Protective role of aqueous medicinal herbal extracts against oxidative stress on Glucose-6-phosphate dehydrogenase activity and red blood cells fragility

Wajdy Al-Awaida[1]*, Muhanad Akash[1,2] , Baker Jawabrah Al-Hourani[3]

[1]Department of Biology and Biotechnology, American University of Madaba, Madaba, Jordan; [2]On Sabbatical leave from Faculty of Agriculture, The University of Jordan, Amman, Jordan;[3]Department of Basic Sciences and Humanities, American University of Madaba, Madaba, Jordan
 w.alawaida@aum.edu.jo

Abstract: We investigated the protective effect of the aqueous extracts of *Salvia oficinalls, Thymus vulgaris* and *trigonella Foem–graecum* against oxidative stress on Glucose-6-phosphate dehydrogenase (G6PD) activity and red blood cells (RBC) hemolysis. The NO donor compounds nitrosocysteine and nitrosoarginine caused strong inhibition on G6PD activities. Those oxidative compounds promoted RBC hemolysis in parallel to their inhibition extents on G6PD activities. The complete protection of both G6PD activity and red blood cells hemolysis against oxidative stress induced by NO donor compounds was achieved by preincubation with the above mentioned aqueous extracts. Those extracts were able to protect hemolysis in parallel to their activation extents on G6PD activities. Compared to nitrosoarginine, nitrosocysteine showed more effect on both G6PD activity and RBC hemolysis. Thus, more aqueous extracts concentration is needed for protection against nitrosocysteine.

Keyword: *Salvia oficinalls; Thymus vulgaris; trigonella Foem–graecum extracts;* Glucose-6-phosphate dehydrogenase; Nitrosocysteine; Nitrosoarginine; aqueous extract.

1. Introduction:

Glucose-6-phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway, with main physiological function to produce NADPH and pentose sugars (**Sodeinde, 1992; Mehta, 1994; Prchal and Gregg, 2005).** G6PD is not the sole NADPH producing enzyme in nucleated cell, but in red blood cell only G6PD enzyme can generate NADPH **(Scriver, 1995).** The NADPH plays important role in erythrocytes, by preserving the integrity of red blood cell membrane sulfhydryl groups and detoxifies harmful hydrogen peroxide and oxygen radicals. Also it is used in the regeneration of reduced glutathione which prevents hemoglobin denaturation (**Ciftci et al., 2004; Abboud and Awaida, 2010)**.

Hemolysis is the destruction or removal of red blood cells from the circulation before the completion of their 120 days normal life span (**Beutler, 1959; Bowman et al., 2004)**. Decrease in G6PD enzyme activity leads to decrease in NADPH and reduced Glutathione. It is believed that a lack of reduced glu-tathione availability causes early hemolysis in the spleen (**Tian et al., 1999)**. G6PD deficient erythrocytes are sensitive to oxidative stress (**Gerli et al., 1982)**.

A deficiency in G6PD activity was connected with increases accumulation of cellular reactive oxygen species (ROS) while an overexpression of G6PD activity decreases the accumulation of these species in response to oxidative stress (**Leopold et al., 2003; Fico et al., 2004)**. Sodium nitroprusside, spermidine and nitroso derivatives are nitro compounds that act as oxidants (**Kuhn and Arthur., 1997; Gobble et al., 1997; Xian et al., 2000; Abboud and Awaida, 2010)**. Nitric oxide (NO) and its derivatives directly inactivate Glutathione peroxidase enzyme, resulting in an increase of intra-cellular peroxides that are responsible for cellular damage (**Asahi et al., 1997)**.

Herbs and spices have been widely used as food flavors for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical cures for delaying aging and biological tissue deterioration (**Frankel, 1996)**. *Salvia oficinalls* is an old medicinal plant, which is rich source of different chemical constituents including terpenoids, polyphenol as well as essential oils (**Wang et al., 2000; Bozin et al., 2007)**. *Salvia officinalis* extract is used to stabilize fat and fat-containing food against oxidation (**Schwarz and Ternes., 1992)**. The main antioxidant compounds in the sage are carnosic acid and carnosol rosmarinic acid (**Cuvelier et al., 1996)**.

Thymol and carvacrol were found to be the major components of *Thymus vulgaris* essential oil. However, the thymol showed more effectiveness than carvacrol in preventing oxidation during the autoxidaion of lipids (**Yanishlieva, 1999; El-**

Nekeety et al., 2011). Aqueous extract of thyme showed a degree of reductive and radical scavenging capacity **(Dorman et al, 2003)**. Fenugreek (*trigonella Foem–graecum*) being rich in phytochemical components have encouraged its use as medicinal plant (**Zaman et al 1998; Dixit et al, 2005**). Simultaneous administration of aqueous extract of fenugreek seeds with ethanol for 60 days in rats prevented the enzymatic leakage and rise in lipid peroxidation and enhanced antioxidant potential thus offering protection against ethanol toxicity **(Thirunavukkarasu et al., 2003)**. This work aims to investigate protective role of aqueous medicinal herbal extracts against oxidative stress on both Glucose-6-phosphate dehydrogenase activity and RBC fragility

2. Materials and methods

The protocol for this study was approved by the Ethics Committee of the science faculty of American University of Madaba (AUM) and all subjects gave their informed consent.

Preparation of human erythrocytes

Fresh heparinized blood samples from healthy subjects or patients were centrifuged under cooled conditions for 5 min at 1000 rpm. After removing plasma, the washed buffy coat pellet was resuspended in phosphate buffered saline (PBS) at pH 7.4 **(Masahiko et al., 2002)**.

Analysis of nitric Oxide (NO) donor compounds (Nitroso amino acids) induced hemolysis

The erythrocyte suspension was incubated with different concentration of the oxidizing agent for 60 min at 37 °C. The sample was then centrifuged at 1000 rpm for 10 min and the absorbance of the supernatant at 415 nm was measured spectrophotometrically. The extent of hemolysis was expressed as a percentage value relative to complete hemolysis of similar sample (blank) in deionized water. A control experiment was conducted under similar conditions without adding oxidizing agent.

Preparation of enzyme extract and determination of enzyme activity

Fresh unhemolyzed blood sample was collected and kept no longer than 7 days at 2–8 °C before being processed for G6PD extraction. A blood sample of 200 μL was washed 3 times with 2 mL aliquots of saline solution. The washed pellet was re-suspended in solution of digitonin (0.02%) and sodium azide (0.1% w\v) then allowed standing for 15 min at 2–8 °C. After centrifugation the supernatant was used as an enzyme preparation (enzyme extract) within 2 h of the extraction procedure. Activity of the enzyme G6PD was measured kinetically. An extracted fresh enzyme of 15 μL was added to a cuvette with 900 μL of 50 mM Tris buffer at pH 7.6 containing 1mM

$MgCl_2$ and a substrate glucose 6-phosphate at 500 μm concentration. The reaction was initiated by adding $NADP^+$ (250 μm) and the rate of increase in absorbency at 340 nm due to the conversion of NADP+ to NADPH was determined for a period of 2 min **(Tian et al., 1994)**.

Preparation of nitric Oxide (NO) donor compounds (Nitroso amino acids 10 mM):

The Nitroso amino acids (10 mM) were prepared freshly according to Hogg's method **(Hogg, 1999)**.

Preparation of aqueous medicinal herbal extracts:

Leaves from *Salvia officinalis*, *Thymus vulgaris* and seeds from Fenugreek (*trigonella Foem – graecum*) were harvested and dried in the dark at 35°C, before being stored at -20°C in paper bags until analysis. The aqueous extracts were prepared by boiling the dried plant material with 12 times the volume of water (wt/vol) for 1 h. After filtration and concentration of the supernatant under water suctioning, the sample was dried in oven at 50 °C **(Dragland et al., 2003).**

Antioxidants protection against the nitric Oxide (NO) donor compounds (Nitroso amino acids) caused inhibition of G6PD activity:

Aqueous extract with different concentrations (0.01%, 0.02%, 0.03%, 0.08%, 0.1%) were preincubated for 5 minutes with 15μl of G6PD enzyme before adding nitric Oxide (NO) donor compounds. Each measurement was repeated three times and the mean values were calculated.

Statistical analysis

Descriptive statistical analysis was performed with SPSS.

3. Results

Effects of nitric oxide (NO) donor compounds on G6PD activity:

The effects of NO donor compounds nitrosoarginine and nitrosocysteine on G6PD activity were investigated. Each oxidant was added to the enzyme assay at concentration range of 0.01-2 mM. Before testing nitroso derivatives, the effects of their corresponding amino acids (arginine and cysteine) on enzyme activity were examined. Neither cysteine nor arginine produced effects on G6PD activity (Figures 1). In contrast, the nitric oxide (NO) donor compounds nitrosoarginine and nitrosocysteine showed inhibition effects on G6PD activities (Figures 2). The calculation of I_{50} values (concentration of the compound that gives 50% inhibition in enzyme activity) showed 950 µm for nitrosoarginine and 300 µm for nitrosocysteine.
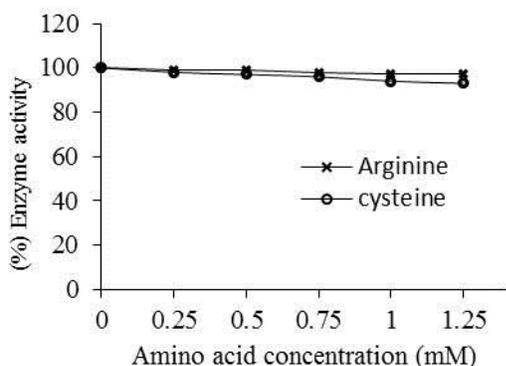
Figure 1: Effect of amino acids cysteine and arginine on G6PD enzyme activity. Different concentrations of amino acids cysteine and arginine were added directly to the assay G6PD enzyme. Using control experiments without amino acids (100% activity).
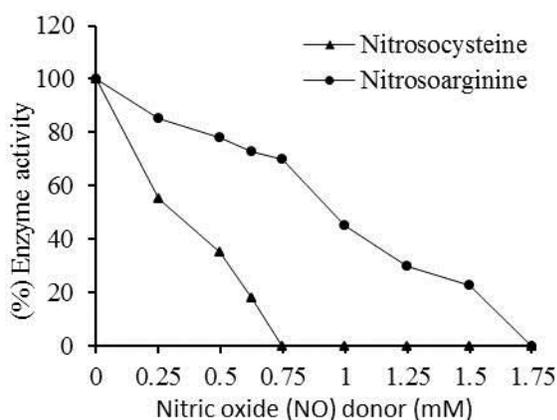
Figure 2: Effect of nitric oxide (NO) donors on G6PD activity. Different concentrations of nitrosoarginine and nitrosocysteine were added directly to the assay G6PD enzyme. Using control experiments without nitric oxide (NO) donors compounds (100% activity).

Effect of preincubation time on the reversibility of G6PD inhibition by nitric oxide (NO) donors compounds:

Nitric oxide (NO) donor compounds showed inhibition actions on G6PD activities. The objective was to investigate the reversibility of G6PD inhibition by these compounds after long preincubation time. Figure 3 shows that the G6PD inhibition by these compounds was not altered after long preincubation time. At the end of this period, the G6PD activity remained at a level of less than 60% from the control of enzyme activity without adding nitric oxide (NO) donor compounds.
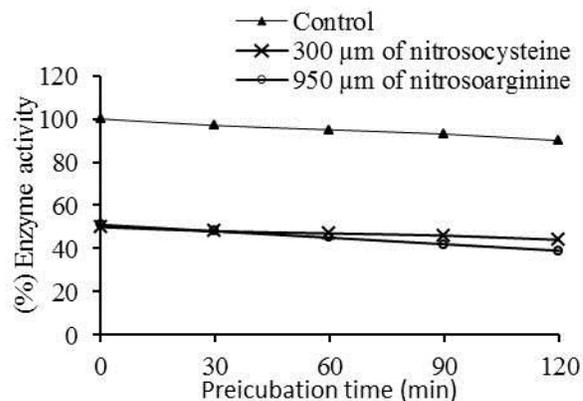
Figure 3: Effect of preincubation time on reversibility of G6PD inhibition by 950 µm nitrosoarginine and 300 µm nitrosocysteine. Using control experiments without nitric oxide (NO) donors compounds (100% activity).

Effects of nitric oxide (NO) donor compounds **on the fragility of red blood cells**

The effects of arginine, cysteine, nitrosoarginine and nitrosocysteine compounds on the fragility of red blood cells were investigated. Figure 4 indicates that the compounds arginine and cysteine did not have hemolysis effect on the fragility of red blood cells. In contrast, The NO donor compounds nitrosocysteine and nitrosoarginine induced concentration dependent red blood cells hemolysis (Figure 5). Control experiments in the absence of NO donor compounds indicated no hemolysis of RBC from either normal or G6PD deficient patients during the period of incubation. The calculation of I_{50} values (concentration of the compound that induces 50% hemolysis of RBC) showed 1100 µm for nitrosoarginine and 750 µm for nitrosocysteine
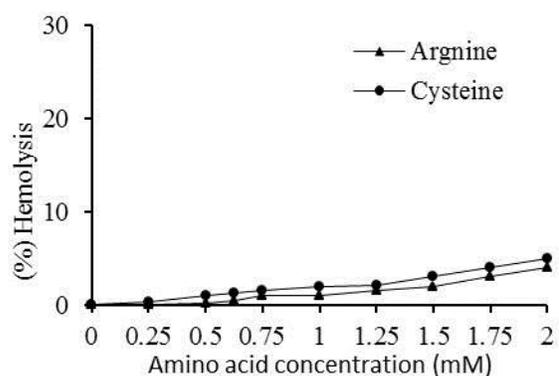
Figure 4: Effect of amino acids on the hemolysis of red blood cells. 10% RBC suspension was incubated for 60 min with different concentration of cysteine and arginine.
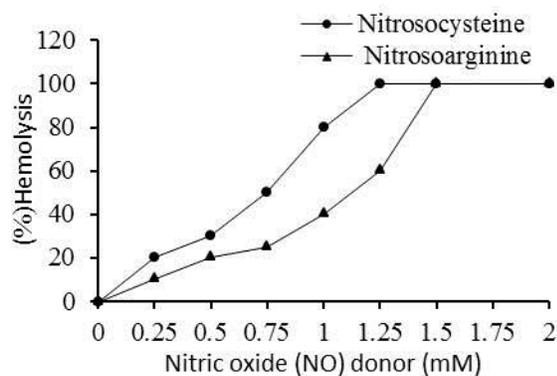
Figure 5: Hemolysis of RBC by (NO) donors: 10% RBC suspension was incubated for 60 min with different concentration of nitrosoarginine and nitrosocysteine. A control sample subjected to similar conditions but without (NO) donors showed insignificance hemolysis during the same period of incubation.

Effect of aqueous medicinal herbal extracts on G6PD activity completely inhibited by nitric oxide (NO) donors:

Figures 6 and 7 show the effect of preincubated G6PD enzyme with different aqueous herbal extract concentrations from *Salvia oficinalls, Thymus vulgaris* and *trigonella Foem–graecum* before testing the enzyme with the 1.75 mM of nitrosoarginine and 0.75 mM of nitrosocysteine, respectively. All above aqueous herbal extracts managed to produce 100% protection of the enzyme from the inhibition by nitrosoarginine and nitrosocysteine and sometimes led to activation of G6PD activity compared with the control. It should be noted that when the NO donors were preincubated first with the enzyme, no protection could be detected with aqueous herbal extracts.
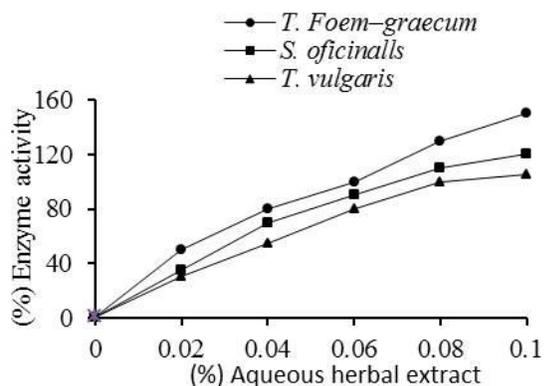
Figure 6: Protection of G6PD inhibition, each aqueous herbal extract was pre-incubated at different concentrations for 5 min with G6PD enzyme before adding the 1.75 mM of nitrosoarginine to inhibit G6PD activity (100%) as described in Fig. 2. When the NO donors were preincubated first with the enzyme, no protection could be detected with herbal extracts.
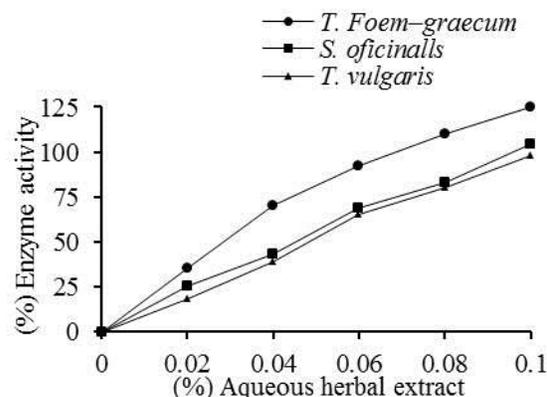
Figure 7: Protection of G6PD inhibition, each aqueous herbal extract was pre-incubated at different concentrations for 5 min with G6PD enzyme, before adding the 0.75 mM of nitrosocysteine to initiate 100% inhibition in G6PD activity. When the NO donors were preincubated first with the enzyme, no protection could be detected with herbal extracts and 0.75 mM of nitrosocysteine.

Protection of RBC hemolysis

The protection of RBC hemolysis was examined using different concentrations of aqueous herbal extract from *Salvia oficinalls, Thymus vulgaris* and *trigonella Foem–graecum*. Various concentrations of aqueous herbal extracts had neutral effects on RBC hemolysis (Figure 8). Furthermore, each aqueous herbal extract was preincubated with RBC for 5 min before the hemolysis was initiated by NO donors. Figure 9 and 10 show these aqueous herbal extracts were able to protect the RBC from hemolysis by nitrosoarginine and nitrosocysteine, respectively.
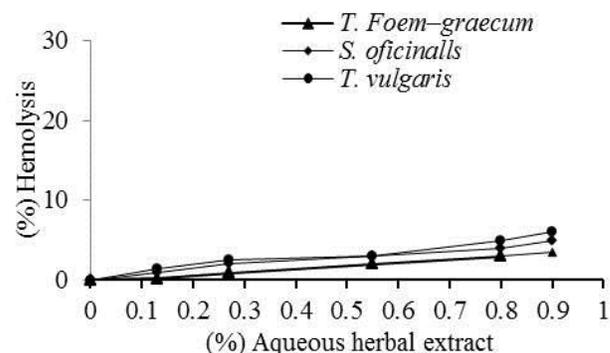
Figure 8: Effect of aqueous herbal extracts on the hemolysis of red blood cells. 10% RBC suspension was incubated for 60 min with different concentration of aqueous extracts.
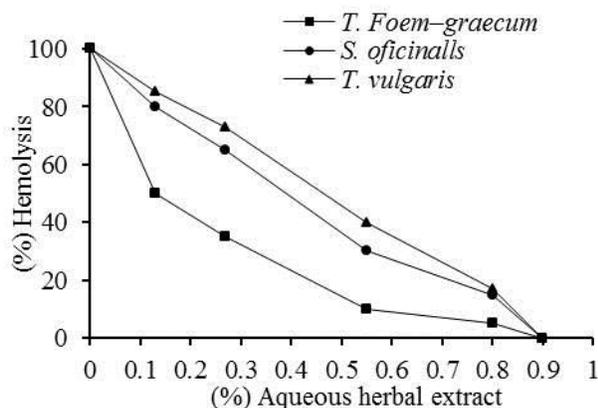
Figure 9: Protection of RBC hemolysis, each aqueous herbal extract was pre-incubated at different concentrations for 5 min with fresh RBC suspension, before adding 1.5 mM of nitrosoarginine to initiate complete hemolysis as described fig.5.
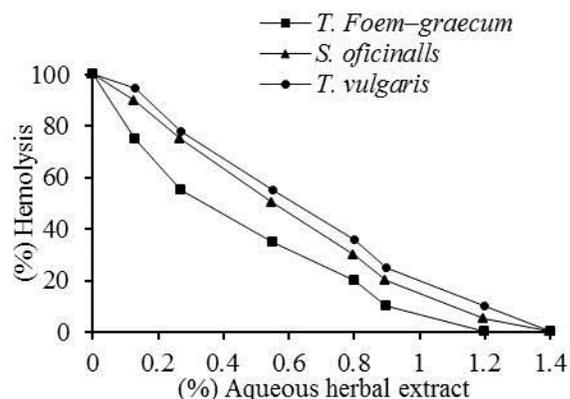
Figure 10: Protection of RBC hemolysis, each aqueous herbal extract was pre-incubated at different concentrations for 5 min with fresh RBC suspension, before adding 1.25 mM of nitrosocysteine to initiate complete hemolysis as described in fig.5.

4. Discussion:

As an antioxidant enzyme, G6PD is present in all human cells but it is particularly essential to red blood cells because these cells have no alternative source for producing NADPH (**Luzzatto, 1967; Beutler, 1994**). The reduced glutathione (GSH) and NADPH are dependent on the availability of G6PD activity, however both are considered to have contribution in the resistance of RBC against oxidative damage (**Gerli et al., 1982; Abboud and Awaida., 2010**). A decrease in G6PD enzyme activity leads to decrease in NADPH and reduced Glutathione. It is believed that a lack of reduced glutathione availability causes early hemolysis in the spleen (**Tian et al., 1999; Leopold and Loscalzo., 2005**). It has been documented that G6PD structure possesses two critical sulfhydryl groups that may

play important roles in the enzyme catalytic function (**Desforges et al., 1960**). It is possible that one of these enzymatic sulfhydryl groups is attacked by oxidant compounds. Enzyme inhibitions by NO donor compounds commonly imply the oxidation of specific tyrosine residues (**Yamakura et al., 1998**) or cysteine residual groups (**Spooren et al., 1998; Gow and Ischiropoulos., 2001**) and both residues have been established in the crystal structure of human G6PD (**Shannon et al., 2000**). Also, the toxicity of these NO donor compounds on erythrocytes have been documented when peroxynitrite formed in vivo via the reaction of nitric oxide and the superoxide anion, was found to cause high rate of hemolysis on bovine erythrocytes (**Wrobel et al., 2003**). Several enzymes such as 5-nucleotidase (**Siegfried et al., 1996**) and tryptophan hydroxylase (**Kuhn and Geddes, 1999**) are inactivated through S-nitrosylation interaction between oxidant and reactive enzymes sulfhydryde group. Usually these oxidants are NO generator that initiates the S- nitrosylation step followed by oxidation of sulfhydryde groups to Disulfide Bridge. This might be applied for the action of nitrosoarginine, nitrosocysteine oxidants on G6PD activity.

The irreversibility of G6PD inhibition by NO donor compounds was not altered after long preincubation time, the conversion of enzymatic thiols to disulfides may explain the irreversible inhibition observed by all these inhibitors. **Zhou et al (2000)** found that dopamine β-hydroxylase enzyme was irreversibly inhibited by diethylamine..

An imbalance between cellular pro-oxidant and antioxidant levels leads to the oxidative stress resulting in tissue damage. The antioxidant enzyme interacts directly with reactive oxygen species (ROS) to convert them to non-radical products. We had previously demonstrated that the overproduction of these radicals by cigarette smoking has an inhibitory effect on the enzymes responsible for removal of ROS such as CAT, G6PD and GPx (**Al-Awaida et al., 2013**).

Preincubated G6PD enzyme with aqueous herbal extract were able to protect the enzyme activity against inhibition and overcome red blood hemolysis effects due to richness of these extracts with antioxidants compound. **Abboud and Awaida, 2010** showed the NO donor compounds promoted RBC hemolysis in parallel to their inhibition extents on G6PD activities. A decrease in G6PD enzyme activity was connected with increases accumulation of cellular reactive oxygen species (ROS) while an overexpression of G6PD activity decreases the accumulation of these species in response to oxidative stress (**Leopold et al., 2003; Fico et al.,**

2004). Nitrosocysteine showed more effect than nitrosoarginine on both G6PD activity and red blood cells hemolysis. Thus, more aqueous extracts concentration is needed for protection against nitrosocysteine. This result indicate the nitrosocysteine release more nitric oxide. *Salvia oficinalls, Thymus vulgaris* and *trigonella Foem–graecum* have been widely used as a source of natural antioxidants. *Salvia oficinalls* is an old medicinal plant, which is rich source of different chemical constituents including terpenoids, polyphenol as well as essential oils **(Wang et al., 2000; Bozin et al., 2007)**. *Salvia officinalis* extract is used to stabilize fat and fat-containing food against oxidation **(Schwarz and Ternes, 1992)**. The main antioxidant compounds in the sage are carnosic acid and carnosol rosmarinic acid **(Cuvelier et al 1996)**. Thymol and carvacrol were the major components of Thymus vulgaris essential oil. However, the thymol was more effective than carvacrol in preventing oxidation during the autoxidaion of lipids **(Yanishlievaet al., 1999; El-Nekeety et al, 20**

11). Aqueous extract of thyme showed a degree of reductive and radical scavenging capacity **(Dorman et al, 2003)**. Fenugreek *(trigonella Foem–graecum)* being rich in phytochemical components have encouraged its use as medicinal plant **(Zaman et al., 1998; Dixit et al., 2005)**.

Simultaneous administration of aqueous extract of fenugreek seeds with ethanol for 60 days in rats prevented ethanol toxicity **(Thirunavukkarasu et al., 2003).**

5- Conclusion

The NO donor compounds (nitrosocysteine and nitrosoarginine) caused strong inhibition on G6PD enzyme activities, these NO donor compounds promoted RBC hemolysis in parallel to their inhibition extents on G6PD enzyme activities. The protection of both G6PD enzyme activity and red blood cell hemolysis against oxidative stress were achieved by preincubation with aqueous extracts from *Salvia oficinalls, Thymus vulgaris* and *trigonella Foem–graecum*. Those extracts were able to protect hemolysis in parallel to their activation extents on G6PD activities. The nitrosocysteine showed more effect than nitrosoarginine on both G6PD inhibition and red blood cells hemolysis. Thus, more aqueous extracts concentration is needed for protection against nitrosocysteine.

Corresponding Author:
Dr. Wajdy Al-Awaida
Assistant Professor, Biochemistry
Department of Biology and Biotechnology, American University of Madaba, Madaba, Jordan
E-mail: w.alawaida@aum.edu.jo

References
1. Abboud A, Awaida W. Synchrony of G6PD activity and RBC fragility under oxidative stress exerted at normal and G6PD deficiency. Clinical Biochemistry 2010; 43: 455–460.
2. Al-Awaida W, Abuereish G, Shraideh Z. Correlation of antioxidant and NADPH generating enzymes activities with tissues inflammation during smoke exposure of rats and smoke. ROAVS 2013; 3(2): 41-49.
3. Asahi M, Fujii J, Takao T, Kuzuya T, Hori M, Shimonishi Y, et al. The oxidation of selenocysteine is involved in the inactivation of glutathione peroxidase by nitric oxide donor. *J Biol Chem* 1997; **272**: 19152–7.
4. Beutler E. G6PD deficiency. Blood 1994; 84:3613.
5. Beutler E. The hemolytic effect of primaquine and related compounds: a review. Blood 1959; 14:103-109.
6. Bowman ZS, Oatis JE, Whelan JL, Jollow DJ, McMillan DC. Primaquine Induced Hemolytic Anemia: Susceptibility of Normal versus Glutathione-Depleted Rat Erythrocytes to 5-Hydroxyprimaquine. J Pharmacol Exp Ther 2004; 309:79-85.
7. Bozin B, Dukic NM, Samojlik I, Jovin E. Antimicrobialand Antioxidant Properties of Rosemary and Sage *(Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae)* Essential Oils. J. Agric. Food Chem 2007; 55:7879–7885.
8. Ciftci M, Ciltas A, Erdogan O. Purification and characterization of glucose 6-phosphate dehydrogenase from rainbow trout (Oncorhynchus mykiss) erythrocytes. Vet Med – Czech 2004;49:327–33.
9. Cuvelier M E, Richard H, Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. J. Am. Oil Chem. Soc. 1996; 73: 645−652.
10. Desforges JF, Kalaw E, Gilchrist P,Inhibition of glucose-6-phosphate dehydrogenase by hemolysis inducing drugs. J. Lab. Clin.Med 1960; 55(5): 757-766.
11. Dixit PP, Ghaskadbi S, Hari M, Devasagayam TPA. Antioxidant properties of germinated fenugreek seeds. Phytother. Res 2005;19:977–983
12. Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chem 2003; 83(2): 255-262.
13. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J Nutr* 2003; 133: 1286-1290.

14. El-Nekeety AA, Mohamed SR, Hathout AS, Hassan NS, Aly SE, Abdel-Wahhab MA. Antioxidant properties of Thymus vulgaris oil against aflatoxin-induce oxidative stress in male rats. Toxicon 2011; 57:984-91

15. Fico A, Paglialunga F, Cigliano L, Abrescia P, Verde P, Martini G, Iaccarino I, Filosa S. Glucose-6-phosphate dehydrogenase plays a crucial role in protection from redox-stress-induced apoptosis. Cell Death Differ 2004; 11: 823-831.

16. Frankel EN. Antioxidants in lipid foods and their impact on food quality. Food Chem 1996;57: 51-55.

17. Gerli GC, Beretta L, Bianchi M, Agostoni A, Gualandri V, Orsini GB. Erythrocyte superoxide dismutase, catalase, and glutathione peroxidase in glucose-6-phosphate dehydrogenase deficiency. Scand J Haematol 1982; 29: 135-40.

18. Gobbel, GT, Chan, TY, Chan, PH. Nitric oxide- and superoxide-mediated toxicity in cerebral endothelial cells. J Pharmacol Exp Ther 1997; 282:1600-1607.

19. Gow AJ, Ischiropoulos H. Nitric oxide chemistry and cellular signaling. J Cell Physiol 2001;137:277–82.

20. Hogg N. The kinetics of S-transnitrosation-A reversible second-order reaction. Anal Biochem 1999; 272: 257–262.

21. Kuhn DM and Geddes TJ. Peroxynitrite inactivates tryptophan hydroxylase via sulfhydryl oxidation. Coincident nitration of enzyme tyrosyl residues has minimal impact on catalytic activity. J Biol Chem 1999; 274:29726 –29732.

22. Kuhn DM, Arthur RE, States JC. Phosphorylation and activation of brain tryptophan Hydroxylase: identification of serine -58 as a substrate Site for protein Kinas A. J Nevrochem 1997; 68: 2220-2223

23. Leopold JA, Loscalzo J. Oxidative Enzymopathies and Vascular Disease. Arterioscler Thromb Vasc Biol 2005; 25:1332.

24. Leopold JA, Zhang Y-Y, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arterioscler Thromb Vasc Biol 2003; 23:411-7.

25. Luzzatto L. Regulation of the activity of glucose-6-phosphate dehydrogenase by NADP+ and NADPH. Biochim Biophys Acta. 1967;146:18–25.

26. Masahiko T, Akira A, Emiko K, et al. Antioxidant protection of propofol and itsrecycling in erythrocytes membranes. Am J Respir Crit Care Med 2002; 165:54–60.

27. Mehta AB. Glucose-6-phosphate dehydrogenase deficiency. Postgrad Med. J *1994;* 70:871-877.

28. Prchal JT, Gregg XT. Red Cell Enzymes. Hematol 2005; 1: 19-23.

29. Schwarz, K, Ternes W. Antioxidative constituents of *Rosmarinus officinalis and Salvia officinalis. Z. Lebensm.* Unters. Forsch 1992; 195:95–98.

30. Scriver CR."Glucose-6-Phosphate Dehydrogenase Deficiency." In: The metabolic and molecular bases of inherited disease. 7th ed. McGraw- Hill 1995; 3367-98.

31. Shannon WN, Gover S, Lam VMS, et al. Human glucose-6-phosphate dehydrogenase: the crystalstructure reveals a structural NADP+ molecule and provides insights into enzyme deficiency. Structure 2000;8:293–303.

32. Siegfried G, Amiel C, and Friedlander G. Inhibition of ecto-5-nucleotidase by nitric oxide donors: implications in renal epithelial cells. J Biol Chem 1996; 271: 4659–4664.

33. Sodeinde O. Glucose-6-phosphate dehydrogenase deficiency. Baill Clin Hematol 1992; 5:367-382.

34. Spooren AA, Evelo CT. Only the glutathione-dependent enzymes are inhibited by haematotoxic hydroxylamines. Hum Exp Toxicol 1998;17:554–9.

35. Thirunavukkarasu V, Anuradha CV, and Viswanathan P. Protective effect of fenugreek (Trigonella foenum graecum) seeds in experimental ethanol toxicity.Phytother Res 2003; 17:737–743.

36. Tian WN, Braunstein LD, Apse K, Pang J, Rose M, Tian X, Stanton C. Importance of glucose-6-phosphate dehydrogenase activity in cell death. Am J Physiol Cell Physiol 1999; 276: 1121-31.

37. Tian WN, Pignatare JN, Stanton RC. Signal transduction proteins that associate with the platelet-derived growth factor (PDGF) receptor mediate the PDGFinduced release of glucose-6-phosphate dehydrogenase from permeabilized cells. J Biol Chem 1994; 269:14798–805.

38. Wang M, Kikuzaki H, Zhu N, Sang S, Nakatani N, Ho CT. Isolation and structural elucidation of two new glycosides from sage (Salvia officinalis L.). J Agric Food Chem 2000; 48(2): 235-8.

39. Wrobel A, Lukaszynska B, Jadwiga KJ. The effect of peroxinitrate and some antioxidants on the rate of osmotic hemolysis of bovine erythrocytes. Cell Mol Biol Lett 2003;8:455–60.

40. Xian M, Chen X, Liu Z, Wang K, Wang PG. Inhibition of paoain by S-nitrosthiols. The Journal of Biological Chemistry. J Biol Chem 2000; 275(27):20467-73.

41. Yamakura F, Taka H, Fujimura T, et al. Inactivation of human manganesesuperoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine34 to 3-nitrotyrosine. J Biol Chem 1998;273:14039–85.

42. Yanishlieva NV, Marinova EM, Gordon MH, Raneva VG. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 1999; 64:59–66.

43. Zaman MM, Rouf MA, Haque S, Khan LR, Chowdhury NA, Razzaque SA, Yoshiike N, Tanaka H. Does rheumatic fever occur usually between the ages of 5 and 15 years? Int J Cardiol 1998;66:17–21.

44. Zhou X, Espey MG, Chen JX et al: Inhibitory effects of nitric oxide and nitrosative stress on dopamine-beta-hydroxylase. J Biol Chem, 2000; 275: 21241–46.

1/23/2014