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Cell cell death communication by signals passing through non-aqueous environments

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ABSTRACT

Investigation of ultraweak photon emission (UPE) in living mater was started in 1920-s by Russian biologist A. Gurwitsch discovered mitogenetic radiation (MGR), ultraviolet light emitted from growing organisms that stimulated similar organisms to grow. MGR attracted enormous interest of the scientific community resulting in many scientific publications confirming occurrence of permanent and spontaneous biophoton emission during natural metabolic processes in diverse living organisms. However, along with studies showing the existence of intercellular electromagnetic communication resulted in various responses of detector cells there have been numerous unsuccessful attempts to confirm the biological significance of MGR. Here we reported strong evidence for non-chemical intercellular signaling leading to biological cellular response. We found the ability of various cell types under conditions of oxidative stress induced by p-benzoquinones to generate death signals, which can affect target cells over long distances through non-aquatic environments resulting in morphological alterations and viability loss. We show that detector cells may distinguish and respond the same way to death signals transmitted from various type of inducer cells and pharmaceuticals may interrupt cellular death responses. These findings provide strong support for the view that non-chemical signals can provide a prompt and synchronic response of cell ensembles to noxious stimuli.

1. Introduction

Investigation of ultraweak photon emission (UPE) in living mater was started in 1920-s by Russian biologist A. Gurwitsch discovered mitogenetic radiation (MGR), ultraviolet light emitted from growing organisms that stimulated similar organisms to grow [1,2,3]. MGR attracted enormous interest of the scientific community resulting in many scientific publications confirming occurrence of permanent and spontaneous biophoton emission in the range of a few units to several thousand photons during natural metabolic processes in diverse living organism [4,5,6]. As early as 1934, it was hypothesized that MGR originates from recombination of free radicals [7]. The following studies revealed that the emergence of biophotons is due to the bioluminescent radical and non-radical reactions of reactive oxygen and nitrogen species [8,9,10]. However, biophotons may originate from a coherent emission mechanism and chromatin was suggested as one of the most essential sources of biophoton emission [11,12]. These processes are activated at the time of cellular death, resulted in that radiation is much stronger than that emitted under normal conditions [13]. However, along with studies showing the existence of intercellular electromagnetic communication resulted in the stimulation of cell division, neutrophils activation, etc., there have been numerous unsuccessful attempts to confirm the biological significance of MGR [4,6,14]. Here we reported strong evidence for non-chemical intercellular signaling leading to biological cellular response. We found the ability of various cell types under conditions of oxidative stress induced by p-benzoquinones to generate death signals, which can affect target cells over long distances through non-aquatic environments. These findings provide strong support for the view that biophotons may have biological significance.

2. Results and discussion

To clarify possibility non-chemical cell to cell communication, human HaCaT keratinocytes were plated (Fig. 1) and treated as described in “Methods” section. Cells were cultured 24 h and cells viability were analyzed. Almost complete loss in viability of HaCaT cells incubated with trimethyl-p-benzoquinone (cumoquinone) compared with viability of cells in rows 2 and 10 (approximate control) was observed (Fig. 2). However, much to the surprise of the research team, cells in the wells of neighboring rows (5 and 7) also showed significant morphological alterations and viability loss (Fig. 2). Similar results

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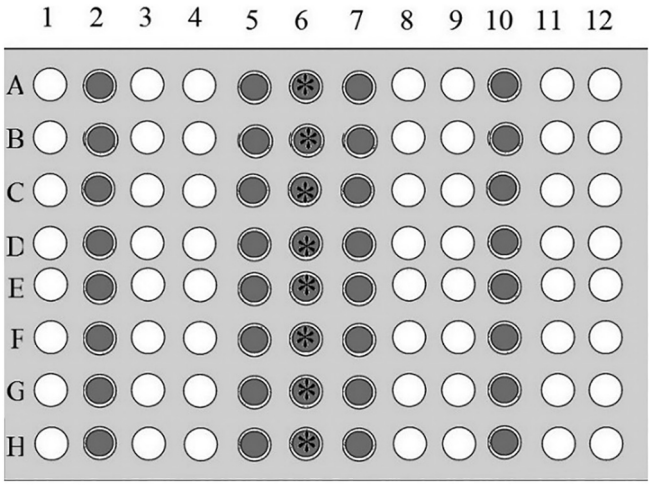

Fig. 1. The schematic view of the wells and rows arrangement on the multiwell plate. Filled circles indicate wells were plated with cells. Row 6 (*) - inductor, rows 5, 7 - detector, rows 2 and 10 - approximate control.

were found with middle passages of lung fibroblasts and MCF 7 breast cancer cells though no death of MDA-MB-231 breast cancer cells was found in the wells of neighboring rows (Fig. 2 right panel).

The same experimental protocol was followed for tetramethyl-p-benzoquinone (duroquinone). The presence of this compound in culture medium at a concentration 200 μM resulted in inducer cells death but a loss in viability of neighboring (detector) cells was much lower than that in experiments with cumoquinone (23.2 ± 7.9% $p < 0.001$ and 11.2 ± 8.2% $p > 0.05$ for HaCaT cells and fibroblasts respectively). Thus, the above data clearly show that cell to cell communication may be performed by signals, which have the potential of spreading through non-aquatic environments and particularly through plastic material. Consequently it must be admitted that such signals are non-chemical and cells (inductor) can emit electromagnetic waves in the spectral range above 300 nm (optical transparency of the used plate plastic, data not shown) and this radiation can be received by remotely located cells (detector) as a signal to initiate cell death. Thus, our data clearly indicate that, along with stimulating effects on proliferation and cellular activity [4–6,15] non-chemical signaling can result in cell death. This signaling seems to be very unusual but this is rational explanation of the above results. One of the essential conditions for initiating of non-chemical signaling is severe oxidative stress in inducer cells. Cellular oxidative stress may be defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or oxidative damage to biomolecules [16]. Numerous exposome factors (chemical, physical, and microbial) can lead to oxidative-mediated stress in tissues and cells [17]. Farhadi at al. revealed that non-chemical intercellular signaling between mechanically separated intestinal epithelial cells resulting in activation of NFκB pathway in detectors cells may be activated by exposing inducer cells to high concentration of H_2O_2 [18]. Apparently, for the biochemical response of the detector cells certain conditions are necessary, in particular, coherent electromagnetic fields appears to play an important role in this phenomena [19,20]. Quinones are widely used as anticancer, antibacterial or antimalarial drugs and as fungicides. Oxidative stress arises when the quinone is reduced by reductases to a semiquinone radical, which reduces oxygen to superoxide radicals and reforms the quinone. Most quinones form GSH conjugates which also undergo redox cycling and oxygen activation [21]. Among p-benzoquinone derivatives, trimethyl-p-benzoquinone and tetramethyl-p-benzoquinone has been reported to possess high cytotoxic activity and ability to induce oxidative stress [22]. In the present study, addition of each of the substances into culture media caused cellular oxidative stress and near complete cell death after 24 h of treatment. However cumoquinone treated cells were far more efficient as inductors of death signals compared with duroquinone. This finding predicates that benzoquinones participate in emitting of death signals not only as nonspecific inducers of oxidative stress but also may specifically enhance non-chemical intercellular signaling.

Next, we wished to determine whether this signaling could affect cells that were even further separated. Unlike the previous experiment with cumoquinone treated human HaCaT keratinocytes, detector cells were plated not only in wells of rows 5, 7 (1 cm apart from inducer cells), but also in wells of rows 4, 8 (2 cm apart from inducer cells). The effect could be seen in detector cells as far as 2 cm away from inducer cells (Table 1).

To reveal cell-type specificity of this signaling, HaCaT and MCF 7 cells were plated in two 96 well plates in such a way that on the first plate HaCaT cells were grown in wells of rows 2, 5, 6 and MCF 7 cells were grown in wells of rows 7 and 10; on the second plate HaCaT cells

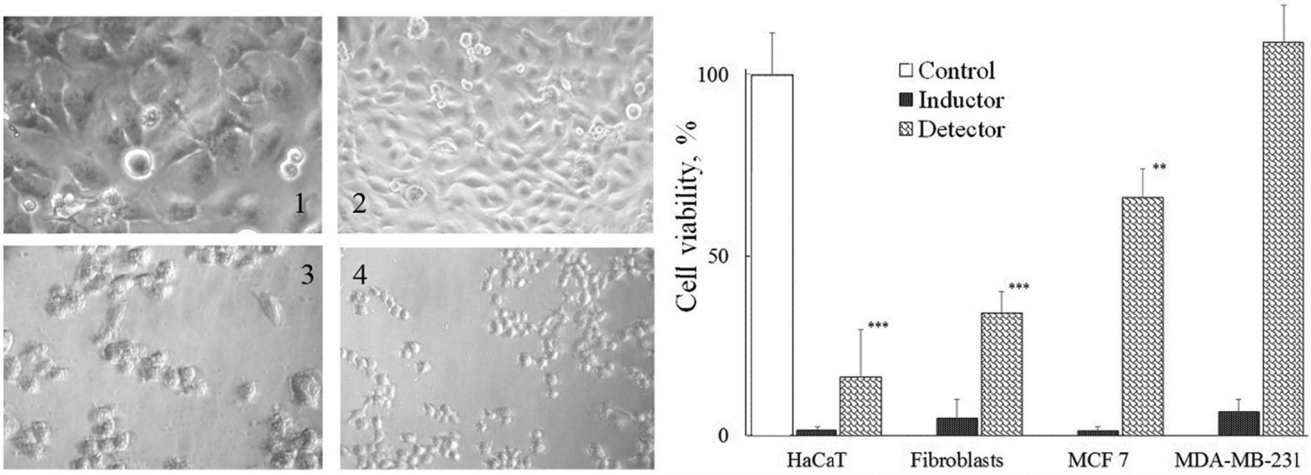

Fig. 2. Representative phase contrast images of control HaCaT cells (1, 2) and detector HaCaT cells (3, 4) at an optical zoom 300 (1, 3) and 200 (2,4) (left panel) and viability of control, inducer and detector cells after 24 h exposition under conditions given in "Methods" (right panel) ** – $p < 0.0001$; *** $p < 0.00001$ versus control.

Table 1
Non-chemical death signals emitted by inducer HaCaT cells can affect target HaCaT cells over 2 cm distance.

Experimental conditions	24 h viability of cells, %
Approximate control	100.0 ± 11.5
Inductor	1.5 ± 1.1
Detector (1 cm from inducer cells)	16.5 ± 10.5***
Detector (2 cm from inducer cells)	70,5 ± 14.0**

** – $p < 0.0001$; *** $p < 0.00001$ versus control.

were grown in wells of rows 2, 5 whereas MCF 7 cells were grown in wells of rows 6, 7 and 10. Then, complete media in all wells was replaced by serum free media. Additionally, fresh media added to the wells of row 6 contained 200 μM cumoquinone. With such an experimental design, HaCaT operated as an inductor on the first plate and MCF 7 cells played the same function on the second.

From the results presented in Table 2, it may be concluded that detector cells can distinguish and respond the same way to death signals transmitted from various type of inducer cells. However, there is significant difference between normal and cancer cells, namely the death response of detector cells to non-chemical death signals was less pronounced in the case of MCF 7 and absent in the case of MDA-MB-231. We can confidently assume that death of detector cells is due to apoptosis and this assumption is in good agreement with earlier findings indicating that epithelial malignant cancer cells are often found to be highly resistant to apoptosis induction by chemotherapeutic drugs and radiation [23–25].

To show that combined signaling pathway including non-chemical and biochemical arms may be pharmacologically targeted, detector cells were treated with caspase-3 inhibitor quercetin[26] and epicatechin [27] and with silibinin, which has been reported to influence apoptosis through the activation of DNA repair machinery [28,29]. Compounds were added to the medium of detector cells (HaCaT) simultaneously with the addition of cumoquinone to HaCaT cells employed as inductor. It was found that quercetin and silibinin at concentration 50 μM significantly increased viability of detector cells (Table 3). Thus, pharmaceuticals may interrupt death signaling and prevent the apoptotic response of detector cells.

Miller and Web [30] explained non-chemical cell–cell communication as function of genome that can directly send and receive the electromagnetic information. Recently it was suggested that electroacoustic resonances between similar DNA sequences form the basis of signaling within the genome and coordinate the function of the cell [31–34]. We can hypothesize that non-chemical death signals emitted by inducer cells may be received by detector cells through sequence-specific resonance signaling in the genome which in turn trigger apoptosis. Obviously, further integrated, comprehensive studies are needed to verify these hypotheses and to clarify in detail the mechanism underlying non-chemical signaling are required. Nevertheless, the above results are convincing enough to suggest that animal cells are capable of sending and receiving biologically significant non-chemical signals, which can provide a prompt and synchronic response of cell ensembles to noxious stimuli.

Table 2
Lack of cell-type specificity in non-chemical cell to cell death communication.

Inducer cells (24 h viability, %)	Detector cells, 24 h viability %	
	HaCaT cells	MCF 7 cells
HaCaT cells (1.0 ± 2.0)	5.0 ± 5.5	57.0 ± 6.0**
MCF-7 cells (1.5 ± 1.0)	10.0 ± 9.5	66.0 ± 8.0**

**$p < 0.0001$ versus HaCaT cells.

Table 3
Influence of pharmaceuticals on death response of detector cells (HaCaT).

Experimental conditions	24 h viability of cells, %
Approximate control	100.0 ± 10.5
Inductor	1.6 ± 1.2
Detector	25.0 ± 9.5
Detector + 50 μM quercetin	62 ± 5.5**
Detector + 50 μM silibinin	63 ± 6.5**
Detector + 50 μM epicatechin	31 ± 9.0

**$p < 0.0001$ versus detector.

3. Methods

The immortalized human keratinocyte cell line HaCaT gifted from N. E. Fusenig (Deutsches Krebsforschungszentrum, Heidelberg, Germany), human breast cancer cells MCF 7 and MDA-MB-231 purchased from the American Type Culture Collection, human lung fibroblasts obtained from the Cell Bank of Belorussian Research and Practical Center for Epidemiology and Microbiology were maintained in Dulbecco's modified Eagle's medium (DMEM) purchased from Lonza (1% glucose) supplemented with 10% heat-inactivated fetal bovine serum. Non-chemical cell to cell communication was studied using the following protocol: cells were plated in 96-wells plate (Sarstedt) as shown in Fig. 1 and allowed to grow to confluence in complete media. Then, complete media in all wells excepting eight wells of row 6 was replaced by serum free media with 2% ethanol. Media in wells of row 6 was replaced with serum-free media containing 200 μM duroquinone or cumoquinone, added as ethanol solution. The final concentration of ethanol in the medium was 2%. Commercial duroquinone purified by recrystallization and cumoquinone, prepared by controlled oxidation of commercial 2,3,5-trimethylhydroquinone [35,36] were kindly provided by the lab of Prof. Oleg Shadyro (Chemical department, Belarusian State University). In some experiments, serum-free medium added to wells of row 7 was supplemented with quercetin, epicatechin or silibinin (Sigma-Aldrich). Cell morphology and cell viability were analyzed 24 h after media change. Cell viability was determined by quantitative PrestoBlue Cell Viability Assay (Invitrogen Life Sciences, Paisley, United Kingdom). Cell morphology was evaluated by inverted phase contrast microscopy (Zeiss, Germany).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.G. Gurwitsch, Die natur desspezifischen erregers der zellteilung, Arch. Mikrosk. Anat. Und. Entw. Mech. 100 (1923) 11–40.
[2] A. Gurwitsch, Physikalisches über mitogenetische Strahlen, Archiv f mikr Anat u Entwicklungsmechanik 103 (3-4) (1924) 490–498, https://doi.org/10.1007/BF02107498.
[3] Reiter, T. Gábor, D. Zellteilung und Strahlung: Sonderheft der Wissenschaftlichen Veröffentlichungen aus dem Siemens-Konzern. Springer-Verlag, Berlin (1928). http://dx.doi.org/10.1007/978-3-642-50832-5
[4] I. Volodyaev, L.V. Beloussov, Revisiting the mitogenetic effect of ultra-weak photonemission, Front. Physiol. 6 (2015) 241, https://doi.org/10.3389/fphys.2015.00241.
[5] M. Cifra, P. Pospsil, Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications, J. Photochem. Photobiol. B. XX (14) (2014), https://doi.org/10.1016/j.jphotobiol.2014.02.009. pii: S1011-1344(14) 00046-3.
[6] M. Rahnama, J.A. Tuszynski, I. Bókkon, M. Cifra, P. Sardar, V. Salari, Emission of mitochondrial biophotons and their effect on electrical activity of membrane via microtubules, J. Integr. Neurosci. 10 (1) (2011) 65–88, https://doi.org/10.1142/S0219635211002622.
[7] A.G. Gurwitsch, L.D. Gurwitsch, Ultra-violet chemi-luminescence, Nature 143 (3633) (1939) 1022–1023, https://doi.org/10.1038/1431022b0.

[8] I. Kruk, K. Lichszteld, T. Michalska, J. Wronska, M. Bounias, The formation of singlet oxygen during oxidation of catechol amines as detected by infrared chemiluminescence and spectrophotometric method, Z. Naturforsch. [C] 44 (1989) 895–900, https://doi.org/10.1515/znc-1989-11-1203.
[9] M. Nakano, Low-level chemiluminescence during lipid peroxidations and enzymatic reactions, J. Biolumin. Chemilumin. 4 (1) (1989) 231–240, https://doi.org/10.1002/bio.1170040133.
[10] B.P. Watts, M. Barnard, J.F. Turrens, Peroxynitrite-dependent chemiluminescence of amino acids, proteins, and intact cells, Archiv. Biochem. Biophys. 317 (2) (1995) 324–330, https://doi.org/10.1006/abbi.1995.1170.
[11] F.A. Popp, Properties of biophotons and their theoretical implications, Indian J. Exp. Biol. 41 (5) (2003) 391–402. PMID: 15244259.
[12] F. Popp, K. Li, Hyperbolic relaxation as a sufficient condition of a fully coherent ergodic field, Int. J. Theor. Phys. 32 (1993) 1573–1583. https://doi.org/10.1007/.
[13] J. Slawinski, Electromagnetic radiation and the afterlife, J. Near-Death Stud. 6 (2) (1987) 79–94, https://doi.org/10.1007/BF01073390.
[14] A.A. Gurwitsch, A historical review of the problem of mitogenetie radiation, Experientia 44 (1988) 545–550, https://doi.org/10.1007/BF01953301.
[15] D. Fels, Endogenous physical regulation of population density in the freshwater protozoan Paramecium caudatum, Sci. Rep. 7 (1) (2017) 13800, https://doi.org/10.1038/s41598-017-14231-0.
[16] H. Sies, Chapter 13 - oxidative stress: eustress and distress in redox homeostasis in stress: physiology, biochemistry, and pathology, Academic Press, Oxford, 2019, pp. 153–163. http://dx.doi.org/10.1016/B978-0-12-813146-6.00013-8.
[17] F. Gagné, Chapter 6 - Oxidative Stress, in Biochemical Ecotoxicology, Academic Press, Oxford, 2014, pp. 103–115. http://dx.doi.org/10.1016/B978-0-12-411604-7.00006-4.
[18] A. Farhadi, C. Forsyth, A. Banan, M. Shaikh, P. Engen, J.Z. Fields, A. Keshavarzian, Evidence for non-chemical, non-electrical intercellular signaling in intestinal epithelial cells, Bioelectrochemistry 71 (2) (2007) 142–148, https://doi.org/10.1016/j.bioelechem.2007.03.001.
[19] Popp FA Some Essential Questions of BP Research and possible Answers. In: Popp, F.A., Li, K.H., Gu, Q.(eds.): Recent advances in biophoton research and its applications. World Scientific Publishing, Singapore 1992, pp. 445–456. http://dx.doi.org/10.1142/9789814439671_0001.
[20] R.P. Bajpai, S. Kumar, V.A. Sivadasan, Biophoton emission in the evolution of a squeezed state of frequency stable damped oscillator, Appl. Math. Comput. 93 (2-3) (1998) 277–288, https://doi.org/10.1016/S0096-3003(97)10117-5.
[21] P.J. O'Brien, Molecular mechanisms of quinone cytotoxicity, Chemico-Biol. Interact. 80 (1) (1991) 1–41, https://doi.org/10.1016/0009-2797(91)90029-7.
[22] G.A. Moore, L. Rossi, P. Nicotera, S. Orrenius, P.J. O'Brien, Quinone toxicity in hepatocytes: Studies on mitochondrial Ca^{2+} release induced by benzoquinone derivatives, Archiv. Biochem. Biophys. 259 (2) (1987) 283–295, https://doi.org/10.1016/0003-9861(87)90495-4.
[23] C. Kihara, T. Tsunoda, T. Tanaka, H. Yamana, Y. Furukawa, K. Ono, et al, Prediction of sensitivity of esophageal tumors to adjuvant chemotherapy by cDNA microarray analysis of gene-expression profiles, Cancer Res. 61 (2001) 6474–6479. PMID: 11522643.
[24] T. Watanabe, Y. Komuro, T. Kiyomatsu, T. Kanazawa, Y. Kazama, J. Tanaka, T. Tanaka, Y. Yamamoto, M. Shirane, T. Muto, H. Nagawa, Prediction of sensitivity of rectal cancer cells in response to preoperative radiotherapy by DNA microarray analysis of gene expression profiles, Cancer Res. 66 (7) (2006) 3370–3374, https://doi.org/10.1158/0008-5472.CAN-05-3834.
[25] M. Todaro, Y. Lombardo, M.G. Francipane, M.P. Alea, P. Cammareri, F. Iovino, A. B. Di Stefano, C. Di Bernardo, A. Agrusa, G. Condorelli, H. Walczak, G. Stassi, Apoptosis resistance in epithelial tumors is mediated by tumor-cell-derived interleukin-4, Cell Death Differ. 15 (4) (2008) 762–772, https://doi.org/10.1038/sj.cdd.4402305.
[26] B. Ossola, T.M. Kääriäinen, A. Raasmaja, P.T. Männistö, Time-dependent protective and harmful effects of quercetin on 6-OHDA-induced toxicity in neuronal SH-SY5Y cells, Toxicology 250 (1) (2008) 1–8, https://doi.org/10.1016/j.tox.2008.04.001.
[27] N. Katunama, A. Ohachi, E. Sano, N. Ishimaru, Y. Hayashi, E. Murata, Catechin derivatives: specific inhibitor for caspases-3, 7 and 2, and the prevention of apoptosis at the cell and animal levels, FEBS Lett. 580 (2006) 741–746, https://doi.org/10.1016/j.febslet.2005.12.087.
[28] S. Narayanapillai, C. Agarwal, G. Deep, R. Agarwal, Silibinin inhibits ultraviolet B radiation-induced DNA-damage and apoptosis by enhancing interleukin-12 expression in JB6 cells and SKH-1 hairless mouse skin, Mol. Carcinog. 53 (6) (2014) 471–479, https://doi.org/10.1002/mc.22000.
[29] S.K. Katiyar, S.K. Mantena, Meeran, S.M. Silymarin, Protects epidermal keratinocytes from ultraviolet radiation-induced apoptosis and DNA damage by nucleotide excision repair mechanism, PLoS One 6 (6) (2011) e21410, https://doi.org/10.1371/journal.pone.0021410.
[30] R.A. Miller, B. Webb, Embryonic holography: an application of the holographic concept of reality, DNA Decipher J. 2 (2) (2012). http://www.dnadecipher.com/index.php/ddj/article/view/26.
[31] I. Savelev, M. Myakishev-Rempel, Possible traces of resonance signaling in the genome, Progress Biophys. Mol. Biol. 151 (2020) 23–31, https://doi.org/10.1016/j.pbiomolbio.2019.11.010.
[32] E. Del Giudice, P.R. Spinetti, A. Tedeschi, Water dynamics at the root of metamorphosis in living organisms, Water 566–586 (2012), https://doi.org/10.3390/w2030566.
[33] A. De Ninno, M. De Francesco, Water molecules ordering in strong electrolytes solutions, Chem. Phys. Lett. 705 (2018) 7–11, https://doi.org/10.1016/j.cplett.2018.05.044.
[34] P. Kurian, A. Capolupo, T.J.A. Craddock, G. Vitiello, Water-mediated correlations in DNA-enzyme interactions, Phys. Lett. A 382 (1) (2018) 33–43, https://doi.org/10.1016/j.physleta.2017.10.038.
[35] W. Flaig, T. Ploetz, H. Biergans, Zur Kenntnis der Huminsäuren. XIV Mitteilung. Bildung und Reactionen einiger Hydroxy-chinone, Justus Liebigs Annalen der Chemie. 597 (1955) 197–219.
[36] O.I. Shadyro, I.P. Edimecheva, G.K. Glushonok, N.I. Ostrovskaya, G.I. Polozov, H. Murase, T. Kagiya, Effects of phenolic compounds on reactions involving various organic radicals, Free Radic. Res. 37 (10) (2003) 1087–1097, https://doi.org/10.1080/10715760310001600417.