Bioorganic Chemistry 50 (2013) 26–33

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Evaluating the antioxidative activity of diselenide containing compounds in human blood

Joanna Saluk *, Michał Bijak, Paweł Nowak, Barbara Wachowicz

Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland

ARTICLE INFO

Article history:
Received 4 March 2013
Available online 26 July 2013

Keywords:
Antioxidant
Diselenide derivatives
Ebselen

ABSTRACT

This study was designed to determine and compare the antioxidant effects of synthetic organoselenium compounds. In experimental trials three different diselenides were used: bis(2-hydroxyphenyl) diselenide, bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide and bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide. The compounds were screened for antioxidant activities in human blood under oxidation stress conditions. Oxidative stress was induced *in vitro* in human blood platelet samples and in plasma by 0.1 mM peroxynitrite ($ONOO^-$) or by Fe^{2+}. In experimental trials the levels of chosen oxidative stress markers (TBARS, $O_2^{\cdot-}$, and protein carbonyl groups) were significantly decreased by the action of the tested compounds. The antioxidative properties and the changes in proteins and lipids in the presence of new synthesized selenoorganic compounds were studied *in vitro* and compared with activity of ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) – a classical antioxidant, well known as the most important glutathione peroxidase mimetic agent. Our results indicate that the tested diselenides have distinctly protective effects against oxidative alterations of biomolecules caused by $ONOO^-$ and Fe^{2+} in blood platelets and in plasma. Therefore it seems that not only ebselen with a wide spectrum of therapeutic actions but also other organoselenium compounds can be considered in the future as active pharmacological agents.

1. Introduction

Selenium had been regarded as a toxic element for many years but later studies have proven its essentiality since selenium deficiency can cause severe disturbances in organisms [1]. The positive and negative effects of both inorganic and organic compounds have been studied and the organic compounds of selenium have been shown to be more efficient and less toxic [2]. Thus, numerous investigations have been performed to find the most effective synthetic selenoorganic compound which could be a dietary supplement [3]. Among other issues, the question of the relationship between selenium supplementation and oxidative balance has been investigated [4]. Our work was carried out with the aim of studying the possible application of three organic compounds with abis-phenyl-diselenide-like structure as preventive agents against disturbance in antioxidant status of blood elements. These Se-compounds are not a source of dietary selenium in human, but they can be used as drugs, like ebselen.

The discovery of the role of reactive oxygen species (ROS) and position of antioxidants in acute (e.g. inflammation) or chronic degenerative diseases including cardiovascular diseases [5], is a medical revolution that promises a new age of health. The alteration of structure and function in plasma and in platelet biomolecules caused by exogenous ROS occurs as a result of pathologically enhanced peroxynitrite (PN; $ONOO^-$) production, mainly in vessel walls [6]. $ONOO^-$ is a potent biological oxidant, generated in vessel wall, and implicated in several inflammatory disorders and atherosclerosis [7]. PN is a mediator of toxicity in pathological processes with strong oxidizing properties toward biological molecules, including thiols, ascorbate, lipids, amino acids and nucleotides,and it can cause strand breaks in DNA [6]. The exposure of proteins to this potent oxidant results in the oxidation of cysteine, methionine and tryptophane, the fragmentation of protein molecules and the formation of carbonyl groups. PN promotes nitration (incorporation of a nitro $-NO_2$ group) of aromatic and aliphatic residues. Particularly, protein tyrosine residues appear to be main targets for $ONOO^-$-mediated nitrations with nitrotyrosine and dityrosine formation in response to biological generation of $ONOO^-$ [8]. The oxidation of proteins by PN disturbs tertiary structure of proteins responsible for altered signaling pathways and may lead to the pathogenesis of various diseases [9].

* Corresponding author. Address: Department of General Biochemistry, Institute of Biochemistry, University of Lodz, Pomorska 141/143, 90-236, Poland. Fax: +48 42 635 44 84.
E-mail address: juszczak@biol.uni.lodz.pl (J. Saluk).

0045-2068/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bioorg.2013.07.003

Ebselen is one of the synthetic antioxidants with a wide spectrum of therapeutic actions. It is used as a drug in clinical trials against stroke, reperfusion injury with antiatherosclerotic and renoprotective effects. It is a potent scavenger of peroxynitrite, hydroperoxide as well as hydroperoxides of membrane-bound phospholipids.

In the present study our experiments have been designed to investigate and compare the role of the three different synthetic diselenides: bis(2-hydroxyphenyl) diselenide, bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide and bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide (Fig. 1) in the defence of blood platelet and plasma components against oxidative damages caused by PN using the measurement of the level of well-known oxidative stress biomarkers. We measured the concentration of thiobarbituric acid reactive substances (TBARS) – the marker of lipid peroxidation. The strength of selenoorganic compounds against protein oxidation induced by $ONOO^-$ was evaluated by proteomic techniques targeting protein carbonylation. The formation of carbonyl groups was used as a marker of protein oxidation. Amino acid modifications may be responsible for the loss of somebiochemical and physiological protein functions and lead to the pathological consequences [10]. We provoked platelet and plasma lipid peroxidation also by using Fe^{2+}. The toxicity of iron is attributed to its ability to reduce molecular oxygen and in consequence to form partially reduced oxygen species. Iron involved in free radical production and iron-chelate complex is capable to form strong oxidants, which can oxidize numerous biomolecules [11]. Moreover, we studied the effect of tested compounds on blood platelet activation corresponding to thrombin-induced arachidonic acid pathway. During thrombin-induced platelet activation endogenous arachidonic acid is liberated from membrane phospholipids and rapidly metabolised by cyclooxygenase (COX), mainly to thromboxane A_2 (TXA_2) and prostaglandins (PGs) simultaneously with MDAformation [12]. The amount of MDA formed during enzymatic peroxidation of arachidonic acid reveals that the arachidonate pathway is associated with the superoxide anion ($O_2^{-\cdot}$) generation [13]. During the enzymatic oxidation step of arachidonic acid in platelets by COX-1, $O_2^{-\cdot}$ may be generated as a by-product of ROS. $O_2^{-\cdot}$ production in activated platelets is mainly dependent on NADPH oxidase or xanthine oxidase [14].

The aim of our study was to investigate the antioxidative properties of diselenide derivatives due to their ability to inhibit carbonyl group generation and Fe^{2+}-induced lipid peroxidation. The antiplatelet activities of tested compounds were estimated by reduction of superoxide anion generation and the inhibition of MDA formation.

2. Materials and methods

2.1. Materials

Peroxynitrite was synthesized according to the method of Pryor and Squadrito [15]. Freeze fractionation (−70 °C) of the PN solution formed a yellow top layer, which was retained for further studies. The top layer typically contained 80–100 mM PN as determined spectrophotometrically at 302 nm in 0.1 M NaOH (ε_{302nm} = 1679 M^{-1} cm^{-1}).

Ebselen, Thrombin, Cytochrome c, Rabbit anti-DNPH antibodies, Anti-rabbit antibodies, TBA were purchased from Sigma (St. Louis, MO, USA). Selenoorganic compounds (Fig. 1) were synthesized in the Department of Organic Chemistry, Biochemistry and Biotechnology, Wroclaw University of Technology (Wroclaw, Poland) and were donated by Prof. J. Mlochowski. Stock solutions of selenoorganic compounds were made in 50% dimethylosulphoxide (DMSO) at a concentration of 100 μM (the final concentration of DMSO in the samples was less than 0.5%) and kept frozen. All samples were incubated with corresponding volumes of DMSO, served as a control and were handled in an identical manner. Transport effects (0.5% DMSO) under the conditions of our model of experiments were negligible. All other reagents were analytical grade and were provided by commercial suppliers.

2.2. Isolation of blood platelets and plasma

Human blood was obtained from young (20–25 years) healthy, non-smoking men and collected into ACD solution (1.36% citric acid/2.5% sodium citrate/2% dextrose; 5:1 v/v). Blood platelets and plasma were isolated by differential centrifugation of blood [16]. The platelets were counted by the photometric method according to Walkowiak et al. [17]. The final platelet concentration was about 4×10^8 platelets/ml. The entire platelet washing procedure was performed in plastic tubes and carried out at room temperature. Washed human platelets were suspended in the modified Tyrode's Ca^{+2}/Mg^{+2} free buffer (127 mM NaCl, 2.7 mM KCl, 0.5 mM NaH_2PO_4, 12 mM $NaHCO_3$, 5 mM HEPES, 5.6 mM glucose, pH 7.4).

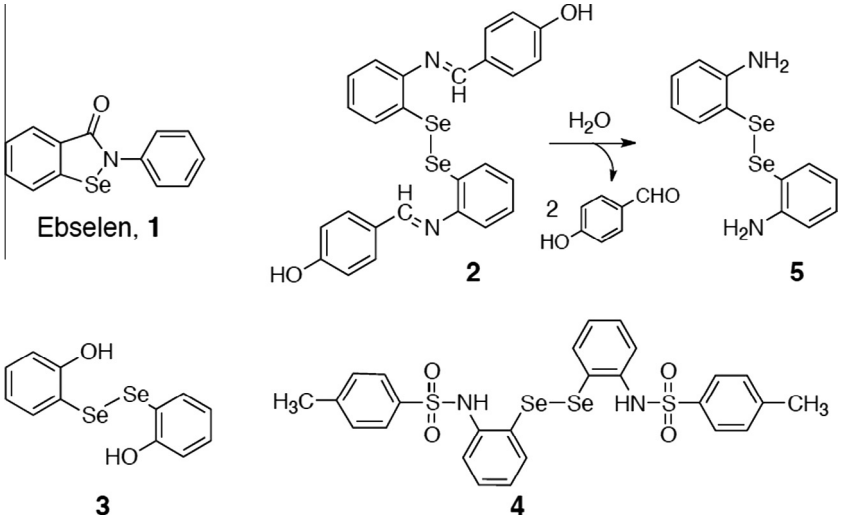

Fig. 1. Ebselen and diselenide containing compounds used in this study. (1) – ebselen; (2) – bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide; (3) – bis(2-hydroxyphenyl) diselenide; (4) – bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide; and (5) – bis(2-aminophenyl) diselenide.

The study was performed under the guidelines of the Helsinki Declaration for Human Research and approved by the committee on the Ethics of Research in Human Experimentation at the University of Łódź (KBBN-UŁ/II/21/2011).

2.3. Sample preparation

Samples of platelet suspensions or plasma were pre-incubated (37 °C, 20 min) with selenoorganic compounds (at final concentrations of 1–100 μM), and then were treated with $ONOO^-$ (0.1 mM, 2 min, 37 °C) or with Fe^{2+} (37 °C, 20 min.) or with thrombin (6 U/ml, 37 °C, 5 min). In platelets samples (prepared as mentioned before) the levels of MDA/TBARS and $O_2^{-\cdot}$ were measured. In prepared plasma samples TBARS and carbonyl groups were estimated. To determine the level of carbonyl groups in platelets proteins, prepared samples were additionally dissolved in lysis buffer (2% Triton-X-100, 100 mM EDTA and 100 mM Tris–HCl; pH 7.4) [18] and platelet lysates were used.

2.4. Thiobarbituric acid reactive substances estimation

Samples (plasma or platelet suspensions) without (control) or after exposure to tested selenium compounds and Fe^{2+} were mixed with an equal volume of 15% (w/v) cold TCA in 0.25 M HCl and with an equal volume of 0.37% (w/v) TBA in 0.25 M HCl. Then samples were immersed in a boiling water bath for 10 min. After cooling and centrifugation absorbance at 535 nm was measured. Results were expressed as nmoles of TBARS per mg of proteins [19].

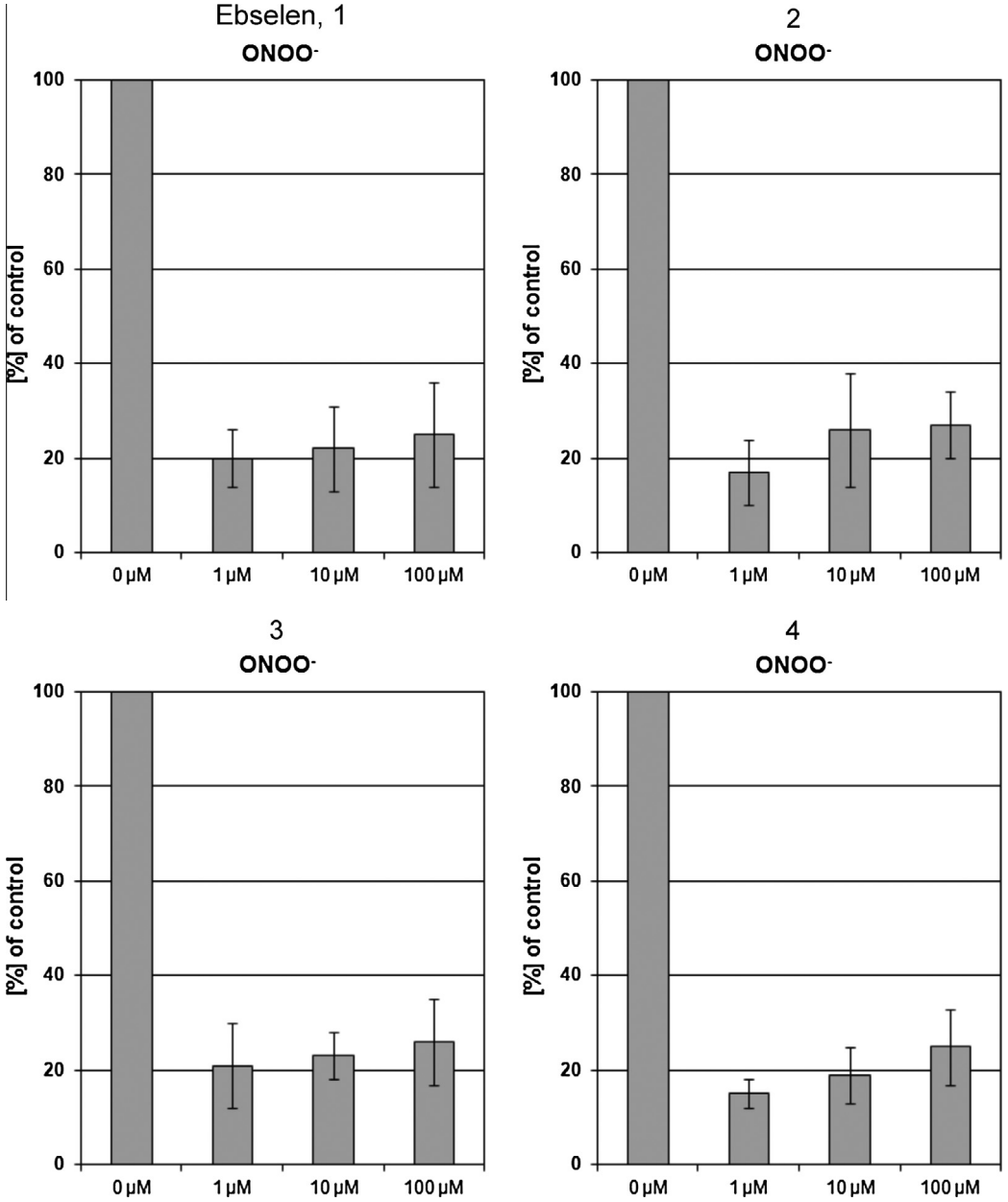

Fig. 2. The effects of selenocompounds on carbonyl group formation in blood platelet proteins. (1) – Ebselen; (2) – bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide; (3) – bis(2-hydroxyphenyl) diselenide; and (4) – bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide. The results were measured as nmol carbonyl groups/mg of platelet proteins and expressed as percentage of that recorded for 100 μM $ONOO^-$-induced carbonylation. The effects of all tested compounds at three different concentrations were statistically significant compared to the control according to Kruskal–Wallis test, $p < 0.0001$.

2.5. Production of malondialdehyde (MDA) in blood platelets stimulated by thrombin

Samples of blood platelet suspensions non-treated (control) or treated with tested selenium were incubated with thrombin and further procedure was the same as TBARS estimation [20].

2.6. Superoxide anion generation

Cytochrome c reduction method [21] was used to test the $O_2^{-\cdot}$ generation in control and in platelets pre-incubated with tested compounds and incubated with thrombin. For that an equal volume of Tyrode's buffer, containing cytochrome c (160 μM), was added to suspension of platelets. After incubation, the platelets were sedimented by centrifugation at 2000*g* for 5 min and the supernatants were transferred to cuvettes. Reduction of cytochrome c was measured spectrophotometrically at 550 nm. To calculate the molar concentration of $O_2^{-\cdot}$ an extinction coefficient for cytochrome c of 18 700 M^{-1} cm^{-1} was used.

2.7. Detection of protein carbonyl groups in human plasma and blood platelets by an ELISA method

Detection of carbonyl groups by an ELISA method in plasma and blood platelets proteins (control or pretreated with tested compounds and then with $ONOO^-$) was carried out according to a method described by Buss et al. [22] modified by Almadari et al. [23]. Concentration of protein in supernatants of platelet lisates were estimated by BCA method. 200 μl of diluted standards, platelet protein samples (5 μg protein per 1 ml PBS) and PBS as blank were added into wells. Plasma or platelet lysate proteins reacted with dinitrophenylhydrazine (DNPH) and then were non-specifically

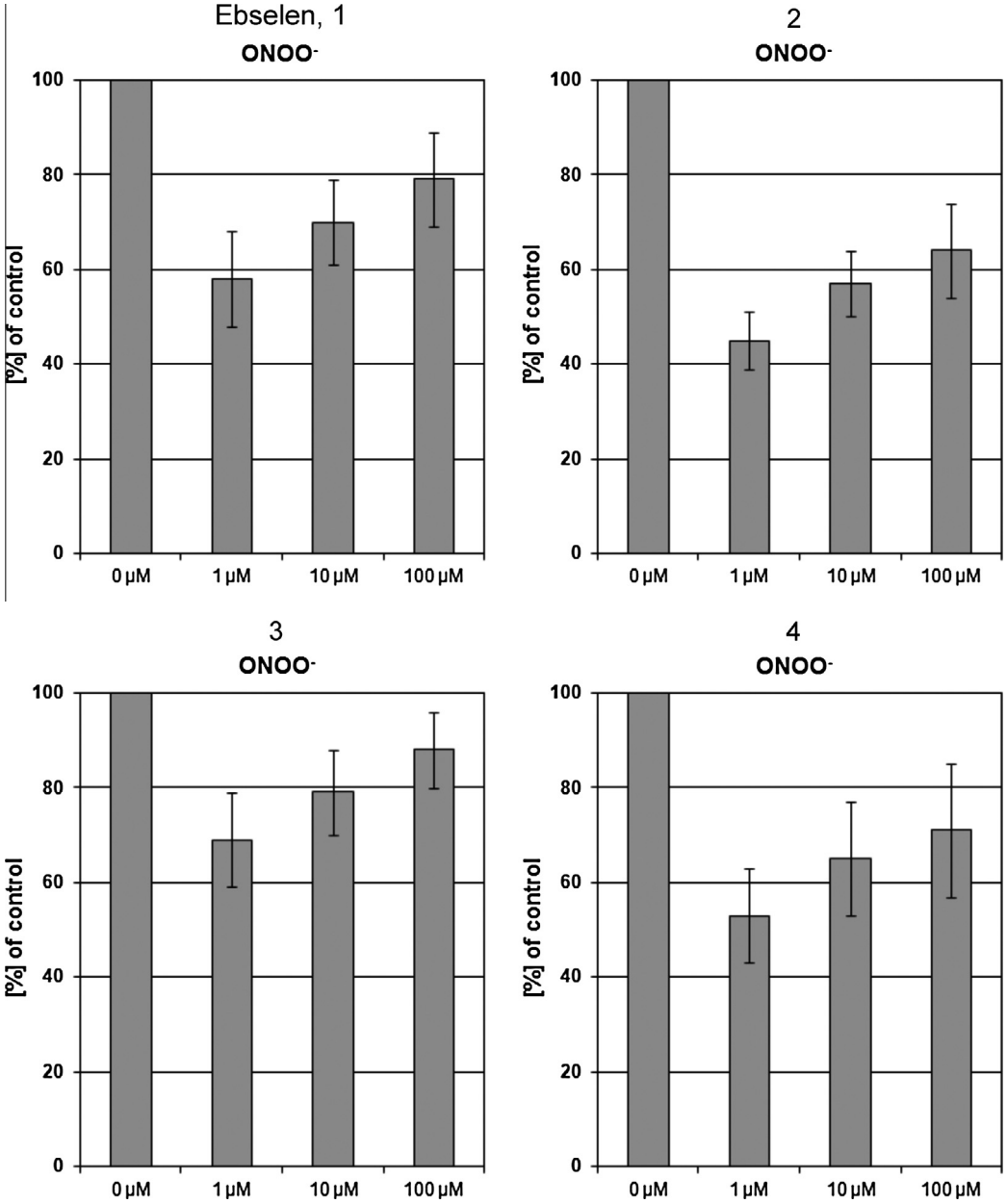

Fig. 3. The effects of selenocompounds on carbonyl group formation in plasma proteins. (1) – Ebselen; (2) – bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide; (3) – bis(2-hydroxyphenyl) diselenide; and (4) – bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide. The results were measured as nmol carbonyl groups/mg of plasma proteins and expressed as percentage of that recorded for 100 μM $ONOO^-$-induced carbonylation. The effects of all tested compounds at three different concentrations were statistically significant compared to the control according to Kruskal–Wallis test, $p < 0.0001$.

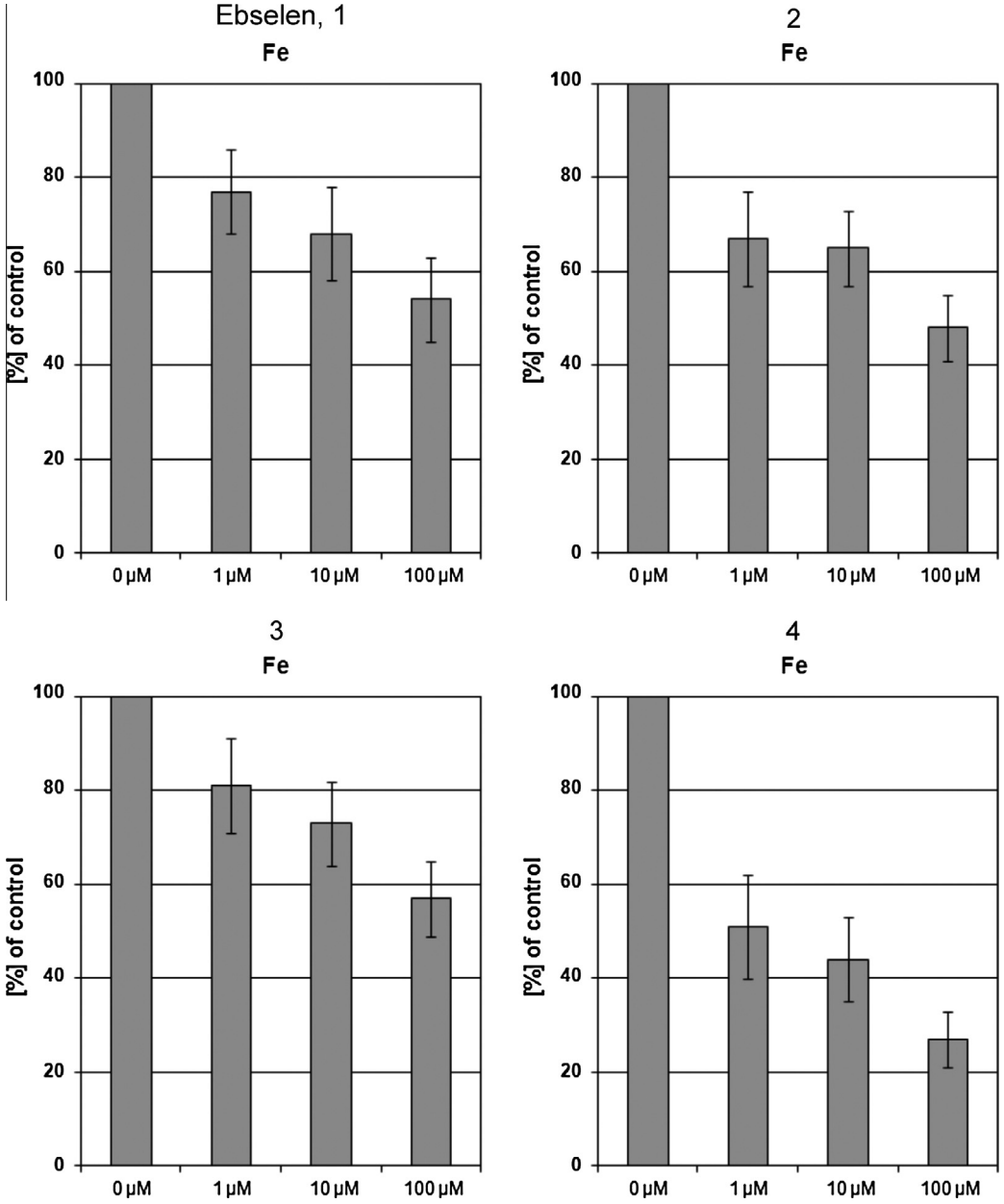

Fig. 4. The effects of selenocompounds on the lipid peroxidation induced by Fe^{2+} in blood platelets. (1) – Ebselen; (2) – bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide; (3) – bis(2-hydroxyphenyl) diselenide; and (4) – bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide. The results were measured as nmol TBARS/mg of platelet proteins and expressed as percentage of that recorded for Fe^{2+}-induced peroxidation. The effects of all tested compounds at three different concentrations were statistically significant compared to the control according to Kruskal–Wallis test, $p < 0.0001$.

adsorbed to an ELISA plate. The anti-dinitrophenylhydrazine (anti-DNPH) antibodies were used to detection of carbonyl groups according to Almadari et al. [23] procedure.Hydrogen peroxide oxidized fibrinogen (10 nmol of carbonyl groups/mg of fibrinogen) was prepared for use in the standard curve. The linearity of the ELISA method was confirmed by the construction of a standard curve (nmol carbonyl groups/mg of Fg). The amount of carbonyl groups present in Fg after treatment with PN (0.1 mM) was determined spectrophotometrically as described by Levine et al. [24].

2.8. Data analysis

All the values in this study were expressed as mean ± SD. Results were analyzed under the account of normality with Shapiro–Wilk test and Equality of Variance with Levene test. The significance of differences between the values was analyzed depending on the Levene test by ANOVA followed by Tukey multiple comparisons test or Kruskal–Wallis test [25–27]. A level $p < 0.05$ was accepted as statistically significant.

3. Results

The antioxidative properties of three different types of diselenide derivatives were studied and compared with the activity of ebselen. All synthetic organoselenium compounds were tested at a dose range of 1–100 μM and all of them displayed antioxidative effects. The carbonyl group formation induced by $ONOO^-$ in platelet (Fig. 2) or plasma proteins (Fig. 3) in the presence of organoselenium compounds was distinctly inhibited *in vitro*, as it was determined by ELISA method. The protective effects of all investigated compounds were dose-dependent and statistically significant. The antioxidative effects of blood platelet protein

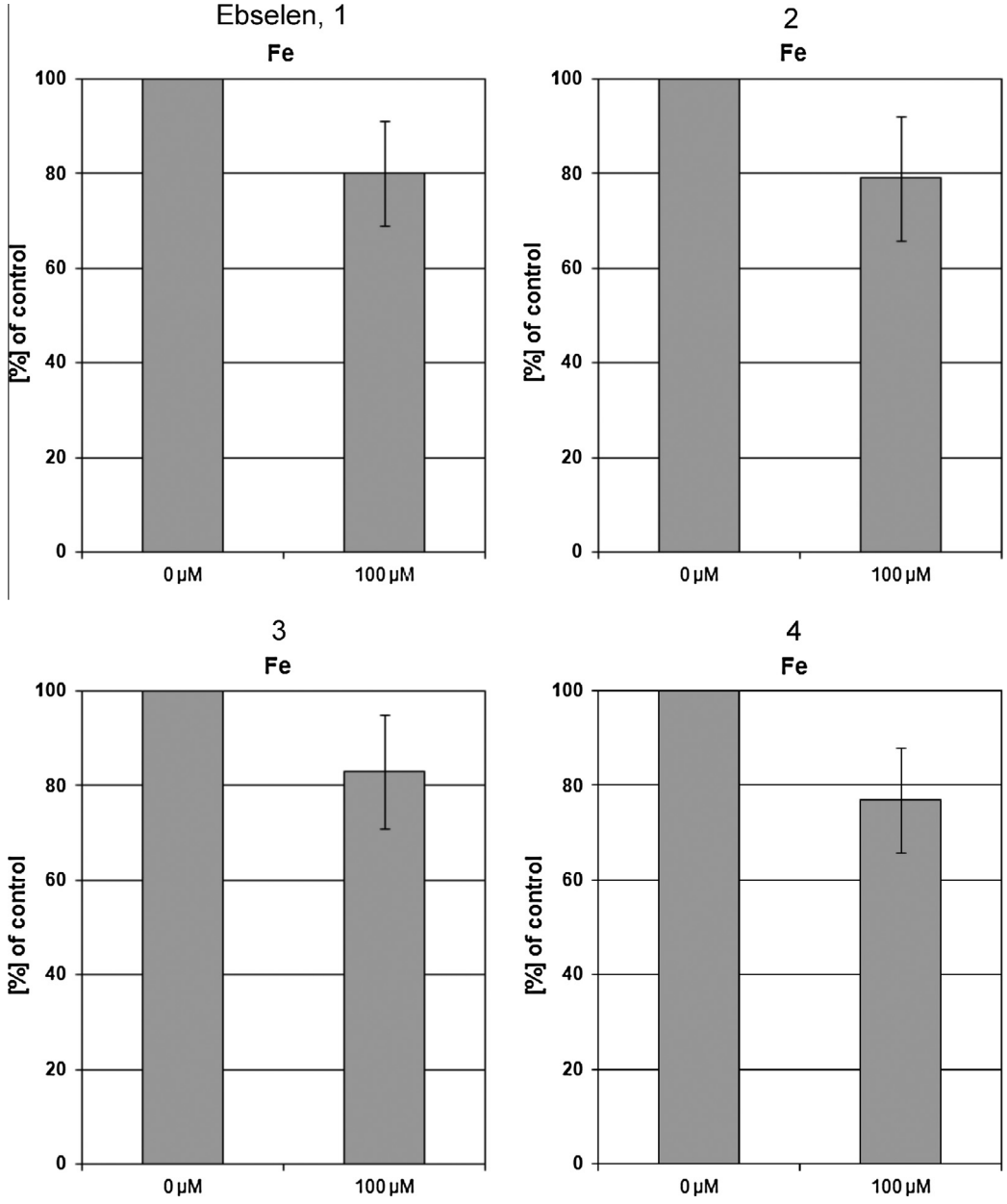

Fig. 5. The effects of selenocompounds on the lipid peroxidation induced by Fe^{2+} in plasma. (1) – Ebselen; (2) – bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide; (3) – bis(2-hydroxyphenyl) diselenide; (4) – bis[2-(4-methylphenylsulfonylamino)phenyl] diselenide. The results were measured as nmol TBARS/mg of plasma proteins and expressed as percentage of that recorded for Fe^{2+}-induced peroxidation. The effects of all tested compounds were statistically significant according to Tukey test, $p < 0.05$.

carbonylation were significant ($p < 0.05$, Fig. 2), whereas the protection of plasma protein against oxidation measured by inhibition of carbonyl group formation was not so strong (Fig. 3). Compared to ebselen, tested compounds displayed stronger inhibitory effects in $ONOO^-$-induced protein carbonylation in plasma as well as in platelets (Figs. 2 and 3). Among three synthesized selenoorganic compounds the weakest protective action against oxidative challenge afforded by $ONOO^-$ was compound 3 (Figs. 2 and 3).

As shown in Fig. 4 all diselenide derivatives at the concentrations of 1–100 μM reduced lipid peroxidation in blood platelets treated with Fe^{2+}; plasma lipid peroxidation was slightly diminished only at the highest concentrations of diselenides (Fig. 4). The activity of ebselen was very similar to 3, which among selenoorganic analogues possessed the weakest defense properties against Fe^{2+} toxicity (Figs. 4 and 5).

The presence of selenium organocompounds slightly inhibited COX-mediated arachidonic acid pathway in blood platelets stimulated by thrombin (Fig. 5). Generation of superoxide anion in platelets was reduced only by 100 μM concentration of compound 2 (Fig. 6A). Among tested compounds only compound 3 caused a significant decrease of lipid peroxidation measured by MDA production (Fig. 6B). The thrombin activation of blood platelets was not inhibited by ebselen (data not presented).

4. Discussion

Ebselen and its analogues are catalysts for the decomposition of peroxynitrite [28]. Ebselen protected induced by peroxynitrite single-strand break formation in DNA more effectively than its sulfur analogue [29]. Ebselen and its analogues also protect against lipid peroxidation induced by multiple inductors (transition metals, ascorbate or ADP) [30]. Iwaoka and Tomoda tested many diselenide analogues as a glutathione peroxidase analogue. Other studies

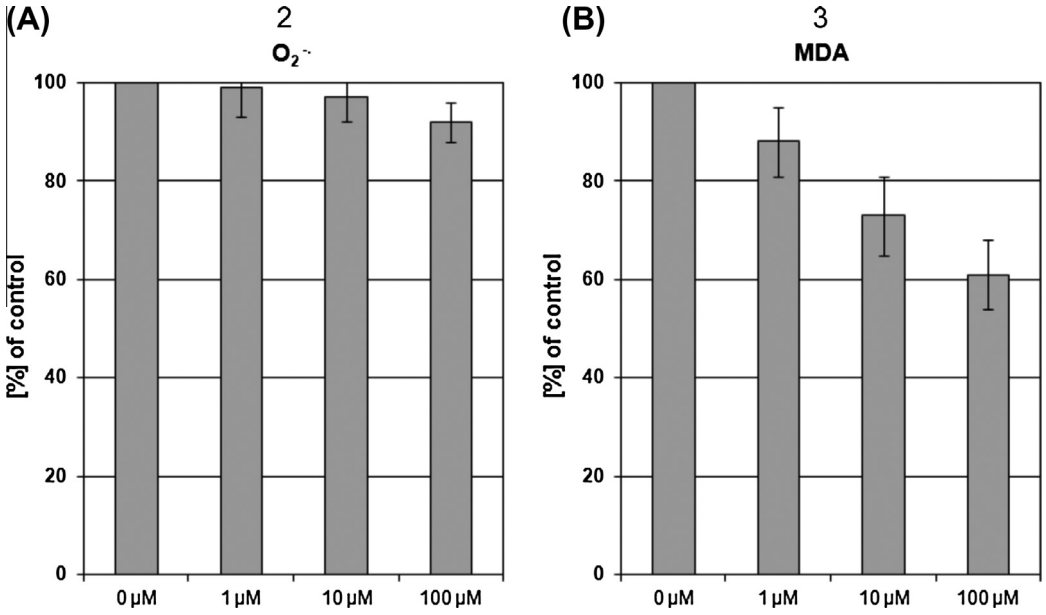

Fig. 6. The effects of selenocompounds on thrombin-induced blood platelet activation. (A) The effect of bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide on level of $O_2^{\cdot-}$ in blood platelets. The results were measured as nmol $O_2^{\cdot-}$/mg of platelet proteins and expressed as percentage of that recorded for thrombin-induced stimulation. The effect of highest concentration (100 μM) was statistically significant according to Tukey test $p < 0.05$. (B) The effect of bis(2-hydroxyphenyl) diselenide on level of TBARS (Malondialdehyde – MDA) in blood platelets. The results were measured as nmol TBARS/mg of platelet proteins and expressed as percentage of that recorded for thrombin-induced stimulation. The effects of all tested compounds at three different concentrations were statistically significant compared to the control according to Tukey test, at concentration 1 μM $p < 0.05$, at concentration 10 μM and 100 μM $p < 0.0001$.

also shows that synthetic diselenides imitate glutathione peroxidase [31–33]. Diphenyl diselenide also protect against peroxynitrite-mediated endothelial cell death [34].

The current studies examine the antioxidative effects of synthetic organoselenium compounds such as diselenide derivatives and compare to ebselen in oxidative stress model of blood components.

Among the studied compounds only compound 3 exhibited antioxidant property similar to ebselen activity, whereas compounds 2 and 4 demonstrated stronger antioxidant potential. Bis{[2-(4-hydroxybenzyl)imino]phenyl} diselenide (compound 2) might be unstable in aqueous solution and may hydrolyze into bis(2-aminophenyl) diselenide (compound 5) (Fig. 1). This might indicate that in our study we observed effect of compound 5 not 2.

Blood platelets play a central role in the pathogenesis of atherosclerosis and thrombosis. Platelet adhesion and aggregate formation are critical events that occur in unstable coronary syndromes. Coronary atherosclerosis is the primary cause of heart diseases in industrialized nations. It is well recognized that platelets play a key role in thrombotic vascular occlusion at the ruptured coronary atherosclerotic plaque, leading to acute ischemic episodes, the acute coronary syndromes (ACSs) [35]. The activity of glutathione peroxidase containing selenium, in platelets is particularly high and is very sensitive to the requirement of selenium. This sensitivity could explain why the selenium-deficiency caused the increase of platelet aggregation, thromboxane B_2 production and synthesis of the lipoxygenase-derived compounds in blood platelets. The selenium administration could increase plateletglutathione peroxidase activity and inhibit platelet hyperaggregation and leukotriene synthesis [36]. Ebselen and three new diselenide derivatives were screened for their antiplatelet activity and their ability to diminish a thrombin-provoke platelet responses. During thrombin-induced platelet activation endogenous arachidonic acid is liberated from membrane phospholipids by phospholipases, and rapidly metabolised by COX, mainly to proaggregatory factor – TXA_2 simultaneously with MDA formation. The amount of MDA formed in the arachidonic acid cascade is a marker of platelet [12]. Thus our studies were focused on protective effects of tested compounds on arachidonic cascade with MDA production in blood platelets stimulated by thrombin. Ebselen has been demonstrated to inhibit biosynthesis of TXA_2 due to the modulation of the COX pathway inplatelets [37]. Although literature data have indicated mostly the potential usefulness of ebselen as a therapeutic agent for the prevention of thrombotic events, the current study showed that arachidonic acid pathway in platelets and TXA_2 generation was markedly inhibited by compound 3. Arachidonic acid pathway is associated with the superoxide anion generation [13]. Upon platelet stimulation by thrombin, during the enzymatic oxidation of arachidonic acid, the generation of superoxide from oxygen occurs due to NADPH oxidase and xanthine oxidase [14]. Even though ebselen has been shown to be an inhibitor of leukocyte NADPH oxidase [38], we demonstrate that $O_2^{\cdot-}$ generation in blood platelets was only slightly abolished by compound 2, whereas other compounds had no preventive effects.

Ebselen is believed to display particularly beneficial effects due to its potential medical applications [39]. Ebselen is well known to be effective in protecting of various compounds from being oxidized or nitrated by $ONOO^-$ [40]. Ebselen has radical scavenging properties, and it is reported as a PN scavenger [41] with capacity to react with the PN radical intermediates [42]. Ebselen is a GPx mimetic compound [43] but some authors hypothesized that its reactivity with PN mimics a peroxynitrite reductase activity of selenoproteins [44]. The radical scavenging activity was also confirmed for other low-molecular-weightselenocompounds, including diselenides, which displayed the most pronounced effect [45]. Detailed mechanistic studies show that ebselen and its analogues react directly with PN to generate highly unstable selenoxides that undergo a rapid hydrolysis to seleninic acids [28]. Because ebselen has been shown to have protective effects against $ONOO^-$ action we studied the activity of different selenocompounds on the oxidative damages of biomolecules caused by PN *in vitro*. Our results demonstrate that all tested compounds protected proteins against destruction induced by $ONOO^-$ as manifested by a significant decrease of carbonyl group formation in blood platelets as well as in

plasma proteins. In our study we found the inverse correlation between selenium compound concentrations and the inhibition of protein carbonylation. Theantiplatelet action of ebselen is known to be due to its inhibition of platelet aggregation and decrease of P-selectin exposition on platelet surface [46]. Our results revealed that ebselen as well as other tested selenoorganic compounds were able to drastically reduce the oxidative effect of PN on platelet proteins. The results of presented study suggest that the selenoorganic derivatives of ebselen (compounds 2 and 4), can scavenge $ONOO^-$ more efficiently than ebselen.

The antioxidant activity of selenocompounds is dependent on substituents on the aromatic ring. Therefore, the compounds containing an aromatic ring with more substituents e.g. compounds 2 and 3 possess the better antioxidant activity. The chemical structure of compounds has an important role in establishing its applications as a biological antioxidant [47]. The other data confirm our suggestion that the antioxidant activity is sensitive to substituents on the aromatic ring [48] and indicate also that antioxidant activity of selenoorganic compounds is significantly dependent on the electronic effects of the substituents on the aromatic ring [49]. The comparison of the level of carbonyl groups generated in plasma proteins after $ONOO^-$ action in the presence of studied compounds confirmed thatpreventive effects of both ebselen and compound 4 were similar, whereas two other compounds were more efficient in reducing of protein oxidation.

Antioxidative properties of all tested compounds could be also observed in a model of Fe^{2+}-induced lipid peroxidation in blood platelets and in plasma samples. In our experiments we confirmed that ebselen has an antioxidant effect but less efficient than compounds 2 and 4. The results obtained with the model of lipid peroxidation derived from the reaction of hydroxyl radical with linolic acid confirmed the highest antioxidant effect of diselenide derivatives among tested selenoorganic compounds. Ebselen exerted its antioxidant capacity not only by the GPx-mimicking activity, but also by catalyzing the reduction of peroxide and inhibiting the formation of oxidant species [45]. We demonstrated that diselenide derivatives exhibited the same profiles and should be used as candidates for pharmacological investigation. All tested compounds reduced in the dose-dependent manner the lipid peroxidation inblood platelets, but they were less efficient in inhibition of plasma lipid peroxidation, and displayed an inhibitory activity only at the highest concentration. Contrary to diphenyl diselenide derivatives, in our experimental protocol ebselen did not show a significant antioxidant effects.

5. Conclusion

In summary, the strong antioxidative properties of diselenide derivatives are the most important findings of the present study. Their antioxidative activities appear to be more powerful than commonly reported activity of ebselen. Our data demonstrate that not only ebselen but also diphenyl diselenides possess radical scavenging properties and are able to inhibit protein carbonylation and lipid peroxidation and this way these compounds might have biological relevance. The further studies are required to establish if these compounds like ebselen might be used as pharmacological tools.

Acknowledgment

This work was supported by the Grant 506/810 from the University of Lodz.

References

[1] M. Navarro-Alarcon, M.C. Lopez-Martinez, Sci. Total Environ. 249 (2000) 347–371.
[2] R.L. Payne, L.L. Southern, Poult. Sci. 84 (2005) 898–902.
[3] T. Rezanka, K. Sigler, Phytochemistry 69 (2008) 585–606.
[4] J.B. Taylor, J.W. Finley, J.S. Caton, J. Anim. Sci. 83 (2005) 422–429.
[5] E. Lubos, D.E. Handy, J. Loscalzo, Front. Biosci. 13 (2008) 5323–5344.
[6] V. Darley-Usmar, B. Halliwell, Pharm. Res. 13 (1996) 649–662.
[7] L. Liaudet, G. Vassalli, P. Pacher, Front. Biosci. 14 (2009) 4809–4814.
[8] B. Alvarez, R. Radi, Amino Acids 25 (2003) 295–311.
[9] T. Grune, L.O. Klotz, J. Gieche, M. Rudeck, H. Sies, Free Radic. Biol. Med. 30 (2001) 1243–1253.
[10] J.R. Mazzulli, R. Hodara, L. Summer, H. Ischiropoulos, Protein Rev. 24 (2007) 123–133.
[11] K.D. Welch, T.Z. Davis, S.D. Aust, Arch. Biochem. Biophys. 397 (2002) 360–369.
[12] A. Ryningen, H. Holmsen, in: H. Gundu, R. Rao (Eds.), Platelet Physiology and Pharmacology, Kluwer Academic Publishers, Norwell, 1999, pp. 1–22.
[13] C. Kim, J.Y. Kim, J.H. Kim, BMB Rep. 41 (2008) 555–559.
[14] B. Wachowicz, B. Olas, H.M. Zbikowska, A. Buczynski, Platelets 13 (2002) 175–182.
[15] W.A. Pryor, G.L. Squadrito, Am. J. Physiol. 268 (1995) L699–L722.
[16] B. Wachowicz, J. Kustron, Cytobios 70 (1992) 41–47.
[17] B. Walkowiak, E. Michalak, W. Koziolkiewicz, C.S. Cierniewski, Thromb. Res. 56 (1989) 763–766.
[18] J. Saluk-Juszczak, B. Olas, P. Nowak, A. Staron, B. Wachowicz, Nutr. Metab. Cardiovasc. Dis. 18 (2008) 422–428.
[19] B. Wachowicz, Cell Biochem. Funct. 2 (1984) 167–170.
[20] J. Saluk-Juszczak, B. Wachowicz, W. Kaca, Microbios 103 (2000) 17–25.
[21] B. Jahn, G.M. Hansch, Int. Arch. Allergy Appl. Immunol. 93 (1990) 73–79.
[22] H. Buss, T.P. Chan, K.B. Sluis, N.M. Domigan, C.C. Winterbourn, Free Radic. Biol. Med. 23 (1997) 361–366.
[23] D.H. Alamdari, E. Kostidou, K. Paletas, M. Sarigianni, A.G. Konstas, A. Karapiperidou, G. Koliakos, Free Radic. Biol. Med. 39 (2005) 1362–1367.
[24] R.L. Levine, J.A. Williams, E.R. Stadtman, E. Shacter, Methods Enzymol. 233 (1994) 346–357.
[25] M. Bijak, M. Bobrowski, M. Borowiecka, A. Podsedek, J. Golanski, P. Nowak, Fitoterapia 82 (2011) 811–817.
[26] M. Bijak, J. Saluk, M.B. Ponczek, P. Nowak, Phytother. Res. 27 (2013) 71–76.
[27] M. Bijak, J. Kolodziejczyk-Czepas, M.B. Ponczek, J. Saluk, P. Nowak, Int. J. Biol. Macromol. 51 (2012) 183–187.
[28] K.P. Bhabak, A.A. Vernekar, S.R. Jakka, G. Roy, G. Mugesh, Org. Biomol. Chem. 9 (2011) 5193–5200.
[29] H. Masumoto, H. Sies, Chem. Res. Toxicol. 9 (1996) 262–267.
[30] G. Mugesh, W.W. du Mont, H. Sies, Chem. Rev. 101 (2001) 2125–2179.
[31] J. Liu, G. Luo, X. Ren, Y. Mu, Y. Bai, J. Shen, Biochim. Biophys. Acta 1481 (2000) 222–228.
[32] L. Shen, K.M. Shin, K.T. Lee, J.H. Jeong, Arch. Pharm. Res. 27 (2004) 816–819.
[33] S.W. Lv, X.G. Wang, Y. Mu, T.Z. Zang, Y.T. Ji, J.Q. Liu, J.C. Shen, G.M. Luo, FEBS J. 274 (2007) 3846–3854.
[34] A.F. Bem, B. Fiuza, P. Calcerrada, P.M. Brito, G. Peluffo, T.C. Dinis, M. Trujillo, J.B. Rocha, R. Radi, L.M. Almeida, Nitric Oxide 31 (2013) 20–30.
[35] S. Massberg, C. Schulz, M. Gawaz, Semin. Vasc. Med 3 (2003) 147–162.
[36] D. Vitoux, P. Chappuis, J. Arnaud, M. Bost, M. Accominotti, A.M. Roussel, Ann. Biol. Clin. 54 (1996) 181–187.
[37] G. Perona, R. Schiavon, G.C. Guidi, D. Veneri, P. Minuz, Thromb. Haemost. 64 (1990) 312–318.
[38] M. Parnham, H. Sies, Expert Opin. Investig. Drugs 9 (2000) 607–619.
[39] N. Pawlas, A. Malecki, Pharmacol. Rep. 59 (2007) 708–714.
[40] H. Masumoto, R. Kissner, W.H. Koppenol, H. Sies, FEBS Lett. 398 (1996) 179–182.
[41] A. Daiber, M.H. Zou, M. Bachschmid, V. Ullrich, Biochem. Pharmacol. 59 (2000) 153–160.
[42] G.L. Squadrito, W.A. Pryor, Free Radic. Biol. Med. 25 (1998) 392–403.
[43] L.O. Klotz, K.D. Kroncke, D.P. Buchczyk, H. Sies, J. Nutr. 133 (2003) 1448S–1451S.
[44] H. Sies, V.S. Sharov, L.O. Klotz, K. Briviba, J. Biol. Chem. 272 (1997) 27812–27817.
[45] M.A. Mouithys-Mickalad, J.M. Faez, L. Chistiaens, S. Kohnen, C. Deby, M. Hoebeke, M. Lamy, G. Deby-Dupont, Redox. Rep. 9 (2004) 81–87.
[46] N. Lindenblatt, W. Schareck, L. Belusa, R.M. Nickels, M.D. Menger, B. Vollmar, Thromb. Haemost. 90 (2003) 882–892.
[47] L. Tiano, D. Fedeli, A.M. Santroni, M. Villarini, L. Engman, G. Falcioni, Mutat. Res. 464 (2000) 269–277.
[48] L. Savegnago, C.R. Jesse, A.V. Moro, V.C. Borges, F.W. Santos, J.B. Rocha, C.W. Nogueira, Pharmacol. Biochem. Behav. 83 (2006) 221–229.
[49] V. Galet, J.L. Bernier, J.P. Henichart, D. Lesieur, C. Abadie, L. Rochette, A. Lindenbaum, J. Chalas, J.F. Renaud de la Faverie, B. Pfeiffer, J. Med. Chem. 37 (1994) 2903–2911.