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# Regulation of Glutathione in Health and Disease with Special Emphasis on Chronic Alcoholism and Hyperglycaemia Mediated Liver Injury: A Brief Perspective

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**Abstract** Glutathione (GSH) plays a major role in the cell due to its antioxidant properties. The transcription factor Nrf2 plays an important role in the synthesis of GSH in the cell. Increased cellular GSH content leads to greatly decreased oxidative stress and toxicity. Alcoholism and hyperglycaemia both lead to liver injury, and GSH plays a crucial role in ameliorating the hepatic damage under these pathophysiological conditions. Further, GSH transferase which is involved in drug detoxification through conjugation of xenobiotics with GSH exhibits polymorphic forms which affect its detoxification efficacy. Further, GSH supplementation through NAC and UDCA and GSH depletion through BSO and DEM lessen and aggravate cellular dysfunction, respectively. Therefore, GSH regulation which occurs through several mechanisms has a very important role in maintaining or disrupting the oxidative environment of the cell.

**Keywords** Glutathione · Liver · Alcoholism · Hyperglycaemia

## Introduction

Glutathione (GSH) was first discovered in yeast cell extract and was named as 'labile hydrogen' which reduced sulphur to hydrogen sulphide [49]. Later it was revealed to have two amino acid moieties (glutamate and cysteine) and was named as 'glutathione' which denotes the glutamate and thiol residues [49, 109]. Finally, the original structure of GSH was revealed as a tripeptide consisting of glutamate, cysteine and glycine [50].

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## Physiological Role of GSH

Glutathione is a tripeptide thiol consisting of glutamine, cysteine and glycine. The cysteine residue in GSH contributes to its reducing property. Glutathione is mainly involved in eliminating hydrogen peroxide ($H_2O_2$) which is catalyzed by GSH peroxidase, thus preventing cellular lipid peroxidation (Fig. 1). During this process the reduced GSH is converted to its oxidized form (GSSG). The oxidized form is then recycled back to its reduced form (GSH) by GSH reductase at the cellular expense of NADPH.

## Glutathione Synthesis

The transport of the precursor amino acids through different specific amino acid transporters systems: alanine, serine and cysteine preferring (ASC), cystine glutamate preferring ($X_c^-$), leucine preferring (L) and asparagine preferring (N) is essential for GSH synthesis [7]. Among the three precursor amino acids, cysteine proves to be the rate-limiting factor for GSH synthesis since its intracellular availability is lower than the Km value of γ-glutamyl cysteine synthase (GCS), whereas glutamate and glycine are abundant in the cell [7, 70]. Intracellular cysteine availability is obtained either from cysteine, cystine or methionine/serine transport (transsulphuration pathway) through the involvement of any one of the above-mentioned amino acid transporters.


**Fig. 1** Mode of action of glutathione

After cysteine is transported inside the cell, it is linked to glutamate in a distinct covalent bond which is catalyzed by the enzyme GCS, where the γ-carbon in glutamate is involved in covalent bond with the amino group of cysteine; therefore, the name γ-glutamylcysteine. The distinct covalent bond between the glutamate and the cysteine protects GSH against protease activity. GSH synthetase catalyzes the second step where glycine is added to the γ-glutamylcysteine to form GSH (Fig. 2). The GCS is regulated by feedback mechanism, i.e. the binding of GSH to its glutamate binding site inhibits the further synthesis of the enzyme [98].

## Regulation of GSH Synthesis: Nrf2, a Vital Player

GSH synthesis in the cells is highly dependent on the expression and the activity of GCS. The increased GCS expression increases the intracellular GSH level. Further, the nuclear factor erythroid 2-related factor 2 (Nrf2) is involved in the regulation of GCS expression. The translocation of Nrf2 into the nucleus activates the expression of the GCS through the antioxidant response element (ARE) [65, 105].

Under normal physiological condition Nrf2 is bound to its negative regulator Kelch-like ECH-associated protein 1 (Keap1). When activated, Nrf2 gets released from Keap1 and is translocated to nucleus. Nrf2 signals redox changes (accumulation of GSSG) and induces GSH synthesis by activating GCS [47, 127].

The redox status of the cell is one of the factors which determine the rate of GSH synthesis by activating the γ-glutamyl cysteine expression through Nrf2 activation [105]. The increased level of reactive oxygen species (ROS) sensitizes the cell to GSH synthesis via Nrf2 signalling [29]. Nrf2-induced GCS expression and increased GSH level is also regulated by insulin [62].

The activation of Nrf2 as a protective response is highly dependent on the severity of cellular toxicity [96]. Under conditions with overwhelming nitrosative stress, the signalling kinase pathway phosphoinositide-3-Kinases/v-Akt murine thymoma viral oncogene (PI3K/AKT) is involved in the activation of Nrf2 and the Nrf2-dependent genes [66].

The ethanol-induced CYP2E1 expression in the cell activates Nrf2 and increases GSH synthesis in hepatocytes [17]. The Nrf2-mediated protective response is increased under elevated CYP2E1 expression in liver cells [17]. Further, 4-hyroxynonenal (HNE), a major lipid peroxidation

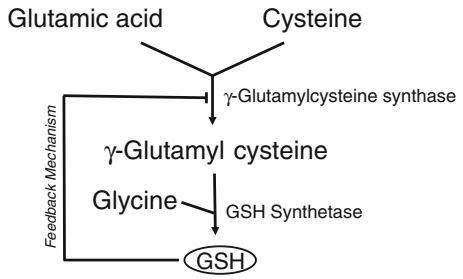


**Fig. 2** Glutathione synthesis

product, induces Nrf2 signalling and the expression of GSH-synthesizing enzymes, thereby increasing the level of GSH in the cells [29].

The c-Jun NH(2)-terminal protein kinases (JNK) 1 and 2 and glycogen synthase kinase 3 alpha and 3 beta (GSK-3alpha and -3beta) are the negative regulators of GSH synthesis, since their upregulation affects Nrf2 activation and the expression of γ-glutamylcysteine synthase which results in decreased GSH level [108]. On the other hand, PI3K/AKT signalling is involved in the activation of Nrf2 and Nrf2-dependent genes [66] via inhibition of GSK-3beta [104].

## GSH and JNK Pathway

Oxidative stress-induced altered redox potential sensitizes the cells to apoptosis by activation of the MAPK/JNK pathway [69]. The activation of the pro-apoptotic signalling pathway JNK is enhanced by GSH depletion by buthionine sulphoximine (BSO) under oxidative stress [86]. The depletion of GSH with BSO increases hydrogen peroxide in the cell and enhances JNK activation [115]. *N*-acetyl cysteine (NAC), the donor for cysteine moiety of GSH prevents HNE-induced JNK activation, which suggests that GSH plays a crucial role in regulating JNK activation [115]. This is further confirmed as NAC also inhibits drug-induced JNK and ERK2 activation [60].

## Increased GSH Levels in the Cell: Its Beneficial Effects

The increased GSH level by activation of the GCS subunits enhances mitochondrial stability [71]. The oxidative stress-induced caspase 3 activation and apoptosis is prevented by the increased GSH synthesis and GSH level [112]. The increase in intracellular GSH level attenuates methylglyoxal-induced toxicity [63].

The cysteine donor NAC blunts ethanol-mediated toxicity, lipid accumulation, and oxidant stress in CYP2E1-overexpressing E47 cells [124]. NAC exerts, proportional to its concentration, a dual role simultaneously increasing

both proliferation and apoptosis in HepG2 and 3B cells and interestingly, the levels of protein-bound GSH are decreased by NAC [1]. NAC treatment in rats prevents high sucrose-induced oxidative stress, as assessed by blood and tissue GSH and carbonyl levels, glucose intolerance, impaired postprandial glycemic control, and a decrease in muscle and liver insulin-induced activation of insulin receptor substrate 1 and Akt [9].

The impaired synthesis and increased loss and degradation of GSH appear to contribute to a decrease in GSH level in streptozotocin-treated diabetic rat liver [39]. NAC is able to partially protect from oxidative stress and GSH decrease, while enhancing GSH synthesis and restricting GSH loss [39].

The co-administration of NAC, Coenzyme Q10 and the SOD mimetic MnTBAP enhances the expression of mitochondrial complex I subunits, and reduces ROS production, oxidized/reduced GSH ratio, mitochondrial dysfunction and cell death induced by D-galactosamine in the cultured hepatocytes isolated from liver resections [42]. The primary hepatocytes isolated from mice lacking hepatocyte growth factor receptor c-Met (Met-KO) exhibit increased sensitivity to Fas-mediated apoptosis [40]. NAC significantly reduces Jo2-induced cell death and conversely, BSO completely abolishes the protective effects of NAC in Met-KO hepatocytes [40].

UDCA reduces p53 transcriptional activity, thereby preventing its ability to induce Bax expression, mitochondrial translocation, cytochrome c release and apoptosis in primary rat hepatocytes [2]. UDCA treatment leads to a significant increase in the proliferative activity in liver histology in 40 % partially hepatectomized rats [8].

UDCA inhibits both a decrease in the GSH level and an increase in the ROS in HepG2 cells exposed to excessive iron [4]. UDCA increases the gene expression of the catalytic- and modifier-units of glutamine-cysteine ligase (GCL), a key enzyme in GSH synthesis [4]. UDCA increases the GSH synthesis through the activation of the PI3K/Akt/Nrf2 pathway [4].

The pretreatment of cultured rat hepatocytes with UDCA significantly prevents the decrease in viability due to $H_2O_2$ or cadmium administration [77]. The amounts of GSH and protein thiol increase significantly and the mRNA levels of gamma-glutamylcysteine synthetase and metallothionein are significantly higher in UDCA-treated hepatocytes than in controls [77].

UDCA+Vitamin E improves not only aminotransferase levels and liver histology of patients with NASH, but also decreases hepatocellular apoptosis and restores circulating levels of adiponectin [6]. In liver biopsies obtained from NAFLD morbid obese patients undergoing bariatric surgery, miR-34a, apoptosis and acetylated p53 increase with disease severity, while sirtuin 1 (SIRT1) diminishes [15]. UDCA inhibits the miR-34a/SIRT1/p53 pathway in the rat liver in vivo and in primary rat hepatocytes [15].

## Decreased GSH Levels in the Cell: Its Injurious Effects

The BSO-induced GSH depletion enhances mitochondrial structural aberrations by affecting the thiol redox potential, which is prevented by the overexpression of BOLA1, a mitochondrial protein [121]. The treatment of cultured mouse hepatocytes with TNF-alpha plus 0.25 or 0.5 mmol/L DEM leads to incremental cell death in the form of apoptosis associated with increased caspase activities, release of cytochrome c, and DNA laddering [81].

Increased cell death along with increased levels of ROS and mitochondrial 3-nitrotyrosine and 4-hydroxynonenal protein adducts; and decreased mitochondrial aconitase activity and mitochondrial membrane potential are observed in HepG2 lines overexpressing CYP2E1 in mitochondria (mE10 and mE27 cells) treated with BSO as compared with cells transfected with empty vector (pCI-neo) [5]. The BSO toxicity is higher in CYP2E1-expressing E47 HepG2 cells compared to the control cells [53].

The BSO treatment of HepG2 cells that causes marginal GSH deficiency increases ceruloplasmin synthesis due to increased transcription mediated by activator protein (AP)-1-binding site [110]. In higher GSH deficiency (>40 %) with increased ROS generation, ceruloplasmin expression is decreased [110]. Thus, GSH deficiency leads to dual mechanisms in regulation of hepatic iron homoeostasis [110].

BSO treatment causes a significant reduction of the total GSH in liver (−70 %), which is attributable to the diminished levels of the reduced GSH (GSH, −71 %) in rats [12]. The BSO-induced GSH deficiency lowers hepatic triglyceride concentrations via influencing lipogenesis [12]. The authors speculate that the reduced activity of the redox-sensitive protein tyrosine phosphatase (PTP)1B and the higher concentration of irreversible oxidized PTP1B could be, at least in part, responsible for this effect [12].

The treatment of cells expressing cytochrome P450 2E1 (CYP2E1) −E47 cells with BSO results in apoptosis as well as necrosis [124]. The activity of caspase 3, but not caspases 1, 8, or 9, is increased in the BSO-treated E47 cells, and damage to mitochondria appears to play a role in the CYP2E1- and BSO-dependent toxicity, because mitochondrial membrane potential is found to be decreased in the process [124].

The DEM treatment of HepG2 cells causes an immediate and sustained loss of intracellular GSH, with a concomitant increase in GSSG. From 6 to 12 h after exposure, there is a substantial increase in the percentage of cells undergoing S phase arrest and apoptosis [14]. The genes

for inhibitors of the cell cycle (CDKN1, CDKN4D and ATM) are induced, whereas cyclins (proliferating cell nuclear antigen (PCNA), cyclin A, cyclin D1 and cyclin K) are downregulated during the period from 6 to 20 h [14]. Likewise, pro-apoptotic genes such as the caspases (CASP9, CASP3 and CASP2) and apoptotic protease activating factor (APAF) are induced during the same period [14]. However, a study reported that GSH depletion with BSO also activates Nrf2 signalling as an adaptive response in murine embryonic fibroblasts [64].

## Alcohol Metabolism in Liver

The liver functions as the body's major homoeostatic regulator of several cellular components. Alcohol (ethanol) crosses gastrointestinal tract by simple diffusion into the blood stream [35]. Significant amount of ethanol is subjected to the first phase metabolism by gastric mucosal lining [56]. The diffused alcohol is taken to the liver by hepatic vein. Ethanol in liver sinusoids is diffused into the hepatocytes and gets oxidized to acetaldehyde by alcohol dehydrogenase (ADH). The conversion of ethanol to acetaldehyde by ADH utilizes $NAD^+$ and releases NADH [103]. Acetaldehyde is converted to acetate by acetaldehyde dehydrogenase, in mitochondria, which also requires $NAD^+$ and releases NADH. Acetate, an unstable compound, is readily converted into $H_2O$ or $CO_2$.

The ADH-mediated metabolism of ethanol affects the hepatocytes in two ways: cellular redox imbalance through increased NADH production and acetaldehyde–protein adduct formation. The excess release of NADH affects fatty acid metabolism which results in the accumulation of triglyceride in hepatocyte [101]. It has been shown that alcohol-induced free fatty acid accumulation is prevented with the overexpression of Nrf2 [125].

In addition to redox changes, formation of acetaldehyde mediates ADH-induced ethanol metabolic toxicity [24]. Acetaldehyde forms adduct with cellular proteins, membrane lipids and DNA which potentiates liver injury and hepatocellular carcinoma [106]. The increased acetaldehyde also sensitizes the hepatocytes against TNF-α by affecting the GSH transport of mitochondria [68]. It has also been observed that acetaldehyde activates transcription factors—nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and activator protein 1 (AP-1) in liver cells (HepG2) [100].

Under chronic alcohol consumption the microsomal ethanol-oxidizing enzyme—CYP2E1 is activated [67]. The CYP2E1-induced ethanol oxidation to acetaldehyde releases superoxide anion which results in oxidative stress [67]. E47 cells exhibit increased oxidative stress by higher ROS level and lipid peroxidation which results in loss of cell viability [122, 22]. Consistently, CYP2E1-induced oxidative stress is modulated by the CYP2E1 inhibitors such as diallyl sulphide (DAS) and phenethyl isothiocyanate (PIC) in both in vivo and in vitro conditions [80, 16]. Thus, CYP2E1 plays a crucial role in the development of alcoholic liver damage mainly through oxidative stress.

## In Vitro Evidences for Ethanol-Inducible CYP2E1 and ADH Activity

Several in vitro models have been established to study the adverse effects of ADH and CYP2E1 in liver cells. Human hepatoma cells (HepG2) are widely used as an in vitro model to study the effects of several xenobiotics in hepatocytes [37, 38]. Ethanol-induced cellular toxicity has been extensively studied in HepG2 cells transfected with human ADH- and/or CYP2E1-expressing genes.

The effects of acetaminophen toxicity and the role of CYP2E1 in the process have been studied in MVh2E1-9 (CYP2E1-transfected HepG2 cells) cells [26]. The ethanol-induced altered fatty acid metabolism has been studied utilizing the non-transfected HepG2 cells [3].

HepG2 cells, transfected with human CYP2E1-expressing gene, (E47 cells) have been established to study CYP2E1-dependent ethanol hepatotoxicity [122, 22]. The proliferation rate of CYP2E1-overexpressing HepG2 cells is slower than the non-CYP2E1 expressing control HepG2 cells along with increased oxidative stress [22].

The ethanol-inducible CYP2E1-induced generation of ROS and oxidative stress has been well observed in HepG2 E9 cells [122]. HepG2 cells stably expressing CYP2E1 undergo apoptosis, which is not observed in HepG2 cells without CYP2E1 expression under ethanol treatment [122].

It has also been shown that ethanol in CYP2E1-overexpressing HepG2 cells (E47 cells) activates NF-Kappa B and AP-1 [100]. Endoplasmic reticulum dysfunction, which results in aggregation of unfolded proteins, is one of the possible mechanisms of CYP2E1-induced cytotoxicity [28]. Further, CYP2E1-induced cytotoxicity sensitizes the hepatocytes against TGF-beta 1 [128].

The CYP2E1 induced increases oxidative stress in the hepatocytes, activates fibrotic signals by enhancing proliferation of stellate cells and increases collagen type 1 protein [83]. The transcriptional activation of laminin in stellate cells is also enhanced by CYP2E1-induced oxidant stress in the hepatocytes [84]. CYP2E1 under GSH depletion activates ERK MAPK pathway in HepG2 cells [41].

Hence, in order to study the specific effect of combined ADH and CYP2E1 activity on chronic alcohol treatment in hepatocytes, VL-17A cells, which are HepG2 cells overexpressing both ADH and CYP2E1, have been established [31].

## The Role of Hyperglycaemia in Mediating Liver Injury

Oxidative stress is associated with hyperglycaemia-mediated liver injury and as evident from the following studies, the depletion in GSH level forms an important component of the oxidative insult. Obesity and diabetes are associated with non-alcoholic steatohepatitis. Non-alcoholic steatohepatitis and alcoholic steatohepatitis share similar pathogenic mechanisms including increased expression of CYP2E1 [120]. Consistently, increased oxidative stress, and accumulation of malondialdehyde and 4-hydroxynonenal are observed in the hepatocytes of diabetic rats [114]. The generation of ketone bodies under obesity and type 2 diabetes could lead to CYP2E1 induction [99]. Further, high glucose sensitizes HepG2 cells towards apoptosis [18] and induces the expression of CYP2E1 in VL-17A cells [21]. Significant level of increased expression of ADH is also observed under high glucose treatment in VL-17A cells [20].

Reduction in GSH is observed in the liver in streptozotocin (STZ)-induced rats and the oral supplementation of esculetin to diabetic rats for 45 days significantly brings it back near normalcy [93]. The addition of sodium selenate to metformin is able to restore the hepatic GSH back to normal levels in a type 2 diabetes model which was achieved by feeding the rats with high-fat, high-fructose diet for 8 weeks followed by a low dose of STZ [102].

The diminished activities of antioxidant enzymes and reduced GSH in STZ-induced diabetic rats are improved upon the administration of a tetra hydroxy flavone fisetin [94]. Alpha-lipoic acid, a naturally occurring compound possessing antioxidant activity, administration practically normalises the activities of the indicators of hepatocellular injury, alanine and aspartate aminotransferases; lowers oxidative stress, as observed by the thiobarbituric acid-reactive substance assay; restores the reduced GSH:GSSG ratio; and increases the protein sulphhydryl group content in a rat model of STZ-induced diabetes [30]. The total thiol and GSH levels in livers are markedly reduced in untreated STZ-induced diabetic rats; however, these parameters are increased in the diabetic rats following melatonin treatment [61]. The liver from STZ-induced diabetic rats exhibits a significant decrease in GSH and GSH-S-transferase, and treatment with resveratrol abrogates the decrease [46].

The gerbil *Psammomys obesus*, a unique model of nutritional diabetes resembling the disease in humans, displays many metabolic disturbances (hyperinsulinaemia, hyperglycaemia and dyslipidaemia) which is coupled with a decline in GSH and reduced GSH peroxidase activity; and treatment with silibinin alleviates most of the metabolic defects and largely restores antioxidant status [10]. The oral administration of interferon tau decreases ratios of the hepatic oxidized GSH to reduced GSH in Zucker diabetic fatty rats [111].

Oleanolic acid inhibits increased cellular and mitochondrial ROS production in obese diabetic db/db mice and the authors speculate that Nrf2–GCLc-mediated stabilization of mitochondrial GSH pool may be involved in the protective actions of oleanolic acid [118]. However, the hepatic levels of cysteine and its metabolites, such as hypotaurine, taurine, and GSH, are increased despite inhibition of the transsulphuration of homocysteine to cysteine in non-obese type-2 diabetic Goto-Kakizaki rats [57]. The elevated hepatic taurine and GSH levels may be attributed to the upregulation of cysteine dioxygenase expression and the increased cysteine availability for GSH synthesis [57].

Methylglyoxal (MGO) is a dicarbonyl that reacts with amino acids and nucleic acids to form advanced glycation end products, which may contribute to diabetes and its cardiovascular complications [76]. MGO detoxification through the glyoxalase (GLO) pathway is GSH-dependent [76]. Hepatic GSH is 68–71 % lower at 6–12 h following BSO administration to rats, and MGO is 27 % higher at 12 h [76]. At 12 h, hepatic D-lactate is 13 % lower and GLO activity is 52 % lower following BSO, which is fully restored by the exogenous addition of GSH [76]. The hepatic GSH is inversely related to hepatic MGO and positively correlates with the hepatic GLO activity whereas the hepatic GLO activity is positively correlated with the hepatic D-lactate [76]. Thus, GSH depletion in vivo increases the hepatic MGO accumulation by impairing its GSH-dependent, GLO-mediated detoxification to D-lactate independent of oxidative stress [76]. Further, MG is believed to cause insulin resistance by inducing inflammation and pancreas damage [63]. In MG-induced diabetic rats, ankaflavin elevates the GSH levels in liver and pancreas of MG-induced rats [63].

## ADH/CYP2E1 and GSH

In the hepatocytes, chronic alcohol exposure causes an increase in reduced (GSH) and a decrease in oxidized GSH (GSSG) [48]. In contrast, 40 % of loss in GSH is observed in liver of rats fed alcohol and high-fat diet, despite increased expression of transcription factors—NF-kB, ARE and AP-1 which regulate GSH synthesis [70]. The loss of induction of the γ-glutamyl cysteine synthase-light subunit (GCS-LS) is considered to be the responsible factor for loss of liver GSH level even under ethanol treatment [70]. It has also been shown that acetaldehyde plays a major role in the depletion of GSH in the isolated hepatocytes [117]. The mitochondrial depletion of GSH is

prevented under overexpression of Nrf2 in a mouse model of alcoholic liver injury [125].

The CYP2E1-induced oxidative stress increases the intracellular GSH level by increasing the activation of GCS through Nrf2 nuclear translocation [74, 75, 84, 17]. The exogenous induction of GSH depletion causes mitochondrial-dependent apoptosis in CYP2E1-expressing HepG2 cells (E47 cells) [123]. Similarly, GSH is increased in VL-17A cells exposed to high glucose [21] or chronic ethanol [19] and it is increased to an intermediate extent in VL-17A cells subjected to chronic ethanol plus high glucose [20].

## Status of GSH in In Vivo Models

The liver content of GSH is lower in mice fed methionine–choline-deficient diet for 4 and 6 weeks exhibiting induced nonalcoholic fatty liver disease [54]. 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency is a disorder biochemically characterized by the predominant accumulation of 3-hydroxy-3-methylglutarate (HMG), 3-methylglutarate (MGA), 3-methylglutaconate and 3-hydroxyisovalerate in tissues and in vivo administration of HMG and MGA significantly decrease GSH concentration in liver in young rats [25].

The hepatic S-adenosylmethionine, cysteine and GSH levels, reduced in the rats receiving a liquid ethanol diet for 6 weeks, are increased by betaine supplementation [57]. Further, cysteine dioxygenase is downregulated, which appears to account for the increment in the cysteine availability for GSH synthesis in the rats supplemented with betaine [57]. In another study involving ageing rats, doubling the dietary intake of cysteine (free cysteine) through long-term dietary fortification in old rats increases the cysteine and GSH pools in liver thus alleviating age-associated low-grade inflammation and resulting in global physiological benefits [116].

A study demonstrated that the hypocholesterolaemic effect of rice protein is attributable to inducing antioxidative response and depressing oxidative damage in adult rats fed cholesterol-free/enriched diets [13]. After 2 weeks of feeding rice protein in adult rats fed with and without cholesterol, significantly increased hepatic and plasma GSH contents are observed whereas the hepatic accumulation of GSSG is significantly reduced.

Although GSH content does not change significantly in the intact rat liver after intraperitoneal NAC administration, the cysteine content increases rapidly [126]. However, in liver depleted of GSH with DEM, NAC administration restores GSH contents [126]. A similar phenomenon is observed in the perfused rat liver where liver perfusion of DEM-injected rats with NAC restores GSH content [126]. The findings of the study indicate that NAC stimulates GSH synthesis in the GSH-depleted intact liver and in the perfused rat liver [126].

GSH depletion—a primary cause of acetaminophen-mediated injury is significantly attenuated in interleukin-4 knockout (IL-$4^{-/-}$) mice and the authors conclude that IL-$4^{-/-}$ mice are protected from acetaminophen-induced liver injury due to the reduced depletion of GSH, which prevents liver damage and tissue inflammation [91]. One of the most abundant dietary polyphenols—chlorogenic acid reverses acetaminophen-decreased liver GSH levels in mice in vivo [52]. Very long chain (C22–C24) ceramides are synthesized by ceramide synthase 2 (CerS2) and a CerS2 null mouse displays hepatopathy because of depletion of C22–C24 ceramides, elevation of C16-ceramide and/or elevation of sphinganine [89]. Unexpectedly, CerS2 null mice are resistant to the acetaminophen-induced hepatotoxicity accompanied by increased level of GSH [89].

The fatty acid synthase inhibitor cerulenin increases hepatic GSH content in some of the steatotic ob/ob mice administered cerulenin [23]. The d-Galactosamine (GalN) and lipopolysaccharide (LPS) treatment that results in hepatic inflammation and subsequent fulminant hepatic failure in mice decreases the liver GSH content which is attenuated by hemin [haem oxygenase-1 (HO-1) inducer], but zinc protoporphyrin [(HO-1) inhibitor] reverses the effects of hemin [59].

## GSH and Drug Detoxification: the Role of GSH S-Transferases

Glutathione S-transferases (GSTs) are involved in drug detoxification through the conjugation of reactive drug metabolites to GSH [34, 58, 85]. The GSTs catalyze the nucleophilic attack of GSH on the electrophilic centre of a number of xenobiotic compounds, including several chemotherapeutic drugs [58, 85]. Apart from conjugation of xenobiotics to GSH, many other activities are also associated with the GSTs, including steroid and leukotriene biosynthesis, peroxide degradation, double-bond cis–trans isomerization, dehydroascorbate reduction, Michael addition, and noncatalytic ‘ligandin’ activity (ligand binding and transport) [85].

The GSTs are present in different subcellular compartments including cytosol, mitochondria, endoplasmic reticulum, nucleus and plasma membrane [97]. The regulation and function of the GSTs have implications in cell growth, oxidative stress as well as disease progression and prevention [97]. The genetically determined deficiencies in GSTs might be a risk factor for the idiosyncratic adverse drug reactions resulting from the formation of reactive drug metabolites [34]. Altered GST expression has been implicated in hepatic, cardiac and neurological diseases [97].

Mitochondria-specific GSTK has also been implicated in obesity, diabetes and related metabolic disorders [97]. In context of this review, it is interesting to note that studies have shown that silencing the GSTA4 (GST alpha) gene results in mitochondrial dysfunction, as is also seen in GSTA4 null mice, which could contribute to insulin resistance in type 2 diabetes [97].

The ability of four recombinant human GSTs (hGST A1-1, hGST M1-1, hGSTP1-1 and hGST T1-1) to catalyze the GSH conjugation of reactive metabolites of clozapine, formed in vitro by human and rat liver microsomes and drug-metabolizing P450 BM3 mutant—P450 102A1M11H, has been studied [33]. In the presence of three of the GSTs, hGSTP1-1, hGST M1-1 and hGST A1-1, the total GSH conjugation is strongly increased in all bioactivation systems tested [33]. The highest activity is observed with hGSTP1-1, whereas hGST M1-1 and hGST A1-1 show slightly lower activity [33]. Interestingly, the addition of hGSTs results in major changes in the regioselectivity of GSH conjugation of the reactive clozapine metabolite, possibly due to the different active site geometries of hGSTs [33]. The authors conclude that the human GSTs may play a significant role in the inactivation of reactive intermediates of clozapine [33].

hGSTP1-1 is polymorphic in the human population with a number of single nucleotide polymorphisms that yield an amino acid change in the encoded protein [34]. Three allelic variants of hGSTP1-1 containing an Ile105Val or Ala114Val substitution, or a combination of both, have been most widely studied and show different activities when compared to wild-type hGSTP1-1*A (Ile105/Ala114) [34]. The ability of these allelic variants to catalyze the GSH conjugation of reactive metabolites of acetaminophen, clozapine and diclofenac formed by bioactivation in in vitro incubations by human liver microsomes and drug-metabolizing P450 BM3 mutants has been studied [34]. The different hGSTP1-1 mutants show slightly altered regioselectivities in the formation of the individual GSH conjugates of clozapine which suggests that the binding orientation of the reactive nitrenium ion of clozapine is affected by the mutations [34]. For diclofenac, a significant decrease in activity in GSH conjugation of diclofenac 1′,4′-quinone imine is observed for variants hGSTP1-1*B (Val105/Ala114) and hGSTP1-1*C (Val105/Val114) (Dragovic et al.). However, since the differences in total GSH conjugation activity catalyzed by these allelic variants are not higher than 30 %, the differences in inactivation of reactive intermediates by hGSTP1-1 are not likely to be a major factor in determining interindividual difference in susceptibility to adverse drug reactions induced by the drugs studied [34].

In the presence of GSH, the chemotherapeutic drug chlorambucil (CBL) behaves as an efficient substrate for human GSTA1-1 (hGSTA1-1). In the absence of GSH, CBL acts as an alkylating irreversible inhibitor for hGSTA1-1 [58]. The GSTs are known to be overexpressed in tumor, and naturally occurring isothiocyanates, such as benzyl isothiocyanate (BITC), are effective cancer chemopreventive compounds [95]. The presence of the sulphur atom from the isothiocyanate moiety in BITC-SG conjugate is found to be crucial for its irreversible inhibition of GST P1-1 [95].

Icariside II activates the nuclear translocation of Nrf2 and upregulates the expression of Nrf2-related antioxidant protein HO-1 and GST in HepG2 cells [45]. Further, icariside II significantly increases the phosphorylation levels of ERK1/2, Akt and JNK1/2 suggesting that the Nrf2/ARE pathway plays an important role in the regulation of icariside-mediated antioxidant effects in HepG2 cells [45].

The null mutation of GSTM1 and GSTT1 is reported to correlate statistically with an abnormal increase in the plasma levels of alanine aminotransferase or aspartate aminotransferase caused by troglitazone in diabetic patients [119]. The involvement of the human GST isoforms in the GSH conjugation of reactive metabolites of troglitazone using recombinant GST enzymes has been investigated [88]. Five reported GSH conjugates of reactive metabolites are produced from troglitazone after incubation with liver microsomes, NADPH, and GSH in a GSH concentration-dependent manner [88]. The addition of human recombinant GSTA1, GSTA2, GSTM1 or GSTP1 protein to the incubation mixture further increases the GSH conjugates [88]. Thus, the GST isoforms contribute differently to the GSH conjugation of individual reactive metabolites of troglitazone, and GSTM1 is the most important GST isoform in the GSH conjugation of a specific reactive metabolite produced from the cytotoxic, quinone-form metabolite of troglitazone [88].

The role for GSTs in the detoxification of the reactive metabolite—2-ABT-S-oxide (M1) of zileuton, an agent which targets the leukotriene pathway through inhibition of 5-lipoxygenase (5-LO), has been investigated [55]. The non-enzymatic conjugation with M1 and GSH is accelerated in the presence of GSTA1-1, GSTM1-1 and GSTP1-1 [55]. M1 inhibits GSTM1-1 and GSTP1-1 to a greater extent as compared with GSTA1-1 [55]. In the case of GSTA1-1, the inhibition is observed to be reversible, whereas M1 inhibition of GSTM1-1 and GSTP1-1 is found to be irreversible under identical conditions [55]. Thus, the authors demonstrate the presence of GSTM1-1 in liver and conclude that the alkylation and potential irreversible inactivation of this isoform in vivo could contribute to an understanding of the hepatotoxicity associated with zileuton [55].

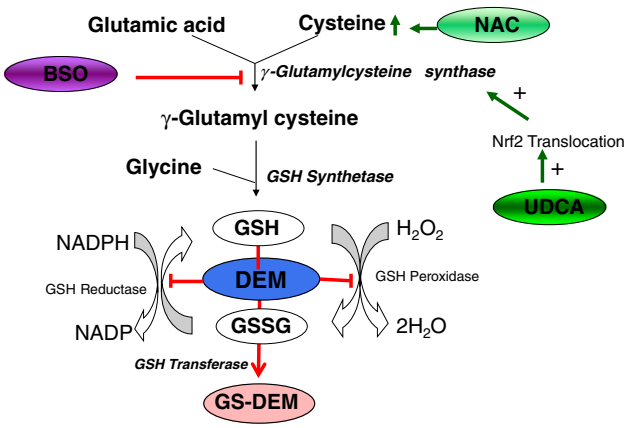


**Fig. 3** Exogenous modulation of glutathione synthesis

## Modulation of Cellular GSH Through Exogenous Agents

The exogenous GSH modulators, such as NAC, BSO, ursodeoxycholic acid (UDCA) and diethyl maleate (DEM), are widely used to alter intracellular GSH in cells [27] and their mechanisms of actions are shown in Fig. 3.

## NAC and UDCA

NAC treatment is found to abrogate acroline-induced hepatotoxicity associated with reduced GSH level, disturbed mitochondrial integrity, endoplasmic reticulum and JNK activation [78]. The long-term exposure of alcohol is found to sensitize the hepatocytes against TNF-induced toxicity by inhibition of Nf-κB associated with increased lipid peroxidation and accumulation of 4-HNE [32]. The treatment of the alcohol-treated hepatocytes with NAC has a protective action against ethanol-induced Nf-κB inhibition by reducing lipid peroxidation-induced 4-HNE accumulation [32].

NAC provides the cysteine precursor for GSH synthesis and increases intracellular GSH level [51]. The ethanol-induced cytotoxicity in CYP2E1-overexpressing HepG2 cells (HepG2 E9 cells) is abrogated by NAC treatment [122]. Further the cytotoxicity of acetaminophen is abrogated by NAC in HepG2 cells [26].

UDCA, a hydrophilic bile salt, enhances the expression and nuclear translocation of Nrf2 thereby increasing expression of GSH synthesizing enzymes and the GSH level through PI3K/Akt pathway [87, 4]. Thus, UDCA prevents $H_2O_2$- or ethanol-induced toxicity in rat hepatocytes by increasing the intracellular GSH level [77, 79]. UDCA also enhances the hepatic membrane stability against oxidative insult [72].

## BSO and DEM

BSO, an inhibitor of the GCS, has been used extensively to study the role of GSH in CYP2E1-induced toxicity in HepG2 cells [44, 75, 73]. The S-alkyl moiety of the sulphoximine binds at the active site of the gamma-glutamyl cysteine synthetase that normally binds the acceptor amino acid [43, 44].

Besides, BSO involved in the depletion of GSH increases oxidative stress in the cells through increased sensitivity of the cells to $H_2O_2$ and several other oxidative stress-inducing agents [27]. GSH depletion with BSO is effective in HepG2 cells overexpressing CYP2E1 than HepG2 cells devoid of ADH and CYP2E1 expression [22]. BSO-induced GSH depletion in CYP2E1-overexpressing HepG2 cells (E47 cells) activates NF-Kappa B and AP-1 [100]. BSO-induced depletion of GSH in CYP2E1-expressing HepG2 cells causes differential induction of apoptosis and necrosis associated with decreased mitochondrial membrane stability and increased caspase 3 activity [123]. The cytotoxicity of acetaminophen is enhanced by BSO in HepG2 cells [107].

Another mode of GSH depletion occurs through GSH transferase-mediated reactions where DEM, an electrophilic reagent and a mildly reactive alpha, β unsaturated carbonyl compound conjugates with GSH which is catalyzed by GSH transferase which leads to the formation of hydrophilic GSH conjugates and causes rapid depletion of GSH [11, 92, 27] [90]. DEM has been shown to cause cytoskeleton disruptions in Clara cells which include cell swelling and membrane bleb formation [90], and disruption in actin and tubulin filaments in hepatocytes [36, 82]. DEM-induced GSH depletion causes lipid peroxidation-induced cell death in hepatocyte [113].

## Future Directions and Perspectives

It would be interesting to investigate whether GSH supplementation or its depletion ameliorates or aggravates hyperglycaemia- or chronic alcohol-mediated oxidative stress and injury in liver, respectively, and to what degree do the GSH-modulating agents affect the damage due to the parent toxins, i.e. high cellular concentrations of alcohol or glucose.

## Conclusions

As evident from the literature, GSH plays a crucial role in maintaining the antioxidant balance and lowering the oxidative stress in the cell. The rate-limiting step in GSH biosynthesis is catalyzed by GCS, and GSH biosynthesis is

regulated by Nrf2 which itself is regulated by several factors. GSH besides attenuating oxidative stress also has several other beneficial effects in the cell which include decreases in apoptosis and methylglyoxal-induced toxicity. The metabolism of alcohol by ADH and CYP2E1 leads to increased acetaldehyde and ROS formation. Several in vitro studies have shown the roles of ADH and CYP2E1 in ethanol metabolism. Further, hyperglycaemia is emerging as an important player in aggravating liver injury. Both hyperglycaemia and chronic alcoholism induce ADH and CYP2E1. Several in vivo studies utilizing animal models have shown the essential role of GSH in lowering cellular oxidative stress. The depletion in the hepatic GSH level is an important mechanism for hyperglycaemia-mediated oxidative injury. Further, impairment of the activity of GSH transferase or polymorphisms in the different isoforms of GSH transferase lead to decreased drug detoxification. The depletion of GSH potentiates CYP2E1-mediated oxidative stress and injury in liver cells. The GSH donors—NAC and UDCA increase the beneficial effects of GSH in different cell culture and animal models and the opposite effects were observed with the agents causing GSH depletion—BSO and DEM. Hence, GSH regulation in the cell occurs through diverse mechanisms with Nrf2 playing a key role in the process.

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