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The interaction of glutathione and thymoquinone and their antioxidant properties

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ABSTRACT

Protein S-glutathionylation has been regarded as one of the main mechanisms that control cell signaling and metabolic regulation. Several diseases including cardiovascular diseases, diabetes, neurodegenerative diseases and many types of cancer have been certainly found to be related with S-glutathionylation. It can be regarded as a promising area for therapeutic and diagnostic approaches in many diseases. In this mini review, we aimed to summarize and compare the interaction of glutathione (GSH) and thymoquinone (TQ), and elucidate the possible synergistic mechanisms of antioxidant and other beneficial effects.

Keywords: Glutathione, glutathionylation, thymoquinone, oxidative stress

INTRODUCTION

Glutathione (GSH) is a rather simple and major intracellular thiol molecule ($C_{10}H_{17}N_3O_6S$; molecular weight 307.32 g mol^{-1}) containing a tri-peptide (gamma-glutamyl-cysteinyl-glycine). GSH serves important roles in biological oxidation-reduction processes, as a cofactor for many enzymes, and a detoxifying and free radical scavenging agent (1). As shown in **Figure 1**, its metabolic pathway includes several enzymes and it must be continually synthesized in many human cells, mainly erythrocytes (red blood cells, RBC). GSH also helps the transport of amino acids across the cell membranes and serves as an agent that forms and maintains disulfide bonds within proteins. Glutathione is one of the main components that take the parts of antioxidative barricade against electrophiles and reactive oxygen species (ROS). Redox-active thiols (-SH) of cysteine serves as an antioxidant part of GSH. When GSH meets some molecules that needs to be reduced, it has been oxidized reciprocally. The reason why GSH possesses antioxidant properties is that it has a group that is vulnerable to be oxidized during the reduction of target molecules by GSH itself (1,2). One of the most known primary bioactive constituents of the black seeds' (Nigella Sativa) volatile oils is thymoquinone (TQ). Many kinds of in vivo and in vitro disease models have been designed to investigate the effectiveness of TQ and found that it is potentially a promising pharmacological agent that has therapeutic effects (3,4). The empirical formula [**Figure 2**] and molecular weight of bioactive phytochemical TQ (2-Isopropyl-5-methylbenzo-1,4-quinone) is $C_{10}H_{12}O_2$ and 164.20 g mol^{-1} (5). The in vitro and in vivo studies have been focused on anticancer, anti-inflammatory, and antioxidant activities of TQ. Moreover, TQ can preserve various enzymes that have antioxidant properties such as catalase, glutathione-S-transferase (GST) and glutathione peroxidase (GPX) and, on the other hand, it might act as free radical scavengers (6). Because GSH also forms disulfide bonds with cysteine residues in proteins, protein glutathionylation (PSSG) might provide a potential mechanism for metabolic regulation and cell signaling (7). Currently, explicit evidence shows that many human diseases such as cancer and diabetes have protein S-glutathionylation (PSSG). The purpose of this review is to give a brief information and compare the GSH and TQ interactions and elucidate the possible mechanism of antioxidant and other therapeutic effects.

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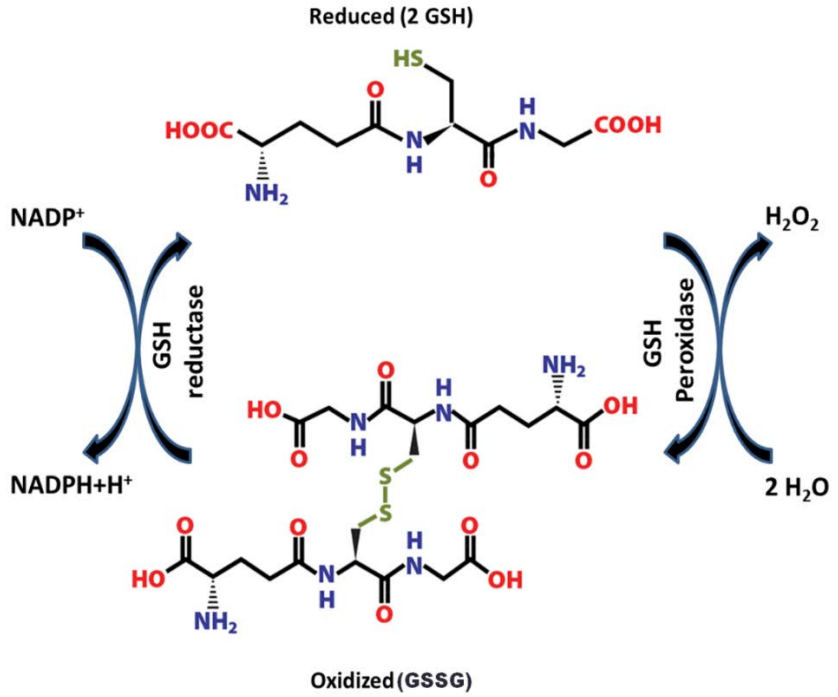

Figure 1: *Representation of glutathione GSH as a redox buffer in biological systems. The redox capacity of cell is reflected by the ratio of GSH/GSSG. Oxidation/reduction reactions involving GR and GPX are used to keep the ratio in balance. ROS or RNS-induced changes which may ultimately decrease GSH level and lead to cell death via apoptosis or necrosis*

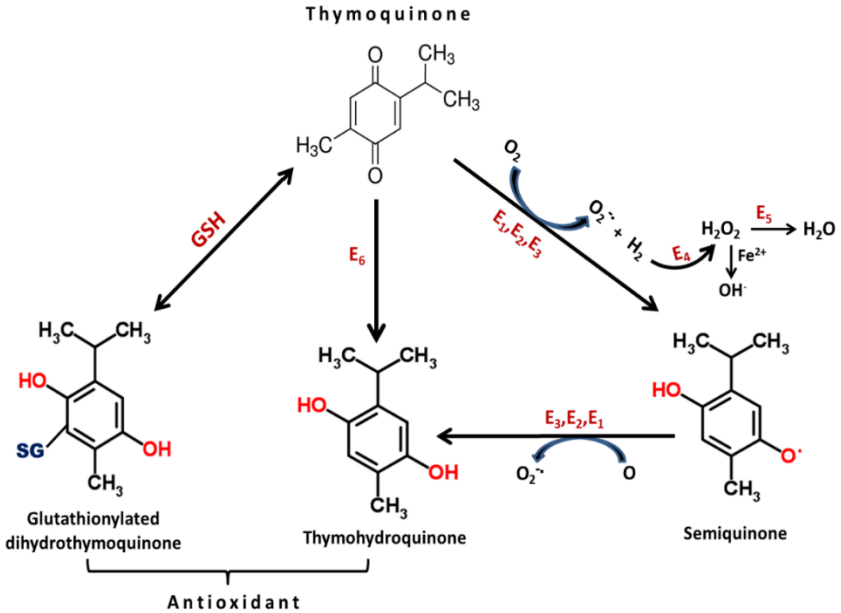

Figure 2: *Thymoquinone (TQ) and its oxidation-reduction cycling mechanism. A one-step two-electron reduction (E6) or by each of the one electron reduced in two successive steps (E1, E2 ve E3) reduction enzymatic reactions plays a role converting TQ into thymohydroquinone. To generate glutathionylated-dihydrothymoquinone, glutathione and TQ reacts with each other nonenzymatically and a reduction reaction is taken place. E1: NADPH cytochrome reductase, E2: NADH cytochrome b5 reductase, E3: NADH-ubiquinone oxidoreductase, E4: Superoxide dismutase, E5: Catalase, E6: NADPH quinone oxidoreductase*

PHYSIOLOGICAL IMPORTANCE OF GLUTATHIONE

Reduced GSH is one of the most common non-protein thiol in mammalian cells. It protects hemoglobin and other critical RBC proteins from peroxidative injury. GSH is an important intracellular reductant and antioxidant, helping to

maintain essential –SH group of enzymes in their reduced state. Exogenous and endogenous electrophilic species may react with cysteine thiol as a nucleophile. Hereby, reactive nitrogen species (RNS) and ROS are continually attacked by GSH in spontaneous and catalytic reactions (6). GSH also plays a role in the detoxification of xenobiotics and heavy metals (7). Inequalities in production of GSH and accompanying enzymes have been suggested in several situations because ROS is responsible for some human disease pathologies and some sort of cellular redox signaling-glutathionylation (8,9). De novo and salvage synthesis pathways are responsible for intracellular GSH homeostasis. The redox capacity of cells is determined by the ratio of GSH/GSSG. For instance, H_2O_2 can be turned to water by variety of ways; reduced GSH is oxidized by giving oxidized GSH (GSSG) as a product and then is reduced back to GSH by a reverse reaction called GSH cycle; glutathione reductase (GR), GPX and GST as well as GSH synthetase, glutamate cysteine ligase and glutaredoxin are included in the specified enzymatic system (**Figure 1**) (9). This cycle of oxidation/reduction reactions is primarily mediated by GPX and GR enzymes, and the redox balance is protected at the end. GSH is also important in the phase II metabolism (catalyzed by GST) of the compounds that have electrophilic characteristics, composing glutathione S-conjugates which are ultimately excreted in bile and urine. Additionally, a metabolic cycle involving GSH as a carrier has been implicated in the transport of some amino acids across membranes in the kidney. On the other hand, apoptosis and necrosis could be induced by RNS/ROS-related changes that lead to decrease in GSH level (7,9). Oxidative or nitrosative stress may be responsible for redox modulation of low pKa proteins that have cysteine moieties. The cysteine amino acids in a protein can be oxidized to sulfonic, sulfenic, and sulfinic acids. In order to create S-glutathionylated proteins via GR, GST or by using non-enzymatic pathways, sulfinic and sulfenic acid residues of proteins can be conjugated or reduced to GSH (10).

THE S-GLUTATHIONYLATION OF PROTEINS

One of the most significant post-translational modifications is reversible protein S-glutathionylation which contributes to the protection of cysteine groups of proteins from destructive irreversible oxidations and allows the management of signaling pathways to happen during alterations in RNS/ROS homeostasis (11). The formation of mixed disulfide protein-glutathione in leukocytes has been developed by the researchers as an important and original signaling paradigm that has a capacity to provide a new molecular approach for the relationship between oxidative stress especially in metabolic disorders and cardiovascular diseases (12). The reversible binding of GSH to protein protection thiols (PSH), called protein S-glutathionylation, is essential for the storage of GSH, protein S-glutathionylation itself, and PSH stability against irreversible oxidation. Explicit interaction between GSH and partially oxidized PSH, disulfide/thiol exchange between GSSG and PSH, GSH thiyl radical, or reactions between PSH and S- nitroso thiols are all results in S-glutathionylated protein. In fact, disulfide/thiol exchange has been regarded as an incredible intracellular mechanism for S-glutathionylation because Cys moieties has a tremendous redox potential and most cells export GSSG as a kind of protection device against ROS-induced stress. Following reestablishment of a reducing GSH/GSSH ratio, enzyme-dependent or -independent systems can reverse S-glutathionylation (13).

The distinct potential of oxidation for generation of the mixed disulfide and the GSH/GSSG ratio determines the glutathionylation status of a protein-SH. From this point of view, it can be proposed that to run the conversion of protein-SH to protein-SSG by 50%, intracellular GSH/GSSG ratio have to be diminished markedly (13). **Table 1** evaluates the reversible S-glutathionylation of specific proteins by giving a list of criteria (6). As seen in **Table 2**, depicting information and data on protein-SSG production as a candidate mechanism of regulation of cellular functions of the glutathionylated proteins can be possible by the criteria mentioned (8,14). Intracellular GSH levels is determined by two important factors; the rate of de novo GSH synthesis and GSH export to extracellular space. The cell membrane is not permeable to GSH; rather there are some enzymes and GSH transporters that has an impact on facilitating catalysis and uptake by the cells. For the catabolism of GSH, a salvage pathway is preferable by the cells and for further de novo synthesis, the constitutive amino acids are needed (14). The diseases originated from the pathologic processes connected to oxidation/reduction imbalance are related to exposure of ROS or RNS. Particular cysteine residues in target proteins can be differentially oxidized by ROS and the damage may be mitigated or mediated by the reversible S-glutathionylation process. A net negative charge and a tripeptide that cause a functional and structural change in target protein has been added by a post-translational modification. S-glutathionylation can be used as a biological shift for a couple of important oxidative signaling events because of reversibility (11,15).

Table 1: *Criteria for S-Glutathionylation as a regulatory mechanism*

S-Glutathionylation is taken place at a separate site and changes the function of the modified protein
S-Glutathionylation is taken place at a relatively high GSH/GSSG ratio (physiological conditions)
S-Glutathionylation is taken place within undamaged cells in response to a physiological stimulus, and provokes a physiological response
Formation of specific proteins-SSG is taken place by an efficient and rapid mechanism
Reversing of S-glutathonylation reaction is taken place by an efficient and rapid mechanism

Table 2: *Proteins characterized as susceptible to glutathionylation*

Proteins reported to undergo *S*-glutathionylation and implicated in Diabetes	Alcohol dehydrogenase, Cu, Zn-SOD, malate dehydrogenase, fatty acid binding protein, cathepsin K, calbindin, creatine kinase, glycogen phosphorylase b, HSP60, pro-caspase 3, GAPDH, hemoglobin
Mitochondrial proteins regulated by *S*-glutathionylation	Actin, G6PDH, α-KGDH, Ras, SERCA, Complexes I, II and IV
Examples of other proteins	Vimentin, myosin, tropomyosin, aldolase, enolase, adenylate kinase, triosephosphate isomerase, pyrophosphatase, thioredoxin-phosphoglycerate kinase, protein disulfide isomerase, 6-phospho-gluconolactonase, HSP70, galectin

Table 3: *Important diseases associated with S-Glutathionylation*

Diseases	**Clinical examples**
Cancer	
Cardiovascular diseases	Myocardial infarction, preconditioning, cardiac hypertrophy, atherosclerosis
Diabetes Mellitus	
Lung diseases	Tobacco exposure, inflammation, chronic obstructive pulmonary disease, fibrotic and granulomatous diseases
Neurodegenerative diseases	Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, Freidreich's ataxia, Huntington's disease

S-GLUTATHIONYLATION AS MOLECULAR MECHANISM FOR HEALTH OUTCOMES: POTENTIAL CLINICAL SIGNIFICANCE

Protein glutathionylation is significant consequence of many diseases and it is increased in endothelial cells under conditions of diabetes and cardiovascular diseases (CVD). The reversible formation of protein S-glutathionylation as an important signaling mechanism in CVD arranges some physiological pathways connected to cardiovascular homeostasis, such as protein synthesis, glycolysis, oxidative phosphorylation, myocyte contraction and response to insulin (16,17). Several cellular processes are regulated by redox homeostasis and several pathologies of human diseases are linked to abnormalities in pathways that control reductive and oxidative conditions, especially age-related diseases. A reduced intracellular environment is also provided by the reduced GSH as a most prevalent biological thiol. Cellular regulatory events, protein structure/function, and even human diseases have been influenced by S-glutathionylation cycle. There are clear evidences that S-glutathionylation is one of the underlying factors in several human diseases. Protein S-glutathionylation increase was demonstrated in aging human lenses and cataractogenesis (18). At the site of pathology, some of the proteins located in human lenses such as α- and β-crystallins were found to be increased. Disulfides, mixed disulfides, and disulfide-cross-linked aggregates form both disulfide-cross-linked aggregates and mixed disulfides, which support cataract severity, are found in cataract tissue. The fact that protein-thiol mixed disulfides might have some important roles in brunescence development and cataractogenesis in human lenses indicates that there is a strong correlation between opalescence intensity and lens nuclear color with protein S-glutathionylation. On the other hand, there is a great interest for glutathionylation. There has been a remarkable increase in the number of glutathionylated proteins in neurodegenerative diseases (19). Lately, inferior parietal lobule of Alzheimer's patients was found to be connected with specific PSSG and, a specific PSSG has been determined in the same brain lobule area in Alzheimer's disease (**Table 3**) (13,15). Since PSSG probably regulates cellular signaling pathways and protein functions that might play a critical role in pathogenesis of these diseases, it is now considered as an important therapeutic target for drug invention. As a matter of fact, original analogs of GSH molecule have been studied for using in breast cancer as adjuvant therapy (20). Utilization of several antioxidant molecules including GSH has been considered as a next phase of the treatment of cardiovascular diseases (7). Additionally, the same researchers suggested that these antioxidant molecules would improve the pathophysiological progress that are dominated by S-glutathionylation including metabolism, cellular growth, inflammation, and contraction (7).

MECHANISMS OF OXIDO-REDUCTION CYCLING OF THYMOQUINONE

TQ is converted into thymohydroquinone through a two-step one electron reduction or one-step two electron reduction enzymatic reactions. Various reductases such as mitochondrial complex I (NADH ubiquinone oxidoreductase), NADH cytochrome b5 reductase (microsomal), and microsomal NADPH cytochrome P450 reductase has been used for one electron reduction of TQ. NAD(P)H quinone acceptor oxidoreductases, a type of flavoenzymes located in the cytosol, are alternative enzymes that catalyze two electron reduction of TQ to yield instant thymohydroquinone synthesis which shows a strong antioxidant effect (6). Via a nonenzymatic reduction by using GSH, TQ can also be converted to glutathionylated-dihydrothymoquinone (**Figure 2**) (21). The reduced end products of TQ have great antioxidant properties. Likewise, superoxide dismutase (SOD) and catalase enzymes can detoxify superoxide anion radicals which are produced by a pathway where reduced TQ has been oxidized. The accumulation of superoxide radicals in absence of detoxification system might contribute to the prooxidant effect of TQ (15,21).

EFFECTS OF GLUTATHIONYLATED-DIHYDROTHYMOQUINONE

TQ was found to inhibit SOD glycation after its in vitro incubation with two alternative molecules, methylglyoxal or glucose and to retain its antioxidant activity. Hydroxyl and carbon-centered radicals can also be scavenged by TQ as evaluated by 1',1-diphenyl-2-picrylhydrazyl (DPPH) and iron-catalyzed destruction of deoxyribose (6). TQ is known as a scavenger for the hydroxyl and carbon-centered radicals. Additionally, it also shortened the progress of ROS-facilitated stress by non-enzymatic reactions including the GSH cycle to yield glutathionylated-dihydrothymoquinone (GS-TQ). GSH and TQ can enter the reaction quickly and spontaneously resulting in dihydrothymoquinone-thioether formation (22). Khalife et al. (23) suggested that TQ reacts chemically with GSH, NADH and NADPH. These reactions that occur at physiologically stable conditions results in reduced substances such as GS-TQ and dihydrothymoquinone, respectively. Thymoquinone exhibits lesser scavenging activity than dihydrothymoquinone and GS-TQ; it is so impressive to detect that the reduced end-products apparently exhibit an antioxidant quantity equivalent to Trolox. These findings display a probable nonenzymatic intracellular activation of TQ dependent on NADH, GSH or NADPH that can serve as a 'cellular switch' enable to harmonize intracellular antioxidant contents. Free radicals can be removed when these metabolites are formed, which ultimately show close similarities with other endogenous antioxidant molecules such as GSH and SOD and prevent lipid peroxidation. Hemoglobin and myoglobin with different redox states are easily react with these forms of molecules that result in recovery of these compounds from oxidative stress (22,23).

The modulation of biochemical and physiological processes involved in ROS generation is achieved by TQ which is exerting its biological functions. Increase in antioxidant enzyme amounts/activities such as SOD, CAT, GST, and quinone reductase as well as GSH levels and/or inhibition of lipid peroxidation and superoxide radical production have been seen in normal tissues after TQ administration (24,25). The capacity and function of different cell signaling molecules including cysteine residues that have redox properties might be modulated by TQ because of the capability to bind thiol residue of cysteine amino acids. On the other hand, TQ causes the generation of ROS and diminishes GSH amounts in a dose-dependent manner. Arslan et al. reported that TQ application reversed GSH depletion and reduced malondialdehyde levels as well as gastric ulcer index (26). There is a similarity to a category of compounds which is called Michael reaction acceptors. They have been known as a group of compounds that perform enzyme inductions, which ultimately protect cells from toxicity and chemical carcinogenesis. Because cellular microenvironment is so important for a molecule for being an antioxidant and prooxidant, TQ has been found to have this type of dual role (15,27). TQ shows multiple pharmacological activities such as antidiabetic, hepatoprotective, neuroprotective, antitumor, anti-inflammatory, and antioxidant (21,28).

MODULATION OF GLUTATHIONE AND ANTI-OXIDANT ENZYMES BY THYMOQUINONE

Free radicals from different sources are neutralized by important antioxidant enzymes. The effectiveness of TQ is also dependent on these enzymes. TQ is able to reverse the decreased activities of CAT, GST, and GPX, as well as reduced GSH levels in kidney and liver tissues in streptozotocin-induced diabetic rats (29). Also TQ has a recovery effect on reduced GSH, GPX, and CAT levels/activities in kidney tissue of gentamycin-treated rats (30). TQ dose was also reported to be inversely correlated with liver GSH levels (31). Contrary to this finding, TQ was found to relieve hepatotoxicity induced by carbon tetrachloride as shown by decline in the levels of serum enzymes and restoration of GSH amount in the liver (32). It is demonstrated that TQ has hepatoprotective effect by preventing tert-butyl hydroperoxide-induced depletion of GSH in isolated rat hepatocytes (33).

As a multifunctional compound, TQ is announced to be protective in experimental animals against chemical carcinogenesis. Induction of detoxifying enzymes such as quinone reductase and GST is the possible underlying mechanism of TQ protection on these enzymes (33). TQ has been investigated for its effects on GSH which is a preferable compound in detoxification of drugs and found that it was a good choice to prevent destruction of related tissues caused by ROS. Furthermore, this topic should be investigated in detail to be able to describe its role in possible carcinogenesis or inflammation suppression. In two different studies, elevation in GST and quinone reductase in mice liver were determined after TQ administration, possibly explaining the protective role of TQ against chemical toxicity and carcinogenesis (31,32). Lately, Goyal et al. suggested that TQ can manage various pathological conditions by attenuating oxidative stress status of cells by inducing GSH (34).

CONCLUSION

TQ is a unique natural product that has a capacity to inhibit protein-protein interactions by targeting several different proteins. It gives reactions with antioxidant enzymes, NADH, GSH, and NADPH to produce the two potent antioxidants, GS-TQ and dihydrothymoquinone, respectively. These metabolites show powerful scavenging activity for organic radicals and ultimately prevent lipid peroxidation by replacing other antioxidants such as GSH and SOD. Current data reviewed in this commentary may provide a useful background for the understanding GSH and TQ interaction, and elucidate their possible mechanism of antioxidant and other therapeutic effects. Although further studies are required, collective effect of these two prominent molecules might have a capacity to open new therapeutic targets.

REFERENCES

1. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol 2003;66(8):1499-503. https://doi.org/10.1016/S0006-2952(03)00504-5
2. Ribas V, García-Ruiz C, Fernández-Checa JC. Glutathione and mitochondria. Front Pharmacol 2014;5:151. https://doi.org/10.3389/fphar.2014.00151
3. Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology 2005;49(4):456-64. https://doi.org/10.1016/j.neuropharm.2005.04.004
4. Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol 2003;26(2):87-98. https://doi.org/10.1081/DCT-120020404
5. Woo CC, Kumar AP, Sethi G, Tan KH. Thymoquinone: potential cure for inflammatory disorders and cancer. Biochem Pharmacol 2012;83(4):443-51. https://doi.org/10.1016/j.bcp.2011.09.029
6. Diaz-Vivancos P, de Simone A, Kiddle G, Foyer CH. Glutathione-linking cell proliferation to oxidative stress. Free Radic Biol Med 2015;89:1154-64. https://doi.org/10.1016/j.freeradbiomed.2015.09.023
7. Pimentel D, Haeussler DJ, Matsui R, Burgoyne JR, Cohen RA, Bachschmid MM. Regulation of cell physiology and pathology by protein S-glutathionylation: lessons learned from the cardiovascular system. Antioxid Redox Signal. 2012;16(6):524-42. https://doi.org/10.1089/ars.2011.4336
8. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother 2003;57:145-55. https://doi.org/10.1016/S0753-3322(03)00043-X
9. Xiong Y, Uys JD, Tew KD, Townsend DM. S-glutathionylation: from molecular mechanisms to health outcomes. Antioxid Redox Signal 2011;15(1):233-70. https://doi.org/10.1089/ars.2010.3540
10. Oter O, Jin S, Cucullo L Damien Dorman HJ. Oxidants and antioxidants: friends or foes? Oxid Antioxid Med Sci 2012;1(1):1-4. https://doi.org/10.5455/oams.080612.ed.001
11. Grek CL, Zhang J, Manevich Y, Townsend DM, Tew KD. Causes and consequences of cysteine S-glutathionylation. J Biol Chem. 2013;288(37):26497-504. https://doi.org/10.1074/jbc.R113.461368
12. Ullevig SL, Kim HS, Short JD, Tavakoli S, Weintraub ST, Downs K, Asmis R. Protein S-glutathionylation mediates macrophage responses to metabolic cues from the extracellular environment. Antioxid Redox Signal 2016;25(15):836-51. https://doi.org/10.1089/ars.2015.6531
13. Dalle-Donne I, Milzani A, Gagliano N, Colombo R, Giustarini D, Rossi R. Molecular mechanisms and potential clinical significance of S-glutathionylation. Antioxid Redox Signal 2008;10(3):445-473. https://doi.org/10.1089/ars.2007.1716

14. Ghezzi P. Protein glutathionylation in health and disease. Biochim Biophys Acta 2013;1830(5):3165-72. https://doi.org/10.1016/j.bbagen.2013.02.009
15. Schneider-Stock R, Fakhoury IH, Zaki AM, El-Baba CO, Gali-Muhtasib HU. Thymoquinone: fifty years of success in the battle against cancer models. Drug Discov Today 2014;19(1):18-30. https://doi.org/10.1016/j.drudis.2013.08.021
16. Sanchez-Gomez FJ, Espinosa-Diez C, Dubey M, Dikshit M, Lamas S. S-glutathionylation: relevance in diabetes and potential role as a biomarker. Biol Chem 2013;394(10):1263-80. https://doi.org/10.1515/hsz-2013-0150
17. Pastore A, Piemonte F. Protein glutathionylation in cardiovascular diseases. Int J Mol Sci 2013; 14(10):20845-76. https://doi.org/10.3390/ijms141020845
18. Cooper AJ, Pimto JT, Callery PS. Reversible and irreversible protein glutathionylation: biological and clinical aspects. Expert Opin Drug Metab Toxicol 2011;7(7):891-910. https://doi.org/10.1517/17425255.2011.577738
19. Cha SJ, Kim H, Choi HJ, Lee S, Kim K. Protein glutathionylation in the pathogenesis of neurodegenerative diseases. Oxid Med Cell Longev 2017. https://doi.org/10.1155/2017/2818565
20. Garrett T, Tew KD, and Townsend DM. Protective effects of a glutathione disulfide mimetic (NOV-002) against cisplatin induced kidney toxicity. Biomed Pharmacother 2010;64(1):73-6. https://doi.org/10.1016/j.biopha.2009.09.009
21. Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res 2015;95-96:138-58. https://doi.org/10.1016/j.phrs.2015.03.011
22. Khalife KH, Lupidi G. Nonenzymatic reduction of thymoquinone in physiological conditions. Free Radic Res 2007;41(2):153-61. https://doi.org/10.1080/10715760600978815
23. Khalife KH, Lupidi G. Reduction of hypervalent states of myoglobin and hemoglobin to their ferrous forms by thymoquinone: the role of GSH, NADH and NADPH. Biochim Biophys Acta 2008;1780(4):627-37. https://doi.org/10.1016/j.bbagen.2007.12.006
24. Banerjee S, Padhye S, Azmi A, Wang Z, Philip PA, Kucuk O, Sarkar FH, Mohammad RM. Review on molecular and therapeutic potential of thymoquinone in cancer. Nutr Cancer 2010(7);62:938-946. https://doi.org/10.1080/01635581.2010.509832
25. Woo CC, Kumar AP, Sethi G, Tan KH. Thymoquinone: potential cure for inflammatory disorders and cancer. Biochem Pharmacol 2010;83(4):443-51. https://doi.org/10.1016/j.bcp.2011.09.029
26. Arslan SO, Gelir E, Armutcu F, Coskun O, Gurel A, Sayan H, Celik IL. The protective effect of thymoquinone on ethanol-induced acute gastric damage in the rat. Nutr Res 2005;25(7):673-80. https://doi.org/10.1016/j.nutres.2005.06.004
27. Dinkova-Kostova AT, Massiah MA, Bozak RE, Hicks RJ, Talalay P. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. Proc Natl Acad Sci 2001;98(6):3404-9. https://doi.org/10.1073/pnas.051632198
28. Kundu J, Chun KS, Aruoma OI, Kundu JK. Mechanistic perspectives on cancer chemo-prevention/chemotherapeutic effects of thymoquinone. Mutat Res 2014;768:22-34. https://doi.org/10.1016/j.mrfmmm.2014.05.003
29. Sankaranarayanan C, Pari L. Thymoquinone ameliorates chemical induced oxidative stress and β-cell damage in experimental hyperglycemic rats. Chem Biol Interact 2011;190(2-3):148-54. https://doi.org/10.1016/j.cbi.2011.02.029
30. Sayed-Ahmed MM, Nagi MN. Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats. Clin Exp Pharmacol Physiol 2007;34(5-6):399-405. https://doi.org/10.1111/j.1440-1681.2007.04560.x
31. Nagi MN, Almakki HA. Thymoquinone Supplementation induces quinone reductase and glutathione transferase in mice liver: Possible role in protection against chemical carcinogenesis and toxicity. Phytother Res 2009;23(9):1295-8. https://doi.org/10.1002/ptr.2766
32. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. Cell Biochem Funct 2002;20(2):143-51. https://doi.org/10.1002/cbf.968

33. Sayed-Ahmed MM, Aleisa AM, Al-Rejaie SS, Al-Yahya AA, Al-Shabanah OA, Hafez MM, Nagi MN. Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. Oxid Med Cell Longev 2010;3(4):254-61. https://doi.org/10.4161/oxim.3.4.12714
34. Goyal SN, Prajapati CP, Gore PR, Patil CR, Mahajan UB, Sharma C, Talla SP, Ojha SK. Therapeutic potential and pharmaceutical development of thymoquinone: A multitargeted molecule of natural origin. Front Pharmacol 2017;8:656. https://doi.org/10.3389/fphar.2017.00656

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