeo 300 µg/ml | 3000 cells 968 breaks | 33.4 | 5.7 | 3.8 | 9.6 | 4.9 | - | 6.1 | 4.3 | 5.1 | 4.2 | 5.7 | 6.6 | 6.0 | - | 4.6 | - |
| Sal 100 µg/ml →4h→Zeo 300 µg/ml | 1250 cells 275 breaks | 81.0 | - | - | - | - | 11.9 | - | - | - | - | - | - | 7.1 | - | - | - |
| Sal 100 µg/ml →0h→Zeo 300 µg/ml | 1250 cells 314 breaks | 67.0 | - | - | - | - | - | 11.0 | - | - | - | - | - | 11.0 | - | - | 11.0 |

"Aberration hot spots" (AHS) were evaluated in barley metaphase chromosomes of the first mitosis after treatment (Fig. 7). Zeocin treatment alone induced twelve AHS, whereas the sage extract showed a dose-dependent manner of "hot spot" formation: Salvia at a concentration of 10 µg/ml induced no AHS; at 50 µg/ml, one AHS; and at 100 µg/ml, three AHSs (see Table I). All AHS were isochromatid breaks. The yield of "aberration hot spots" was decreased when both experimental designs with conditioning and challenge treatment were applied. Nearly 2.5-fold reduction of AHS was observed after treatment with Salvia extract and zeocin without any inter-treatment time. A six-fold decrease in the level of AHS was achieved when sage extract was applied as a low-toxic conditioning treatment prior to zeocin challenge and 4-hour inter-treatment time compared to zeocin treatment only. Zeocin induced specific ''aberration hot spots'' in barley which were either limited to heterochromatin-containing segments around the centromers (50%) or to terminal segments (50%). After additional treatment with sage extract the breaks do not more concerns to proximal heterochromatin-areas near (Fig. 7). This is a visible improvement because aberrations within proximal heterochromatin-containing segments and centromers seem to be more harmful for survival than those induced in gene-rich regions (Künzel *et al*. 2001).

The reduced clusters of AHS were located mostly in terminal chromosome segments (see Table I). The change in the location of AHS (proximal or terminal chromosomal regions) seems to be agent specific. In our previous study (Kopaskova *et al.* 2012) we found that extract from *Lilium candidum* reduced the yield of AHS in barley significantly, but did not affect their location. On the other hand, *Papaver rhoeas* extract (Gateva *et al*., 2014) showed characteristics similar to those of *Salvia officinalis* extract, i.e. reduction of proximal heterochromatin-rich region as "aberration hot spots".

The spectrum of induced chromosome aberrations depends on the experimental design and the test system. The treatments applying both schemes of experimental designs – with 4-hour inter-treatment time and without any inter-treatment time between conditioning treatment with sage extract and challenge treatment with zeocin induced a more diverse spectrum of chromosome aberrations in human lymphocytes than in *Hordeum vulgare* (data not shown).

## IV. Discussion

Oxidative stress results from overproduction of ROS and also from imbalance between their production and the organism's ability to detoxify them or repair the DNA damage. ROS possess high reactivity and could affect cellular functions and integrity. Oxidative stress can cause injuries in DNA such as DSBs, changes in major and minor grooves, changes in double helix, and could destroy proteins and lipids (Al-Saadi *et al.*, 2012). It can also cause DNA–DNA and DNA–protein cross links and mismatches between nucleotide pairing (Aust and Eveleigh, 1999).

The biological activities of medical herbs such as *Salvia officinalis* that may protect cells against oxidative stress-induced damage are being investigated (Xavier *et al.*, 2009, Al-Ezzy *et al*., 2011). In order to regulate the application of herbs in medicine it is essential to study their safety. Attention is particularly paid for the herbs used in traditional medicine to have low cytoxicity and genotoxicity. Many plants used in traditional and folk medicine have been reported to be potentially cytotoxic, mutagenic, and/or carcinogenic (Mengs, 1988, Ferreira-Machado *et al*, 2004, Gadano *et al*., 2006). For example, Ewadh *et al*. (2013) showed that notwithstanding their remedial properties, some extracts may cause harmful side effects.

The results presented here show no or just a low cytotoxic effect of Sage water extract in the concentrations applied by us. The clastogenic effect of *Salvia officinalis* extract was clearly dose-dependent in both test-systems. Barley root tip meristem cells could be considered to be more tolerant to the sage extract than human lymphocytes. Our data are in agreement with Vujošević and Blagojević (2004), who showed that sage extract used at a high concentration (100 µl/kg) is cytotoxic in a mammalian test system *in vivo*. According to Dogan (2004) and Lima *et al*. (2004), the use of higher doses of *S. officinalis* is not recommended because its essential oil contains tannins, toxic thujone and eugenol compounds. Contradictory results also exist. Mathew and Thoppil (2012 b) reported no genotoxicity of *S. officinalis* extract. According to the authors, administration of *S. officinalis* extract alone does not produce any significant variation of micronucleated polychromatic erythrocytes compared with the control. Al-Ezzy *et al*. (2010) showed that aqueous extract of sage has the ability to increase the mitotic index in bone marrow cells in albino male mice as compared to the negative control and in mice treated with cytosar drug that caused reduction in mitotic index.

In the present study, our interest was also focused on the anti-cytotoxic and anti-genotoxic potential of sage water extract against the DSBs inducer zeocin. It is well known that unrepaired DSBs can lead to cancer, serious diseases and/or cell death (Rich *et al*., 2000, Alfadda and Sallam, 2012). In our study, we observed a protective effect of *S. officinalis* extract against zeocin. The cytotoxic and clastogenic effects of the radiomimetic were decreased when *S. officinalis* water extract was applied as a conditioning treatment with a higher but low-toxic concentration prior to challenge treatment with a high toxic concentration of zeocin and 4 h inter-treatment time in both test systems. Our results are in agreement with the study of Mathew and Thoppil, (2012 a) on Swiss albino mice, where pre-treatment with methanol extract of *S. officinalis* at higher concentrations 50, 75 and 100 mg/kg was shown to lead to a significant decrease in the frequency of methyl parathion induced micronuclei and DNA damage assessed by comet assay. In another study, Mathew and Thoppil (2012 b) showed that pre-treatment with extracts of three Salvia species decreased not only the micronuclei in polychromatic

erythrocytes, but also the chromosome aberrations induced by methyl parathion. The data presented by us are also in accordance with the study of Alkan *et al*. (2012) where *S. officinalis* methanolic extract reduced the micronucleated polychromatic erythrocytes and increased the ratio of polychromatic erythrocytes to normo-chromatic erythrocytes induced by cyclophosphamide in Wistar albino rat bone marrow. Hudecova *et al*. (2012) showed that plant extracts from *Gentiana asclepiadea* and *Armoracia rusticana* applied in non-toxic concentrations in split treatment with zeocin decrease the DNA damage caused by zeocin to more than 50% and enhance the adaptive response (AR) in human lymphocytes. Our data are in agreement with these results and are also in accordance with our previous study where conditioning treatment with *Papaver rhoeas* extract followed by 4 h inter-treatment time and zeocin challenge treatment showed induction of AR (Gateva *et al*., 2014). The results from our present study that the genotoxic effect of the radiomimetic was decreased more than 2-fold, indicate AR induction after conditioning treatment with sage extract, 4 h inter-treatment time and challenge treatment with zeocin. Many studied show that AR could activate DNA repair networks (Miura *et al*., 2004), *de novo* protein synthesis (Tosello *et al*., 2007), and antioxidants (Achary 2009).

Based on the reconstructed barley karyotype, our results could allow for a valuable detailed interpretation of the chromosome-specific effect of the Salvia extract applied as conditioning treatment followed by challenge treatment with ROS inducer zeocin. Stoilov *et al*. (2013) investigated the potential of different cytological reconstructed barley karyotypes to overcome single- and double-strand DNA breaks induced by bleomicyn, a well known ROS inducer. In some cases there were remarkably increased aberration yields, on the one hand, but on other hand, higher sensitivity was not related to any specific clastogenic response of particular chromosome regions. In our previous studies we reported specific ‘‘aberration hot spots’’ in barley induced by the ROS inducer paraquat (Jovtchev *et al*., 2010; Gateva 2012). They are mostly limited to heterochromatin-rich segments and only isochromatid breaks appeared as aberration clustering. In the present investigation we found that treatment with zeocin induced 12 specific AHS, equally located in heterochromatin-containing regions and terminal segments. When conditioning treatment with *S. officinalis* water extract was given prior to zeocin challenge treatment, the AHS were decreased significantly (2.5- to 6-fold) compared to those observed following zeocin treatment alone. The fact that after the conditional treatment the heterochromatin-regions no longer appeared to be an “aberration hot spot,” underlines the specificity of the plant extracts. This is particularly interesting, considering the fact that the reduced aberration frequency by other plant extracts and/or mutagens is not imperatively connected to the chromosome segments were AHS are manifested (Kopaskova *et al*., 2012; Stoilov *et al*., 2013).

Our data here also showed that the cytotoxic and genotoxic effects of zeocin were also decreased in samples without any inter-treatment time between the sage extract treatment and zeocin in both test systems. This indicates that the protective effect of sage extract is exhibited irrespective of the experimental design and the test system. This is in correspondence with the study of Vujošević and Blagojević (2004), who obtained that post-treatment with terpenoid fractions of sage suppressed the effects of Mitomycin C in a mammalian system *in vivo*. The authors calculated that the percent of aberrations induced by Mitomycin C alone decreased with increasing the concentrations of sage.

Nikolić *et al*. (2012) showed that multiple mechanisms are involved in the anti-mutagenicity/anti-genotoxicity of sage and basil extracts and pure monoterpenes, using *Escherichia coli* K12 assay and modified standard mutagenicity tests (*E. coli* K12, *Salmonella*/microsome and *S. cerevisiae* reversion assays). Many reports indicated that the anti-mutagenic potential of *S. officinalis* extracts is probably due to the bioactive components present in it (Fleming, 1998; Dent *et al*., 2013). Salvia extracts contain a plethora of compounds including polyphenols, terpenoids and flavonoids (Dogan, 2004; Slamenova *et al*., 2004; Lima *et al*., 2005). These phytochemicals can act as antioxidants (Miura et. al., (2002) and cell prolipherative stimulators (Mathew and Thoppil 2012 a). Sage is a natural source of flavonoids and polyphenolic compounds (e.g., carnosic acid, rosmarinic acid and caffeic acid) possessing strong anti-oxidant, radical-scavenging, and anti-bacterial activities (Cuvelier *et al*., 1994, Dorman *et al*., 2003; Lima *et al*., 2007;–Baranauskiene *et al*., 2011). Weisburger (2001) also reported protective effects of phenolic compounds against deleterious genotoxic carcinogens by scavenging reactive oxygen species (ROS) and enhancing host antioxidant defence systems.

Pradhan and Girish (2006) reported glutathione-enhancing and liver regenerative effects of *Salvia officinalis*. The plant extract had a high free radical scavenger activity expressed as a reactive reaction % in a dose-dependant manner. Knezevic-Vukcevic *et al*. (2007) suggested that the protective effect of sage monoterpenoids was through enhanced recombinational repair and excision repair.

The results from our cytogenetic analysis using chromosome aberrations and micronuclei as endpoints and applying various experimental designs showed a well expressed protective effect of *S. officinalis* water extract against the oxidative stress inducer zeocin. The protective effect was demonstrated in phylogenetically distant test systems that have different susceptibility. The anti-cytotoxic and anti-clastogenic effects of sage extract could indicate AR induction and also correspond to antioxidant properties of plant extract well documented in other studies.

Plant extract from *S. officinalis* could be used as a natural and alternative means of preventing oxidation. The present work could be useful in further health

research programs and prophylactic therapy using natural plant extracts.

## V. Conclusions

1) Sage water extract alone showed no or just low cytotoxic effect and clastogenic effect clearly dependent on the concentration and the test system. Human lymphocytes *in vitro* as a test system were more susceptible to *S. officinalis* extract than *H. vulgare*.

2) *S. officinalis* water extract applied in a higher but low-toxic concentration showed well expressed anti-cytotoxic and anti-clastogenic effect against the radiomimetic zeocin.

3) S. *officinalis* water extract could provide genome protection against the harmful action of genotoxins such as zeocin. The protective effect was manifested irrespective of the experimental design and the test system.

4) The analysis of ‘‘aberration hot spots’’ in the reconstructed barley karyotype MK14/2034 after zeocin treatment showed that ~65% of all isochromatid breaks were clustered within 12 chromosome segments only. All AHS were found to be within heterochromatin-containing or terminal segments. AHS were reduced and no longer located in heterochromatin-rich regions after sage treatment following different experimental designs.

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## References

[1] W. Al-Awaida and M. Akash, “Protective role of aqueous medicinal herbal extracts against oxidative stress on glucose-6-phosphate dehydrogenase activity and RBC fragility,” *Life Sci. J.*, vol. 11, no. 2, pp. 385-391, 2014.

[2] V. M. M. Achary and B. B. Panda, “Aluminum – induced DNA damage and adaptive response to genotoxic stress in plant cells are mediated through reactive oxygen intermediates,” *Mutagenesis*, vol. 24, pp. 1-9, 2009.

[3] R. M. Al-Ezzy, K. W. Al-Samarrae, and A. H. Ad'hiah, “Effect of sage (salvia officinalis) aqueous extract on mitotic index in albino male mice,” *J. Biotechnol. Res.*, vol. 4, no. 1, pp. 36-43, 2010.

[4] R. M. Al-Ezzy, K. W. Al-Samarraei, and A. H. Ad'hiah, “Assessing the anti-mutagenic potentials of sage salvia officinalis L. leaf aqueous extract in cultured blood cells of acute lymphocytic leukaemia patients using the micronucleus formation assay,” *J. Biotechnol. Res.*, vol. 5, no. 2, pp. 25-30, 2011.

[5] A. A. Alfadda and R. M. Sallam, “Reactive oxygen species in health and disease,” *J. Biomed. Biotechnol.*, 2012.

[6] R. A. Agabeyli, “Antimutagenic activities extracts from leaves of the morus alba, morus nigra and their mixtures,” *Int. J. Biol.*, vol. 4, no. 2, pp. 166-172, 2012.

[7] F. Ü. Alkan, F. E. Gürsel, A. Ateş, M. Özyürek, K. Güçlü, and M. Altun, “Protective effects of salvia officinalis extract against cyclophosphamide-induced genotoxicity and oxidative stress in rats,” *Turk. J. Vet. Anim. Sci.*, vol. 36, no. 6, pp. 646-654, 2012.

[8] A. Al-Saadi, M. Al-Terehi, M. A. Kathem, M. Ewadh, Q. Al-Ameri, and H. Zaidan, “Protective effect of origanum vulgare against UV irradiation on DNA,” *J. Biol. Agric. Healthcare*, vol. 3, no. 4, pp. 11-17, 2013.

[9] O. Angelova, E. Miadokova, P. Parvanova, F. Gregan, and S. Chankova, “Differences in the bioactivity of methanolic and water extracts of poppy (papaver rhoeas L.),” *Compt. Rend. Acad. Bulg. Sci.*, vol. 67, no. 2, pp. 243-250, 2014.

[10] E. A. Aust and J. F. Eveleigh, “Mechanisms of DNA oxidation,” *P. Soc. Exp. Biol. Med.*, vol. 222, pp. 246-252, 1999.

[11] P. Badiee, A. R. Nasirzadeh, and M. Motaffaf, “Comparison of salvia officinalis L. essential oil and antifungal agents against candida species,” *J. Pharmaceut. Technol. Drug Res.*, vol. 1, pp. 7, 2012.

[12] J. Behboud, E. Amirreza, and M. Mohammad, “Antibacterial effect of salvia officinalis lam extract,” *Ann. Biol. Res.*, vol. 2, no. 6, pp. 532-535, 2011.

[13] R. Baranauskiene, E. Dambrauskiene, and P. Venskutonis, “Influence of harvesting time on the yield and chemical composition of sage (salvia officinalis L.),” in *Proc. 6th Baltic Conference on Food Science and Technology: Innovations for Food Science and Production*, Jelgava, LLU, May 5-6 2011, pp. 105-109.

[14] R. Capasso, A. I. Izzo, F. Capasso, G. Romussi, A. Bisio, and N. Mascolo, “A diterpenoid from salvia cinnabarina inhibits mouse intestinal motility in vivo,” *Planta Med.*, vol. 70, pp. 375–377, 2004.

[15] T. A. Celik, “Potential genotoxic and cytotoxic effects of plant extracts,” in *A Compendium of Essays on Alternative Therapy*, A. Bhattacharya, Ed., 2012, pp. 233-250.

[16] S. G. Chankova, E. Dimova, M. Dimitrova, and P. E. Bryant, “Induction of DNA double-strand breaks by zeocin in chlamydomonas reinhardtii and the role of increased DNA double-strand breaks rejoining in the formation of an adaptive response,” *Radiat. Environ. Biophys.*, vol. 46, no. 4, pp. 409-416, 2007.

[17] M. E. Cuvelier, C. Berset, and H. Richard, “Antioxidant constituents in sage (salvia officinalis),” *J. Agric. Food Chem.*, vol. 42, pp. 665-669, 1994.

[18] M. Dent, V. Dragović-Uzelac, M. Penić, M. Brnčić, T. Bosiljkov, and B. Levaj, “The Effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (salvia officinalis L.) extracts,” *Food Technol. Biotechnol.*, vol. 51, no. 1, pp. 84–91, 2013.

[19] E. Dimova, M. Dimitrova, D. Miteva, Z. Mitrovska, N. P. Yurina, P. E. Bryant, and S. Chankova, “Does single-dose cell resistance to the radio-mimetic zeocin correlate with a zeocin-induced adaptive response in chlamydomonas reinhardtii strains?” *Radiat. Environ. Biophys.*, vol. 48, no. 1, pp. 77-84, 2009.

[20] Y. Dogan, “A source of healing from past to today: Salvia officinalis,” *J. Nature Environ. Culture*, Izmir, vol. 3, 2004.

[21] H. J. D Dorman, A. Peltoketo, R. Hiltunen, and M. J. Tikkanen, “Characterisation of the antioxidant properties of deodorised aqueous extracts from selected lamiaceae herbs,” *Food Chem.*, vol. 83, pp. 255-262, 2003.

[22] H. Evans, *Handbook of Mutagenicity Test Procedures*, B. Kilbey, L. M. W. Nicols, and C. Ramel, Eds., Elsevier Science Publishers BV, 1984, pp. 405-427.

[23] M. Ewadh, H. Zaidan, Z. Haleem, M. Al-Terehi, and A. Al-Saadi, “Protective activity of some antioxidant plant against hydrogen peroxidase genotoxic effects on human lymphocyte,” *Adv. Life Sci. Technol.*, vol. 13, pp. 34-44, 2013.

[24] M. Farhoudi, S. Ghodratizadeh, and S. Ghodratizadeh, “Effects of salvia officinalis extract on carbon tetrachloride induced hepatotoxicity,” *Glob. Vet.*, vol. 7, no. 4, pp. 353-357, 2011.

[25] S. C. Ferreira-Machado, M. P. Rodrigues, A. P. M.Nunes, F. J. Dantas, *et al*., “Genotoxic potentiality of aqueous extract prepared from chrysobalanus icaco L. leaves,” *Toxicol. Lett.*, vol. 151, no. 3, pp. 481-487, 2004.

[26] J. Fischedick, “Terpenoids for medicine,” dissertation, Chapter 8, Conclusions and Future Perspectives, Leiden University, 2013, pp. 169-176.

[27] T. Fleming, *PDR for Herbal Medicines*, 1st Edn., Montvale, Medical Economics Company, 1998, pp. 1113.

[28] A. B. Gadano, A. A. Gurni, and M. A. Carballo, "Argentine folk medicine: Genotoxic effects of chenopodiaceae family," *J. Ethnopharmacol*., vol. 103, pp. 246-251, 2006.
[29] S. Gateva, G. Jovtchev, M. Stergios, and P. Yonova, "The potential of synthetic thiourea compound to reduce the cytotoxic and genotoxic effects of paraquat in hordeum vulgare and cultured human lymphocytes," *Environ. Toxicol.*, vol. 227, no. 4, pp. 220-228, 2012.
[30] S. Gateva, G. Jovtchev, A. Stankov, and F. Gregan, "Antigenotoxic capacity of papaver rhoeas L. extract," *Int. J. Pharm. Pharmaceut. Sci.*, vol. 6, no. 1, pp. 717-723, 2014.
[31] M. Hamidpour, R. Hamidpour, S. Hamidpour, and M. Shahlari, "Chemistry, pharmacology, and medicinal property of sage (salvia) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer," *J. Tradit. Complement. Med.*, vol. 4, pp. 82-88, 2014.
[32] A. Hudecova, K. Hasplova, L. Kellovska, M. Ikreniova, E. Miadokova, E. Galova, and E. Horvathova, "Gentiana asclepiadea and armoracia rusticana can modulate the adaptive response induced by zeocin in human lymphocytes," Neoplasma, vol. 59, pp. 62-69, 2012.
[33] G. Jovtchev, S. Gateva, M. Stergios, and S. Kulekova, "Cytotoxic and genotoxic effects of paraquat in hordeum vulgare and human lymphocytes in vitro," *Environ. Toxicol.*, vol. 25, no. 3, pp. 294-303, 2010.
[34] A. Khan, N. Rehman, K. M. AlKharfy, and A. H. Gilani, "Antidiarrheal and antispasmodic activities of salvia officinalis are mediated through activation of K+ channels," *Bangl. J. Pharmacol.*, vol. 6, no. 2, pp. 111-116, 2011.
[35] J. Knezevic-Vukcevic, B. Vukovic Gacic, and D. Simic, "Antigenotoxic effect of plant extracts," *Genetika*, vol. 39, pp. 207-226, 2007.
[36] M. Kopaskova, L. Hadjo, B. Yankulova, G. Jovtchev, E. Galova, *et al*., "Extract of lillium candidum L. Can modulate the genotoxicity of the antibiotic zeocin," *Molecules*, vol. 17, pp. 80-97, 2012.
[37] G. Künzel, K. I. Gecheff, and I. Schubert, "Different chromosomal distribution patterns of radiation-induced interchange breakpoints in barley: First post-treatment mitosis versus viable offspring," *Genome*, vol. 44, pp. 128-132, 2001.
[38] C. F. Lima, F. Carvalho, E. Fernandes, M. L. Bastos, P. C. Santos-Gomes, *et al*., "Evaluation of toxic/protective effects of essential oil of salvia officinalis on freshly isolated rat hepatocytes," *Toxicol in Vitro*, vol. 18, pp. 457-465, 2004.
[39] C. F. Lima, P. C. Valentao, P. B. Andrade, R. M. Seabra, M. Fernandes-Ferreira and C. Pereira-Wilson, "Water and methanolic extracts of salvia officinalis protect HepG2 cells from t-BHP induced oxidative damage," *Chem. Biol. Interact.*, vol. 167, pp. 107-115, 2007.
[40] C. F. Lima, P. B. Andrade, M. S. Rosa, M. F. Fernandes, and C. P. Wilson, "The drinking of salvia officinalis infusion liver antioxidant status in miceand rats," *J. Ethnopharmacol.*, vol. 97, pp. 383-389, 2005.
[41] R. Liman, U. G. Gökçe, D. Akyıl, Y. Eren, and M. Konuk, "Evaluation of genotoxic and mutagenic effects of aqueous extract from aerial parts of linaria genistifolia subsp. genistifolia," *Braz. J. Pharmacogn.*, vol. 22, no. 3, pp. 541-548, 2014.
[42] J. Mathew and J. E. Thoppil, "Genotoxicity of methyl Parathion and antimutagenic activity of salvia officinalis L. (sage) extracts in swiss albino mice," *Asian J. Pharm. Clin. Res.*, vol. 5, no. 2, pp. 164-170, 2012.
[43] J. Mathew and J. E. Thoppil, "Investigation of the antimutagenic activity of three Salvia extracts," *Int. J. Pharm. Pharmaceut. Sci.*, 4, no. 3, pp. 225-230, 2012.
[44] R. R. Mendonça-Filho, "Bioactive phytocompounds: New approaches in the phytosciences," in *Modern Phytomedicine: Turning Medicinal Plants into Drugs*, I. Ahmad, F. Aqil, and M. Owais, Eds., Weinheim, Germany: WILEY-VCH Verlag GmbH & Co. KGaA, 2006, ch. 1, pp. 1-25.
[45] U. Mengs, "Toxic effects of sennosides in laboratory animals and in vitro," *Pharmacology*, vol. 36, pp. 180-187, 1988.
[46] Y. Miura, "Oxidative stress, radiation-adaptive stress, and aging," *J. Radiat. Res.*, vol. 45, pp. 357-372, 2004.
[47] K. Miura, H. Kikuzaki, and N. Nakatani, "Antioxidant activity of chemical components from sage (salvia officinalisis L.) and Thyme (thymus vulgaris L.) Measured by the oil stability index method," *J. Agric. Food Chem*., vol. 50, no. 7, pp. 1845-1851, 2002.
[48] I. D. Mierlici, G. Ghioghita, and G. Capraru, "Some cytogenetic effects induced in barley by the treatments with hydroalcoholic rosemary extract," *Analele Ştiinţifice Ale Universităţii Alexandru Ioan Cuza din Iaşi, Sectiunea II A: Genetica si Biologie Moleculara*, vol. 10, pp. 57-61, 2009.
[49] H. D. Nicolella, P. F. de Oliveira, C. C. Munari, and G. F. D. Costa, "Differential effect of manool – A diterpene from salvia officinalis, on genotoxicity induced by methyl methanesulfonate in V79 and HepG2 cells," *Food Chem. Toxicol.*, vol. 72, pp. 8-12, 2014.
[50] B. Nikolić, D. Mitić-Ćulafić, B. Vuković-Gačić, and J. Knežević-Vukčević, "Molecular Mechanisms of Action of Antimutagens from sage (salvia officinalis) and basil (ocimum basilicum)," in *Mutagenesis*, R. Mishra, Ed., Intech, Open Science, 2012, ch. 5, pp. 85-112.
[51] H. Nicoloff, R. Rieger, and G. Russev, "Mutations in RDNA 5. Is translocation involvement of barley nucleus organizer regions influenced by chromatin structure?" *Mutat. Res.*, vol. 181, pp. 147-155, 1987.
[52] N. N. Osman and A. S. Abd El–Azime, "Salvia officinalis L. (sage) ameliorates radiation-induced oxidative brain damage in rats," *Arab. J. Nucl. Sci. Appl.*, vol. 46, no. 1, pp. 297-304, 2013.
[53] A. Patenkovic, M. Stamenkovic-Radak, T. Banjanac, and M. Andjelkovic, "Antimutagenic effect of sage tea in the wing spot test of drosophila melanogaster," *Food Chem. Toxicol.*, vol. 47, pp. 80-183, 2009.
[54] S. C. Pradhan and C. Girish, "Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine," *Indian J. Med. Res.*, vol. 124, no. 5, pp. 491-504, 2006.
[55] G. Ramu, G. K. Mohan, K. N. Jayaveera, S. P. Dhanapal, and G Senthilkumar, "Preliminary phytochemical and antioxidant study of hydroalcoholic extracts from selected genera of Indian lamiaceae," *Asian Pac. J. Trop. Biomed.*, pp. S685-S688, 2012.
[56] K. Rami and Z. Li, "Antimicrobial activity of essential oil of salvia officinalis L. collected in Syria," *Afr. J. Biotech*., vol. 10, pp. 8397-8402, 2011.
[57] Y. Ren, P. J. Houghton, R. C. Hider, and M. J. R. Howes, "Novel diterpenoid acetylcholinesterase inhibitors from salvia miltiorrhiza," *Planta Med.*, vol. 70, pp. 201-204, 2004.
[58] T. Rich, R. L. Allen, and A. H. Wyllie, "Defying death after DNA damage," *Nature*, vol. 407, no. 6805, pp. 777-783, 2000.
[59] R. Rieger, A. Michaelis, I. Schubert, P. Döbel, and H. W. Jank, "Non-random intrachromosomal distribution of chromatid aberrations induced by X-rays, alkylating agents and ethanol in vicia faba," *Mutat. Res.*, vol. 27, pp. 69-79, 1975.
[60] I. Schubert and R. Rieger, "Expressivity of aberration hot spots after treatment with mutagens showing delayed or non-delayed effects," *Mutat. Res.*, vol. 44, pp. 337-344, 1977.
[61] K. Shahrzad, N. Mahya, T. B. Fatemeh, K. Maryam, F. B. Mohammadreza, and M. H. Jahromy, "Hepatoprotective and antioxidant effects of salvia officinalis L. hydroalcoholic extract in male rats," *Chin. Med.*, vol. 5, pp. 130-136, 2014.
[62] D. Slamenova, I. Masterova, J. Labaj, H. Horvathova, P. Kubala, J. Jakubikova, and L. Wsolova, "Cytotoxic and DNA-damaging effects of diterpenoid quinones from the roots of salvia officinalis L. on colonic and hepatic human cells cultured in vitro," *Basic Clin. Pharmacol. Toxicol.*, vol. 94, pp. 282-290, 2004.
[63] L. Stoilov, M. Georgieva, V. Manova, L. Liu, and K. Gecheff, "Karyotype reconstruction modulates the sensitivity of barley genome to radiation-induced DNA and chromosomal damage," *Mutagenesis*, vol. 28, no. 2, pp. 153-160, 2013.
[64] Y. Surh and L. R. Ferguson, "Dietary and medicinal antimutagens and anticarcinogens: Molecular mechanisms and chemopreventive potential - highlights of a symposium," *Mutat. Res.*, vol. 523-524, pp. 1-8, 2003.
[65] T. Todorova, M. Pesheva, F. Gregan, and S. Chankova, "Antioxidant, antimutagenic, and anticarcinogenic effects of papaver rhoeas L. extract on saccharomyces cerevisiae," *J. Med. Food*, 2014.
[66] M. E. Tosello, M. S. Biasoli, A. G. Luque, H. M. Magaró, and A. R. Krapp, "Oxidative stress response involving induction of protective enzymes in candida dubliniensis," *Med. Mycol.*, vol. 45, pp. 535-540, 2007.

[67] M. Vujošević and J. Blagojević, "Antimutagenic effect of extracts from sage (salvia officinalis) in mammalian system in vivo," *Acta Vet. Hungarica*, vol. 52, no. 4, pp. 439-443, 2004.
[68] M. Wang, J. Li, M. Rangarajan, Y. Shao, E. J. LaVoie, H. T-Ch and H. Ch-T, "Anti-oxidative phenolic compounds from sage (salvia officinalis)," *J. Agri. Food Chem.*, vol. 46, pp. 4869-4873, 1998.
[69] J. H. Weisburger, "Antimutagenesis and anticarcinogenesis, from the past to the future," *Mutat. Res.*, vol. 480-481, pp. 23-35, 2001.
[70] C. P. R. Xavier, C. F. Lima, M. Fernandes-Ferreira, and C. Pereira-Wilson, "Salvia fruticosa, salvia officinalis and rosmarinic acid induce apoptosis and inhibit proliferation of human colorectal cell lines: The role in MAPK/ERK pathway," *Nutr. Cancer*, vol. 61, no. 4, pp. 564-571, 2009.
[71] S. Yurtseven, M. Çetin, T. Şengül, and B. Sögüt, "Effect of sage extract (salvia officinalis) on growth performance, blood parameters, oxidative stress and DNA damage in partridges," *South African. J. Anim. Sci.*, vol. 38, no. 2, pp. 145-152, 2008.