Biol Trace Elem Res (2016) 169:174–179
DOI 10.1007/s12011-015-0409-1

Proanthocyanidin Protects Human Embryo Hepatocytes from Fluoride-Induced Oxidative Stress by Regulating Iron Metabolism

Qiang Niu [1,2] · Lati Mu [1,2] · Shugang Li [1,2] · Shangzhi Xu [1,2] · Ruling Ma [1,2] · Shuxia Guo [1,2]

Received: 6 May 2015 / Accepted: 11 June 2015 / Published online: 25 June 2015

Abstract To investigate whether grape seed proanthocyanidin extract (GSPE) antagonizes fluoride-induced oxidative injury by regulating iron metabolism, human embryo hepatic cells (L-02) were incubated with sodium fluoride (NaF, 80 mg/L) and/or GSPE (100 μmol/L) for 24 h. Results showed the glutathione peroxidase (GSH-Px) content, superoxide dismutase (SOD) activity, and total antioxidant capacity (T-AOC) level of the NaF group were significantly lower than that of the control group ($P < 0.05$), while malondialdehyde (MDA) content increased in the NaF group compared with the control group ($P < 0.05$). Moreover, the indexes mentioned above showed opposite changes in the NaF + GSPE group. In addition, iron content significantly increased in the NaF group compared to the control group($P < 0.05$) and significantly decreased in the NaF + GSPE group compared to the NaF group ($P < 0.05$). Furthermore, hepcidin (coded by *HAMP*) messenger RNA (mRNA) expression significantly increased in the NaF group compared to the control group($P < 0.05$) and significantly decreased in the NaF + GSPE group compared to the NaF group ($P < 0.05$). Ferroportin 1 (coded by *FPN1*) mRNA expression significantly decreased in the NaF group compared to the control group ($P < 0.05$) and significantly increased in the NaF + GSPE group compared to the NaF group ($P < 0.05$). These results indicate that GSPE provides significant cellular protection against oxidative stress induced by excessive fluoride via the iron metabolism regulation.

Keywords Fluoride · Grape seed proanthocyanidin extract · Oxidative stress · Iron metabolism · L-02 cells

Introduction

Endemic fluorosis, a disease due to ingestion of excessive amounts of fluorine, is prevalent in many parts of the world, including China, India, Sri Lanka, Senegal, Ghana, South Africa, etc. [1]. Fluorosis can cause damage not only to skeletal tissue and teeth but also to soft tissues, such as the brain, liver, kidney, and spinal cord. Previous studies revealed that continuous intake of excessive fluoride may cause liver oxidative damage [2, 3]. Some researchers believe that fluorine can directly attack antioxidant enzymes, weakening their activities and increasing free radical abundance [4]. Other scholars hold that fluorine can activate the NADH oxidation system, transfer electrons to oxygen, and produce oxygen free radicals [5]. These theories greatly enrich and expand the knowledge regarding oxidative damage induced by fluoride; however, the detailed mechanisms underlying these effects remain to be explored.

Along with understanding Alzheimer's disease and Parkinson's disease, oxidative damage caused by iron metabolism disorder is a subject of research attention. Iron is an essential factor for several important biological activities and biochemical reactions, including oxygen transport, electron transport, and xenobiotic metabolism [6]. However, accumulation of iron within tissues may induce generation of reactive oxygen species (ROS) and thus produce a toxic impact [7]. Therefore, the normal physical level of iron is ensured by rigid regulation of iron metabolism. Iron levels are tightly regulated

Qiang Niu and Lati Mu contributed equally to this work.

✉ Shuxia Guo
20139206@qq.com

[1] Key Laboratory of Xinjiang Endemic and Ethnic Diseases, Ministry of Education, Shihezi University School of Medicine, Shihezi, China

[2] Department of Preventive Medicine, Medical College, Shihezi University, Bei er Road 13, Shihezi 832002, Xinjiang, People's Republic of China

by hepcidin (encoded by *HAMP*), which is a key regulator of iron metabolism produced by hepatocytes [8]. Hepcidin binds to ferroportin 1 (FPN1), the only known iron efflux transporter found in the cell membranes of hepatocytes and macrophages, where it induces internalization and eventual degradation [9]. Thereby, FPN1 decreases further cellular iron export.

A variety of pharmacological strategies have been employed to counter oxidative stress resulting from iron overload. An alternative measure is phytochemical treatment, which is believed to be safer, healthier, and less prone than their synthetic counterparts to produce adverse effects. Grape seed proanthocyanidin extract (GSPE) is a combination of biologically active polyphenolic flavonoids, including oligomeric proanthocyanidins [10]. GSPE has demonstrated a wide spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against free radicals and oxidative stress [11, 12]. The remarkable spectrum of biochemical and cellular functions of GSPE holds promise for the prevention and treatment of various disorders caused by oxidative stress. Recently, some studies have shown that the antioxidant capacity of GSPE is closely related to its iron-chelating function [13]. It is of importance to rationally utilize antioxidant additives to investigate the specific antioxidant mechanisms of GSPE.

There are few studies focused on the capacity of GSPE to antagonize fluoride-induced oxidative injury by regulating iron metabolism. Therefore, in this study, we used human embryo hepatocytes (L-02) to explore the effects of sodium fluoride (NaF) alone, GSPE alone, and NaF in combination with GSPE on oxidative stress, iron content, and messenger RNA (mRNA) expression levels of *HAMP* and *FPN1*, with the goal of providing preliminary, but important information that could lead to the development of new strategies to inhibit or alleviate oxidative damage attributed to fluorosis.

Materials and Methods

Chemicals

Fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI) 1640 medium culture media were obtained from Gibco CRL (Paisley, UK). Taq DNA polymerase, dNTP mix, and SYBR PrimeScript RT-PCR kits were purchased from Takara Bio (Dalian, China). The primers for *HAMP*, *FPN1*, and β-actin were synthesized and purified by Invitrogen Corp. (Shanghai, China). NaF was obtained from Shanghai Chemical Reagent Corp. (Shanghai, China). The assay kits for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) were obtained from the Nanking Jiancheng Bioengineering Research Institute (Nanjing, China). The Olympus-Ckx61 fluorescence microscope was supplied by Olympus (Japan), and fluorescence quantity PCR (7900-HT) was purchased from Applied Biosystems (Foster City, USA). All other chemicals were of analytical grade and obtained commercially.

Cell Culture and Treatment with NaF and/or GSPE

L-02 cells were cultured in RPMI 1640 medium with 110 mg/L sodium pyruvate at 37 °C in a humidified atmosphere with 5 % CO_2. The media were supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, and 10 % FBS.

Exponentially growing cells were divided into four groups: control group (FBS), NaF group (80 mg/L), GSPE group (100 μmol/L), and NaF (80 mg/L) + GSPE (100 μmol/L) group. The doses of NaF and GSPE were selected based on previous studies conducted in our laboratory [14, 15].

Cells were rinsed twice with PBS, trypsinized, centrifuged at 1000×*g* for 5 min, and kept on ice until assays were performed.

GSH-Px, SOD, T-AOC, and MDA Assays

GSH-Px, SOD, T-AOC, and MDA levels were determined using commercially available kits according to the manufacturer's instructions strictly (Nanjing Jiancheng Bioengineering Institute, China). The results of the assays were normalized to the total amount of protein as measured by the bicinchoninic acid (BCA) method.

Determination of Iron Content

The cell suspension was centrifuged at 500×*g* for 5 min at 4 °C and the resulting cell pellet was dissolved in 0.5 mL of cell lysis solution (containing 1 mM Na_2EDTA, 150 mM NaCl, 10 mM PMSF, 10 mM Tris, and 1 mM aprotinin). Cellular iron content was determined using a kit by following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China). Briefly, in acidic buffer solution, the Fe^{3+} of ferritin was reduced to Fe^{2+}, after which Ferene S reacted with Fe^{2+} to produce blue compounds that were measured by colorimetry at 593 nm.

Analysis of mRNA Expression Levels of *HAMP* and *FPN1*

RNA was extracted from cultured L-02 cells using the TRIzol method. The A260/280 ratio was in the range of 1.8–2.0. Real-time PCR (qPCR) was conducted using the SYBR PrimeScript RT-PCR Kit with the manufacturer's protocol. qPCR was performed with SYBR Green using the ABI Prism 7900 Sequence Detection System. To obtain the relative quantitative gene expression values, β-actin was used as an

endogenous control. The primer sequences are listed in Table 1.

Statistical Analysis

Results are expressed as mean ± SD for at least three experiments, performed in triplicate. Data were evaluated statistically using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for independent mean comparisons. The level of significance was set at $P < 0.05$.

Results

Iron Content in L-02 Cells Treated with NaF and/or GSPE

As shown in Fig. 1, the iron content of the NaF group was significantly higher than that of the control group ($P < 0.05$). Furthermore, the iron content of the GSPE group was significantly reduced in comparison with that of the NaF group ($P < 0.05$). In addition, the iron content of the NaF + GSPE group was significantly reduced in comparison with that of the NaF group ($P < 0.05$), but greater than that of the GSPE group ($P < 0.05$).

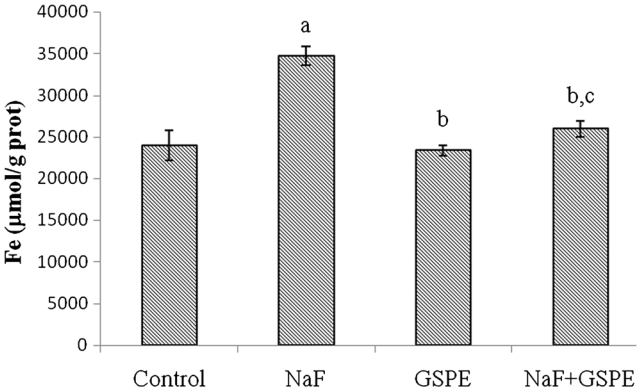

Fig. 1 Effects of NaF and/or GSPE on iron contents. Values are represented as mean ± SD of three independent determinations, assayed in triplicate. The *different lowercase letters* denote significant treatment-related effects ($P < 0.05$), as determined by one-way ANOVA followed by LSD test. *a* $P < 0.05$ vs. control group; *b* $P < 0.05$ vs. NaF group; *c* $P < 0.05$ vs. GSPE group

GSH-Px, SOD, T-AOC, and MDA in L-02 Cells Treated with NaF and/or GSPE

As shown in Table 2, significant decreases in GSH-Px content, SOD activity, and T-AOC level in L-02 cells treated with NaF were observed in comparison with those of the control group ($P < 0.05$), but MDA content was increased significantly in the NaF group in comparison with that of the control group ($P < 0.05$). Furthermore, GSH-Px content, SOD activity, and T-AOC level in L-02 cells treated with GSPE were significantly elevated in comparison with those of the NaF group ($P < 0.05$), while MDA content was decreased significantly in the GSPE group in comparison with that of the NaF group ($P < 0.05$). In addition, GSH-Px content, SOD activity, and T-AOC level in L-02 cells treated with NaF + GSPE were reduced in comparison with those of the NaF group ($P < 0.05$), but MDA content was decreased significantly in the NaF + GSPE group in comparison with that of the NaF group ($P < 0.05$).

mRNA Expression of *HAMP* and *FPN1* in L-02 Cells Treated with NaF and/or GSPE

As shown in Fig. 2, the *HAMP* mRNA expression level of the NaF group was significantly higher than that of the control group ($P < 0.05$). The *HAMP* mRNA expression level of the GSPE group was lower than that of the NaF group ($P < 0.05$). The *HAMP* mRNA expression level of the NaF + GSPE group was lower than that of the NaF group ($P < 0.05$), but higher than that of the GSPE group ($P < 0.05$).

In addition, the *FPN1* mRNA expression level of the NaF group was significantly lower than that of the control group ($P < 0.05$). The *FPN1* mRNA expression level of the GSPE group was higher than that of the NaF group ($P < 0.05$). The *FPN1* mRNA expression level of the NaF + GSPE group was lower than that of the GSPE group ($P < 0.05$), but higher than that of the NaF group ($P < 0.05$).

Table 1 List of primers used for real-time RT-PCR

Accession number	Gene	Oligonucleotide primer 5′-3′	Product size (bp)
NM_021175	HAMP	(F) 5′- ACCAGTGGCTCTGTTTTCCC-3 (R) 5′-AGCAGCCGCAGCAGAAAATG-3	154
NM_014585	FPN 1	(F) 5′- GCAGGAGAAGACAGAAGCAAACT-3′ (R) 5′-TCCATCCCGAAATAAAGCCACA-3′	148
NM_001101	β-actin	(F) 5′-AGCGAGCATCCCCCAAAGTT-3′ (R) 5′-GGGCACGAAGGCTCATCATT-3′	284

Table 2 Effect of NaF and/or GSPE on GSH-Px, MDA, SOD and T-AOC in L-02 cells ($\bar{x} \pm S$, $n = 6$)

Group	GSH-Px (mol/g prot)	MDA (nmol/mg prot)	SOD (U/g prot)	T-AOC (U/g prot)
Control	164.05 ± 7.51	0.03 ± 0.01	11.83 ± 1.12	1.15 ± 0.18
NaF	46.28 ± 8.59*	0.12 ± 0.02*	6.28 ± 0.74*	0.12 ± 0.01*
GSPE	151.76 ± 5.22**	0.04 ± 0.03**	11.96 ± 0.55**	1.48 ± 0.09**
NaF + GSPE	116.84 ± 5.16**, ***	0.05 ± 0.01**	11.57 ± 0.56**	1.13 ± 0.17**, ***

Data are presented as the mean ± SD of three independent determinations in triplicate; *$P < 0.05$, significantly different from control group; **$P < 0.05$, significantly different from NaF group; ***$P < 0.05$, significantly different from GSPE group

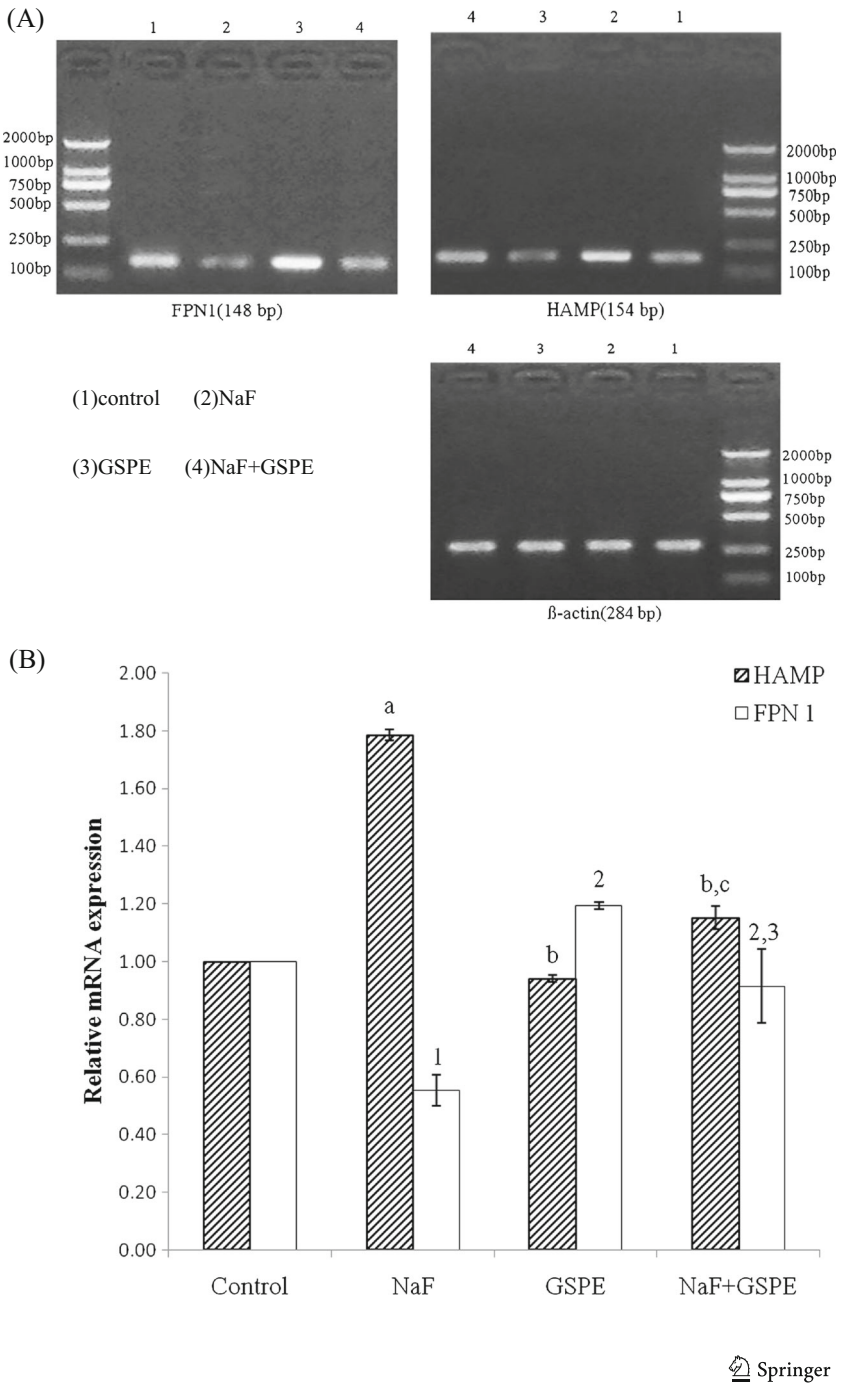

Fig. 2 Effects of NaF and/or GSPE on mRNA expression levels of HAMP and FPN 1 in L-02 cells analyzed by real-time PCR. **a** Results of representative RT-PCR are shown. **b** Values are represented as mean ± SD of three independent determinations, assayed in triplicate. The *different lowercase letters and numbers* denote significant treatment-related effects ($P < 0.05$), determined by one-way ANOVA followed by LSD test. The *lowercase letters* represent the comparison of mRNA expression levels of HAMP among the groups: *a* $P < 0.05$ vs. control group; *b* $P < 0.05$ vs. NaF group; *c* $P < 0.05$ vs. GSPE group. The *Arabic numerals* represent the comparison of the mRNA expression levels of FPN 1 among the groups: *1* $P < 0.05$ vs. control group; *2* $P < 0.05$ vs. NaF group; *3* $P < 0.05$ vs. GSPE group

Discussion

Since ROS were implicated as important pathologic mediators in many disorders, various studies have investigated whether oxidative stress and lipid peroxidation are involved in the pathogenesis of chronic fluorosis. In this study, we observed a significant decrease in T-AOC level, SOD activity, and GSH-Px content, but increased MDA content, in the NaF group. These results indicated that the equilibrium between the oxidative system and antioxidant system in the cells was destroyed by fluoride exposure. Our findings are similar to earlier observations [16, 17]. These findings indicate that oxidative stress plays a vital role in hepatotoxicity induced by excessive fluoride.

For decades, efforts have been made to elucidate the mechanism of oxidative stress caused by fluorosis, achieving substantial progress. Recently, iron overload has aroused researcher interest, as it might be an explanation for oxidative stress resulting from fluorosis. The present results showed that the concentration of hepatic iron was increased significantly in the fluoride-treated group in comparison with that of the control group, demonstrating that iron homeostasis of L-02 cells was disturbed by excessive fluoride. Such an increase in free iron by fluoride could catalyze the Fenton reaction, produce hydroxyl radicals, and subsequently cause oxidative injury. Hepcidin (encoded by *HAMP*) is a liver-derived regulatory hormone that plays a pivotal role in systemic iron homeostasis. The hepcidin peptide regulates systemic iron homeostasis by controlling iron flux into the plasma by binding to its receptor, the iron transporter FPN1 [18, 19]. In the present study, we showed that *HAMP* mRNA expression was significantly upregulated in the fluoride-treated group, while *FPN1* mRNA expression was significantly downregulated. In the fluoride-treated group, hepcidin would be expected to ultimately bind to the iron transporter FPN1 and cause its internalization and degradation. By inhibiting FPN1, hepcidin inhibits iron release into the hepatic portal system, thereby leading to iron overload in L-02 cells.

Fluorosis is irreversible, but preventable by appropriate intervention. One of the best ways to delay or prevent the onset of fluorosis is improve the antioxidant capacity of the body by providing additional radical scavengers [20–22]. GSPE contains several polyphenolic bioflavonoids and has been reported to exhibit a wide range of inhibitory effects against oxygen free radicals. Previous studies revealed that the antioxidant capacity of GSPE is higher than that of vitamin E and C [23, 24]. It is believed GSPE induces antioxidant effects through several mechanisms, including neutralization of free radicals, reduction of peroxide concentrations, and repair of oxidized membranes, all of which alleviate oxidative damage [25–27]. Besides, GSPE shows little toxicity. The LD_{50} value of GSPE is approximately 4 g/kg in male and female rats [28]. Actually, GSPE has been used in Europe and the USA for decades without reported adverse effects. The present study showed that T-AOC level, SOD activity, and GSH-Px content were elevated in the NaF + GSPE group in comparison with those of the NaF group, while MDA content was decreased; these results were similar to those of previous studies. Furthermore, the hepatic iron content of the NaF + GSPE group was significantly lower than that of the NaF group, perhaps because of the antioxidant capacity of GSPE conferred by its iron-chelating abilities. The catechol and dihydroxy phenols of GSPE can chelate iron ions formed during the Fenton reaction to form inert compounds, thus preventing production of free radicals normally caused by iron overload. In addition to its free radical scavenging property, GSPE regulates the expression of a number of genes and regulatory signaling pathways and may thereby prevent cell death. Excitingly, the group coincubated with NaF and GSPE showed significantly upregulated *FPN1* mRNA expression in comparison with that of the NaF group, along with downregulated *HAMP* mRNA expression. These results indicate that GSPE exerts its beneficial effects through its ability to chelate free iron and scavenge H_2O_2 generated by the Fenton reaction, thus triggering *HAMP* reduction and *FPN1* elevation, activating iron efflux channels and leading to the release of iron ions from L-02 cells, thereby effectively relieving oxidative stress due to fluorosis-induced iron overload.

The present results are the first report that NaF-induced oxidative damage in L-02 cells is at least partially caused by abnormal iron homeostasis. More importantly, the findings reported herein demonstrate that GSPE provides significant cellular protection against oxidative stress induced by excessive fluoride via regulation of iron metabolism. Thus, the present study provides preliminary but important data that will facilitate further study of the antioxidant mechanisms of GSPE, while providing valuable evidence and ideas that could improve strategies for preventing and treating fluorosis. Further investigation is required to identify the detailed antioxidative mechanisms underlying the therapeutic effects of GSPE against oxidative stress caused by fluorosis.

Acknowledgments The work was supported by grants from the Science and Technology Research and Development grant of Shihezi University, Xinjiang, China (Project No. 2012ZRKXYQ25), Xinjiang Production and Construction Corps grant, Xinjiang, China (Project No. 2014BA039) and Shihezi University grant, Xinjiang, China (Project No. RCZX201112).

Conflict of Interest The authors declare that they have read the manuscript and agree to its submission to the journal and that the manuscript is original and has not been published elsewhere. The authors declare that they have no competing interests.

References

1. Ozsvath DL (2009) Fluoride and environmental health: a review. Rev Environ Sci Bio/Technol 8(1):59–79
2. M J, Sinha S, Ghosh M, Mukherjee A (2013) Evaluation of multi-endpoint assay to detect genotoxicity and oxidative stress in mice exposed to sodium fluoride. Mutat Res 751(1):59–65
3. Zhang KL, Lou DD, Guan ZZ (2015) Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. Neurotoxicol Teratol. 48:49–55
4. Perumal E, Paul V, Govindarajan V, Panneerselvam L (2013) A brief review on experimental fluorosis. Toxicol Lett. 223(2):236–251
5. Pawłowska-Góral K, Kurzeja E, Stec M (2013) N-acetylcysteine protects against fluoride-induced oxidative damage in primary rat hepatocytes. Toxicol in Vitro. 27(8):2279–2282
6. Ganz T (2013) Systemic iron homeostasis. Physiol Rev. 93(4): 1721–1741
7. Koskenkorva-Frank TS, Weiss G, Koppenol WH, Burckhardt S (2013) The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. Free Radic Biol Med. 65:1174–1194
8. Ganz T, Nemeth E (2011) Hepcidin and disorders of iron metabolism. Annu Rev Med. 62:347–360
9. Altamura S, Kessler R, Gröne HJ, Gretz N, Hentze MW, Galy B, Muckenthaler MU (2014) Resistance of ferroportin to hepcidin binding causes exocrine pancreatic failure and fatal iron overload. Cell Metab. 20(2):359–367
10. Bagchi D, Swaroop A, Preuss HG, Bagchi M (2014) Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: an overview. Mutat Res. 768:69–73
11. Gollucke AP, Peres RC, Odair Jr A, Ribeiro DA (2013) Polyphenols: a nutraceutical approach against diseases. Recent Patents Food, Nutr Agric 5(3):214–219
12. Belviranlı M, Gökbel H, Okudan N, Büyükbaş S (2013) Effects of grape seed polyphenols on oxidative damage in liver tissue of acutely and chronically exercised rats. Phytother Res 27(5):672–677
13. Wu TH, Liao JH, Hsu FL, Wu HR, Shen CK, Yuann JM, Chen ST (2010) Grape seed proanthocyanidin extract chelates iron and attenuates the toxic effects of 6-hydroxydopamine: implications for Parkinson's disease. J Food Biochem 34(2):244–262
14. Niu Q, Liu HL, Guan ZZ, Zeng Q, Guo SX, He P, Guo LJ, Gao P, Xu BY, Xu ZX, Xia T, Wang AG (2012) The effect of c-Fos demethylation on sodium fluoride-induced apoptosis in L-02 cells. Biol Trace Elem Res 149(1):102–109
15. Baselga-Escudero L, Bladé C, Ribas-Latre A, Casanova E, Salvadó MJ, Arola L, Arola-Arnal A (2012) Grape seed proanthocyanidins repress the hepatic lipid regulators miR-33 and miR-122 in rats. Mol Nutr Food Res 56(11):1636–1646
16. Inkielewicz-Stępniak I, Knap N (2012) Effect of exposure to fluoride and acetaminophen on oxidative/nitrosative status of liver and kidney in male and female rats. Pharmacol Rep 64(4):902–911
17. Dubey N, Khan AM, Raina R (2013) Sub-acute deltamethrin and fluoride toxicity induced hepatic oxidative stress and biochemical alterations in rats. Bull Environ Contam Toxicol 91(3):334–338
18. Pietrangelo A (2011) Hepcidin in human iron disorders: therapeutic implications. J Hepatol 54(1):173–181
19. D'Anna MC, Roque ME (2013) Physiological focus on the erythropoietin–hepcidin–ferroportin axis. Can J Physiol Pharmacol 91(5):338–345
20. Sarmadi BH, Ismail A (2010) Antioxidative peptides from food proteins: a review. Peptides 31(10):1949–1956
21. Carocho M, Ferreira IC (2013) A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol 51:15–25
22. Krishnaiah D, Sarbatly R, Nithyanandam R (2011) A review of the antioxidant potential of medicinal plant species. Food Bioprod Process 89(3):217–233
23. Yonguc GN, Dodurga Y, Adiguzel E, Gundogdu G, Kucukatay V, Ozbal S, Yilmaz I, Cankurt U, Yilmaz Y, Akdogan I (2015) Grape seed extract has superior beneficial effects than vitamin E on oxidative stress and apoptosis in the hippocampus of streptozotocin induced diabetic rats. Gene 555(2):119–126
24. Songsermsakul P, Pornphairin E, Porasuphatana S (2013) Comparison of antioxidant activity of grape seed extract and fruits containing high β-carotene, vitamin C, and E. Int J Food Prop 16(3):643–648
25. Cui X, Liu X, Feng H, Zhao S, Gao H (2012) Grape seed proanthocyanidin extracts enhance endothelial nitric oxide synthase expression through 5′-AMP activated protein kinase/Surtuin 1–Krüpple like factor 2 pathway and modulate blood pressure in ouabain induced hypertensive rats. Biol Pharm Bull 35(12):2192–2197
26. Gao Z, Liu G, Hu Z, Li X, Yang X, Jiang B, Li X (2014) Grape seed proanthocyanidin extract protects from cisplatin-induced nephrotoxicity by inhibiting endoplasmic reticulum stress-induced apoptosis. Mol Med Rep 9(3):801–807
27. Bao L, Cai X, Dai X, Ding Y, Jiang Y, Li Y, Zhang Z, Li Y (2014) Grape seed proanthocyanidin extracts ameliorate podocyte injury by activating peroxisome proliferator-activated receptor-γ coactivator 1α in low-dose streptozotocin-and high-carbohydrate/high-fat diet-induced diabetic rats. Food Funct 5(8):1872–1880
28. Hamlaoui S, Mokni M, Limam N, Zouaoui K, Ben Rayana MC, Carrier A, Limam F, Amri M, Marzouki L, Aouani E (2012) Protective effect of grape seed and skin extract on garlic-induced erythrocyte oxidative stress. J Physiol Pharmacol 63(4):381–388