AMERICAN JOURNAL OF RESEARCH IN MEDICAL SCIENCES, 2018
VOL 1, NO. 1: 1–8
10.5455/ajrms.281449

eJManager

ORIGINAL ARTICLE

Open Access

Organ-specific effects of selenium glutathione peroxidases in response to medicinal plant extracts

Janka Vašková[1], Gabriela Mojžišová[2], Ladislav Vaško[1], Michal Stanko[1], Janka Poráčová[3]

[1]Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Tr. SNP 1, 040 66 Košice, Slovak Republic, [2]Department of Experimental Medicine, Faculty of Medicine, Pavol Jozef Šafárik University, Tr. SNP 1, 040 66 Košice, Slovak Republic, [3]Department of Biology, Faculty of Humanities and Natural Sciences, Presov University in Presov, Ul. 17 Novembra 1, 080 01 Presov, Slovak Republic

ABSTRACT

Background: Various plant extracts are used in the prevention and treatment of various disease states, with their beneficial effects mainly attributed to their antioxidant properties.

Materials and Methods: Knowledge of their localisation, the importance of selenium glutathione peroxidases, and the methodological options for their determination, led us to choose to investigate the affects of oregano, stevia, ginseng and agrimonia extracts on plasma, heart and kidney mitochondria under *in vitro* conditions.

Results: The lowest activity of selenium isoforms was observed in the heart mitochondria. In the kidney mitochondria, it was approximately 100-fold higher and the highest was observed in plasma. The same was detected for total glutathione peroxidases in the heart, although those in kidney mitochondria were higher than in plasma. Oregano caused a generally significant increase in selenium isoform activity and a decrease in total glutathione peroxidase activity in the heart, while both decreased in the kidney. Stevia significantly increased the activity of selenium isoforms in the heart at a concentration of 125 μg.ml^{-1}, with while either having no effect, or a slightly opposite effect on total peroxidase activities. The same effect was observed at lower concentrations in the kidney. Conversely, stevia caused a significant reduction in selenium peroxidase activity in the plasma. Ginseng caused a statistically significant increase in selenium isoform activity in the heart but reduced its activity in the kidney and plasma, even in total glutathione peroxidases. Mutually comparable effects were observed with agrimony.

Conclusion: In terms of total glutathione peroxidases, the extracts can be assumed to have significant antioxidant effects. When considering the physiological functions of selenium isoforms, it is possible to predict the ability of oregano and stevia to significantly affect redox signalling pathways, especially in heart mitochondria, and to a lesser extent in the kidney.

ARTICLE HISTORY

Received 3 August 2017
Accepted 30 October 2017
Published

KEYWORDS

agrimony; ginseng; glutathione peroxidase; oregano; stevia

Introduction

Physiologically generated reactive oxygen species (ROS) are predominantly peroxides. Despite their being mediators of physiological processes, at certain concentrations and in certain media, they can exhibit harmful effects. To be specific peroxide reactions, they must be detected by means of proteins with very reactive thiol groups or selenium to transport signals to the traffickers, and then to the effector [1,2], such as glutathione peroxidase (GPx). GPx may function as a sensor as oxidized GPx can also transfer oxidation

Contact Janka Vašková janka.vaskova@upjs.sk Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Tr. SNP 1, 040 66 Košice, Slovak Republic.

equivalents to the target proteins with selectivity for specific protein interactions. GPx enzymes utilize equivalents of GSH to reduce peroxides [3]. Five of eight known isoforms are selenium-dependent with specific sites of action. Regulation of the concentration of hydroperoxide mediators is implicated in many physiological and pathophysiological processes [4-7]. An adequate concentration of hydroperoxide signalling is deduced from frequent dysregulation of the expression of selenium GPx in cancer cells [8]. The role of selenium-containing GPx is obvious but in addition to these are the not inconsiderable cysteine-containing GPx (GPx-like proteins) or non-selenium-dependent GPx.

The essential oils of aromatic and medicinal plants have different biological activity mediated in particular by their antioxidant properties [9,10]. It is clear from observations in folk medicine and scientific studies that their effects differ, even in such small amounts. *Eleutherococcus senticosus* (Rupr. & Maxim.), known as ginseng, native to north-eastern Asia is primarily known as an adaptogen [11]. *Stevia rebaudiana* (Bert.), is a plant native to tropical and subtropical regions of South and Central America. Leaves of stevia are several times sweeter than refined sugar due to steviol glycosides. They have been used in these countries for centuries as a sweetener and also in medicine [12]. *Oregano vulgare* (L.) is an aromatic plant used in many countries in seasoning dishes [13]. It is used in folk medicine for the prevention of respiratory disease, dyspepsia, dysmenorrhea, rheumatoid arthritis and problems of the urinary system [14]. *Agrimonia eupatoria* (L.), is used in folk medicine for the treatment of inflammatory diseases such as haemorrhagic colitis, inflammation of the urinary tract, and liver disease [15].

It was showed that ginseng, stevia and oregano demonstrate a various activity in counteracting reactive oxygen and nitrogen compounds [16]. The removal of peroxides involved in signalling pathways through antioxidant molecules may be specific and, as is clear from the very nature of antioxidant enzymes, dependent on the concentration of redox-active substances or the site of action, and may lead to different conclusions. The aim of this paper is to highlight the different and organ-specific effects of plant extracts in the plasma and the mitochondria of the heart and kidney, and also the very specific responses of selenium glutathione peroxidases in contrast to total glutathione peroxidase activities.

Materials and Methods

In the experiment, biological material was obtained from Sprague-Dawley male rats from the control group, with the first stage of the process approved by State Veterinary and Food Administration of the Slovak Republic no. Ro-2575/14-221. Animals were aged 6 weeks, weighing 250 grams. After sacrificing the animals, blood was taken for plasma extraction. The animals' organs were harvested for heart and kidney mitochondria isolation according to Fernández-Vizzara et al. [17]. The protein content was determined by biuret complex under alkaline conditions by bicinchoninic acid in isolates.

For the analysis, ethanol root extract of Siberian Ginseng (*Eleutherococcus senticosus* (Rupr. & Maxim.)), aqueous extract of stevia leaves (*Stevia rebaudiana* (Bert.)), dry extract of agrimony tops (*Agrimonia eupatoria* (L.)) and oregano essential oils (*Origanum vulgare* (L.)) were purchased from Calendula ojsc (Stará Ľubovňa, Slovakia). The extracts were incubated with plasma and mitochondria at final concentrations of 125, 62.5 a 31.25 $\mu g.ml^{-1}$ for 1 hour.

GPx activities (EC 1.11.1.9) were measured in plasma, heart and kidney mitochondria by means of a kit (Sigma-Aldrich, Germany). The final concentrations of reagents in the test mixture was as follows: 0.25 mM NADPH reagent, 2.1 mM reduced glutathione, 0.5 U/ml glutathione reductase, and 300 μM terc-butyl-hydroperoxide (t-Bu-OOH) for selenium containing GPx or 300 μM cumene hydroperoxide for total GPx acitivities determination. Absorbance was measured at 340 nm using the kinetic program of M501 Single Beam UV/Vis spectrophotometer (Spectronic Camspec Ltd, Leeds, UK).

Results were expressed as milligrams of protein per millilitre of homogenate ($mg\ prot.ml^{-1}$). Statistical significance of the effects of extracts was determined by Student's t-test. Differences between the effects of extracts was found by one-way ANOVA followed by Tukey post-hoc test.

Table 1. The effects of extracts on total GPx and selenium glutathione peroxidases (Se-GPx) in heart mitochondria.

Concentration (μg.ml^{-1})	group/extract	Total GPx (nkat/mg prot)	Se-GPx (nkat/mg prot)	Post-hoc analysis
	control	2.929 ± 0.186	0.072 ± 0.068^{a,b}	$^{a}p = 0.0323$
125	oregano	1.738 ± 0.071	0.969 ± 0.009^{c}	$^{b}p = 0.0177$
	stevia	2.544 ± 0.557	0.288 ± 0.088^{a,c}	$^{c}p > 0.0000$
	ginseng	3.688 ± 0.601	0.313 ± 0.124^{b,c}	
	agrimony	2.575 ± 0.088	0.238 ± 0.071^{c}	
	control	2.929 ± 0.186	0.072 ± 0.068	$^{a,b,c}p = 0.0010$
62.5	oregano	1.175 ± 0.159	0.706 ± 0.080^{a,b,c}	$^{d}p = 0.0064$
	stevia	3.075 ± 0.495	0.019 ± 0.009^{a,d}	
	ginseng	2.875 ± 0.742	0.050 ± 0.018^{b}	
	agrimony	1.694 ± 0.256	0.163 ± 0.053^{c,d}	
	control	2.929 ± 0.186	0.072 ± 0.068	$^{a}p > 0.0000$
31.25	oregano	1.406 ± 0.380	0.731 ± 0.009	$^{b}p = 0.0001$
	stevia	1.975 ± 0.177	0.031 ± 0.009	$^{c}p = 0.0020$
	ginseng	2.556 ± 0.256	0.244 ± 0.027	
	agrimony	2.300 ± 0.495	0.088 ± 0.035	

Results

The effect of oregano on Se-GPx activity in heart mitochondria was significantly higher in comparison to controls without addition of any compound ($p < 0.001$) using all three concentrations (Figure 1A). Similarly, significantly higher activities were observed at stevia and ginseng concentrations of 125 μg.ml^{-1}. Remarkably higher Se-GPx activities were observed after incubation with the lowest concentration of ginseng. The opposite effect - a significant reduction in total GPx activity, was observed after oregano essential oil incubation ($p < 0.001$ and $p < 0.01$), also using all three concentrations (Figure 2A). In contrast to Se-GPx, incubation with stevia extract led to a significant decrease in total GPx activities ($p < 0.01$) at the lowest concentration, compared to the control. Agrimony also significantly reduced total GPx activities of the heart mitochondria ($p < 0.05$). One-way ANOVA followed by Tukey post-hoc analysis revealed statistically significant differences in the induction of activities of the individual extracts only for Se-GPx (Table 1). In particular, significant differences were found when comparing the extract of ginseng, oregano and stevia.

The effects of extracts on glutathione peroxidase in the kidney mitochondria differed. Se-GPx activities were significantly decreased by all concentrations (Figure 1B). Higher concentrations of ginseng and agrimony significantly reduced Se-GPx activity but incubation of kidney mitochondria with stevia increased their activity at a concentration of 62.5 μg.ml^{-1}. Total GPx consistently showed statistically lower activity relative to the control in the case of all four extracts and at all concentrations (Figure 2). Post-hoc analysis of the differences between activities revealed differences in the effect of extracts at higher concentrations on total glutathione peroxidase, especially in the case of agrimony and stevia. Lower concentrations (62.5 μg.ml^{-1}) showed a divergence mainly from the effect of ginseng. Significant differences between effects on Se-GPx activities in the mitochondria of kidney were observed among all the extracts and at all concentrations (Table 2B).

Se-GPx activities were significantly reduced by the effect of stevia, ginseng and agrimony, but the effect of oregano was not observed when compared to control in plasma (Figure 1C). No significant differences have been observed in the alteration of total GPx activity or between the effects of the extracts themselves (Figure 2C).

Discussion

Peroxidases are represented numerically in living organisms, whether plant or animal. From the time of their discovery in the 19th century, they have gained an important position in biotechnology and

Table 2. The effects of extracts on total GPx and selenium glutathione peroxidases (Se-GPx) in kidney mitochondria.

Concentration ($\mu g.ml^{-1}$)	group/extract	Total GPx (nkat/mg prot)	Post-hoc analysis	Se-GPx (nkat/mg prot)	Post-hoc analysis
	control	5.175 ± 0.083[a]	[a]p = 0.0083	0.748 ± 0.223[a,b,c]	[a]p = 0.0029
125	oregano	1.163 ± 0.088[b]	[b]p = 0.0069	0.194 ± 0.027[a,d]	[b]p = 0.0279
	stevia	1,031 ± 0.044[c,d]	[c]p = 0.0261	0.956 ± 0.186[d,e,f]	[c]p = 0.0008
	ginseng	1.394 ± 0.221[c]	[d]p = 0.0010	0.356 ± 0.027[b,e]	[d]p = 0.0002
	agrimony	0.619 ± 0.097[a,b,d]		0.088 ± 0.018[c,f]	[e]p = 0.0016 [f]p = 0.0001
	control	5.175 ± 0.083[a]	[a]p = 0.0010	0.748 ± 0.223[a,b,c]	[a]p = 0.0029
62.5	oregano	0.600 ± 0.053	[b]p = 0.0064	0.013 ± 0.001[a,d,e]	[b]p = 0.0279
	stevia	1,444 ± 0.203[b]	[c]p = 0.0016	1.519 ± 0.062[d,f]	[c]p = 0.0008
	ginseng	1.863 ± 0.106[a,b,c]		0.050 ± 0.018[b,f]	[d]p = 0.0002
	agrimony	0.356 ± 0.009[c]		0.400 ± 0.071[c,e]	[e]p = 0.0016 [f]p = 0.0001
	control	5.175 ± 0.083		0.748 ± 0.223[a,b]	[a]p = 0.0017
31.25	oregano	1.163 ± 0.194		0.081 ± 0.044[a,c,d]	[b]p = 0.0035
	stevia	1,106 ± 0.239		1.144 ± 0.115[b,e]	[c]p = 0.0025
	ginseng	1.619 ± 0.345		0.713 ± 0.106[c]	[d]p = 0.0157
	agrimony	1.369 ± 0.080		0.563 ± 0.177[d,e]	[e]p = 0.0053

related research areas [18]. However, it stresses the importance of peroxidases mainly due to their wide distribution among living organisms and participation in physiological processes. In terms of resolved issues, it is an important function of the endogenous antioxidants.

Glutathione peroxidases constitute only a very small group of peroxidases [19]. The catalytic efficiency of some GPx isozymes is dependent on the presence of the trace element, Se. The distribution of these selenoproteins between tissues and within specific subcellular compartments has a significant impact on the physiological functions of individual compartments within cells and relevance to the development of the diseases. Selenoenzyme GPx1 is present in the cytosol and mitochondria. GPx4, with a unique function in its ability to reduce phospholipid hydroperoxides, is responsible for most of the observed antioxidant effects in studies. The distribution of these selenoenzymes affects the level of ROS, the relative usage of glycolysis in a ratio to oxidative phosphorylation as well as the level of redox-sensitive transcription factors (NF-κB), and the resulting number of important cellular processes [20]. GPx3 is specific to the plasma, although it is synthesised in the proximal tubules of the kidney [21]. The acquired knowledge about the location and importance of selenium glutathione peroxidases as well as methodological possibilities has led us to the selection of the studied biological material *in vitro*; namely plasma, heart and kidney mitochondria. We could not proceed to detect the activities of Se-GPx in the liver mitochondria due to methodological differences, notably the use of H_2O_2 instead of tert-butyl hydroperoxide. We observed the lowest activity Se-GPx in the heart mitochondria (Figure 1A). Kidney mitochondria Se-GPx activities have been about 100-fold higher and the highest were observed in plasma (Figure 1B). The specific activities of total GPx were again lowest in the mitochondria of the heart, but higher in kidney mitochondria than in plasma (Figure 2). Differences in activities showed that Se-GPx activities represented a minor portion (less in heart than kidney) in mitochondria, but in the plasma are responsible for a substantial part of the observed GPx activities. This is in line with the place of production and action of selenoenzymes. However, the object of most *in vitro* studies are the liver mitochondria, or treatment methods containing H_2O_2 (cumene hydroperoxide) as a substrate. Therefore comparison of Se-GPx and total GPx activities in plasma, heart and kidney mitochondria could not be performed.

Treatment of disease states based on the imbalance of ROS production and degradation through drugs is economically disadvantageous for the end-user. It is therefore easier to investigate and use plant material for that purpose, which are more easily accessible. Various plant compounds form components of a balanced diet and the

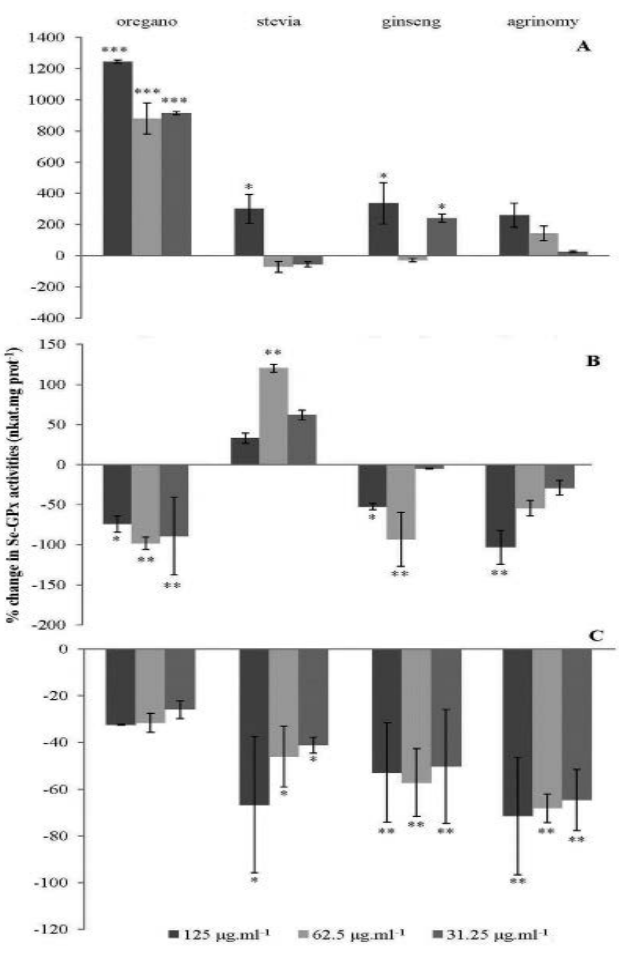

Figure 1. Comparison of changes in the activities of selenium glutathione peroxidases affected by selected plant extracts in comparison to the activities of selenium glutathione peroxidases without effect in heart mitochondria (A), kidney mitochondria (B), and plasma (C). Statistical significance at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

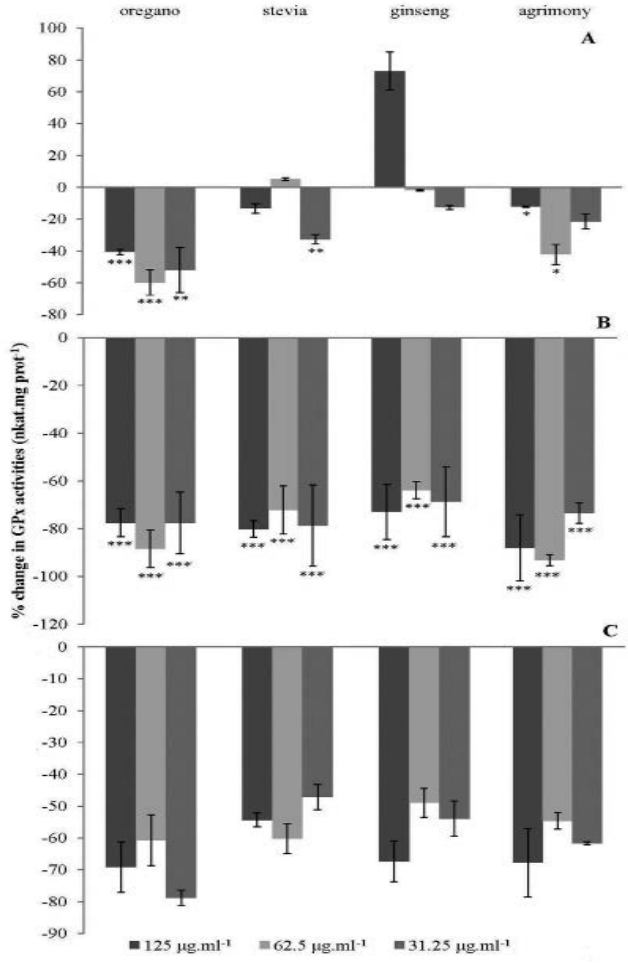

Figure 2. Comparison of changes in the activities of total glutathione peroxidases affected by selected plant extracts in comparison to the activities of total glutathione peroxidases without effect in heart mitochondria (A), kidney mitochondria (B), and plasma (C). Statistical significance at $*p < 0.5$, $**p < 0.01$, $***p < 0.001$.

amount of ethnobotanical studies provide information about their healing effects. These beneficial effects are achieved thanks to the essential active antioxidants, such as flavonoids, flavones, isoflavones, lignans, catechins, and isocatechins [22]. In light of these considerations, we assessed the effect of the extracts from *Oregano vulgare*, *Stevia rebaudiana*, *Eleutherococcus senticosus* and *Agrimonia eupatoria* on selenium and total glutathione peroxidase activities and thus affecting production/decomposition of peroxides and resulted physiological processes. The activities of selenium GPx were significantly increased in heart mitochondria for all oregano concentrations tested, but the total GPx activity constituted a significant decline. A less significant increase in Se-GPx activities was observed in heart mitochondria, especially at the highest concentrations of stevia and ginseng (Figure 1A, 2A). The intensity of the effects of extracts of oregano, sage and ginseng on the Se-GPx was significantly different to heart mitochondria (Table 1). It was shown that reduced GPx1 activity in humans may be an important cardiac risk factor, since it has been shown that, in patients with coronary heart disease, lower GPx1 is associated with an increased risk of cardiovascular events [23]. Detrimental effects of peroxides in cardiac tissue include intracellular acidosis, electrochemical dysfunction, alteration in cardiac action potential, and contractile force inhibition [18,24]. Ardanaz *et al.* [4] using a GPx1-knockout mouse model, provided evidence of cardiac left ventricular hypertrophy

and dysfunction in GPx1$^{-/-}$ mice in the absence of a change in vascular medial hypertrophy. Changes were not associated with increases in collagen deposition and were independent of blood pressure levels. Several studies also pointed out that an increase in GPx1 activity preserved cardiac cells during ischaemia and reperfusion [25]. Our results showed a significant increase in selenium-dependent enzymes in heart mitochondria in response to oregano and a less-pronounced increase affected by highest concentrations of stevia and ginseng. Results indicate specific protective effect even when considering decreased activities of total GPx in heart mitochondria that collectively reflect satisfactory antioxidant protection against H_2O_2.

The addition of oregano caused an overall decrease in selenium and glutathione peroxidases in kidney mitochondria. With the exception of stevia extracts (at a concentration of 62.5 μg.ml^{-1}), which caused a reduction either in the activity of Se-GPx or the total GPx. A difference in efficiency was found among all the extracts for Se-GPx and two concentrations (125 and 62.5 μg.ml^{-1}) for total GPx (Table 2). The functional importance of Se-GPx in the kidney increased especially after confirming the association between GPx1 activity and glomerular injury.

A functional role of prostanoids in glomerular injury was first suggested by studies showing that nonsteroidal anti-inflammatory drugs can alter glomerular haemodynamics and urinary protein excretion in various proteinuric states [26]. Under physiological conditions, prostaglandins and thromboxanes are abundantly produced in kidney, especially PGE2. They significantly contribute to renal fluid metabolism and blood pressure regulation [27]. However, in free radical-catalysed peroxidation of arachidonic acid, the prostaglandin-like compounds isoprostanes are also produced. Hydroperoxy derivatives of arachidonic acid were found to be comparable in their ability to serve as substrates for the peroxidase activities of GPx [28].

Thus can be seen a very important role, particularly as confirmed by studies, of selenium-dependent GPx1 protection against nephropathy by removal of hydroperoxides, 8-isoprostane, 4-hydroxynonenal [5]. In turn, the function of GPx-4 is the regulation of hydroperoxide metabolism and control of leukotriene metabolism via 5-lipoxygenase deactivation by decreasing hydroperoxide levels [29]. None of the extracts used showed a hint of protective effects on kidney mitochondria in terms of the ability to induce an increase in Se-GPx activity, except for stevia at a final concentration of 62.5 μg.ml^{-1}. Total glutathione peroxidase activities were significantly lowered suggesting rather the opposite, reductive stress. An interesting finding is that an extract of oregano was also found to generally cause a decrease in GPx activities in the liver mitochondria and clearly showed cytotoxic effects at concentrations of 125 and 62.5 μg.ml^{-1} [16].

Proximal convoluted tubule cells of the kidney are the main location for the synthesis of selenium-containing GPx3 before its secretion into plasma and extracellular fluids [21]. However, a number of other cell types have been proposed to produce GPx3 based on the presence of mRNA or immunological identification of the protein [30]. Commenting on our results, there were no significant effects on selenium or total GPx in response to oregano. That being said, ginseng, stevia and agrimony expressed almost the same decreasing effects on selenium in plasma or none on the total GPx (Figure 1, 2). Several studies suggest that the reduced activity of GPx selenium levels are predictive of increased risk for cardiovascular disease or for septic patient outcome [31,32]. Oregano alone failed to cause a decrease in plasma Se-GPx activities. All other extracts decreased Se-GPx in plasma while maintaining total GPx activities.

Conclusion

The results of our work suggest that plant extracts affect activities of selenium and total glutathione peroxidases differently, depending on the organ and the concentrations used. For selenium-dependent isoforms, it is particularly evident in the heart mitochondria, and to a lesser extent in the kidney. The effect of ginseng and agrimony can generally be characterized as operating in the same direction on selenium and total glutathione peroxidases. Effects on Se-GPx are different in mitochondrial bodies, and are also different to plasma. In terms of the overall trend of total GPx, the extracts can be understood to have exhibited significant antioxidant effects. In terms of changes of selenium glutathione peroxidase activities and their physiological functions, some extracts

can be understood to have shown an organ-specific ability to influence redox signalling pathways. Specifically, oregano and ginseng in the heart and stevia in the kidney. Considering the effects of oregano on the heart mitochondria and plasma, they have clearly shown interesting and promising effects in the prevention of cardiovascular diseases.

Acknowledgements

This study was supported by the VEGA no. 1/0782/15.

References

[1] Brigelius-Flohé R, Flohé L. Basic principles and emerging concepts in the redox control of transcription factors. Antioxid. Redox Signal. 2011; 15:2335–81.

[2] Forman HJ, Maiorino M, Ursini F. Signaling functions of reactive oxygen species. Biochemistry 2010; 49:835–42.

[3] Flohé L, Toppo S, Cozza G, Ursini F. A comparison of thiol peroxidase mechanisms. Antioxid Redox Signal. 2011; 15:763-80.

[4] Ardanaz N, Yang XP, Cifuentes ME, Huarani MJ, Jackson KW, Liao TD, et al. Lack of glutathione peroxidase 1 accelerates cardiac-specific hypertrophy and dysfunction in angiotensin II hypertension. Hypertension 2010; 55:116-23.

[5] Chew P, Yuen DY, Stefanovic N, Pete J, Coughlan MT, Jandeleit-Dahm KA, et al. Antiatherosclerotic and renoprotective effects of ebselen in the diabetic apolipoprotein E/GPx1-double knockout mouse. Diabetes 2010; 59:3198-207.

[6] Barrett CW, Ning W, Chen X, Smith JJ, Washington MK, Hill KE, et al. Tumor suppressor function of the plasma glutathione peroxidase gpx3 in colitis-associated carcinoma. Cancer Res. 2013; 73:1245-55.

[7] Jin RC, Mahoney CE, Coleman Anderson L, Ottaviano F, Croce K, Leopold JA, et al. Glutathione peroxidase-3 deficiency promotes platelet-dependent thrombosis in vivo. Circulation 2011; 123:1963-73.

[8] Brigelius-Flohé R, Kipp AP. Physiological functions of GPx2 and its role in inflammation-triggered carcinogenesis. Ann N Y Acad Sci. 2012; 1259:19-25.

[9] Mechírová E, Domoráková I, Danková M, Danielisová V, Burda J. Effects of noradrenalin and EGb 761 pretreatment on the ischemia-reperfusion injured spinal cord neurons in rabbits. Cell Mol Neurobiol. 2009; 29:991-8.

[10] Silva MP, Martinez MJ, Casini C, Grosso NR. Tocopherol content, peroxide value and sensory attributes in roasted peanuts during storage. Int J Food Sci Tech. 2010; 45:1499–1504.

[11] Davydov M, Krikorian AD. Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. (Araliaceae) as an adaptogen: a close look. J Ethnopharmacol. 2000; 72:345–93.

[12] Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ehnopharmacol. 2004; 92:1–21.

[13] Quiroga PR, Grosso NR, Lante A, Lomolino G, Zygadlo JA, Nepote V. Chemical composition, antioxidant activity and anti-lipase activity of Origanum vulgare and Lippia turbinata essential oils. Int J Food Sci Tech. 2013; 48:642–9.

[14] Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines, Ed.: Fleming T. 2nd edition. Montvale, NJ: Medical Economics Company, 2000.

[15] Copland A, Nahar L, Tomlinson CT, Hamilton V, Middleton M, Kumarasamy Y, et al. Antibacterial and free radical scavenging activity of the seeds of Agrimonia eupatoria. Fitoterapia 2003; 74: 133-5.

[16] Vaško L, Vašková J, Fejerčáková A, Mojžišová G, Poráčová J. Comparison of some antioxidant properties of plant extracts from Origanum vulgare, Salvia officinalis, Eleutherococcus senticosus and Stevia rebaudiana. In Vitro Cell Dev Biol Anim. 2014; 50: 614-22.

[17] Fernández-Vizzara E, Ferrín G, Pérez-Martos A, Fernandéz-Silva P, Zeviani M, Enríquez JA. Isolation of mitochondria for biogenetical studies: An update. Mitochondrion 2010; 10:253-62.

[18] Azevedo AM, Martins VC, Prazeres DM, Vojinović V, Cabral JM, Fonseca LP. Horseradish peroxidase: a valuable tool in biotechnology. Biotechnol Annu Rev. 2003; 9:199-249.

[19] Maiorino M, Ursini F, Bosello V, Toppo S, Tosatto SC, Mauri P, et al. The thioredoxin specificity of Drosophila GPx: a paradigm for a peroxiredoxin-like mechanism of many glutathione peroxidases. J Mol Biol. 2007; 365:1033–46.

[20] Bera S, Weinberg F, Ekoue DN, Ansenberger-Fricano K, Mao M, Bonini MG, et al. Natural allelic variations in glutathione peroxidase-1 affect its subcellular localization and function. Cancer Res. 2014; 74:5118–26.

[21] Avissar N, Ornt DB, Yagil Y, Horowitz S, Watkins RH, Kerl EA, et al. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. Am J Physiol. 1994; 266:C367–75.

[22] Subhasree B, Baskar R, Laxmi Keerthana R, Lijina Susan R, Rajasekaran P. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chem. 2009; 115:1213-20.

[23] Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, et al. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med. 2003; 349:1605-13.

[24] Ferdinandy P, Danial H, Ambrus I, Rothery RA, Schulz R. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. Circ Res. 2000; 87:241–7.
[25] Tanguy S, Toufektsian MC, Besse S, Ducros V, De Leiris J, Boucher F. Dietary selenium intake affects cardiac susceptibility to ischemia/reperfusion in male senescent rats. Age Ageing 2003; 32:273-8.
[26] Cybulsky AV. Cyclooxygenases, prostanoids, and glomerular injury: complex relationships. J Am Soc Nephrol. 2007; 18:367-8.
[27] Hao CM, Breyer MD. Roles of lipid mediators in kidney injury. Semin Nephrol. 2007; 27:338-51.
[28] Musee J, Marnett LJ. Prostaglandin H synthase-2-catalyzed oxygenation of 2-arachidonoylglycerol is more sensitive to peroxide tone than oxygenation of arachidonic acid. J Biol Chem. 2012; 287:37383-94.
[29] Chen CJ, Huang HS, Chang WC. Depletion of phospholipid hydroperoxide glutathione peroxidase up-regulates arachidonate metabolism by 12S-lipoxygenase and cyclooxygenase 1 in human epidermoid carcinoma A431 cells. FASEB J. 2003; 17:1694-96.
[30] Arthur JR. The glutathione peroxidases. Cell Mol Life Sci. 2000; 57:1825-35.
[31] Holley AS, Harding SA, Sasse A, Miller JH, Larsen PD. Reduced glutathione peroxidase activity predicts increased cardiovascular risk following an acute coronary syndrome. Int Cardiovasc Forum J. 2016; 6:61-5.
[32] Kočan L, Vašková J, Vaško L, Simonová J, Simon R, Firment J. Selenium adjuvant therapy in septic patients selected according to Carrico index. Clin. Biochem. 2014; 47:44-50.