The Pharmaceutical and Chemical Journal, 2020, 7(2):125-131

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Increased Oxidative Injury in Erythrocytes after Paraquat Exposure is correlated with Declined Enzyme Activity and Reduced Zinc Level

Min-Tzu Chen[1], Chien-Chi Liu[2], Ming-Cheng Lin[3,*]

[1]Department of Medical Laboratory Science and Biotechnology, Chung Hwa University of Medical Technology, Tainan, Taiwan
[2]Department of Nursing, National Taichung University of Science and Technology, Taichung, Taiwan
[3]Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung, Taiwan
*Corresponding author: Dr. Ming-Cheng Lin, Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung, 406, Taiwan
Tel: 886-4-2239-1647; Fax: 886-4-2239-6761; E-mail: mclin@ctust.edu.tw

Abstract Non-selective contact herbicide of paraquat (PQ) is worldwide used for controlling wild plants in agriculture due to its enormous properties such as cheaper price and effective. PQ toxicity has been evidenced in involving the generation of reactive oxygen species (ROS).Zinc (Zn) can exert protective ability in cells due to its anti-oxidative property. This present research attempted to realize whether PQ-induced oxidative stress in erythrocyte is responsible for the alterations of Zn level together with antioxidant activity. Experimental rats were divided into four groups of 10 each and treated with a single, intraperitoneal injection of PQ at the dosages of 0, 10, 20, or 40 mg/kg, respectively. Erythrocytes were obtained via cardiac puncture after 24 h post-injection. Experimental results showed that an opposite correlation between PQ-treated dosage and Zn level were observed in erythrocytes. The oxidative biomarker of the malondialdehyde (MDA) level was increased in parallel with PQ-administrated dosage. Meanwhile, a negative correlation between PQ dosage and enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) were found in the present work. Taking together, it seems likely to suggest our present findings that increased oxidative injury after paraquat exposure in erythrocytes is correlated with reduced Zn concentration and decreased enzyme activity.

Keywords Oxidative injury, Paraquat, Zinc, Malondialdehyde, Enzyme activity

Introduction

Non-selective contact herbicide of paraquat (PQ) is extensively used to remove wild plants in the public environment [1-3]. Based on its cheaper price as well as fast action, PQ is still commonly used in agriculture worldwide [4-6]. Study has clearly suggested that PQ toxicity is involved in the generation of toxic reactive oxygen species (ROS) [7-8]. Animal study shows that a large number of ROS produced by PQ can bind to biomolecules such as nucleic acid, protein and lipid membrane. Given the fact, further adversely oxidative lesion such as lipid peroxidation occurs [6-9].

It has been recognized that essential metal of Zinc (Zn) possesses a large spectrum of beneficial roles for cellular metabolism. In addition to this, other beneficial effect such as anti-inflammation and anti-oxidation has been reported previously [10-12]. Former definite animal experiment indicates that a statistical decrease of the Zn concentration was observed in human disorders such as cerebral ischemia [12-14]. In this regards, it is thereby obvious to note that decreased Zn concentration is disadvantageous and harmful to the live organisms.

Antioxidant enzyme of SOD, CAT, as well as GPX, has been recognized in attenuating ROS-induced oxidative stress and protects the cells from further oxidative damage [15-17]. In contrast, decrease in which activity is realized to be deleterious to the cells [16-17]. PQ intoxication mainly results in hypoxia due to the damage of erythrocytes [17]. Previous study has suggested that erythrocyte is known as a suitable in vivo model for assessing ROS-mediated cellular lesions [15-17]. This present study wanted to investigate whether PQ-induced oxidative stress in erythrocyte is correlated with the changes of the Zn level and antioxidant activity.

Materials and Methods

Animal preparation and harvest of erythrocytes

In this study, forty male Sprague-Dawley rats, weighing from 220-270 g were encompassed in this work. All animals were purchased from the National Laboratory Animal Breeding and Research Center (Taiwan). The protocol of this present work was approved by the Institutional Animal Care and Use Committee (IACUC). All rats were housed under controlled conditions (22 ± 2°C, 50 ± 20% relative humidity, 12-h light-dark cycle). One week after caging, rats were randomly divided into four groups of 10 each and injected intraperitoneally (i.p.) with a single dose of 0 (saline as control), 10, 20, or 40 mg/kg of PQ purchased from Sigma Chemical Co (St. Louis, MO, USA), respectively. Twenty-four hours after treatment, the whole blood samples were collected in heparin-containing anticoagulant tubes via directly cardiac puncture technique. The harvested blood samples were immediately centrifuged at 4°C for 10 min at 650 g and the plasma was discarded. The erythrocyte samples were obtained after washed for three times with ice-cold isotonic saline solution and centrifuged at 4°C for 15 min at 650 g in the present work.

Measurement of the malondialdehyde (MDA) concentration in erythrocytes

The end-product of malondialdehyde (MDA) from lipid peroxidation was measured to act as a marker of oxidative stress. The thiobarbituric acid (TBA) reagent was purchased from E. Merck (Germany) and used for MDA analysis. In short, the MDA level was assayed according to the method of Sunderman et al. [18]. The 1, 1, 3, 3-tetra ethoxy propane (TEP) reagent was used as a standard solution in the reaction with thiobarbituric acid-reactive substance (TBARS). Presently, 2 ml of erythrocyte sample was used, and this method is rely on the determination of the pink color that is produced by the interaction of TBA with the MDA. The spectrophotometer (Thermo Scientific Multiskan Spectrum, USA) was used to measure the MDA level at the wavelength of 532 nm in the present work. Hemoglobin (Hb) assay kit (Cayman Chemical Company, USA) was used and the concentration was determined by the instrument of spectrophotometer at a wavelength of 540 nm.

Determination of the Zinc level in erythrocytes

The harvested erythrocytes were digested with ultra-pure grade solution of nitric acid for essential metal analysis. All containers were soaked with 50% nitric acid purchased from Merck (Germany) and rinsed with ultra-pure deionized water collected from the Milli-Q system (Millipore, USA) followed by drying for later use. The standard solutions (1000 μg/ml) of analyzed metals were purchased from Merck (Germany) and dissolved in ultra-pure grade solution of nitric acid. Essential metal level of Zn was assayed at the wavelength of 213.9nm via SavantAA Z graphite furnace atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) in the present work.

Analysis of the antioxidant enzyme activity

The obtained erythrocytes were lysed in four times its volume of ice-cold water and were centrifuged at 4°C for 15 min at 10000 g. After washing completely, the supernatants were collected for enzyme activity analysis. In short, the SOD activity was assayed based on the method of the Cayman superoxide dismutase assay kit (Cayman Chemical Company, USA). The tetrazolium salt was utilized for the measurement of superoxide radicals produced by xanthine oxidase and hypoxanthine. The Cayman catalase assay kit (Cayman Chemical Company, USA) was used to determine the CAT activity. The principle of CAT activity is based on the reaction of the enzyme with methanol in the presence of hydrogen peroxide. The produced formaldehyde wasanalyzed with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole as the chromogen. The detective principle of the GPX activity is that the GPX catalyzed the oxidation of glutathione by cumenehydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted into a reduced form with concomitant oxidation of NADPH to $NADP^+$. The resulting reduce in the absorbance at the wavelength of 340 nm was assayed. The above enzyme activities were measured by spectrophotometer (Thermo Scientific Multiskan Spectrum, USA) and expressed in terms of U per g of Hb. The experimental values were expressed as mean ± S.D. and the obtained data were analyzed using the statistical tests of one-way analysis of variance (ANOVA). Once the obtained data showed significant differences among the subject means, each subject was compared using the Fisher's least significant difference (FLSD) test. The p-value of less than 0.05 in the present work was considered statistical difference.

Results

Essential metal value of Zn in erythrocytes

Essential metal concentration of Zn was 0.48 ± 0.02, 0.45 ± 0.02, 0.41 ± 0.02, and 0.31 ± 0.02 μg/gHb in PQ-treated dosages of 0, 10, 20, and 40mg/Kg, respectively (Figure 1). Prominently, an opposite association was seen between PQ-administrated dosage and Zn concentration.

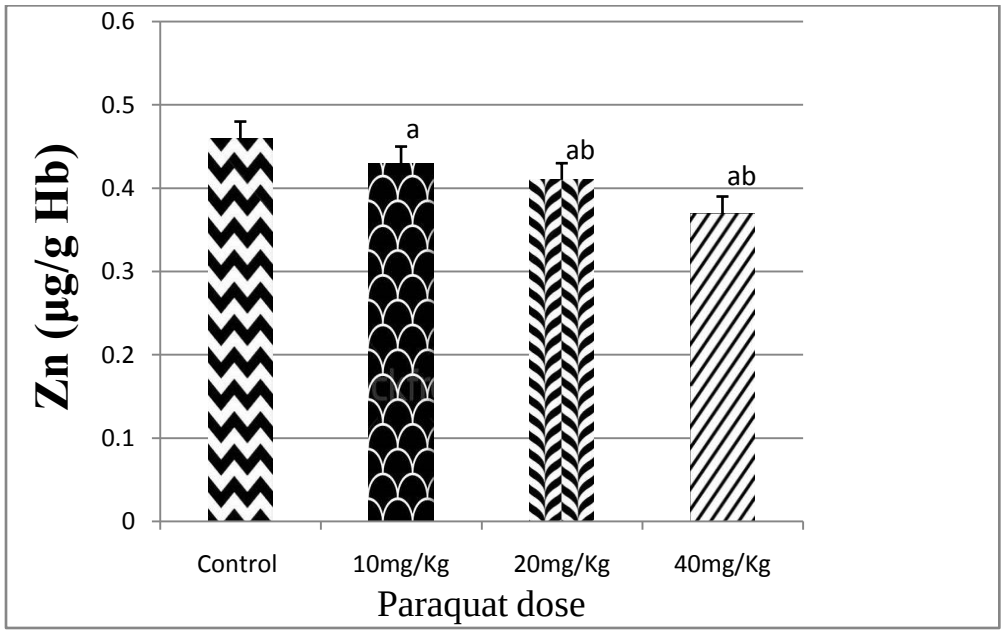

Figure 1: Profiles of Zn level in erythrocytes. Data were expressed as mean ± S.D. (N=40)

One-way ANOVA followed by Least Significant Difference was used presently.

a: Significant difference ($p<0.05$) from control subjects. b: Significant difference ($p<0.05$) from 10mg/kg of paraquat-treated group.

Enzyme activity of SOD, CAT, and GPX in erythrocytes

First of all, the SOD activity in PQ-treated dosages of 0, 10, 20, and 40mg/Kg was 905± 71, 750 ± 80, 774± 90, and 646± 69U/g Hb respectively (Table 1). The CAT activity in control and PQ-treated dosage of 10, 20, and 40mg/Kg was 150± 14, 137 ± 12, 113 ± 10, and100± 6 U/g Hb, respectively as listed in Table 1. Finally, the GPX activity in PQ-treated dosages of 0, 10, 20, and 40mg/Kg was 28.3± 2.6, 25.2± 3.3, 20.5± 2.6, and 20.7± 2.5, respectively (Table 1). Obviously, PQ significantly ($P < 0.05$) reduced these three enzyme activities in erythrocytes in a dose-dependent manner.

Table 1: Antioxidant enzyme activity of CAT, SOD, and GPX in the erythrocytes

Antioxidant enzyme	Paraquat-treated dosage (mg/kg of body weight)			
	Control	**10**	**20**	**40**
SOD (U/g Hb)	905±71	750±80[a]	774±90[a;b]	646±69[a;b]
CAT (U/g Hb)	150±14	137±12[a]	113±10[a;b]	100±6[a;b]
GPX (U/g Hb)	28.3±2.6	25.2±3.3[a]	20.5±2.6[a;b]	20.7±2.[a;b]

[a]Significant difference from the control subjects ($p < 0.05$).

[b]Significant difference from10mg/kg of paraquat-treated group ($p < 0.05$)

Malondialdehyde (MDA) concentration in erythrocytes

As shown in Figure 2, the MDA value in PQ-administrated dosages of 0, 10, 20, and 40mg/Kg was 0.85±0.05, 1.06± 0.05, 1.40±0.06, and 1.76±0.08μmol/gHb, respectively. Obviously, the MDA level was significantly ($P < 0.05$) increased in paralleled with PQ-administrated dosage.

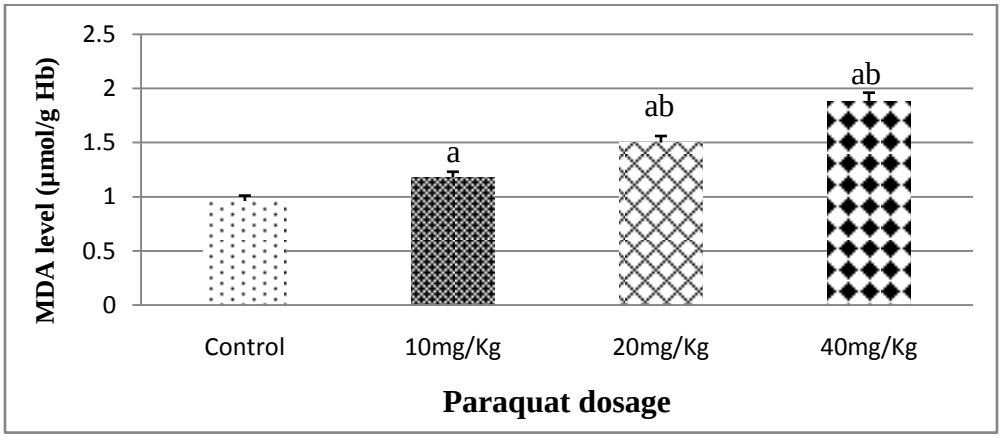

Figure 2: Profiles of MDA level in erythrocytes

Data were expressed as mean ± S.D. (N=40). One-way ANOVA and Least Significant Difference test was used. a: Significant difference ($p < 0.05$) from control subjects. B: Significant difference ($p < 0.05$) from 10mg/kg of paraquat-treated group.

Discussion

It has been well-recognized that essential metal of Zn is required for all living organisms for their biological functions [10]. Based on its anti-oxidative ability, the essential metal of Zn can attenuate ROS-induced lesion [11]. In turn, depletion of the Zn level has been documented in declining the above protective ability [10-11]. Preceding in vivo study has suggested that depletion of the Zn level is responsible for an increased inflammatory effect and elevated lipid peroxidation effect observed in rats [11]. Another animal research has suggested that bovine with tropical theileriosis is correlated with a decreased Zn concentration [12]. In vivo study has indicated that deficiency in Zn level can induce oxidative stress and results in AP-1 activation in 3T3 cells [13]. Furthermore, the clinical investigation has revealed that patients with liver hepatitis or liver cirrhosis emerge Zn deficiency [14]. Our experimental result showed that intracellular Zn concentration in erythrocytes was opposite to PQ-treated dosages as

listed in Figure 1. Diminished Zn concentration seems may enhance oxidative stress and promotes further oxidative injury. These adverse effects may be due to a weak anti-oxidative ability. As a result, further deleterious lipid peroxidation effect and oxidative cellular injury occur, and our experimental result was in agreement with the previous research.

PQ herbicide is commonly used in agriculture and the public environment due to its enormous benefits such as fast action, cheaper price, as well as rapid deactivation once contacts with soils [1-2]. It has been documented that PQ poisoning may generate ROS including superoxide radicals, hydrogen peroxide, as well as hydroxyl radical [2-3]. In vivo study has pointed out that erythrocyte is the most sensitive cell in the bloodstream after PQ ingestion [3]. PQ intoxication not only can interact with the membrane lipid of poly-unsaturated fatty acid but also can promote deleteriously lipid peroxidation effect [4]. Our experimental observations demonstrate that the MDA was positively increased in parallel with the PQ-administered dosage as showed in Figure 2, and the trend of our present finding was consistent with the former investigation.

Normally, a balance between oxidants and anti-oxidants is strictly controlled within the cells. As mentioned previously, oxidative stress is also realized as an imbalance between the intensity of ROS and its elimination. In other words, proper antioxidant activity is beneficial for the cells in scavenging toxic ROS. There are three major antioxidant enzymes which are existed in erythrocytes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [15-17]. The SOD enzyme catalyzes the toxic superoxide radicals into the oxygen and the hydrogen peroxide [16-17]. CAT is found abundantly in erythrocytes and it can convert hydrogen peroxide into oxygen and water [18]. The GPX enzyme converts the toxic hydrogen peroxide into the non-toxic water and oxygen [19]. The previous study has pointed out that erythrocytes exposed to the PQ can generate toxic free radicals and contributing lipid peroxidation as presented by an elevated MDA level [20]. Prominently, our present data showed that the activities of these three antioxidants were opposite to the PQ-administrated dosage as listed in Table 1. Former in vivo study has proposed that cells expose to higher PQ dosage significantly inhibited the SOD activity [21]. Meanwhile, administration of mice with PQ results in a decreased SOD activity [22-23]. Therefore, it seems reasonable to state our present finding here that PQ exposure can inhibit SOD activity. In vivo investigation has indicated that CAT enzyme is mainly existed in erythrocytes and its activity can be inhibited by PQ [24]. Our experimental results suggested that a negative correlation was seen between the CAT activity and PQ-treated dosage. Similar to the patterns of SOD and CAT, the GPX activity in erythrocytes was opposite to the PQ-treated dosage. Previous studies have suggested that PQ exposure may result in a decreased GPX activity and this effect is due to the enzyme inactivation [25-27]. Accordingly, our experimental observations suggest the trend that PQ exposure may exactly inhibit these three enzyme activity in a dose-dependent phenomenon.

Conclusions

Our present investigation suggests that proper zinc concentration and antioxidant activity are important in preventing erythrocytes from further oxidative attack and injury. Conversely, perturbation of which levels is thinkable to be enhanced the oxidative cellular injuryin erythrocytes. Based on our present findings, it seems possible to note here that attenuated zinc concentration together with declined antioxidant enzyme activity in erythrocytes, at least in part, may be correlated with paraquat toxicity.

References

[1]. Khalighi Z, Rahmani A, Cheraghi J, Ahmadi MR, Soleimannejad K, Asadollahi R, Asadollahi K (2016) Perfluorocarbon attenuates inflammatory cytokines, oxidative stress and histopathologic changes in paraquat-induced acute lung injury in rats. Environ Toxicol Pharmacol 42:9-15.

[2]. Tomita M, Katsuyama H, Okuyama T, Hidaka K, Minatogawa Y (2005) Changes in gene expression level for defense system enzymes against oxidative stress and glutathione level in rat administered paraquat. Int J Mol Med 15(4):689-693.

[3]. Gabryelak T, Klekot J. (1985). The effect of paraquat on the peroxide metabolism enzymes in erythrocytes of freshwater fish species. Comp Biochem Physiol C. 81(2):415-418.

[4]. Luan RL, Meng XX, Jiang W (2016) Protective effects of apigenin against paraquat-induced acute lung injury in mice. Inflammation 39(2):752-758.
[5]. Chen D, Ma T, Liu XW, Yang C, Liu Z (2015) Rapamycin reverses paraquat-induced acute lung injury in a rat model through inhibition of NFκB activation. Int J ClinExpPathol 8(5):4627-4638.
[6]. Medina-Leendertz S, Paz M, Mora M, Bonilla E, Bravo Y, Arcaya JL, Terán R, Villalobos V (2014) Long-term melatonin administration alleviates paraquat mediated oxidative stress in Drosophila melanogaster. Invest Clin 55(4):352-364.
[7]. Minakata K, Suzuki O, Saito S, Kawai K, Horio F (2002) Effects of paraquat on essential antioxidant elements in osteogenic disorder Shionogi rat. J Toxicol Environ Health A 65(2):143-147.
[8]. Xiao ZW, Zhang W, Ma L, Qiu ZW (2014) Therapeutic effect of magnesium isoglycyrrhizinate in rats on lung injury induced by paraquat poisoning. Eur Rev Med PharmacolSci 18(3):311-320.
[9]. Hosamani R, Muralidhara (2013) Acute exposure of Drosophila melanogaster to paraquat causes oxidative stress and mitochondrial dysfunction. Arch Insect BiochemPhysiol 83(1):25-40.
[10]. Powell SR (2000) The antioxidant properties of zinc. *J Nutri* 130(5): 1447S–1454S.
[11]. Fang KM, Cheng FC, Huang YL, Chung SY, Jian ZY, Lin MC (2013) Trace element, antioxidant activity, and lipid peroxidation levels in brain cortex of gerbils after cerebral ischemic injury. Biol Trace Elem Res 152(1):66-74.
[12]. Razavi SM, Nazifi S, Bateni M, Rakhshandehroo E (2011) Alterations of erythrocyte antioxidant mechanisms: antioxidant enzymes, lipid peroxidation and serum trace elements associated with anemia in bovine tropical theileriosis. Vet Parasitol 180(3-4):209-214.
[13]. Golasik M, Przybyłowicz A, Woźniak A, Herman M, Gawęcki W, Golusiński W, Walas S, Krejpcio Z, Szyfter K, Florek E, Piekoszewski W (2015) Essential metals profile of the hair and nails of patients with laryngeal cancer. J Trace Elem Med Biol 31:67-73.
[14]. Sengupta S, Wroblewski K, Aronsohn A, Reau N, Reddy KG, Jensen D, Te H (2015) Screening for Zinc Deficiency in Patients with Cirrhosis: When Should We Start? Dig Dis Sci 60(10):3130-3135.
[15]. Saboori S, Koohdani F, Nematipour E, Yousefi Rad E, Saboor-Yaraghi AA, Javanbakht MH, Eshraghian MR, Ramezani A, Djalali M (2016) Beneficial effects of omega-3 and vitamin E coadministration on gene expression of SIRT1 and PGC1α and serum antioxidant enzymes in patients with coronary artery disease. NutrMetabCardiovasc Dis 26(6):489-494.
[16]. Adewoye EO, Adele BO (2015) Effect of methanol extract of musasapientum leaves on protein glycation and erythrocyte antioxidant status in alloxan-induced diabetic wistar rats. Afr J Med Med Sci 44(3):261-268.
[17]. Oguz O zturk, Saadet Gumus, lu (2004) Changes in glucose-6-phosphate dehydrogenase, copper, zinc-superoxidedismutase and catalase activities, glutathione and its metabolizing enzymes, and lipid peroxidation in rat erythrocytes with age. Exp Gerontology 39: 211–216.
[18]. Carocho M, Ferreira I (2013) A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology51:15-25.
[19]. Kielar F, Helsel M.E, Wang Q, Franz K.J (2012) Prochelator BHAPI protects cells against paraquat-induced damage by ROS-triggered iron chelation. Metallomics 4(9):899-909.
[20]. Pezzoli G, Cereda E (2013) Exposure to pesticides or solvents and risk of Parkinson disease. Neurology 80(22):2035-2041.
[21]. Novaes RD, Gonçalves RV, Cupertino MC, Santos EC, Bigonha SM, Fernandes GJ, Maldonado IR, Natali AJ (2016) Acute paraquat exposure determines dose-dependent oxidative injury of multiple organs and metabolic dysfunction in rats: impact on exercise tolerance. Int J ExpPathol.
[22]. Moses K, Pepple D, Singh P (2015) The Protective Effect of Epigallocatechin-3-gallate on Paraquat-induced Haemolysis. West Indian Med J 64(3):186-188.
[23]. Meitern R, Sild E, Kilk K, Porosk R, Hõrak P (2013) On the methodological limitations of detecting

oxidative stress: effects of paraquat on measures of oxidative status in greenfinches. J ExpBiol 216(Pt 14):2713-2721.

[24]. Li D, Wang W, Shan YJ (2012) Synergy between sulforaphane and selenium in the up-regulation of thioredoxinreductase and protection against hydrogen peroxide-induced cell death in human hepatocytes. Food Chem13:300-307.

[25]. Ray S, Sengupta A, Ray A. (2007) Effects of paraquat on anti-oxidant system in rats. Indian J ExpBiol 45(5):432-438.

[26]. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2006) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Bio 39(1): 44-84.

[27]. Jiasi Li, Wenwu Liu, Suju Ding, Weigang Xu, Yangtai G, John H, Zhang, Xuejun Sun (2008) Hyperbaric oxygen preconditioning induces tolerance against brain ischemia–reperfusion injury by up-regulation of antioxidant enzymes in rats. Brain Res 121019):223-229.