Analysis of radioprotection and antimutagenic effects of *Ilex paraguariensis* infusion and its component rutin

N. Bracesco¹, V. Sosa¹, L. Blanc¹, V. Contreras¹, E.C. Candreva¹, V.A. Salvo², S. Hocart⁴, B. Mechoso¹ and E. Nunes¹

¹Radiobiology Laboratory, Department of Biophysics, Faculty of Medicine, University of the Republic, Montevideo, Uruguay
²Cancer Research Laboratory, Ponce School of Medicine Health Sciences, Ponce, Puerto Rico
³Cardiovascular Research Laboratory, Ponce School of Medicine Health Sciences, Ponce, Puerto Rico
⁴Peptide Research, Department of Medicine, Tulane University, New Orleans, LA, USA

Abstract

DNA repair pathways, cell cycle checkpoints, and redox protection systems are essential factors for securing genomic stability. The aim of the present study was to analyze the effect of *Ilex paraguariensis* (Ip) infusion and one of its polyphenolic components rutin on cellular and molecular damage induced by ionizing radiation. Ip is a beverage drank by most inhabitants of Argentina, Paraguay, Southern Brazil, and Uruguay. The yeast *Saccharomyces cerevisiae* (SC7Klys 2-3) was used as the eukaryotic model. Exponentially growing cells were exposed to gamma rays (γ) in the presence or absence of Ip or rutin. The concentrations used simulated those found in the habitual infusion. Surviving fractions, mutation frequency, and DNA double-strand breaks (DSB) were determined after treatments. A significant increase in surviving fractions after gamma irradiation was observed following combined exposure to γ + R, or γ + Ip. Upon these concomitant treatments, mutation and DSB frequency decreased significantly. In the mutant strain deficient in MEC1, a significant increase in γ sensitivity and a low effect of rutin on γ-induced chromosomal fragmentation was observed. Results were interpreted in the framework of a model of interaction between radiation-induced free radicals, DNA repair pathways, and checkpoint controls, where the DNA damage that induced activation of MEC1 nodal point of the network could be modulated by Ip components including rutin. Furthermore, ionizing radiation-induced redox cascades can be interrupted by rutin potential and other protectors contained in Ip.

Key words: Radiation damage; *Ilex paraguariensis* infusion; Rutin; Radioprotection; Antimutagenic effects; *Saccharomyces cerevisiae*

Introduction

*Ilex paraguariensis* St. Hill., *Aquifoliaceae* (Ip) is a widely distributed tree in Southern Brazil, North-eastern Argentina, Paraguay, and Eastern Uruguay. Its dried leaves are used to prepare a traditional infusion (“mate”). The “mate” drinking habit has been popular for centuries, and was adopted from the native inhabitants of the region (“guaraní” Indians) (1).

In the past 25 years, the properties of Ip have been increasingly studied and the subject of several publications. Antioxidant properties (using chemical models and ex vivo lipoprotein studies), vaso-dilating and lipid reduction properties, antimutagenic effects (depending on used model), anti-glycation effects, and weight reduction properties (2–9) have been reported.

Given the known effects of ionizing radiation as an inducer of reactive oxygen species (OH−, superoxide radicals, and H₂O₂) in intracellular media, we hypothesized that either Ip infusion or some of its components could counteract radiation damage at the cellular and molecular levels by trapping free radicals through modulation of DNA repair pathways. The putative protective effect could be detected in vivo by analyzing cell proliferation, mutagenesis, and DNA damage in irradiated cell populations of the eukaryotic model *Saccharomyces cerevisiae*.

Previous work at our laboratory showed that both caffeine and high temperatures had mutagenic effects on cell populations, which decreased significantly in the presence of Ip infusion (4). This antimutagenic effect of Ip was attributed in part to the presence of B-complex vitamins in the infusion through modulation of error-free DNA repair (4,10,11). Furthermore, it was indicated that one or more Ilex components (i.e., polyphenols and vitamins) could induce cellular defense mechanisms such as DNA protection in a similar way as alpha-tocopherol (12,13).

The aim of present work was to analyze the putative protective role of Ip infusion (“mate”) and one of its
polyphenolic components - the bioflavonoid rutin (quercetin-3-O-rutinoside) - on cellular and molecular damage induced by ionizing radiation. Importantly, the used concentrations simulated those found in the habitual infusion.

Material and Methods

Yeast strains and growth conditions
The following Saccharomyces cerevisiae haploid strains were used in the present analysis: SC7K lys2-3, wild type SJR751 (MATa ade2-101 his3 Δ200 ura3 ΔNco lys2 ΔBgl CAN1), and the corresponding mutant strains: smt1 (MATa ade2-101 his3 Δ200 ura3 ΔNco lys2 ΔBgl CAN1 smt1 Δ::Kan) and smt1/mec1 (MATa ade2-101 his3 Δ200 ura3 ΔNco lys2 ΔBgl CAN1 smt1 Δ::Kan mec1 Δ::Hyg) (14).

Yeast cells were grown to exponential phase (N=1, 2 × 10^7 cells per mL) at 30°C with aeration by shaking, in liquid nutrient medium YPD (1% yeast extract, 2% peptone, and 2% glucose (Sigma-Aldrich, USA).

Extraction of “yerba mate” by liquid chromatography and mass spectrometry
Yerba mate (77.784 g, Canarias S.A., Uruguay) was added to hot water (70°C 500 mL) and mixed for 15 min. Filtrate was collected with a glass wool plug, and then filtered with a 0.2 μm filter for sterilization. The mate was concentrated by rotary evaporation under reduced pressure and lyophilized to yield a brown powder (22.17 g, 28.5%).

Rutin analysis by liquid chromatography and mass spectrometry
Rutin hydrate (Sigma-Aldrich) was dissolved in methanol and diluted with water to give four separate standards containing 0.1, 0.125, 0.25 and 0.5 μg in 10 μL. Each standard (10 μL) was analyzed on a Shimadzu LCMS 2010A (Japan) using a diphenyl column (250 × 4.6 mm, 5 μm pore size, Vydac, (USA), a buffer system of 0.005% formic acid in water and B solution, and acetonitrile (0–50% B) over 30 min at a flow rate of 1 mL/min.

The dried Ip leaves (50 g) were added to 250 mL of distilled water at 70°C, and left to stand for 15 min, simulating the classic preparation. Thereafter, the infusion was sterilized with a fiberglass filter (2.7 μm). Final treatment concentration was 10%. Rutin hydrate (Sigma-Aldrich, USA) was diluted in distilled water, sterilized by filtration and used at the concentration found in the Ip infusion (40 μg/mL) (15). Both products at the indicated concentrations were added separately to liquid YPD cultures 1 h before irradiation (room temperature). Ip infusion was also added 5 min before irradiation in order to elucidate the kinetics of the putative protection. Exposure times corresponded approximately to 0.33 and 0.03 of cell cycle duration.

Irradiation was performed in Compton effect range, with a ^60Co source (Nordion 220, (Canada), mean photon energy E=1.25 MeV, and dose rate of 13.4 kGy/h. The dosimetry was performed with a polymethyl methacrylate Harwell Amber S 3042 dosimeter (United Kingdom). Absorbed dose was 0 < D < 200 Gy. Samples were irradiated in YPD liquid medium with or without Ip or R. After treatment, controls and treated samples were kept on ice before further processing. Relative surviving fraction was determined as a function of the absorbed doses. Based on survival curves (Figure 1) an absorbed dose of 200 Gy was selected for combination treatments. Aliquots of cells were plated in solid nutrient medium YPDA: YPD + 2% agar (DIFCO Laboratories, USA) and incubated at 30°C for 72 h. Survival was calculated as surviving fraction: S(x,y) = Ns/No, where Ns is the number of surviving cells capable of generating visible clones/mL; No is the total number of treated cells/mL; x the absorbed dose of radiation; and y the doses of the putative protectors (16,17).

To determine mutation frequency, cell samples of SC7K lys2-3 were plated after treatment on omission media (OM: 2% dextrose, 0.67% nitrogenous base yeast (Sigma-Aldrich, USA), 2% agar) (17,18) and incubated at 30°C for 21 days. Thereafter, the number of revertants lys → LYS were scored (12,13). Mutation frequency M(x,y) and mutation yield Y(x,y) were calculated as: M(x,y) = Nm / Ns; Y(x,y) = Nm / No, where Nm is the number of mutants per mL, No is the number of treated cells per mL, x the absorbed dose of radiation, and y the dose of modulators (16,19,20).

Figure 1. Surviving fractions as a function of γ-ray absorbed dose (Grays: Gy). Error bars indicate 95% binomial confidence intervals.
DNA double-strand breaks (DSB) determination

After exposure to treatments, nuclear DNA was isolated in agarose plugs after enzymatic treatment with lyticase and proteinase K (Sigma-Aldrich, USA) and submitted to pulse field electrophoresis (24 h, 3 steps; GeneLine II, Beckman, USA) (21). Analysis of DNA bands was performed by ImageJ software (NIH, USA).

Statistical analysis

Data from at least three reproducible experiments are reported as means ± SD. Binomial or Poisson confidence limits were determined and plotted in figures. Where not shown, confidence limits are of the size of the symbols.

Results

The analyzed samples showed high concentrations of polyphenols in Ip infusion as well as caffeine and other methylxanthines.

The characterization of Ip infusion and rutin quantification were performed using positive and negative ion mass chromatograms of Ip positive and negative ion; MS and UV spectra were recorded. Rutin eluted at 14.4 min and the area under the negative ion peak at 609.05 Da was determined to give a linear four-point calibration curve with an \( r^2 = 0.9876 \) (Supplementary Figures S1, S2, and S3).

Survival

Figure 1 shows surviving fraction as a function of \( \gamma \)-ray absorbed dose. An absorbed dose of 200 Gy giving a surviving fraction of 0.20 ± 0.02 in SC7K lys2-3 strain was selected for combined treatments of \( \gamma \)-rays either with Ip infusion or with rutin. The surviving fractions of irradiated (200 Gy) cells and respective controls are shown in Figure 2. A statistically significant increase was observed in cell survival after combined treatments compared to \( \gamma \)-ray acting as single agent. In the Ip + \( \gamma \) treatment group, the observed surviving fraction was significantly lower with addition 5 min before irradiation compared to addition 1 h before irradiation. The control samples and those treated either with Ip or with rutin showed similar surviving fractions (1.0 ± 0.02).

In the SJR151 and sml1 strains after 200 Gy exposure, surviving fractions were 0.20 ± 0.02 and 0.16 ± 0.03, respectively (Figure 3). Furthermore, both strains showed similar responses to \( \gamma \) treatment plus Ip and rutin as SC7K lys2-3 (compare Figure 2 and 3). Meanwhile, and as expected, sml1/mec1 mutant strain showed a significant increase in radiation sensitivity: \( S = 0.0023 ± 0.0028 \). The effects of \( \gamma \) exposure plus either Ip or rutin showed a low and not significant sensitivity increase compared to results using wild type SC7K lys2-3 (Figure 3).

Mutagenesis determination

In order to analyze the differential effect of each treatment on induced mutagenesis, cell samples of SC7K lys2-3 were plated on omission medium, and mutation frequency as a function of absorbed dose was determined (Figure 4). An exponential increase in mutation frequency was observed. Figure 5 indicates the significant antimutagenic effect of rutin and Ip present in the nutrient medium one hour before irradiation (200 Gy).

DNA double-strand breaks determination

Cell samples irradiated in the aforementioned conditions and respective controls were submitted to pulsed field electrophoresis after DNA isolation. In SC7K lys2-3, Ip as well as rutin resulted in protection against DNA breakage if present during irradiation. Thus, a decrease in \( \gamma \)-ray induced DSBs was observed, associated with the increased surviving fractions. Importantly, neither Ip nor rutin induced significant DSBs compared to the untreated control sample (Figure 6).

To elucidate the role of Ip and rutin in the observed increase in radiation resistance, we analyzed the corresponding effect on chromosomal fragmentation after irradiation in the mutant strain sml1/mec1 defective in MEC1/hATR gene.

Regarding induced DSB after 200 Gy, no significant effect of either rutin or Ip was observed compared to the corresponding wild type as well as to the sml strain (Figure 7).
The present results showed that Ip infusion and Ip component rutin had a significant protective effect against ionizing radiation in cell populations of haploid S. cerevisiae if added 1-h before irradiation and during it at concentrations corresponding to the habitual mate infusion. Importantly, Ip infusion showed relatively high concentrations of polyphenols including rutin. In fact, both natural products induced a significant increase in the survival probability of irradiated cell populations, as well as a significant decrease in the mutation frequency and in DSBs induced by gamma irradiation. Shorter exposure times to the infusion or to rutin showed a lower effect. Importantly, the spontaneous mutation frequency was not significantly altered upon single treatments.

Ionizing radiation at used energies and in aqueous nutrient media induced primary and secondary ionizations determining the formation of free radicals (reactive oxygen species and reactive nitrogen species - ROS and NRS) and oxidative damage in proteins, lipids, and DNA. These types of damage have been extensively investigated in different contexts (22 for review). Part of these modifications are reversible through red-ox mechanisms, or can be repaired by enzymatic networks, and part undergo cell
death (lack of proliferation ability in optimal conditions, Poisson distribution). Induced lethal genomic events (including DSB, chromosomal aberrations, and lethal mutations) in eukaryotic cells depend on a linear quadratic relationship in absorbed dose, where the probability of repair is an interactive factor (23).

In case of mutant strain sml1/mec1 deficient in MEC1, a significant increase in radiation sensitivity was observed. Regarding surviving fractions and chromosomal fragmentation after gamma exposure upon Ip or rutin addition, a low, not significant effect was observed, indicating the important role of MEC1 in the observed radiation response.

Other authors have shown that resveratrol, a polyphenol synthesized in several plants including *Ilex paraguariensis*, can modulate DSB repair and proliferation in lymphoblastic cell lines. It was suggested that this modulation depends on ATM/ATR-p53 cell cycle control and repair pathways (24). Importantly, these pathways have structural and functional homology between animal cells and yeast. However, other authors found an increase in chromosomal aberrations and the induction of micronuclei after resveratrol exposure (24–26).

The different and somewhat contradictory effects of some plant components on cell death and genomic stability could depend on the use of different media and concentrations (27). Several studies have indicated an important role of natural products, such as tea and its polyphenolic components, as radical scavengers, antioxidants, and anti-inflammatory chemopreventive agents. Tea infusions (*Camellia sinensis*) and tea polyphenols have been shown to inhibit tumor growth at different localizations and in several animal models. This inhibition was associated with decreased cell proliferation, increased apoptosis, and modulation of transduction cascades (27–30 for review). Thus, the potential use of tea, polyphenols, and other tea-derived products for cancer prevention is presently in continuous investigation. In this context, it was reported that peroxynitrite and lipoxygenase-induced oxidation in human LDL is inhibited by “mate” extracts and quercetin (2,31–33).

The present results indicated the central role of the phosphoinositol (PI3) kinase Mec1 in the activation of repair pathways involved in radiation resistance and their increase in the presence of Ip and rutin observed in wild type. Presence of these natural products previously to and during irradiation is essential to explain the observed increase in radiation resistance that depends on DNA repair transfunctions and protection through radical scavenging.

Alternative checkpoints function (CHK1 and RAD53) determine division delay and activate DSBs repair pathways (homologous recombination and non-homologous end-joining) (14,34,35). Our results indicated the important role of PI3 kinase Mec1 in genome stability after radiation damage. In fact, upon mutation of MEC1, an increase in radiosensitivity, as measured by surviving fraction, and a significant increase in DSB induction was observed. Furthermore, and regarding radiation resistance, error-prone or error-free Rad6 and Rad18 dependent translesion synthesis (TLS) play an important role upon single and double strand breaks as well as upon base damage (21,24,30). Induced base oxidative damage (i.e., 8-oxoG) in DNA is counteracted by base excision repair (BER), as well as by components of mismatch repair (MMR) and nucleotide excision repair (NER) pathways in connection to the recombination repair pathway (14,30 for review, 34–37).

The antioxidants contained in the Ip infusion can partially change the generation and fate of free radicals induced by radiation, depending on their concentration and redox potential of the involved components. The interference at redox cascades can take place at different compartments (i.e., at mitochondrial, and nuclear levels) as well as in the extracellular medium. The present results showed that the addition of Ip 5 min before irradiation only provided a mild protective effect, indicating that the time required for transport and intracellular distribution of the antioxidants play an important role in the observed radioprotective effects.

Based on the present and previous results on radiation resistance pathways, it is proposed that PI3 kinase Mec1...
acted as a nodal point in the regulatory network of transduction events elicited by direct and indirect oxidative effects of ionizing radiation. Importantly, it is known that ATR is a human MEC1 homolog.

Since protection against ionizing radiations and DNA repair are current important topics regarding human health and radioecology, more investigations are needed to further elucidate the involved mechanisms at molecular, cellular, and system levels.

Supplementary material

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Acknowledgments

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References

1. Ricca J. El Mate. Los secretos de la infusión. Desde la cultura nativa hasta nuestros días, 3rd ed., El Mendrugo: Montevideo, Uruguay; 2005.
2. Bracesco N, Sánchez AG, Contreras V, Menini T, Gugliucci A. Recent advances on Ilex paraguariensis research: minireview. J Ethnopharmacol 2011; 136: 378–384, doi: 10.1016/j.jep.2010.06.032.
3. Heck CI, de Mejia EG. Yerba mate tea (Ilex paraguariensis): a comprehensive review on chemistry, health implications, and technological considerations. J Food Sci
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1. Candreva EC, Keszenman DJ, Barrios E, Gelós U, Nunes E. Mutagenicity induced by hyperthermia, hot mate infusion, and hot caffeine in Saccharomyces cerevisiae. Cancer Res 1993; 53: 5750–5753.

2. Markowicz D, Moura D, Lobato R, de Oliveira P, Lima M. Yerba mate: pharmacological properties, research and biotechnology. Med Aromat Plant Sci Biotechnol 2007; 1: 37–46.

3. Peralta IN, de Oliveira Machado M, Becker AM, de Morais EC, Becker AM, de Morais EC, de Andrade F, et al. Mate tea (Ilex paraguariensis) enhances the gene modulation and activity of paraxanthine-2: in vitro and in vivo studies. Nutrition 2012; 28: 1157–1164, doi: 10.1016/j.nut.2012.04.011.

4. Klein GA, Stefanuto A, Boaventura BC, de Morais EC, Cavalcante Lda S, de Andrade F, et al. Mate tea (Ilex paraguariensis) improves glycemic and lipid profiles of type 2 diabetes and pre-diabetes individuals: a pilot study. J Am Coll Nutr 2011; 30: 320–332, doi: 10.1080/07315724.2011.10719975.

5. Boaventura BC, Di Pietro PF, Stefanuto A, Klein GA, de Morais EC, de Andrade F, et al. Association of mate tea (Ilex paraguariensis) intake and dietary intervention and effects on oxidative stress biomarkers of dyslipidemic subjects. Nutrition 2012; 28: 657–664, doi: 10.1016/j.nut.2011.10.017.

6. Nunes E, Candreva C, Keszenman EC. The mutagenic effect of elevated temperatures in yeast is blocked by a previous heat shock. Mutat Res 1993; 289: 165–170, doi: 10.1016/0027-5107(93)90086-O.

7. Bracesco N, Dell M, Rocha A, Behtash S, Menini T, Gugliucci A, et al. Antioxidant activity of a botanical extract preparation of Ilex paraguariensis: prevention of DNA double-strand breaks in Saccharomyces cerevisiae and human low-density lipoprotein oxidation. J Altern Complement Med 2003; 9: 379–387, doi: 10.1089/10755530376551606.

8. Pawar W, Jingjing L, Patel N, Kaur N, Doetsch P, Shadel G, et al. Check point kinase phosphorylation in response to endogenous oxidative DNA damage in repair-deficient stationary-phase Saccharomyces cerevisiae. Mech Ageing Dev 2009; 130: 501–508, doi: 10.1016/j.mad.2009.06.002.

9. Menini T, Heck C, Schulze J, de Mejia E, Gugliucci A. Protective action of Ilex paraguariensis extract against free radical inactivation of paraxanthine-1 in high-density lipoprotein. Planta Med 2007; 73: 1141–1147, doi: 10.1055/s-2007-981585.

10. Haynes RH. Formal, empirical and mechanistic equations in cellular radiation biology. In Kiefer J (Editor), Quantitative mathematical models in radiation biology, Springer Verlag, Berlin: 1988; p 181–189, doi: 10.1007/978-3-642-46656-4.

11. Lillo O, Bracesco N, Nunes E. Lethal and mutagenic interactions between γ-rays, cisplatin and etoposide at the cellular and molecular levels. Int J Radiat Biol 2011; 87: 222–230, doi: 10.3109/09553002.2010.518207.

12. Rose M, Winston F, Hieter P. Methods in yeast genetics, A laboratory course manual. Cold Spring Harbor Laboratory Press: New York, USA, 1990.

13. Severgini A, Lillo O, Nunes E. Analysis of bleomycin-induced mutagenic function related to the P50S (=XS9) gene of Saccharomyces cerevisiae. Environ Mol Mutagen 1991; 18: 102–106, doi: 10.1002/em.2850180204.

14. Ager DD, Haynes RH. Quantitative aspects of the interactive killing effects between X rays and other mutagens in microorganisms. Radiat Res 1988; 115: 124–140, doi: 10.1037/3577061.

15. Keszenman DJ, Candreva EC, Nunes E. Cellular and molecular effects of bleomycin are modulated by heat shock in Saccharomyces cerevisiae. Mutat Res 2000; 459: 29–41, doi: 10.1016/S0921-8779(99)00056-7.

16. Reisz JA, Bansal N, Qian J, Zhao W, Furdui MC. Effects of ionizing radiation on biological molecules. Mechanisms of damage. Antiox Redox Signaling 2014; 21: 260–292, doi: 10.1089/ars.2013.5489.

17. Chawick K, Leenhoux H, Wijngaard E, Sijmens M. DNA double-strand breaks and their relation to cytotoxicity. In: In Kiefer J (Editor), Quantitative Mathematical models in radiation biology, Springer Verlag, Berlin: 1988; p 149–158.

18. Gatz SA, Wiesmüller L. Take a break- resveratrol in action on DNA. Carcinogenesis 2008; 29: 321–332, doi: 10.1093/carcin/bgm276.

19. Cosentino C, Gricco D, Costanzo V. ATM activates the pentose phosphate pathway promoting anti-oxidant defense and DNA repair. EMBO Journal 2011; 30: 546–555, doi: 10.1038/embob.2010.330.

20. Matsuoka A, Lundin C, Johansson F, Sahlin V, Fukuhara K, Sjöberg BM, et al. Correlation of sister chromatid exchange lethal killing effects between X rays and other mutagens in microorganisms. Environ Mol Mutagen 1994; 23: 170–180, doi: 10.1038/emboj.2010.330.

21. Friedberg E, Walker G, Siede W, Wood RD, Schultz RA, Meselson M. DNA Repair and Mutagenesis, Second ed. American Society for Microbiology: Washington, DC, 2006.

22. Yang C, Wang V, Lu G, Picinich SC. Cancer prevention by Ilex paraguariensis extract against free radical inactivation of paraoxonase-1 in high-density lipoprotein oxidation. J Altern Complement Med 2003; 9: 379–387, doi: 10.1089/10755530376551606.

23. Owuor ED, Kong AN. Antioxidants and oxidants regulated signal transduction pathways. Biochem Pharmacol 2002; 64: 765–770, doi: 10.1016/S0006-2952(02)01137-1.

24. Kong AN, Rong Y, Hebbar V, Chen V, Owuor V, Hu R, et al. Signal transduction events elicited by cancer prevention compounds. Mutat Res 2001; 480: 231–241, doi: 10.1016/S0027-5107(01)00182-8.

25. Friedberg E, Walker G, Siede W, Wood RD, Schultz RA, et al. DNA Repair and Mutagenesis, Second ed. American Society for Microbiology: Washington, DC, 2006.

26. Yang C, Wang V, Lu G, Picinich SC. Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 2009; 9: 429–439, doi: 10.1038/nrc2641.

27. Gugliucci A, Menini T. The botanical extracts of Achyranthes satureoides and Ilex paraguariensis prevent methylglyoxal-induced inhibition of plasminogen and antithrombin III.
Life Sciences 2002; 72: 279–292, doi: 10.1016/S0024-3205 (02)02242-7.

33. Gugliucci A, Stahl AJC. Low density lipoprotein oxidation is inhibited by extracts of *Ilex paraguariensis*. *Biochem Mol Biol Int* 1995; 35: 47–56.

34. Nunes E, Candreva EC, Bracesco N, Sánchez A, Dell M. HDF1 and RAD17 genes are involved in DNA double-strand break repair in stationary phase *Saccharomyces cerevisiae*. *J Biol Phys* 2008; 34: 63–71, doi: 10.1007/s10867-008-9105-0.

35. Bracesco N, Candreva EC, Keszenman D, Sánchez AG, Soria S, Dell M, et al. Roles of *Saccharomyces cerevisiae* RAD17 and CHK1 checkpoint genes in the repair of double-strand breaks in cycling cells. *Radiat Environ Biophys* 2007; 46: 401–407, doi: 10.1007/s00411-007-0119-y.

36. van Loon B, Markkanen E, Hübscher U. Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. *DNA Repair* 2010; 9: 604–616, doi: 10.1016/j.dnarep.2010.03.004.

37. Biehs R, Steinlage M, Sibata A, Jeggo P, Lobrich M. DNA double-strand break resection occurs during non-homologous end joining in G1 but is distinct from resection during homologous recombination. *Molecular Cell* 2017; 65: 671–684, doi: 10.1016/j.molcel.2016.12.016.