

**CLINOPODIUM VULGARE L. EXTRACT COUNTERACTS
DNA DAMAGING ACTION OF RADIOMIMETIC ZEOCIN
ACCELERATING DSBs REJOINING IN SACCHAROMYCES
CEREVISIAE**

Teodora Todorova¹, Iviyana Ivanova^{1,2}, Petya Parvanova¹,
Stephka Chankova¹✉

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Abstract

The aim of the present work was to evaluate the potential of *Clinopodium vulgare* water leaves extract to decrease the DNA damaging action of zeocin and to throw more light on the mode of action.

Single and combined treatments with 10, 100, and 1000 µg/ml plant extract and 100 µg/ml zeocin were performed on 551 (haploid) and D7ts1 (diploid) yeast strains. The protective potential, the magnitude of the repair capacity, and the role of incubation conditions were studied based on double strand breaks' (DSB) induction and rejoining.

The results provided new evidence that despite the different ploidy both strains are DSBs repair proficient but differ in their repair capacity although comparable levels of initially induced DSBs were measured. The diploid strain D7ts1 was found to possess better-expressed repair capacity than the haploid strain 551. The combined treatment with *Clinopodium vulgare* L. extract at concentrations' range of 10–1000 µg/ml and zeocin resulted in a significant decrease in DSBs levels. It could be suggested that priming the cells with various concentrations of the extract results in an acceleration of DSBs rejoining when 30 min recovery time at optimal experimental conditions is given. When incubation is performed on ice the repair processes are impeded but not fully blocked. Such a study confirms that the difference in the repair capacity of

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the cells could be one of the possible mechanisms, participating in cell protection against different DNA damaging factors. The combined application of *Clinopodium vulgare* L. extract at concentrations range of 10–1000 µg/ml protects yeast DNA against zeocin-induced DSBs by acceleration of DSB rejoining.

Key words: *Saccharomyces cerevisiae*, *Clinopodium vulgare* L., acceleration of DSB rejoining, repair capacity, double strand breaks

Introduction. The modulation of the genotoxic potential of various environmental stressors by using biologically active natural compounds is one of the main strategies for genome protection. A lot of information concerning the mechanisms of action of natural products of plant origin has been gathered: anti-oxidative and/or regeneration of other antioxidants; inactivation of free radicals; chelation of heavy metals; inhibition of cell proliferation; modulation of signal transduction; regulation of transcription; DNA protective effect by stimulating DNA repair processes, etc. [1, 2].

Plant products are known as rich sources of antioxidants. Nevertheless, data concerning their ability to modulate DNA damaging action of various stressors is scarce. Wild basil (*Clinopodium vulgare* L.) is a worldwide-spread plant commonly used in Balkan Peninsula folk medicine for the treatment of various diseases [3, 4]. The main chemical constituents [4, 5] and in vitro antioxidant, antibacterial, anti-inflammatory, plasmid DNA protective, and anticancer activity of the extract have already been identified [3, 4, 6]. Previously it was shown by us that *Clinopodium vulgare* extract may protect plasmid DNA [6]. No other data were found concerning such activity. Based on this finding and the already reported chemical composition [4, 5] here we hypothesize that *Clinopodium vulgare* water leaves extract may possess DNA protective capacity against DNA damaging action of zeocin.

The aim of the present work was to evaluate the potential of *Clinopodium vulgare* water leaves extract to decrease the DNA damaging action of zeocin and to throw more light on the mode of action.

Materials and methods. Two *S. cerevisiae* strains with similar cell survival susceptibility to zeocin [7, 8] were used: haploid – 551 rho+ and diploid – D7ts1.

The cell cultures were cultivated at a temperature of 30 °C, 200 rpm, and their growth was determined spectrophotometrically by measuring the optical density at a wavelength of 600 nm (OD₆₀₀).

The radiomimetic zeocin (Invivogen) was used as a double-strand break inducer [7–9].

The lyophilized *Clinopodium vulgare* L. leaves water extract was prepared and kindly provided by BARDAROV [5]. Protocol for working solutions has been described previously [6].

Single treatment experiments. *Clinopodium vulgare* L. leaves extract treatment. Cell suspensions (1×10^6 cells/ml) at the end of exponential and the beginning of stationary phase were treated with three extract concentrations – 10,

100, and 1000 µg/ml for 1 h at 30 °C with aeration (on a rotary shaker). After incubation, cells were harvested and washed.

Zeocin (Zeo) treatment. Cell suspensions were treated with 100 µg/ml Zeo for 1 min on dark and ice (to prevent DNA repair) as described previously by us [7, 8].

Combined treatment experiments. Two steps procedure was used – single treatment with *Clinopodium vulgare* L. leaves extract as described above, followed by zeocin treatment without washing between them. After the procedure, cells were harvested, and the pellet was washed with cooled sterile MQ H₂O. Further, samples were split into three series: (1) without recovery time (–RT) after Zeo treatment; (2) with 30 min recovery time (+RT) at optimal for cell growth conditions (30 °C, 200 rpm) given after the Zeo treatment; (3) with 30 min RT on ice. Samples were then subjected to constant field gel electrophoresis.

Constant field gel electrophoresis for measurement of double-strand breaks induction. Levels of DSBs were measured by Constant Field Gel Electrophoresis (CFGE) [7, 8]. Gene Tool Analyzer G: Box Syngene was used to measure the ethidium bromide fluorescence. To quantify the induced double-strand break levels, the fraction of DNA released (FDR) was calculated following the formula described in [10].

Statistical data processing. All data are presented as mean values from at least three independent experiments. Values calculated after single treatments are presented as a fraction of DNA released (FDR). Results after combined treatment are presented as normalized FDR [9]. GraphPad Prism software version 6.04 (San Diego, USA) was used for statistical data processing. The magnitude of DSB repair capacity was calculated as a ratio between FDR measured in samples with and without 30 min recovery time [11].

Results and discussion. DNA susceptibility of strains and repair capacity after a single treatment with 100 µg/ml Zeo and different concentrations of wild basil leaves extract. A single treatment with 100 µg/ml Zeo resulted in around 2-fold higher DSB levels compared to untreated control samples without statistically significant differences between both strains. Effect of recovery time at optimal for cell growth conditions – 30 °C, 200 rpm) given 30 min (+RT) after single Zeo treatment was clear. The levels of DSB were reduced as follows: from 0.265 to 0.221 for strain 551 ($p < 0.05$), and from 0.243 to 0.173 for D7ts1 ($p < 0.01$) (Fig. 1A, B).

Further, the repair capacity (Table 1) of both strains was calculated. Data in Table 1 illustrate two main findings – both strains are DSBs repair proficient, but differ in their DSBs repair capacity despite the comparable levels of initially induced DSBs. These results are in agreement with the previous ones obtained on *Chlamydomonas reinhardtii*, where the wild-type 137C and the cross-resistant strains AK-9-9 and H-3 possessed approximately similar DNA susceptibility measured as levels of initially induced DSBs but differ in their repair capacity magnitude [12].

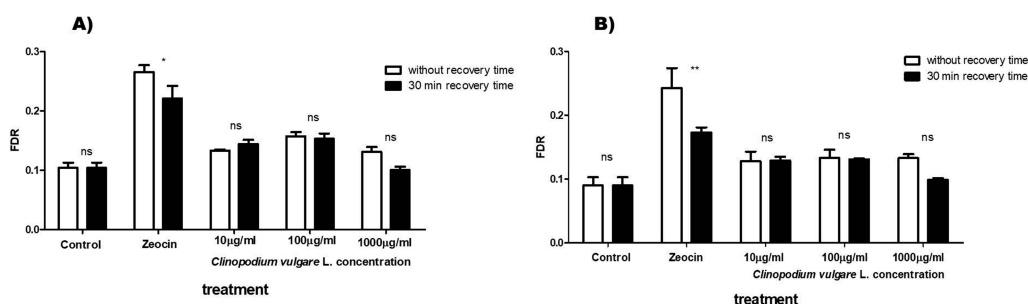


Fig. 1. DNA susceptibility and the role of recovery time after a single treatment with 100 µg/ml Zeo, and various concentrations of *Clinopodium vulgare* L. extract – 10, 100, and 1000 µg/ml on *Saccharomyces cerevisiae* strain 551 (A) and D7ts1 (B). Where the standard errors are not presented, they are equal to or smaller than the symbols of the graphic

T a b l e 1

Repair capacity of *Saccharomyces cerevisiae* strains presented as a ratio of the FDR measured after a single treatment with 100 µg/ml Zeo with and without 30 min recovery time

Strain	Repair capacity
551	1.199
D7ts1	1.405

No concentration or the genotype's dependent statistically significant differences were identified after a single treatment with *Clinopodium vulgare* leaves extract (Fig. 1A, B). The DSB levels were very close to those measured in the control – untreated samples.

The effect of *Clinopodium vulgare* leaves extract priming on the induced by Zeo DSBs. Three priming concentrations of *Clinopodium vulgare* leaves extract were tested. A similar around 2-fold statistically significant decrease of DSB levels was measured for both strains in samples without RT (Fig. 2A, B) in comparison with those after single zeocin treatment ($p < 0.0001$). No effect of the magnitude of concentration was found.

It could be suggested that priming treatment with *Clinopodium vulgare* extract may protect the yeast DNA from the damaging action of Zeo. This is in agreement with our previous data that treatment with the extract could protect plasmid DNA in vitro against DNA oxidative damage even when the lowest concentration of 10 µg/ml was applied [6]. The present results on both strains provide in vivo confirmation of this observation. No genotype specificity was obtained.

Is the DNA protective potential of *Clinopodium vulgare* leaves extract affected by the recovery time? To clarify this question further experiments were performed having in mind that strains were found to possess very similar DNA susceptibility, expressed as comparable levels of initially induced DSBs but different repair capacity.

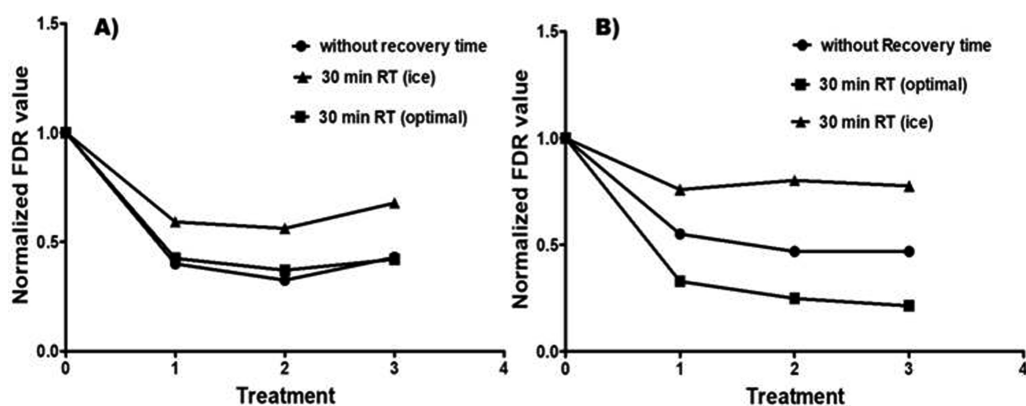


Fig. 2. DSBs levels measured immediately (●), and 30 min at optimal conditions (■) or on ice (▲) after the combined treatment with extract concentrations in the range of 10 (1), 100 (2), and 1000 (3) µg/ml, and 100 µg/ml Zeo of strain 551 (A) and D7ts1 (B). Where the standard errors are not presented, they are equal to or smaller than the symbols of the graphic

The results obtained in strain 551 provided evidence that the DSB levels measured after 30 min RT (optimal conditions) were comparable with those measured immediately after the treatment (Fig. 2A). It could be suggested that either 30 min RT is not enough to activate the DNA repair processes in strain 551 or the extract is not able to trigger the DNA repair.

The DSB levels measured in the diploid strain after 30 min RT at optimal conditions were statistically significantly lower in comparison with those calculated immediately after the treatment (Fig. 2B). Analysing curves in Fig. 2 it could be concluded that priming the cells with various concentrations of the extract results in an acceleration of DSBs rejoining when 30 min recovery time at optimal experimental conditions is given. These results are in good agreement with the studies of [16, 17].

Based on these data it could be suggested that the diploid strain possesses dose-dependent well-expressed repair capacity at optimal conditions when priming with different concentrations of *C. vulgare* is applied. The highest repair capacity was calculated after combined treatment with 1000 µg/ml plant extract and Zeo (Table 2) which correlated well with 1.5-fold increased levels of ROS induced by the application of 1000 µg/ml plant extract (data not shown). Probably this small amount of ROS is good enough to trigger DSBs DNA repair mechanisms.

Further, to clarify whether 30 min RT on ice could fully prevent DSBs repair potential of strains further experiments were performed. Genotype-dependent differences in the DSB levels were obtained after the combined treatment and 30 min RT on ice. Although the DSBs levels were higher than those in samples measured immediately after the combined treatment, they were lower than those in single Zeo-treated samples (Fig. 2A, B). Genotype-dependent decrease of DSB levels was measured – around 1.6-fold in strain 551 and 1.3-fold in D7ts1 (Fig. 2A, B).

T a b l e 2

Repair capacity of *Saccharomyces cerevisiae* D7ts1 30 min after combined treatment at optimal conditions with *C. vulgare* extract at concentrations range 10–1000 µg/ml and 100 µg/ml Zeo at optimal conditions

<i>Clinopodium vulgare</i> L. concentrations (µg/ml) + 100 µg/ml zeocin			
Repair capacity			
Strain	10	100	1000
D7ts1	2.351	2.651	3.081

Our results show that the DSB repair is impeded when samples are on ice, but probably not fully blocked as the DSB levels are lower than those induced after a single Zeo treatment. Such observation is in accordance with our previously published data on *Chlamydomonas reinhardtii* [9]. No effect of the concentration was found on both strains when incubation was performed on ice.

The question of whether a cell's resistance depends on the level of initially induced DSBs or the cell's repair capacity is still under discussion [9,11,12,14,15]. Our previous research as well as other authors revealed that the DNA repair processes could be increased by natural products [15–18]. The present work is a confirmation of this finding.

Conclusion. The current study provides new evidence that despite the different ploidy of the strains D7ts1 and 551, both are DNA repair proficient towards single zeocin treatment. They are found to differ in their repair capacity, which confirms the suggestion that the potential to repair DNA damage is one of the driving events involved in the formation of DNA resistance. Additionally, the combined application of *Clinopodium vulgare* L. extract at concentrations range of 10–1000 µg/ml protects yeast DNA against zeocin-induced DSB by acceleration of DSB rejoining. Experimental confirmation is provided that when *S. cerevisiae* strains are incubated on ice the repair processes are impeded but probably not fully blocked.

Our finding contributes to the scarce knowledge concerning the application of plant extracts for the modulation of DNA repair.

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¹*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,
2 Gagarin St, 1113 Sofia, Bulgaria
e-mails: tedi_todorova@yahoo.com, iv4ity@abv.bg, petq_parvanova@abv.bg,
stephanie.chankova@yahoo.com*

²*Faculty of Biology, Sofia University "St. Kliment Ohridski",
8 Dragan Tsankov Blvd, 1164 Sofia, Bulgaria
e-mail: iv4ity@abv.bg*