Chemistry Research Journal, 2016, 1(4):147-153

Available online www.chemrj.org

Review Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Cytoprotective, Conjugative and Antioxidant Activities of Glutathione; and Its Role in Removal of Toxic Metabolites and Protein Protection: A Review

Idris Z Sadiq[1], Pankaj T[1], Amir R Khan[1], Naziru D[2], Safiyanu I[1], Abdulkadir R Salisu[3]

[1]Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, GautamBudha Nagar, India
[2]National Biotechnology Development Agency, Abuja-Nigeria
[3]Department of Environmental Science, College of Environmental Studies, Kaduna Polytechnic, Kaduna Nigeria

Abstract The purpose of this paper is to examine the role of glutathione in cytoprotection, xenobiotics metabolism and conjugation of reactive metabolites as well as its role in Protein s- glutathionylation induced by reactive oxygen and nitrosative species. We reviewed published literatures addressing the xenobiotics detoxification, involvement of methylglyoxal in glycation and cellular damage as well as role of protein s-glutathionylation in oxidative stress caused by reactive oxygen and nitrosative stress. Glutathione plays critical role in xenobiotics detoxification. It also plays a role in conjugation of toxic metabolites generated in the cell during phase I reactions. It participates in glyoxylase system, protein regulation and expression of gene through disulfide exchange reactions, metabolism of estrogens, prostaglandins and leukotrienes, maturation of iron-sulfur clusters of diverse proteins and operation of certain transcription factors. Glutathione plays indispensable role in cytoprotection, detoxification, protein protection and redox-signalling. It role is well noted in conjugation of highly reactive metabolites. Excess advanced glycation end products have been linked to the development of diabetes and methylgyoxal levels were found to be high in the cerebrospinal fluid of diabetic patients. Recently, Protein S-glutathionylation is appearing as a critical signaling mechanism in cardiovascular diseases.

Keywords GSH, Glyoxylase system, Methylglyoxal, Detoxification, Glutathionylation, Xenobiotics

Abbreviations
AGEs = Advance Glycation End-products
DHAP = Dihydroxy Acetone phosphate
EC = Enzyme Commission
G3P = Glyceraldehydes 3 Phosphate
GCL = Gamma Glutamylcysteinyl Ligase
GlxI = Glyoxylase I
GlxII = Glyoxylase II
Grx = Glutaredoxin
GS = Glutathione synthetase
GSH = Reduce Glutathione
GSSG = Oxidized Glutathione
MGS = Methylglyoxal Synthetase

NO = Nitric Oxide
PKa = Acid Ionization Constant
PSH = Sulfhydryl Groups of Proteins
RNS = Reactive Nitrosative Species
ROS = Reactive Oxygen Species
UDP=Uridine 5'-Diphosphophate

Introduction

Glutathione (GSH) is a tripeptide made up of three amino acids namely, glycine, cyteine and glutamate. It is a water soluble antioxidant that plays a critical role in protecting the cell against oxidative damage and highly reactive oxygen species [1]. Glutathione is extensively distributed in tissues of living organisms (plants, animals and microorganisms). It is normally present in millimolar concentration [1] with highest concentration in the liver. It is the most common thiol present in the cells and the most plentiful low molecular weight tripeptide [1-2]. It makes a significant amount (about 90%) of the total non-protein in most cells [2-3] and also plays important role in detoxification of xenobiotics and peroxides into neutral or less toxic compounds through the catalysis by glutathione-s-transferase and glutathione peroxidase [2-5]. The tripeptide participated in other cellular reactions, like the glyoxylase system, reduction reaction of ribonucleotides into corresponding deoxyribonucleotides, protein regulation and expression of gene through disulfide exchange reactions [4], participates in the metabolism of estrogens, leukotrienes, and prostaglandins, maturation of iron-sulfur clusters of diverse proteins, and in the operation of certain transcription factors [1]. The tripeptide is present in the cells either as a reduced (GSH) or oxidized (GSSG) form. Keeping a balanced ratio between reduced and oxidized form of GSH is important to the cell survival and depletion of GSH increases the risk of cell for oxidative damage [5].

Biosynthesis of Glutathione

GSH is synthesized in the cells from the three amino acids glycine, cysteine and glutamic acid [5]. it is synthesized in a two-step energy dependent process [6]. The Synthesis of GSH takes place in the cytosol [7] and requires the participation of two important cytosolic enzymes; L glutamylcysteine synthetase also known as glutamate cysteine ligase(GCL) (GSH1; EC 6.3.2.2.) and GSH synthetase (GS) (GSH2; EC 6.3.2.3.) [5-7]. In the first step, Glutamate and cysteine are combined by the enzyme glutamylcysteine synthetase to form-glutamylcysteine. In the second step, glutamylcysteine is then combined with glycine by glutathione synthase at the expense of two ATP molecules to yield GSH [6] (figure 1). The bioavailability of cysteine is the rate limiting for the synthesis of GSH [5,7]. Cysteine and oxidized form of amino acid are transported into the cell through sodium dependent and independent Transporters [5-8]. The level of GSH inside the cell is much greater than that of cyteine, thus, GSH act as a repository and convey form of cyteine moieties. GSH is synthesized within the cells and exported from the cells; glutamyltranspeptidase, an enzyme localized in the external surface of cell membranes coordinates it's breakdown. Export of GSH functions in inter-organ and intra-organ transfer of cysteine moieties, in the protection of cell membranes, and as part of a pathway of transport for cysteine and probably other amino acids [9]. GSH is under tight homeostatic control both within and outside the cell [5, 10]. A good balance is achieved through synthesis of GSH; it's recycling from GSSG/oxidized glutathione, and its utilization.

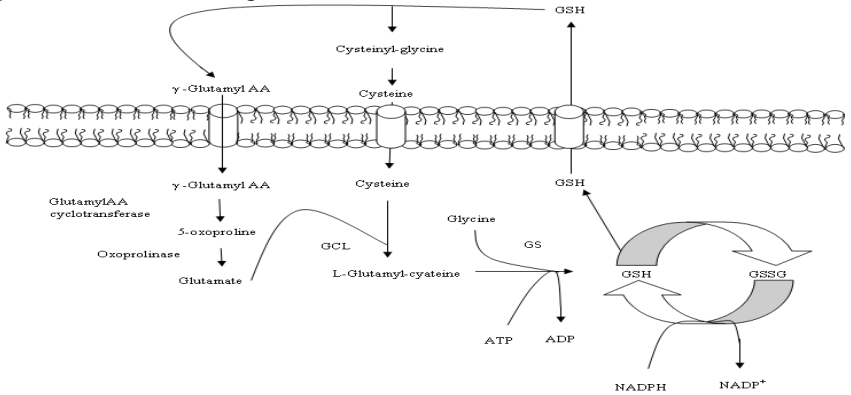

Figure 1: Biosynthesis of glutathione; glutamate and cyteine combined to form γ-glutamylcysteine in the cytosol catalyzed by the enzyme glutamylcyteine ligase (GCL). The γ-glutamylcyteine is then combined with glycine to form GSH catalyzed by the enzyme GSH synthetase (GS). The whole process is energy dependent and two molecules of ATP are consumed per GSH synthesized. The bioavailability of cysteine is the rate limiting in the synthesis of GSH.

Role of Glutathione in Detoxification of Xenobiotics

Xenobiotics are foreign chemicals which are not normally present in a biological system. All organisms are exposed frequently and inescapably to xenobiotics, which include both artificial and natural chemicals such as drugs, pesticides ,industrial chemicals, pollutants, alkaloids, secondary metabolites of plant, and toxins generated by molds, plants, and animals and pyrolysis products in cooked food [11]. Glutathione plays vital role in conjugation of these xenobiotics and act as critical antioxidant neutralizing the free radicals produced in phase I metabolism[12]. Reduce glutathione(GSH) glutathione, In the process of neutralizing the free radicals, is however, oxidized to glutathione disulfide(GSSG). The involvement of GSH in several intracellular detoxification routes is well noted [12-13]. Some of the routes in which glutathione is involved includes; protecting the formation of covalent bounds between alkylating compounds [13-14] and cellular genetic materials (DNA and RNA) and prevents mutation and cancerous potentials of genes, the conjugation reaction with electrophiles, especially epoxides of cyclic and acyclic organic compounds. The conjugative activity of GSH to electrophiles may occur with or without the facilitation of Glutathione-S-transferase (GST); an enzyme with ability to conjugate xenobiotics with reduced form of glutathione [12-14].The principal function of GSH is to remove toxic metabollites thereby preventing oxidative damage through the exchange of NADPH and $NADP^+$ [12-13]. Xenobiotic metabolism occurs in two phases in the cells (figure 2). The first phase involves biotransformation of the xenobiotics catalyzed by phase I drug metabolizing enzymes like cytochrome P450, monooxygenases, epoxide hydrolases, esterases and amidases. The second phase consist of mainly detoxification, in which xenobiotics are conjugation by phase II metabolising enzymes, such as Glutathione-*S*-Transferase (GST), UDP-glucuronosyltransferases, and sulfotransferases. The initial detoxification process may generate electrophiles or nucleophiles that are often carcinogenic or toxic [13-14] for example, toxicant such as nicotine and acetaminophen, as well as the procarcinogenic substances, benzene and polyaromatic hydrocarbons [15].

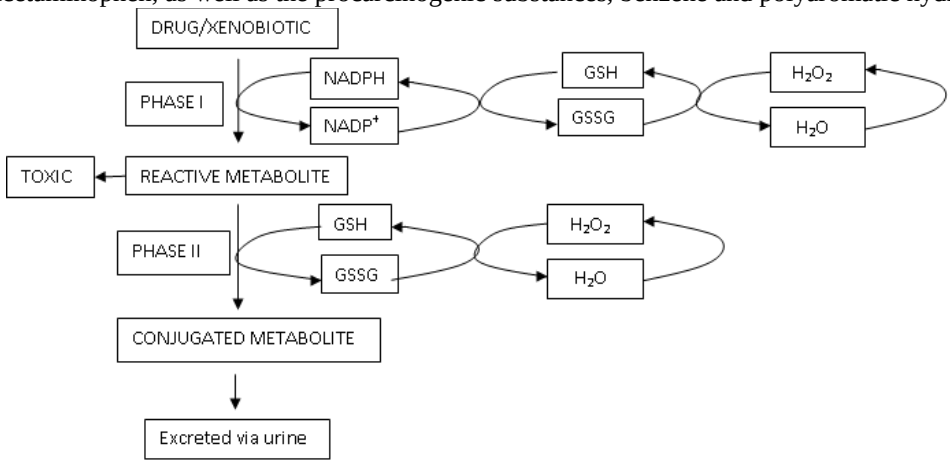

Figure 2: metabolism of xenobiotics: the role of glutathione in conjugation of reactive metabolite, thereby protecting cells from damage.

The Glyoxylase System and the Glutathione

The glyoxylase system is a system made of up two enzymes namely glyoxylase I (GlxI) and glyoxylase II (GlxII) all of which participated in the conversion of methylglyoxal, a highly reactive acyclic oxoaldehyde to D-lactate [16]. The system is mediated by glutathione, an endogenous tripeptide charged with responsibility of detoxification of toxic substances. The GlxI carry out the transformation of hemithioacetal (a product from methylglyoxal and glutathione) to S-D-lactoylglutathione. The second enzyme GlxII transformed S-D-lactoyl-glutathione into D-lactate and glutathione. Glyoxalase system play role in detoxification of methylglyoxal, a by-product of glycolysis which is highly reactive [16]. Glycation of important macromolecules such as DNA causes cell damaged. Therefore, conversion of methyglyoxal into D-lactate is an important detoxification reaction mediated by glutathione to protect cells from oxidative damaged [16-17] (figure**:** 3). The extreme accumulation of advanced glycation end products (AGEs) has been linked to the development of diabetes and methylgyoxal levels were found to be high in the cerebrospinal fluid of diabetic individuals[18]. Methylglyoxal has been suggested to be generated mostly during glycolysis, by nonenzymatic conversion of triose phosphates, specifically DHAP, which occurs spontaneous [17, 19-21]. When insufficient inorganic phosphate is available, Methylglyoxal has been suggested to be produced from the triose phosphate intermediate, particularly dihydroxyacetone phosphate (DHAP) [22-23]. Sources other than that from DHAP which are believed to generate lower amounts of methylglyoxal, include intermediates of protein and

fatty acid metabolism, such as aminoacetone formed from l-threonine and glycine [24-25], and acetone [26-27]. Reduced glutathione (GSH) plays a crucial role by binding methylglyoxal and presenting it to glyoxalase I. Hence, sufficient availability of GSH is imperative in keeping low levels of Methylglyoxal in the body. Enzymes involved in the synthesis and recycling of GSH, such as glutathione peroxidase and glutathione reductase are also essential in the metabolism of Methylglyoxal [28-31]. Research conducted in plants and animal have suggested that glyoxylase system might be connected with regulation of cell proliferation and cell maturation as well as vesicle mobilization and disease process such as tumor growth and diabetes mellitus [32].

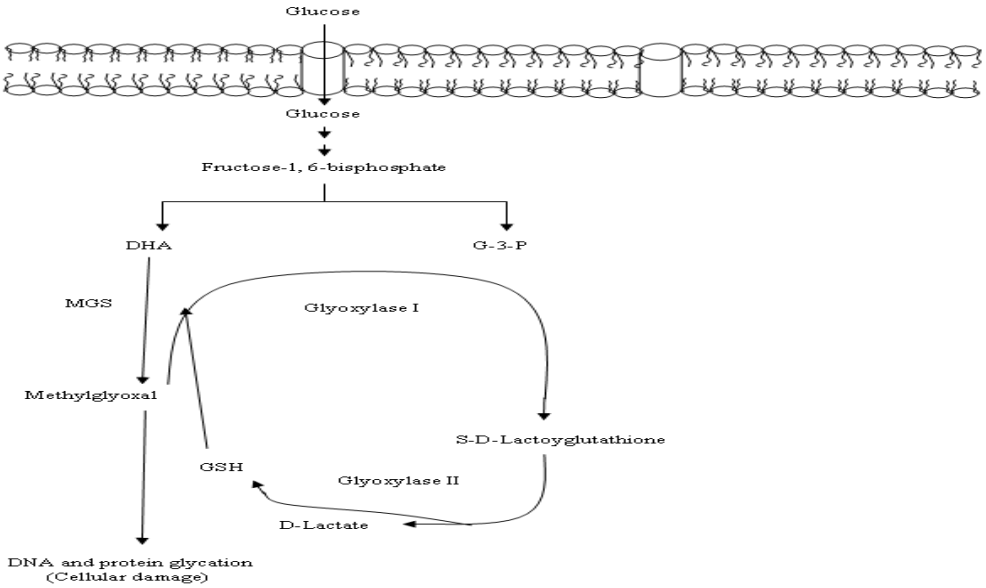

Figure 3: Role of glutathione in glyoxylase system in detoxification of reactive metabolites. In the diagram, the detoxification of methylgyoxal is carried out in the glyoxylase system which is mediated by glutathione. Glucose is first imported into the cell by glucose transporter 1(GLU1). The glucose undergo glycolysis by glycolytic enzymes to produced fructose 1, 6-bisphosphate which is cleaved into glyceraldehydes- phosphate 3- (G-3-P) and Dihydroxy acetone phosphate (DHAP). The methylglyoxal is generated from DHAP and it is converted into S-D-LactoylGlutathione by Glx1 and subsequently into D-lactate by GlxII and free GSH is released.

Protein S-Glutathionylation

The term protein S-glutathionylation refers to the process whereby GSH bind to proteins which occurs during oxidative stress caused by reactive oxygen species (ROS) and reactive nitrosative species (RNS)[6, 33-35]. Protein modification by ROS/RNS may befacilitated directly or indirectlyvia reaction with secondary by-products of oxidative stress. The effects that may result due to protein modification include; formation of cross-linked protein aggregates, cleavage of the polypeptide chain, and oxidation of amino acid side chains as well. Non-reversible modifications instigated by ROS/RNS, for example, protein carbonylation, are usuallylinked with permanent loss of protein function and may results inincrease accumulation of the damaged proteins into cytoplasmic inclusions, as reported in agerelated neurodegenerative disorders [33]. Several roles of Protein s-glutathionylation has been identified and a number of vital role in regulation of transcription factors and enzyme activity as well as protection of proteins against oxidation by cystein residues has been suggested [6, 34]. Firstdefined as a result of oxidative stress, S-glutathionylation was later identified to play major regulatory functions as well as in post-translational modification [34] many research has been directed towards the investigation of protein S-glutathionylation due to tremendous understandingin the role of ROS and NO in signal transduction and probably by the reversibility of protein S-glutathionylation [34-36]. Protein glutathionylation may be a means by which cells protect protein thiol groups from irreversible oxidation or store GSH during oxidative stress and has attracted particular interest as a post-translational modification in which enzyme activities can be regulated [34]. Reversibility of both glutathionylation and deglutathionylation can be catalyzed by family of disulfide enzymes such as oxidoreductases, glutaredoxin and to a less extent thioltransferase, which in particular catalyses exchange of thiol disulfide and in turn oxidizes protein using GSSG or reduce protein using GSH [35-36]. During oxidative and nitrosative stress, protein S-glutathionylation may possibly acts as molecular mechanism by which GSH performed redox-dependent signaling molecule resulting in transcriptional activation of many genes [6] Protein s-glutathionylation might betransducer of

redox signals generated by ROS and NO species probably due to activation of either glutathione or protein thiols by oxidants in order to keep on reacting with sufhydryls. Due to the highly reducing nature of intracellular environment (except in certain organelles such as endoplasmic reticulum), only small number of proteins are subjected to s-glutathionylation. The reactivity of thiols group has been suggested to be determined factor for the global susceptibility and site specificity for protein s-glutathionylation [34-36]. The relevant factors in protein susceptibility for S glutathionylation which were thought to be thiol steric accessibility and thiol pKa; depend solely on protein folding as well as surrounding area to the side chains of basic amino acids, respectively. The biochemical mechanisms of protein-S-glutathionylation proposed so far are based on thiol protein or GSH pool redox modifications which may be triggered through ROS or NO derived species. The most reported and investigsted mechanisms of protein S-glutathionylation are based on either thiol disulfide exchange through protein thiolate and glutathione disulfide (GSSG) or the reaction between an oxidized thiol to sulfenic acid with the reduced form of GSH [34-36]. Protein *S*-glutathionylation is now appear as a critical signaling mechanism in cardiovasculardiseases, as it regulates many physiological processes involved in cardiovascularhomeostasis, including myocyte contraction, oxidative phosphorylation, protein synthesis,vasodilation, glycolytic metabolism and response to insulin[36].A considerable amount of glutathione reversibly bound to sulfhydryl groups of proteins (PSH) via S- glutathionylation to form S- glutathionylated proteins (PSSG) (figure 4). In human disease, PSSG have been exploring as biomarker of oxidative stress [37]. There have been report of increase S-Glutathionylhaemoglobin in both type I and type IIdiabetes, hyperlipidemia, uremia associated with haemodialysis or peritoneal dialysis, and in smokers and has been suggested as biomarker of oxidative stress for the whole body [37].

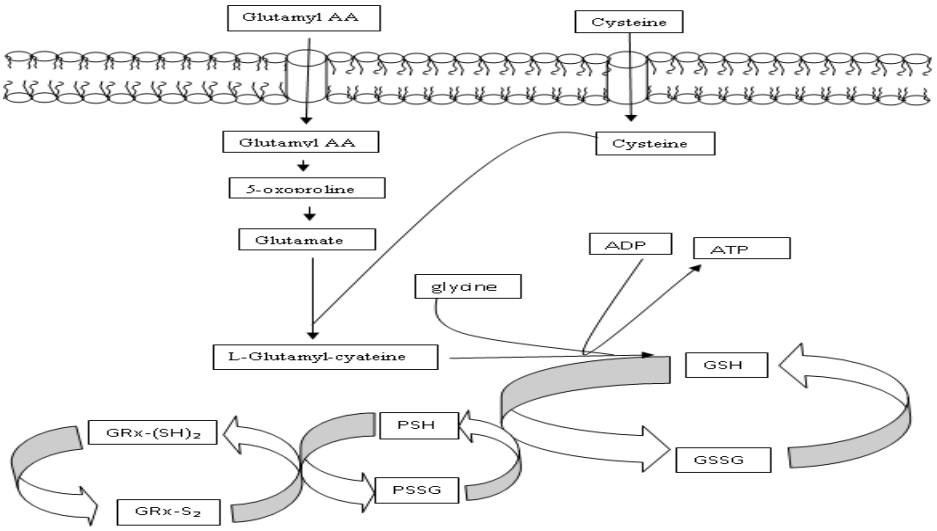

Figure 4: the role of glutathione in protein s- glutathionylation: in the diagram, glutathione synthesized from three amino acids, glutamate, cyteine and glycine, binds to the protein during oxidative and nitrosative stress. In the process, reduced glutathione (GSH) is converted into oxidized glutathione (GSSG). In the same vein, glutathione binds to sulfhydryl groups of proteins (PSH) via S-glutathionylation to generate S-glutathionylated proteins (PSSG). Glutaredoxin (GRx) regulates activities of many redox sensitive enzymes such as glutathione reductase which is essential for the GSH/PSH redox cycle and maintains adequate levels of reduced cellular GSH.

Conclusion

Glutathione plays an important role in cytoprotection, detoxification, protein protection and redox-signalling. It role is well noted in conjugation of highly reactive metabolites. Excess advanced glycation end products have been linked to the development of diabetes and methylgyoxal levels were found to be high in the cerebrospinal fluid of diabetic patient. Recently, Protein S-glutathionylation is appearing as a critical signaling mechanisms in cardiovascular diseases.

References

1. Lushchak, V I. Glutathione homeostasisand functions: potential targets for medical interventions.J Amino Acids,2012; 736-837.
2. ColowickS P, Kaplan N O. (Eds.), Academic Press, New York. Methods in Enzymol, 1985; 105, 113-26.

3. Anderson ME. Glutathione: an overview of biosynthesis and modulation. Chem. Biol Interact, 1998; 1–14, 111–2.
4. Mullineaux P M, Creissen G.Glutathione reductase: regulation and role in oxidative stress. In: Oxidative Stress and the Molecular Biology of Antioxidant Defenses , 1997; 667–713, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
5. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease, Biomed. Pharmacother, 2003; 57,145–55.
6. Singh S, Khan A R, Gupta A. K. Role of glutathione in cancer pathophysiology and therapeutic .Interventions J Exp Ther Oncol, 2012; 4, 303-16.
7. Aoyama K, Watabe M, Nakaki T.Regulation of neuronal glutathione synthesis. J Pharmacol Sci, 2008; 108, 227-38.
8. Bannai S, Christensen HN, Vadgama JV, et al. Amino acid transport systems. Nature,1984; 311-08.
9. Meister A. Glutathione Metabolism and Its Selective Modification. Journal of biological chemistry, 1988; 263, 17205-08.
10. Kidd PM. Glutathione: systemic protectant against oxidative and free radical damage. Altern Med Rev, 1997; 1,155-76.
11. Parkinson A. Biotransformation of xenobiotics, in Casarett and Doull's Toxicology*:* The Basic Science of Poisons(Klaassen CD eds.), 2001; pp 133–224.McGraw-Hill, NewYork.
12. Soleo L, Strzelczyk R. Xenobiotics and glutathione. G Ital Med Lav Ergon, 1999; 21, 302-8
13. Sharma R, Awasthi S, Zimniak P, et al. Transport of glutathione-conjugates in human erythrocytes Acta Biochimica Polonica 2000; 47, 751–62
14. Wu Y, Zhang X, Bardag-Gorce F, *et al*. **Retinoid X receptor alpha regulates glutathione homeostasis and xenobiotic detoxification processes in mouse liver.** *Mol Pharmacol*,2004**; 65,** 550-57.
15. Omiecinski C J, Vanden Heuvel J P, Perdew G H, et al.Xenobiotic Metabolism, Disposition, and Regulation by Receptors: FromBiochemical Phenomenon to Predictors of Major Toxicities.Toxicological Sciences, 2011; 120, 49–75.
16. Bélanger M, Yang J, Petit JM, et al. Role of the glyoxalase system in astrocyte-mediated neuroprotection. J Neurosci, 2011; 31, 18338-52.
17. Thornalley, P. J. Pharmacology of methylglyoxal: Formation, modification of proteinsand nucleic acids, and enzymatic detoxification--a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol, 1996; 27, 565-73.
18. *Chang*WC, *Wu*SC, *Xu*KD, et al. Scopoletin Protects against Methylglyoxal-Induced Hyperglycemia and Insulin Resistance Mediated by Suppression of Advanced Glycation Endproducts (AGEs) Generation and Anti-Glycation. Molecules,2015; 20, 2786-801
19. Phillips S A, Thornalley P J, The formation of methylglyoxal from triose phosphates: investigation using a specific assay for methylglyoxal. Eur. J. Biochem, 1993; 212, 101-05.
20. Sato J, Wang Y M, vanEys J. Methylglyoxal formation in rat liver cells. J Biol. Chem, 1980; 255, 2046-50.
21. Kalapos M P. The tandem of free radicals and methylglyoxalformation in rat liver cells. Chem. Biol. Interact, 2008; 171,251-71.
22. Hopper D J, Cooper R A. The regulation of Escherichia coli methylglyoxal synthase:a new control site in glycolysis? FEBS Lett, 1971; 13,213-16.
23. Ray S, Ray M. Isolation of methylglyoxal synthase from goat liver. J. Biol. Chem, 1981; 256, 6230-33.
24. Lyles G A, Chalmers J. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem. Pharmacol, 1992; 43, 1409-14.
25. Deng Y, Yu P H. Simultaneous determination of formaldehyde and methylglyoxal in urine: Involvement of semicarbazide-sensitive amine oxidase-mediated deamination in diabetic complications. J. Chromatogr. Sci, 1999; 37, 317-22.
26. Casazza J P, Felver M E, Veech R L. The metabolism of acetone in rat. J. Biol. Chem,1984; 259,231-36.
27. Yu P H, Wright S, Fan E H, et al. Physiological and pathological implications of semicarbazide-sensitive amine oxidase. Biochim. Biophys. Acta, 2003;1647, 193-99.
28. Blakytny R, Harding J. Glycation (non-enzymic glycosylation) inactivates glutathione reductase. Biochem. J,1992; 288, 303-07.
29. Wang X, Desai K, Chang T, et al. Vascular methylglyoxal metabolism and the development of hypertension. J. Hypertens, 2005; 23, 1565-73.

30. Wu L, Juurlink B H. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. Hypertension, 2002; 39, 809-14.
31. Thornalley P J, The glyoxalase system:new developmentstowards Functional characterization of a metabolic pathway fundamental to biological life. Biochem. J, 1990; 269, 1-11.
32. Dhar I, Desai K. Aging: Drugs to Eliminate Methylglyoxal, a Reactive Glucose Metabolite, and Advanced Glycation Endproducts, Pharmacology, Luca Gallelli (Eds.), 2012. ISBN: 978-953-51-0222-9, InTech, DOI: 10.5772/34337. Available from: http://www.intechopen.com/books/pharmacology/aging-drugs-to-eliminate-methylglyoxal-a-reactive-glucose-metabolite-and-advanced-glycation-endprodu
33. Giustarini D, Rossi R, and Milzani A, et al. S-Glutathionylation: from redox regulation of protein functions to human diseases. J Cell Mol Med, 2004; 8, 201-12.
34. Demasi M, Netto L E S, Silva G M,et al. Redox regulation of the proteasome via S-glutathionylation. Redox Biol, 2013; 2, 44–51.
35. GhezziP, *Romines B, Fratelli M, et al. Protein glutathionylation: coupling and uncoupling of glutathione to protein thiol groups in lymphocytes under oxidative stress and HIV infection.* Mol Immunol, *2002; 38,773-80.*
36. Pastore A, Piemonte F. Protein glutathionylation in cardiovascular diseases. Int. J. Mol. Sci,2013; 14, 20845–876.
37. Dalle-Donne I, Rossi R, Giustarini D, et al. Is there a role for S-glutathionylation of proteins in human disease? IUBMB Life 2005; 57, 189-92.