Oxidal® Ameliorates the Ty1 Retrotransposition Induced by Methyl Methanesulfonate in *Saccharomyces cerevisiae*

Teodora Todorova¹, Martin Dimitrov¹,², Ignat Ignatov³*, Georgi Gluhchev⁴ and Georgi Dinkov⁵

¹Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.
²Faculty of Biology, Sofia University “St. Kliment Ohridski”, 1164 Sofia, Bulgaria.
³Scientific Research Center of Medical Biophysics (SRCMB), Sofia, Bulgaria.
⁴Institute of Information Technologies, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.
⁵IdeaLabs, LLC, Washington DC, USA.

Authors’ contributions

This work was carried out in collaboration among all authors. Author TT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MD and GD managed the analyses of the study. Author II managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i430211

Editor(s):
(1) Dr. Zahid Anwar, University of Gujrat, Pakistan.
Reviewers:
(1) Syed Umer Jan, University of Balochistan, Pakistan.
(2) S. Danish Kadir, University of Rajshahi, Bangladesh.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56725

Received 09 March 2020
Accepted 13 May 2020
Published 23 May 2020

Original Research Article

ABSTRACT

**Aim:** The aim of the study was to evaluate the potential of Oxidal® to decrease the Ty1 retrotransposition rate in a model system *Saccharomyces cerevisiae*.

**Study Design:** *Saccharomyces cerevisiae* cell suspensions were pre-treated with different concentrations Oxidal® and subsequently treated with 16mM methyl methanesulfonate. (MMS)

**Methodology:** The potential of various concentrations Oxidal® was evaluated based on “spot” test and Ty1 retro-transposition test.

**Results:** Data revealed that only 5% Oxidal® possesses some cytotoxic properties. Lack of Ty1 retro-transposition was observed after single treatment with 1, 2, 5 and 5% Oxidal® concentrations.

*Corresponding author: E-mail: mbioph@abv.bg, mbioph@dir.bg;
1. INTRODUCTION

Environmental pollution is considered as one of the major causes for different types of human cancer – 70-90% [1]. Cancer is characterized with deregulation of the cellular energetics [2]. It is believed that cancer development could be described by three stages: initiation, promotion and progression. Generation of reactive oxygen species (ROS) is proposed to act in all these stages of carcinogenesis [3,4]. Oxidative stress is reported as a crucial mechanism not only for cancer development but also in various pathologies such as cardiovascular diseases, diabetes, rheumatoid arthritis, Alzheimer or Parkinson disease [5]. As cancer is the second leading cause of mortality worldwide [6], many studies have been focused on the evaluation of substances, which may ameliorate the carcinogenic potential of different genotoxins. Thus, it is of a great importance to study compounds and products possessing antioxidant properties.

Oxidal® is a dietary supplement containing three major components – methylene blue, caffeine and salicylic acid. This product has already been reported to possess pronounced inhibitory activity on pathogenic bacterial strains [7,8]. Another study revealed the potential of Oxidal®, catholyte water and nano colloidal silver for prevention again SARS-CoV-2 and related disease COVID-19 [9]. It is believed that the combination of Oxidal® ingredients may increase oxygenation and improve metabolic processes.

Methylene blue is a widely applied dye, which is reported to compete with molecular oxygen for the transfer of electrons [10,11]. This is typical for the enzyme xanthine oxidase, where the dye diverts the electrons’ flow from the metal sulphur center of the enzyme, a place where molecular oxygen is normally converted into superoxide radicals. By this mechanism of competition, the generation of the cytotoxic superoxide radicals is attenuated [10-12]. Aksu et al. [12] reported that methylene blue could attenuate hepatic damage by reduction of the oxidative stress.

Caffeine at very low concentrations has been reported to protect against sporadic Alzheimer’s disease-like pathology by reduction of the cholesterol-induced increase in β-amyloid, phosphorylated tau and oxidative stress levels [13].

Salicylic acid is shown to exhibit anti-proliferative and antitumor activity in vitro and in vivo [14-16]. Additionally, salicylic acid possesses anticancer effect epithelial tissues most probably by inhibition of c-Myc [17].

On the other hand, all the tested concentrations showed promising results against the standard carcinogen methyl methane sulfonate. The most pronounced anti-carcinogenic and cytoprotective effects were observed after pre-treatment with 2.5% Oxidal®, which could be attributed to the antioxidant properties of the combination of ingredients; methylene blue, salicylic acid and caffeine. Further studies could reveal the exact mechanism of action of the supplement and the role of the antioxidant potential.

Conclusion: New data is provided concerning the potential of Oxidal® at low concentrations to protect *Saccharomyces cerevisiae* cells from MMS-induced Ty1 retro-transposition. The cytoprotective properties of the supplement were also obtained. These results could be considered as a basis for further studies revealing the exact mechanisms of cell protection of the Oxidal®. Additionally, our data could also serve as an important step of the in-depth research of a potential antiviral activity.

**Keywords:** Oxidal®; methylene blue; cytotoxic/anticytotoxic; carcinogenic/anticarcinogenic effects; *Saccharomyces cerevisiae*.

**ABBREVIATIONS**

| Acronym  | Description                                      |
|----------|--------------------------------------------------|
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2 |
| COVID-19 | Coronavirus Disease-19                           |
| MMS      | Methyl Methanesulfonate                          |
| ROS      | Reactive Oxygen Species                          |
| Ty       | Transposon of Yeast                              |
| YEPD     | Yeast Extract, Peptone, Dextrose                 |

On the other hand, all the tested concentrations showed promising results against the standard carcinogen methyl methane sulfonate. The most pronounced anti-carcinogenic and cytoprotective effects were observed after pre-treatment with 2.5% Oxidal®, which could be attributed to the antioxidant properties of the combination of ingredients; methylene blue, salicylic acid and caffeine. Further studies could reveal the exact mechanism of action of the supplement and the role of the antioxidant potential.

Conclusion: New data is provided concerning the potential of Oxidal® at low concentrations to protect *Saccharomyces cerevisiae* cells from MMS-induced Ty1 retro-transposition. The cytoprotective properties of the supplement were also obtained. These results could be considered as a basis for further studies revealing the exact mechanisms of cell protection of the Oxidal®. Additionally, our data could also serve as an important step of the in-depth research of a potential antiviral activity.

**Keywords:** Oxidal®; methylene blue; cytotoxic/anticytotoxic; carcinogenic/anticarcinogenic effects; *Saccharomyces cerevisiae*.

**ABBREVIATIONS**

| Acronym  | Description                                      |
|----------|--------------------------------------------------|
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2 |
| COVID-19 | Coronavirus Disease-19                           |
| MMS      | Methyl Methanesulfonate                          |
| ROS      | Reactive Oxygen Species                          |
| Ty       | Transposon of Yeast                              |
| YEPD     | Yeast Extract, Peptone, Dextrose                 |

1. INTRODUCTION

Environmental pollution is considered as one of the major causes for different types of human cancer – 70-90% [1]. Cancer is characterized with deregulation of the cellular energetics [2]. It is believed that cancer development could be described by three stages: initiation, promotion and progression. Generation of reactive oxygen species (ROS) is proposed to act in all these stages of carcinogenesis [3,4]. Oxidative stress is reported as a crucial mechanism not only for cancer development but also in various pathologies such as cardiovascular diseases, diabetes, rheumatoid arthritis, Alzheimer or Parkinson disease [5]. As cancer is the second leading cause of mortality worldwide [6], many studies have been focused on the evaluation of substances, which may ameliorate the carcinogenic potential of different genotoxins. Thus, it is of a great importance to study compounds and products possessing antioxidant properties.

Oxidal® is a dietary supplement containing three major components – methylene blue, caffeine and salicylic acid. This product has already been reported to possess pronounced inhibitory activity on pathogenic bacterial strains [7,8]. Another study revealed the potential of Oxidal®, catholyte water and nano colloidal silver for prevention again SARS-CoV-2 and related disease COVID-19 [9]. It is believed that the combination of Oxidal® ingredients may increase oxygenation and improve metabolic processes.

Methylene blue is a widely applied dye, which is reported to compete with molecular oxygen for the transfer of electrons [10,11]. This is typical for the enzyme xanthine oxidase, where the dye diverts the electrons’ flow from the metal sulphur center of the enzyme, a place where molecular oxygen is normally converted into superoxide radicals. By this mechanism of competition, the generation of the cytotoxic superoxide radicals is attenuated [10-12]. Aksu et al. [12] reported that methylene blue could attenuate hepatic damage by reduction of the oxidative stress.

Caffeine at very low concentrations has been reported to protect against sporadic Alzheimer’s disease-like pathology by reduction of the cholesterol-induced increase in β-amyloid, phosphorylated tau and oxidative stress levels [13].

Salicylic acid is shown to exhibit anti-proliferative and antitumor activity in vitro and in vivo [14-16]. Additionally, salicylic acid possesses anticancer effect epithelial tissues most probably by inhibition of c-Myc [17].

One strong ROS inducer is methyl methane sulfonate (MMS). MMS is an alkylating agent causing mainly base mispairing and replication blocks [18]. Data exists that MMS possesses strong pro-oxidative effect by inducing high levels
of reactive oxygen species (ROS) and carcinogenic effect [19].

Our hypothesis is that the commercial product Oxidal® could reduce the carcinogenic potential of methyl methane sulfonate.

The aim of the study was to evaluate the potential of Oxidal® to decrease the Ty1 retrotransposition rate in a model system Saccharomyces cerevisiae.

Saccharomyces cerevisiae was chosen as a model system due to the genome similarities with this in mammals. The full genome sequence revealed that around 31% of the proteins coded by yeast genes have human homologues. Additionally, around 50% of the genes responsible for various hereditary diseases have yeast orthologues [20,21].

2. MATERIALS AND METHODS

2.1 Strains

Four strains were used for a preliminary evaluation of the potential cytotoxic effect. The strains and their characteristics are described in Table 1.

2.2 Compounds

The dietary supplement Oxidal® (IdeaLabs, LLC, Washington, USA; author GeorgiDinkov) was studied in the present work. The compound contains methylene blue ([7-(dimethylamino)phenothiazine-3-ylidene]-dimethylazanium chloride; C_{16}H_{18}CIN_{3}S) – at concentration 1% (by mass), salicylic acid (2-hydroxibenzoic acid; C_{7}H_{6}O_{3}) - 1% (by mass) and caffeine (1,3,7-trimethylurine-2,6-dione; C_{8}H_{10}N_{4}O_{2}) - 1% (by mass).

2.3 Methods

Cell suspensions were treated with Oxidal®, dissolved in deionized water at various range of concentrations, mentioned in the methodological descriptions, for 60 min at optimal for cell growth conditions (30°C, aeration). Methyl methane sulfonate (Sigma Aldrich), used as a positive control and carcinogenic agent was applied at concentration 16 mM for 30 min.

2.3.1 “Spot” test

The concentrations range was evaluated based on spot test. The cell suspensions of four strains were treated with Oxidal® in the following concentrations: 1, 2, 3, 4 and 5% as described above. After the treatment, cells were washed, diluted to a concentration 1x10^{5} cells/ml and spotted on a solid YEPD (yeast extract, peptone, dextrose) medium. The intensity of the spots was used as preliminary information concerning the potential cytotoxic effect of the compound.

2.3.2 Ty1 transposition assay

Ty1 transposition assay was performed as described by [19,23,26]. Briefly, after single treatment with 1, 2.5 and 5% Oxidal®, or subsequent treatment with MMS, cell suspensions were washed with YEPD medium, and cultivated at t=20°C (optimal conditions for Ty1 transposition) for 24 hours. Appropriate dilutions of cells were then placed on YEPD to evaluate cell survival and on selective medium lacking histidine – for His^{+}transposants. Yeast media were prepared as described by [27].

Table 1. Different strains and their characteristics

| Strain | Description |
|--------|-------------|
| D7ts1  | diploid strain with genotype MATa/α ade2-119/ade2-40 trp5-27/trp5-12 ilv1-92/ilv1-92 ts1/ts1 [22] |
| 551 rho+ | genotype MATa ura3-167 his3Δ200:TymHIS3AI sec53 rho^{+} (National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria, Cat № 8719) [19,23,24] |
| 551 rho- | mutant strain 551 with disrupted or completely missing mitochondrial DNA, developed after treatment with ethidium bromide This study |
| 551 yap1Δ | has a disrupted YAP1 gene and was constructed by transformation of 551 cells with the yap1:: hisG-URA3-hisG cassette; the strain is characterized with a deletion of the yap1 gene, which makes this strain incapable of synthesizing yap1 protein [25] |
Mean transposition rates were determined and results presented as “fold increase of Ty1 transposition rate” related to control sample, taken as 1.00. A fold increase in treated cultures equal to or higher than 2.00 is considered as positive response of the Ty1 assay.

2.4 Statistical Analysis

All results were presented as mean±SEM from at least 3 independent experiments. The statistical analysis was performed by Graphpad Prism 5 software and includes an application of One-way ANOVA with Bonferroni’s post hoc test. P<0.05 was accepted as the lowest level of statistical significance.

3. RESULTS AND DISCUSSION

3.1 Potential Cytotoxicity and Carcinogenicity

3.1.1 “Spot” test

Concerning the potential cytotoxicity, spot test was performed on four strains. Data obtained revealed lack of cytotoxicity despite the concentration applied (Fig. 1). The spot intensity was similar in all the treatments. None of the strains was affected by the supplement. Such result suggests that Oxidal® does not possess cytotoxic effect on Saccharomyces cerevisiae.

It is known that when entering mitochondria, methylene blue (MB) acts like an additional electron source. More importantly, it prevents electron leakage for oxidants formation, the toxic side products in mitochondria (19). Overall, these data support that MB promotes mitochondrial function and reduces ROS production [28,29].

In present case, lack of cytotoxicity in the strains 551 rho- and 551 yap1Δ, may provide evidence for the activity of Oxidal even when the mitochondrial function is impaired.

3.1.2 Survival

Further studies were performed on strain 551rho+. Treatment with the lowest tested concentration – 1% Oxidal® did not result in reduction of the cell survival. The percentage of survived cells was comparable with the control – untreated cells. Statistically significant decrease was observed after the treatment with the highest tested concentration Oxidal® (5%) - 45% (P<0.0001). Statistically significant but biologically insignificant difference between the control - untreated cells and those treated with 2.5% Oxidal® (P<0.01) was also calculated.
Based on these results, it could be suggested that 5% Oxidal® possesses cytotoxic effect on *Saccharomyces cerevisiae* strain 551 rho+ (Fig. 2).

These data do not correspond with the spot test. Such discrepancy could be explained with the specificity of the tests. Spot test is performed for preliminary evaluation of the potential cytotoxicity while cell survival is a more precise method.

3.1.3 Ty1 retro-transposition

The results obtained in the present work showed that none of the concentrations tested increase the Ty1 retrotransposition rate (Fig. 3). Thus, suggesting lack of carcinogenic potential.

It is well known that carcinogens induce the transposition of Ty1 retro-transposon [26]. The specific activation of Ty1 is due to increased synthesis of ROS [19,23,24,30-33]. Based on the results reported here, it could be speculated that Oxidal is not able to induce ROS. Interestingly, the transposition rate after single treatment with 2.5 and 5% resulted in Ty1 retro-transposition rate significantly lower than the spontaneous one. This may indicate full block of the event.

3.2 Potential Cytoprotective Activity and Anti-carcinogenic Effect

3.2.1 Survival

The results obtained revealed that treatment with the standard carcinogen MMS lead to 58% cell survival.

Interesting results were obtained when cells were pre-treated with Oxidal® and then treated with MMS. Data clearly reveal that pre-treatment with all the tested concentrations of Oxidal® and subsequent treatment with MMS resulted in around 82% cell survival. The cell survival was comparable among the three samples (fig. 4). These results suggest that Oxidal® at the tested concentrations protects the cells from the cytotoxic activity of MMS.

3.2.2 Transposition rate

The transposition rate measured after single treatment with 16 mM MMS was 18 (Fig. 5).

Pre-treatment with different concentrations Oxidal® resulted in a dose-dependent reduction of the MMS-induced transposition. Pre-treatment with 2.5% Oxidal® showed around 5-fold decrease in the Ty1 retro-transposition rate while the pre-treatment with 5% Oxidal® resulted in around 6-fold reduction.

Taken into account the survival data, although, 5% Oxidal® possessed well-expressed anti-carcinogenic activity, single treatment of *Saccharomyces cerevisiae* revealed cytotoxic effect.

Based on these results, it could be suggested that Oxidal® at low concentrations possesses anti-carcinogenic properties. The exact mechanism of protection is not fully known. It could be related to a modulation of the oxidative stress by the combination of the three ingredients – methylene blue, salicylic acid and caffeine. It is well known that not all substances with reduction properties could be classified as antioxidants.
Antioxidants could be compounds, which are able to penetrate into the cells and protect the cellular compartments from the oxidative damage. As ROS are characterized with very short half-life and high reactivity, it always should be taken into account the fate of the potential antioxidants in live cells, instead of only in cell lysates or extracts [19]. The present study reveals that Oxidal® protect the cells, which means that it is able to penetrate into the cells.

In the present study, the dietary supplement Oxidal was found to protect the cells from the carcinogenic pro-oxidant methyl methane sulfonate through amelioration of the Ty1 retrotransposition events. This result could also serve as an important step in indepth research of the potential antiviral activity because it is well known that Ty1 retro-transposons are similar to retroviruses such as equine anemia virus, human immunodeficiency virus type 1 (HIV-1) [discussed in 26].

![Fig. 3. Potential carcinogenic effect of different concentrations Oxidal® - 1, 2.5 and 5%, on S. cerevisiae 551rho+ measured as Fold increase transposition rate. Average values ± SEM from at least 3 independent experiments. The significance in differences between negative control - untreated cells and treatment with the different concentrations was calculated by ANOVA with post-hoc test- Bonferroni’s Multiple Comparison Test. Where no error bars are evident, they are equal or less than the symbols](image1)

![Fig. 4. Cell survival (%) after pre-treatment with 1, 2.5 and 5% Oxidal® and subsequent treatment with the standard carcinogen methyl methane sulfonate. Average values ± SEM from at least 3 independent experiments. The significance in differences between positive control – single treatment with MMS and treatment with the different concentrations was calculated by ANOVA with post-hoc test- Bonferroni’s Multiple Comparison Test. Where no error bars are evident, they are equal or less than the symbols](image2)
Fig. 5. Potential anti-carcinogenic effect of different concentrations Oxidal® - 1, 2.5 and 5% against the standard carcinogen methyl methane sulfonate, on S. cerevisiae 551rho+ measured as Fold increase transposition rate. Average values ± SEM from at least 3 independent experiments. The significance in differences between positive control – single treatment with MMS and treatment with the different concentrations was calculated by ANOVA with post-hoc test- Bonferroni's Multiple Comparison Test. Where no error bars are evident, they are equal or less than the symbols.

4. CONCLUSION

New data is provided concerning the potential of Oxidal® at low concentrations to protect Saccharomyces cerevisiae cells from MMS-induced Ty1 retro-transposition. The cytoprotective properties of the supplement were also obtained. These results could be considered as a basis for further studies revealing the exact mechanisms of cell protection of the Oxidal®.

DISCLAIMAR

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

ACKNOWLEDGEMENTS

The research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wu S, Powers S, Zhu W, Hannun YA. Substantial contribution of extrinsic risk factors to cancer development. Nature. 2016;529(7584):43-47. Available: https://doi.org/10.1038/nature16166
2. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011; 144(5):646-674. DOI: 10.1016/j.cell.2011.02.013
3. Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological Interactions. 2006;160(1):1-40. Available: https://doi.org/10.1016/j.cbi.2005.12.009
4. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 2004;44:239-267. DOI:10.1146/annurev.pharmtox.44.101802.121851
5. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 2009;674:36–44. Available: https://doi.org/10.1016/j.mrgentox.2008.09.017
6. Stewart B, Wild CP World cancer report 2014. Health (N Y); 2017.
7. Popova T, Petrova T, Ignatov I, Karadzhov S. Preliminary in vitro investigations on the inhibitory activity of the original dietary supplement oxidal® on pathogenic bacterial strains. Journal of Advances in Agriculture. 2020a;11:37-43. Available: https://doi.org/10.24297/jaa.v11i1.8693

8. Popova T, Petrova T, Ignatov I, Karadzhov S, Dinkov G. Antibacterial activity of the original dietary supplement oxidal® in vitro. Journal of Advances in Agriculture. 2020b;11:71-78. Available: https://doi.org/10.24297/jaa.v11i1.8716

9. Ignatov I. Antiviral effects of nano colloidal silver, water catholyte, oxidal with methylene blue: Possible effects of influence over coronavirus SARS-CoV and SARS-CoV-2 with disease COVID-19. Journal of Health, Medicine and Nursing. 2020;73:96-101.

10. Ignatov I. Antiviral effects of nano colloidal silver, water catholyte, oxidal with methylene blue. Possible effects of influence over coronavirus SARS-CoV and SARS-CoV-2 with Disease COVID-19. Global Congress on Infectious Diseases, Sci Tech Infectious Diseases 2020; 2020.

11. Aksu B, Umit H, Kanter M, Guzel A, Aktas C, Civelek S, Uzun H. Effects of methylene blue in reducing cholestatic oxidative stress and hepatic damage after bile-duct ligation in rats. Acta histochemica. 2010;112(3):259-269. Available: https://doi.org/10.1016/j.acthis.2008.12.002

12. Salaris SC, Babbs CF, Voorhees III WD. Methylene blue as an inhibitor of superoxide generation by xanthine oxidase. A potential new drug for the attenuation of ischemia/reperfusion injury. Biochem Pharmacol. 1991;42:499-506. Available: https://doi.org/10.1016/0006-2952(91)90311-R

13. Aksu B, Inan M, Kanter M, Oz Puyan F, Uzun H, Durmus-Altun G, et al. The effects of methylene blue on renal scarring due to pyelonephritis in rats. Pediatr Nephrol. 2007;22:992-1001. Available: https://doi.org/10.1007/s00467-007-0464-8

14. Prasanthi JR, Dasari B, Marwarha G, Larson T, Chen X, Geiger JD, Ghribi O. Caffeine protects against oxidative stress and Alzheimer's disease-like pathology in rabbit hippocampus induced by cholesterol-enriched diet. Free Radical Biology and Medicine. 2010;49(7):1212-1220. DOI: 10.1016/j.freeradbiomed.2010.07.007

15. Pathi S, Jutooru I, Chadalapaka G, Nair V, Lee SO, Safe S, et al. Aspirin inhibits colon cancer cell and tumor growth and down regulates specificity protein (Sp) transcription factors. PLoS One. 2012;7:e48208. DOI: 10.1371/journal.pone.0048208

16. Law BK, Waltner-Law ME, Entingham AJ, Chytli A, Aake ME, Nørgaard P, et al. Salicylate-induced growth arrest is associated with inhibition of p70s 6k and down-regulation of c-myc, cyclin D1, cyclin A, and proliferating cellnuclear antigen. J Biol Chem. 2000;275:38261–7. DOI: 10.1074/jbc.M005545200

17. Borthwick GM, Johnson AS, Partington M, Burn J, Wilson R, Arthur HM, et al. Therapeutic levels of aspirin and salicylate directly inhibit a model of angiogenesis through a Cox-independent mechanism. FASEB J. 2006;20:2009–16. Available: https://doi.org/10.1096/fj.06-5987com

18. Ai G, Dachini re N, Muley P, Tummala H, Bhat GJ. Aspirin and salicylic acid decrease c-Myc expression in cancer cells: a potential role in chemoprevention. Tumor Biology. 2016;37(2):1727-1738. Available: https://doi.org/10.1007/s13277-015-3959-0

19. Lundin C, North M, Erixon K, Walters K, Jenssen D, Goldman ASH, Helleday T. Methyl methanesulfonate (MMS) produces heat-labile DNA damage but no detectable in vivo DNA double-strand breaks. Nucleic Acids Research. 2005;33(12):3799–3811. DOI: 10.1093/nar/gki681

20. Dimitrov MD, Pesheva MG, Venkov PV. New cell-based assay indicates dependence of antioxidant biological activity on the origin of reactive oxygen species. J Agric Food Chem. 2013;61: 4344–4351. DOI: 10.1021/jf401045w

21. Foury F. Human genetic diseases – A cross-talk between man and yeast. Gene; 1997;195:1–10. DOI: 10.1016/s0378-1119(97)00140-6

22. Hartwell LH. Yeast and cancer. Biosci Rep. 2004;24:523-544. DOI: 10.1071/s010540-005-2743-6
23. Zimmerman FK, Kern R, Rasenberger H. A yeast strain for simultaneous detection of induced mitotic crossingover, mitotic gene conversion and reverse mutation. Mutat Res. 1975;28:381-388. Available:https://doi.org/10.1016/0027-5107(75)90232-8
24. Pesheva M, Krastanova O, Staleva L, Hadzhitodorov M, Venkov P. The Ty1 transposition assay: A new short-term test for detection of carcinogens. J Microbiol Methods. 2005;61:1-8. Available:https://doi.org/10.1016/j.mimet.2004.10.001
25. Todorova T, Pesheva M, Gregan F, Chankova S. Antioxidant, antimutagenic, and anticarcinogenic effects of Papaver rhoeas L. extract on Saccharomyces cerevisiae. Journal of medicinal food. 2015;18(4):460-467. DOI: 10.1089/jmf.2014.0050
26. Stoycheva T, Pesheva M, Venkov P. The role of reactive oxygen species in the induction of Ty1 retrotransposition in Saccharomyces cerevisiae. Yeast. 2010; 27(5):259-267. Available:https://doi.org/10.1002/yea.1749
27. Stoycheva T, Pesheva M, Dimitrov M, Venkov P. The Ty1 retrotransposition short-term test for selective detection of carcinogenic genotoxins. Edited by Margarita Pesheva, Martin Dimitrov and Teodora Stefkova, INTECH: Croatia. 2012;83:610 110. DOI: 10.5772/38095
28. Sherman F, Fink GR, Hicks GB. (Eds.). Laboratory Course Manual for Methods in Yeast Genetics. CSHLP; 1986.
29. Viteri G, Chung YW, Stadtman ER. Effect of progerin on the accumulation of oxidized proteins in fibroblasts from Hutchinson Gilford progeria patients. Mech. Ageing Dev. 2010;131. DOI: 10.1016/j.mad.2009.11.006
30. Lattanzi G, Marmiroli S, Facchini A, Maraldi NM. Nuclear damages and oxidative stress: New perspectives for laminopathies Eur. J. Histochem. 2012;56: 284–288. DOI: 10.4081/ejh.2012.e45
31. Garfinkel D. Retroelements in microorganisms. Retrovirilidae. Plenum Press, New York, Ed., Levy, J.A. 1992: 107–136.
32. Pesheva M, Krastanova O, Stamenova R, Kantardjiev D, Venkov P. The response of Ty1 test to genotoxins. Arch Toxicol. 2008; 82:779-785. Available:https://doi.org/10.1007/s00204-008-0299-5
33. Dimitrov M, Venkov P, Pesheva M. The positive response of Ty1 retrotransposition test to carcinogens is due to increased levels of reactive oxygen species generated by the genotoxins. Arch Toxicol. 2011;85:67–74. Available:https://doi.org/10.1007/s00204-010-0542-8
34. Toshkova R, Zvetkova E, Gluhchev G, Ignatov I, Dinkov G. In vivo effects of cortinon+ on the emergence and progression of experimental graffi tumor in hamsters. International Research Journal of Oncology. 2020;3.

© 2020 Todorova et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.