ORIGINAL ARTICLE

Arch AHS

Archives of Allied Health Sciences 2023; 35(3): 39-47.

Effect of paraquat on red blood cell oxidative stress in individuals with hemoglobin E trait and G6PD deficiency

Nattaphol Prakobkaew[1], Natthapak Sillawatthumrong[2], Supaporn Khamchan[2,3], Surachat Buddhisa[1,3*]

[1] Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand.
[2] Department of Medical Technology, School of Allied Health Sciences, University of Phayao, Phayao, Thailand.
[3] Unit of Excellence in Cellular and Molecular Immunodiagnosis and Therapy, School of Allied Health Sciences, University of Phayao, Phayao, Thailand.

KEYWORDS
Paraquat;
Oxidative stress;
G6PD deficiency;
Hemoglobin E trait.

ABSTRACT
Paraquat is one of the most widely used herbicides globally and is a potent inducer of cellular reactive oxygen species, which cause damage to cells and organs in humans and animals. Recently, paraquat was found to affect normal human and animal red blood cells (RBCs). However, the effects of paraquat on hemoglobin E (Hb E) and glucose-6-phosphate dehydrogenase (G6PD) deficiency, commonly observed in Thailand, are unknown. This study investigated the effects of paraquat on oxidative stress and osmotic fragility in RBCs of individuals with Hb E trait and G6PD deficiency *in vitro*. The RBCs of individuals with Hb E trait and G6PD deficiency and normal controls were exposed to pure or commercially available paraquat dichloride; malondialdehyde (MDA) levels and the osmotic fragility were evaluated after coculture for 24 hours. In all groups, RBCs exposed to paraquat had significantly increased MDA levels compared with those exposed to saline solution (controls). The MDA level of RBCs from individuals with Hb E trait was significantly higher than that of RBCs from normal controls. Furthermore, the initial hemolysis of saline- and paraquat-treated RBCs from individuals with Hb E trait and G6PD deficiency was significantly higher than that of RBCs from normal controls. These data suggest that paraquat induces increased lipid peroxidation in RBCs of individuals with Hb E trait and G6PD deficiency and that RBCs of individuals with Hb E trait and G6PD are slightly more fragile than RBCs from normal individuals; however, paraquat does not affect the osmotic fragility of RBCs.

***Corresponding author:** Surachat Buddhisa, MT, PhD. Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand.
Email address: surachat.bu@buu.ac.th
Received: 3 August 2023/ Revised: 19 September 2023/ Accepted: 23 October 2023

Introduction

Paraquat is a toxic chemical widely used in agriculture as an herbicide for weed and grass control. Extensive paraquat applications and long-term exposure lead to widespread residues in soil, aquatic environments[1], plants, and food grains[2]. Paraquat has been found in both surface water and groundwater; in such environments, it is adsorbed onto particles and sediment and has a half-life between 2 and 820 years[3]. Paraquat has a strong affinity for soil particles and tends to remain in the soil for up to 20 years[4]. Moreover, it can be absorbed from the soil and transferred to agricultural products[2,4] that ultimately enter the food chain[1]. Paraquat can harm the neurological system and lead to dysfunction in the kidneys, liver, heart, lungs, and hematological system in humans and animals through the extensive generation of reactive oxygen species (ROS). Recently, studies in mice have shown that paraquat can induce transient anemia resulting from ROS production, which randomly eliminates young and mature circulating red blood cells (RBCs) and depresses erythropoietic activity in the bone marrow[5-9].

Hemoglobin E (Hb E) trait and glucose-6-phosphate dehydrogenase (G6PD) deficiency are common hemoglobinopathy and enzymopathy in countries in Southeast Asia, including Thailand[10]. Individuals with Hb E trait have higher antioxidant status because of a compensatory antioxidant response brought on by an excessive amount of oxidative stress, which lowers their antioxidant capacity[11]. G6PD deficiency is a human enzymopathy caused by inherited mutations of the X-linked gene *G6PD* that affect the ability of RBCs to respond to oxidative stress, thus making them susceptible to hemolysis. Previous studies have demonstrated that RBCs from individuals with Hb E and G6PD deficiency exhibit increased lipid peroxidation, increased activity of superoxide dismutase and decreased thiol glutathione (GSH) activity at baseline, and red blood cell defects may render RBC more susceptible to oxidative stress[12-14]. Paraquat was expected to increase RBC lipid peroxidation, which would lead to more rigid *RBCs in* individuals with Hb E and G6PD deficiency. In this study, we investigated the effects of paraquat on oxidative stress and osmotic fragility in RBCs of individuals with Hb E trait and G6PD deficiency subjects *in vitro*. Our findings could be useful in educating the public on the possible effects of this herbicide on human health, thereby reducing the negative effects of paraquat.

Materials and methods

Human subjects and blood samples

This study was approved by the Burapha University Ethics Committee for Human Research (Sci 046/2562). All participants provided informed consent. A total of 18 participants-10 healthy individuals, four individuals with G6PD deficiency, and four individuals with Hb E trait-were enrolled in this study. Individuals with underlying diseases, anemia, or taking antioxidant supplements including vitamin C, glutathione, and coenzyme Q10 were excluded. Whole blood (6 mL) was collected; 3 mL was added to lithium-heparin tubes and 3 mL was added to ethylenediamine-tetraacetic acid (EDTA) tubes. EDTA blood samples were analyzed for complete blood count (CBC) by an automated hematology analyzer (Mindray BC-5800, Shenzhen, China); screened for thalassemia with one-tube osmotic fragility (OF) and dichlorophenol-indophenol precipitation (DCIP) tests; Hb typing was performed by cellulose acetate electrophoresis, and G6PD deficiency was assessed with fluorescent spot (FST) testing.

Thalassemia screening and hemoglobin typing

Screening for α-thalassemia, β-thalassemia, and Hb E was carried out with the KKU-OF and KKU-DCIP reagent kits (PCL Holding, Thailand) according to the manufacturer's instructions as described previously[15]. Both tests are interpreted with a negative and a positive control. The Hb types were determined by cellulose acetate electrophoresis (Helena Laboratories, Beaumont, TX, USA). A_2A type Hb with a normal A_2 level (< 3.5%) was used for comparative interpretation. The samples were classified as normal (negative for KKU-OF and KKU-DCIP, A_2A pattern Hb typing) or Hb E trait (negative for KKU-OF, positive for KKU-DCIP, and EA pattern Hb typing).

Fluorescent spot test (FST) for screening of G6PD deficiency

A commercial FST kit (Trinity Biotech) was used to test for G6PD deficiency. The FST test was performed according to the manufacturer's instructions, as described elsewhere[16]. The samples were divided into three groups: normal (strong fluorescence at 5 and 10 minutes); intermediate (poor fluorescence at 5 minutes and moderate fluorescence at 10 minutes); and deficient (no fluorescence at 5 or 10 minutes).

Red blood cell culture

Lithium-heparinized blood samples were centrifuged at 3,000 rpm for 5 minutes. The plasma and buffy coat were removed, and the samples were washed twice with 0.85% saline solution; subsequently, 1 mL of packed RBCs was resuspended in 2 mL of 0.85% saline solution. The RBC count was determined by an automated hematology analyzer. RBCs (1×10^9/mL) were exposed to either 0.85% saline solution as a negative control or paraquat dichloride, either pure (Sigma-Aldrich) or commercially available, at final concentrations of 100 and 200 ug/mL for 24 hours at 37 °C in 5% CO_2.

Determination of malondialdehyde level by thiobarbituric acid reactive substances assay

The MDA levels were measured by using thiobarbituric acid reactive substances (TBARS) assay modified from Klarod et al[17]. A 25 μL of 0.1 mM butylated hydroxyl toluene (Sigma-Aldrich), 250 μL of 5 mM ethylenediamine tetraacetic acid (Sigma-Aldrich), 500 μL of 8.1% (w/v) sodium dodecyl sulfate (Sigma-Aldrich), 500 μL of 10% (w/v) trichloroacetic acid, and 750 μL of 0.67% (w/v) thiobarbituric acid were added to 500 μL of culture supernatant and standard malondialdehyde (MDA) solution. The mixture was heated in a boiling water bath at 95 °C for 30 minutes before being placed in water for 10 minutes. After cooling, the mixture was centrifuged at 3,000 rpm for 15 minutes, and the supernatant was transferred to a flat-bottom plate. The absorbance was measured at 532 nm with microplate reader (Molecular Devices, CA, USA)[17]. Each standard MDA solution and sample was tested in duplicate, and if the measurement values differed by more than 10%, the test was repeated. MDA standard solution was prepared using 1,1,3,3 tetramethoxypropane (Sigma-Aldrich) as the standard. The results were expressed as nM MDA.

Determination of erythrocyte osmotic fragility

RBCs (1×10^9/mL) from whole blood in lithium-heparin were treated with either a 0.85% saline solution or commercially available paraquat (cPQ) at a final concentration of 200 ug/mL at 37 °C in 5% CO_2 for 24 hours. Erythrocyte osmotic fragility (EOF) was assessed by adding 20 μL of treated RBCs to a set of test tubes containing 1.00%, 0.85%, 0.75%, 0.60%, 0.55%, 0.50%, 0.45%, 0.40%, 0.35%, 0.30%, 0.20%, 0.10%, and 0% NaCl (w/v). After 30 minutes of incubation at room temperature, the mixture was centrifuged at 3,000 rpm for 10 minutes. Each sample was tested in duplicate, and the supernatant was transferred to a spectrophotometer (Eppendorf, MA, USA) for absorbance measurement at 540 nm using 1% NaCl as the blank. The percentage of hemolysis was calculated according to the following formula:

Hemolysis (%): = (OD of test/OD of 0% NaCl) × 100

The effect of commercial paraquat on the osmotic fragility of RBCs in individuals with Hb E trait and G6PD deficiency was assessed as 50% hemolysis or median corpuscular fragility (MCF), respectively. The MCF of normal incubated RBCs ranges from 0.46% to 0.59% NaCl[18,19].

Statistical analysis

The results are presented as the mean ± standard error of the mean (SEM) or the median with range after the normality of data was tested using the Shapiro-Wilk normality test. Statistical analysis was performed with tests appropriate to the dataset, as specified in the figure legends, using GraphPad Prism 10 software (GraphPad, San Diego, CA, USA). A *p*-value of < 0.05 was considered statistically significant.

Results

Screening test for thalassemia, Hb E, and G6PD deficiency

All 18 individuals were screened for thalassemia, Hb E trait, and G6PD deficiency. The results showed that the 10 normal controls were negative for thalassemia according to the OF and DCIP tests, had normal fluorescent spot test results, and their Hb type was A_2A (normal). The four participants with Hb E trait had positive DCIP test results and an EA Hb pattern, whereas they had negative OF test results and normal fluorescent spot test results. The four participants had G6PD deficiency, negative for thalassemia screening and normal Hb type results.

Paraquat induced red blood cell oxidative stress in a concentration-dependent manner

To examine the effect of paraquat on oxidative stress in RBCs, normal RBCs were cocultured with pure (pPQ) and commercially available paraquat (cPQ) at different concentrations for 24 hours. The addition of 200 µg/mL pure paraquat and 100 and 200 µg/mL commercially available paraquat significantly increased the MDA level compared with normal saline in a dose-dependent manner (Figure 1). Moreover, the MDA level in RBCs exposed to 200 µg/mL commercially available paraquat was significantly higher than that of RBCs exposed to other paraquat concentrations (Figure1). These data indicate that paraquat affected RBC lipid membrane peroxidation by inducing oxidative stress.

Increased oxidative stress in red blood cells from participants with Hb E trait and G6PD deficiency

Individuals with Hb E trait and G6PD deficiency are susceptible to oxidative stress; therefore, we investigated the effect of paraquat on oxidative stress in RBCs from participants with Hb E trait and G6PD deficiency. The MDA levels of commercially available paraquat-exposed RBCs from all participants (normal controls and participants with Hb E trait and G6PD deficiency) were significantly higher than the MDA levels of saline-exposed RBCs (Figure 2). Interestingly, the MDA levels of commercially available paraquat-exposed RBCs from participants with Hb E trait were significantly higher than those of RBCs from normal controls, but they were not significantly different from MDA levels of RBCs from participants with G6PD deficiency (Figure 2). These data suggest that RBCs from individuals with Hb E trait are susceptible to paraquat-induced oxidative stress.

Effect of paraquat on erythrocyte osmotic fragility (EOF)

Extensive lipid peroxidation in biological membranes causes fluidity loss, decreased membrane potential, increased permeability to ions, and increased rigidity. To test whether the increase in lipid peroxidation after exposure to paraquat affected RBC membrane rigidity, the EOF of commercially available paraquat-treated RBCs was tested. The results demonstrated that the initial hemolysis of saline- and commercially available paraquat-treated RBCs from individuals with Hb E trait and G6PD deficiency was significantly higher compared with those of normal controls. By contrast, the initial hemolysis (Figure 3A–B) and MCF (Figure 3C) of commercially available paraquat-treated RBCs were not significantly different from those of RBCs from normal controls in all groups. The findings showed that RBCs from individuals with Hb E trait and G6PD deficiency were slightly more fragile than those from normal controls, but paraquat did not affect RBC osmotic fragility.

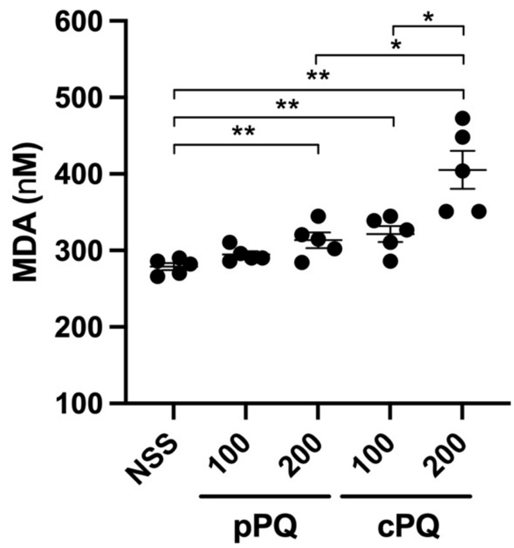

Figure 1 Paraquat induced oxidative stress in RBCs in a concentration-dependent manner.

Note: RBCs of normal controls (n = 5 individuals) were treated with pure paraquat (pPQ) and commercially available paraquat (cPQ). Cell-free supernatants were collected at 24 h and assayed for MDA level by TBARS assay. The results are expressed as mean with standard error of the mean. Statistical significance was determined using paired *t*-test ($^{*}p$-value < 0.05, $^{**}p$-value < 0.01).

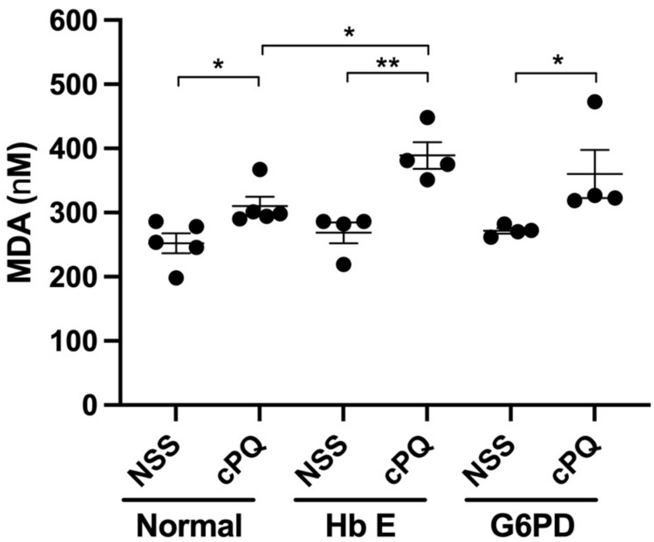

Figure 2 Effect of paraquat on RBC oxidative stress in individuals with Hb E trait and G6PD deficiency.

Note: RBCs of normal controls (n = 5 individuals), individuals with Hb E trait (n = 4 individuals), and individuals with G6PD deficiency (n = 4 individuals) were exposed to 200 µg/mL commercially available paraquat (cPQ) for 24 hours. The results are expressed as median with range. Statistical analysis was performed using the Wilcoxon test or Mann–Whitney U test ($^{*}p$-value < 0.05, $^{**}p$-value < 0.01).

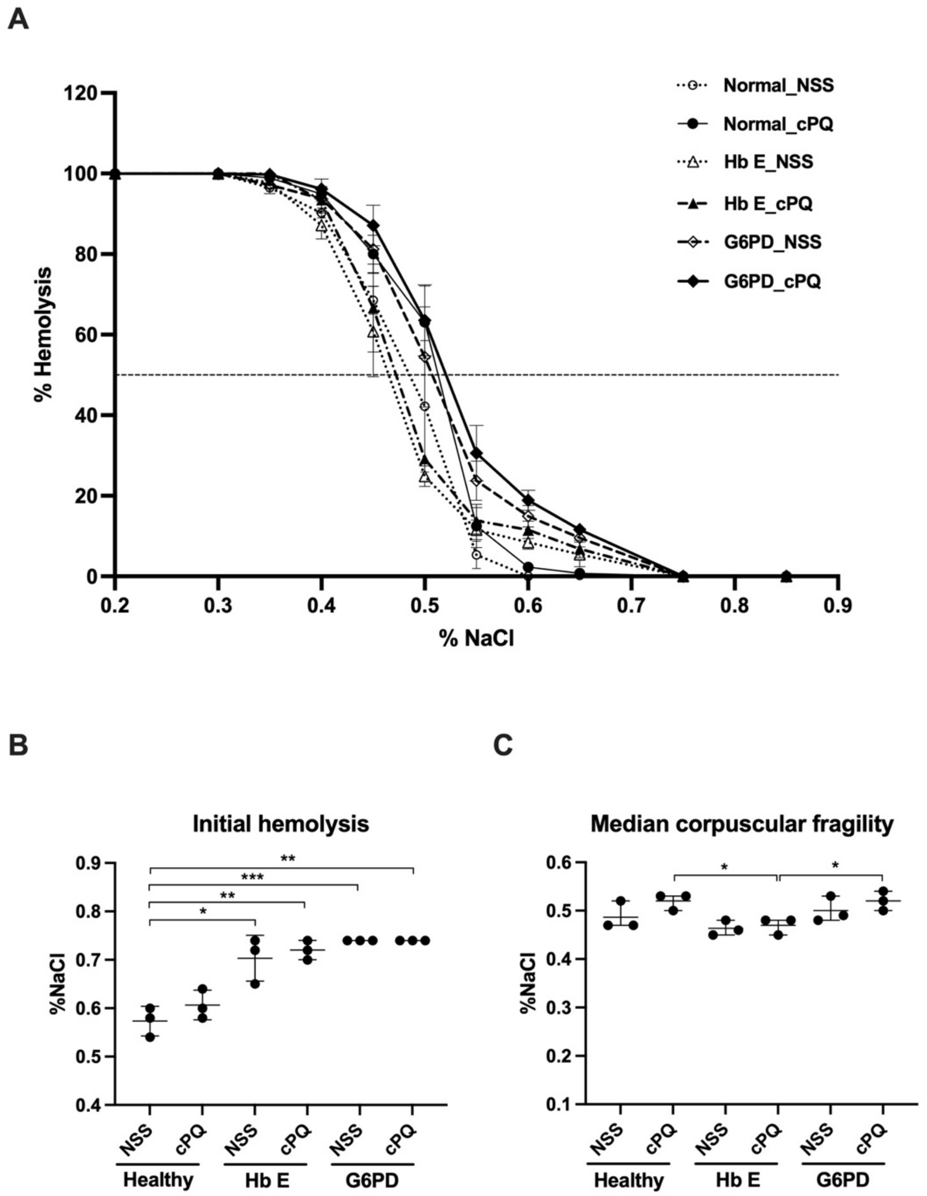

Figure 3 The effect of paraquat on RBC osmotic fragility in individuals with Hb E trait and G6PD deficiency.

Note: RBCs of normal controls and individuals with Hb E trait and G6PD deficiency (n = 3 individuals/group) were exposed to 200 µg/mL commercially available paraquat (cPQ) for 24 hours. A) Erythrocyte osmotic fragility test; B) initial hemolysis; C) median corpuscular fragility. The data are expressed as median with range. Statistical analysis was performed using the Wilcoxon test or Mann–Whitney U test (**p*-value < 0.05, ***p*-value < 0.01, ****p*-value < 0.005).

Discussion

Paraquat is a potent inducer of intracellular ROS[20-22], which impairs tissue and cell function by inducing lipid peroxidation, protein damage, and DNA breakage[9]. Paraquat induced-oxidative stress affects erythropoiesis in the bone marrow and mature RBCs in circulation, resulting in anemia[7,9]. The present study investigated the effect of paraquat on the lipid peroxidation of the RBCs. The results showed that paraquat significantly increased MDA levels in RBCs after 24 hours in a concentration-dependent manner; similar results have been reported in macrophages and peripheral mononuclear cells[23,24]. Additionally, a previous investigation found that erythrocytes treated with paraquat had higher levels of ROS and lower levels of the superoxide dismutase (SOD) enzyme[9]. Erythrocytes may be more susceptible to ROS and lipid peroxidation because of the decreased SOD levels.

Hemoglobinopathy and G6PD deficiency are common in Thailand. Excessively oxidized globin chains in the RBCs of patients with hemoglobinopathy release free iron, leading to the formation of ROS[25]. In a previous study, higher levels of free radicals resulted in decreased glutathione (GSH) levels and increased lipid peroxidation. Moreover, an ROS-induced decrease in Flippase activity resulted in an increase in phosphatidylserine outer membrane ratios, leading to extravascular macrophage destruction in RBCs[26]. Increased ROS levels cause the oxidation of hemoglobin, lipids, and thiol groups in cytoplasmic membrane proteins. Clusters of oxidized band 3 in the membrane bind immunoglobulin G and complement, causing them to become opsonized and undergo erythrophagocytosis[27]. Intravascular hemolysis occurs in RBCs with more severe damage. RBCs from patients with inherited hemolytic anemia are susceptible to damage from oxidative stress. Our results demonstrated that paraquat was associated with significantly higher levels of lipid peroxidation in RBCs from participants with Hb E trait compared with those from normal individuals. The significantly higher MDA level in Hb E trait RBCs was due to hemoglobinopathy; higher levels of free radicals resulted in decreased GSH levels along with increased lipid peroxidation at baseline. Although MDA levels were higher in participants with G6PD deficiency, the difference was not statistically significant because of the small number of individuals who were G6PD deficient.

The effect of paraquat on RBCs was further investigated with the EOF test. RBCs with damaged membranes had a decreased ability to take in water, leading to hemolysis[18]. The plasma membranes in RBCs are mainly affected by elevated ROS, which cause a variety of problems in the membrane, including lipid peroxidation, protein crosslinking, and protein thiol oxidation[28]. All RBCs treated with paraquat exhibited an increase in osmotic fragility. Paraquat-induced ROS could trigger membrane damage and affect hemolysis resistance at high saline solution concentrations. In individuals with G6PD deficiency, the osmotic fragility curve was shifted to the right. RBCs from patients with G6PD deficiency are unable to produce enough GSH to counteract oxidative stress, which increases membrane damage and causes increased osmotic fragility in G6PD-deficient RBCs compared with RBCs from normal individuals. On the other hand, the osmotic fragility curve for Hb E trait was shifted to the left because RBCs from individuals with Hb E trait have slightly lower MCH levels and a higher surface area to volume ratio[15], they are resistant to osmotic lysis. Therefore, MDA levels and the EOF test results indicated that paraquat induced ROS, which damaged RBC membranes and increased lipid peroxidation. The inability of RBCs from individuals with Hb E trait and G6PD deficiency to respond to oxidative stress results in damage to the RBC membrane and more hemolysis compared with RBCs from normal individuals.

Conclusion

According to the findings of this study, paraquat has a diverse effect on RBCs. Commercially available paraquat concentration of 200 µg/mL induces increased lipid peroxidation in RBCs of individuals with Hb E trait and G6PD deficiency and RBCs of individuals with Hb E trait and G6PD are slightly more fragile than RBCs from normal individuals.

Take home messages

Paraquat induced significantly more oxidative stress and initial hemolysis in RBCs from Hb E trait and G6PD deficiency than normal individuals. This indicates that paraquat residue in food and the environment may damage the RBC membrane and lead to more hemolysis in Hb E trait and G6PD deficiency than in normal individuals.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This research project was supported by the Thailand Science Research and Innovation Fund and the University of Phayao (Grant No. FF65-UoE011). The Faculty of Allied Health Sciences, Burapha University. The researchers also would like to sincerely thank all subjects for their kind cooperation.

References

1. Pateiro-Moure M, Nóvoa-Muñoz JC, Arias-Estévez M, López-Periago E, Martínez-Carballo E, Simal-Gándara J. Quaternary herbicides retention by the amendment of acid soils with a bentonite-based waste from wineries. J Hazard Mater 2009; 164(2-3): 769-75.
2. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, et al. Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci 2019; 1(11): 1-16.
3. Watts M. Paraquat. [online] 2011 [cited 2023 Jul 27]. Available from: http://wssroc.agron.ntu.edu.tw/note/Paraquat.pdf
4. Gupta S, Garg NK, Shekhawat K. Regulation of Paraquat for wheat crop contamination. Environ Sci Pollut Res 2022; 29(47): 70909-20.
5. Laohaudomchok W, Nankongnab N, Siriruttanapruk S, Klaimala P, Lianchamroon W, Ousap P, et al. Pesticide use in Thailand: Current situation, health risks, and gaps in research and policy. Hum Ecol Risk Assess 2021; 27(5): 1147-69.
6. Prihartono N, Kriebel D, Woskie S, Thetkhathuek A, Sripaung N, Padungtod C, et al. Risk of aplastic anemia and pesticide and other chemical exposures. Asia-Pacific J Public Heal 2011; 23(3): 369-77.
7. Bhardwaj N, Singh A. Paraquat treatment modulates integrin associated protein (CD47) and basigin (CD147) expression and mitochondrial potential on erythroid cells in mice. Environ Toxicol Pharmacol 2018; 58: 37-44.
8. Ciccoli L, De Felice C, Paccagnini E, Leoncini S, Pecorelli A, Signorini C, et al. Erythrocyte shape abnormalities, membrane oxidative damage, and ß-actin alterations: An unrecognized triad in classical autism. Mediators Inflamm 2013; 2013: 432616
9. Bhardwaj N, Saxena RK. Elimination of young erythrocytes from blood circulation and altered erythropoietic patterns during paraquat induced anemic phase in mice. PLoS One 2014; 9(6): e99364.
10. Kittisares K, Palasuwan D, Noulsri E, Palasuwan A. Thalassemia trait and G6PD deficiency in Thai blood donors. Transfus Apher Sci 2019; 58(2): 201-6.
11. Nanakorn N, Chuechan S. Impaired oxidative stress in Thalassemia-Hemoglobin E traits after acute exhaustive exercise. Sport Sci Health 2022; 18(3): 789-97.

12. Palasuwan A, Soogarun S, Wiwanitkit V, Luechapudiporn R, Pradniwat P, Lertlum T. Preliminary study of the effect of vitamin E supplementation on the antioxidant status of hemoglobin-E carriers. Southeast Asian J Trop Med Public Health 2006; 37: 184-9.
13. Palasuwan A, Soogarun S, Suksom D, Pitaksathienkul C, Rousseau AS. Antioxidant status in hemoglobin E carriers after acute and chronic strenuous exercises. Res Sport Med 2015; 23(4): 351-66.
14. Karadsheh NS, Quttaineh NA, Karadsheh SN, El-Khateeb M. Effect of combined G6PD deficiency and diabetes on protein oxidation and lipid peroxidation. BMC Endocr Disord 2021; 21: 246.
15. Fucharoen G, Sanchaisuriya K, Sae-Ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. Bull World Health Organ 2004; 82(5): 364-72.
16. Prakobkaew N, Buddhisa S. The prevalence of glucose-6-phosphate dehydrogenase deficiency in Trat province, eastern Thailand. Arch Allied Heal Sci 2022; 34(2): 35-43.
17. Klarod K, Singsanan S, Thamwiriyasati N, Ladawan S, Luangpon N, Boonsiri P, et al. Effects of Qigong exercise on muscle strengths and oxidative stress/antioxidant responses in young sedentary females: a quasi-experimental study. J Exerc Rehabil 2020; 16(5): 418-26.
18. Layton M, Roper D. Investigation of the Hereditary Haemolytic Anaemias: Membrane and Enzyme Abnormalities. 12th ed. Dacie and Lewis Practical Haematology. Elsevier Ltd.; 2017.
19. Jaroonwitchawan T, Chaicharoenaudomrung N, Namkaew J, Noisa P. Curcumin attenuates paraquat-induced cell death in human neuroblastoma cells through modulating oxidative stress and autophagy. Neurosci Lett 2017; 636: 40-7.
20. Drechsel DA, Patel M. Chapter 21 Paraquat-Induced Production of Reactive Oxygen Species in Brain Mitochondria. Methods in Enzymology. Methods Enzymol 2009; 456: 381-93.
21. Wang X, Luo F, Zhao H. Paraquat-induced reactive oxygen species inhibit neutrophil apoptosis via a p38 MAPK/NF-κB-IL-6/TNF-α positive-feedback circuit. PLoS One 2014; 9(4): 1-7.
22. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39(1): 44-84.
23. Kim H, Lee SW, Baek KM, Park JS, Min JH. Continuous hypoxia attenuates paraquat-induced cytotoxicity in the human A549 lung carcinoma cell line. Exp Mol Med 2011; 43(9): 494-500.
24. Huang J, Ning N, Zhang W. Effects of paraquat on IL-6 and TNF-α in macrophages. Exp Ther Med 2018; 1783-9.
25. Fibach E, Rachmilewitz E. The Role of Oxidative Stress in Hemolytic Anemia. Curr Mol Med 2008; 8(7): 609-19.
26. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front Physiol 2014; 5: 1-6.
27. Luzzatto L, Ally M, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. Blood 2021; 136(11): 1225-40.
28. Fibach E, Dana M. Oxidative Stress in β-Thalassemia. Mol Diagnosis Ther 2019; 23(2): 245-61.