Published in final edited form as:

Curr Drug Metab. 2015 ; 16(6): 486–503.

Diallyl Sulfide: Potential Use in Novel Therapeutic Interventions in Alcohol, Drugs, and Disease Mediated Cellular Toxicity by Targeting Cytochrome P450 2E1

PSS Rao^{1,II}, Narasimha M Midde^{1,II}, Duane D. Miller¹, Subhash Chauhan¹, Anil Kumar², and Santosh Kumar^{1,*}

¹Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163

²Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64108

Abstract

Diallyl sulfide (DAS) and other organosulfur compounds are chief constituents of garlic. These compounds have many health benefits, as they are very efficient in detoxifying natural agents. Therefore, these compounds may be useful for prevention/treatment of cancers. However, DAS has shown appreciable allergic reactions and toxicity, as they can also affect normal cells. Thus their use as in the prevention and treatment of cancer is limited. DAS is a selective inhibitor of cytochrome P450 2E1 (CYP2E1), which is known to metabolize many xenobiotics including alcohol and analgesic drugs in the liver. CYP2E1-mediated alcohol/drug metabolism produce reactive oxygen species and reactive metabolites, which damage DNA, protein, and lipid membranes, subsequently causing liver damage. Several groups have shown that DAS is not only capable of inhibiting alcohol- and drug-mediated cellular toxicities, but also HIV protein- and diabetes-mediated toxicities by selectively inhibiting CYP2E1 in various cell types. However, due to known DAS toxicities, its use as a treatment modality for alcohol/drug- and HIV/diabetes-mediated toxicity have only limited clinical relevance. Therefore, effort is being made to generate DAS analogs, which are potent and selective inhibitor of CYP2E1 and poor substrate of CYP2E1. This review summarizes current advances in the field of DAS, its anticancer properties, role as a CYP2E1 inhibitor, preventing agent of cellular toxicities from alcohol, analgesic drugs, xenobiotics, as well as, from diseases like HIV and diabetes. Finally, this review also provides insights toward developing novel DAS analogues for chemical intervention of many disease conditions by targeting CYP2E1 enzyme.

Keywords

Diallyl sulfide; CYP2E1; alcohol; cancer

*Address correspondence to this author at the College of Pharmacy, University of Tennessee Health Science Center, 881 Madison Ave, Rm 456, Memphis, TN 38163; Tel: 901-448-7157; ksantosh@uthsc.edu.

^{II}Equal contributions

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

1. INTRODUCTION

Garlic (*Allium sativum*) consumption for its medicinal value has been reported worldwide for over 3000 years. Anticancer effects of garlic and its use for cancer treatment have been documented as early as the ancient civilizations of Egypt and India [1, 2]. Studies over the past few decades have extended the scope of beneficial effects associated with garlic use to include antiseptic, antiviral, bactericidal, fungicidal, anti-diabetic, anti-hypertensive, and several other properties.

The increasing popularity of garlic as an essential dietary supplement has led to formulation of several garlic preparations like garlic essential oil, dehydrated powder, oil macerate, and garlic extract. In addition to several enzymes, amino acids, and minerals, garlic is characterized by high content of organosulfur compounds [3, 4]. On average, a garlic bulb contains about 1% alliin, the chief organosulfur compound. Cutting or crushing of garlic releases the vacuolar enzyme allinase, which rapidly converts alliin to allicin, the chief volatile organosulfur compound. Further metabolism of allicin produces lipid soluble allyl sulfur compounds - diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) (Fig. 1) [5].

Interestingly, the lipid-soluble components of garlic are not easily detected in plasma or urine even at high doses as a result of their rapid metabolism. For example, approximately 99% of allicin is subjected to first-pass metabolism within six minutes of exposure to liver tissue [3, 6]. Likewise, rapid metabolism of allicin is known to occur following incubation with blood over five minutes [7]. Due to its high instability, allicin is quickly converted to several allyl sulfides. Of which, DAS is the principle oil-soluble organosulfur compounds obtained from allicin metabolism. DAS has been studied extensively for its contribution towards the beneficial effects attributed to garlic consumption. The other two garlic-derived allyl sulfides, DADS [8, 9] and DATS [10], have also been shown to possess medicinal benefits, however, their usage is associated with potential toxicity. DATS, for example, has been reported for toxic-ity and mortality at relatively high dose in Swiss mice [5]. Similarly, DADS treatment is associated with decreased cell viability in human primary neurons at relatively high concentration [11]. These observations, in part, have placed greater emphasis on examining the beneficial effects of DAS in several pathological conditions.

Although DAS has been studied extensively with regards to its anticancer activity and as a protective agent, there is no comprehensive review on DAS in the past few years. Based on the reported interactions of DAS with drug metabolizing and anti-oxidant enzymes, DAS can be rationalized to serve as an adjuvant therapy for several conditions (Fig. 2). To further elucidate the potential clinical uses of DAS, this review first summarizes the reports on anti-cancer effects of DAS and its role as a protective agent. The review also focuses on the protective effects of DAS on alcohol, analgesic drugs, and other xenobiotics mediated toxicities by targeting cytochrome P450 2E1 (CYP2E1) enzyme. Since CYP2E1 is also implicated in HIV, diabetes, and Parkinson's disease (PD), this review also briefly discusses potential protective effects of DAS in these conditions. Finally, this review describes a strategy for developing DAS analogs, which possess superior inhibitory properties and

lower substrate capability for CYP2E1 than their parent compound. These compounds are expected to have better protective effects than DAS against alcohol, analgesic drugs, HIV and diabetes mediated toxicity.

2. DAS: ANTICANCER EFFECTS

Garlic-derived allyl sulfides are well-known for their anti-cancer properties [12]. Among allyl sulfides, DAS has been extensively studied for its putative role in anticancer properties (Fig. 3A). In colon epithelial cells, Wargovich and Goldberg [13] reported the ability of DAS to inhibit carcinogen-mediated nuclear damage. In a subsequent study, Hayes, *et al.* [14] demonstrated pretreatment with DAS to be associated with an anti-hepatocarcinogenic effect. Similarly, DAS treatment inhibited the activation of tobacco carcinogen nitrogen-derived nitrosoketone (NNK) and NNK-mediated tumor incidence in lung carcinogenesis mouse model [15]. In rats, aflatoxin-1 and N-nitrosodiethylamine-induced formation of liver preneoplastic foci was blocked by dietary intake of DAS [16].

Several studies have delineated the cellular pathways responsible for cancer preventive actions associated with DAS treatment. In HeLa human cervical cancer cells, for example, treatment with DAS resulted in G₀/G₁ cell cycle arrest and increased expression of pro-caspases [17]. In addition, DAS treatment was shown to enhance the cytosolic levels of mitochondrial enzyme cytochrome c and p53. Induction of p53 expression is a well-established mechanism for apoptosis and cell cycle arrest in association with significant DNA damage [18]. Similarly, activation of cellular components that are known to regulate apoptosis were reported in DAS-treated mouse skin tumors [19, 20]. Furthermore, effectiveness of DAS was evaluated in 7,12-dimethylbenzanthracene (DMBA)-induced skin tumors in mouse model. In agreement with previous observations, DAS treatment resulted in significant induction of p53 expression. In addition, an enhanced ratio of proapoptotic protein bax and antiapoptotic protein bcl-2 was reported in DAS treated animals. Moreover, DAS treatment reversed several effects associated with DMBA-mediated skin carcinogenicity, namely induction of p38 mitogen activated protein kinase, increased PI3K expression, and phosphorylation of signaling molecule Akt. Likewise, DAS-mediated changes in mitochondrial signaling pathway were observed in growth inhibition and apoptosis in anaplastic thyroid cancer cells by Shin, *et al.* [21]. Enhanced bax and reduced bcl-2 expression were also reported in association with significant release of cytochrome c from mitochondria to cytosol following DAS treatment in ARO thyroid cancer cells.

Chiu, *et al.* [22] reported DAS-mediated cell cycle arrest and induction of apoptosis in Ca Ski cervical cancer cells. Decreased viability of Ca Ski cells was observed following enhanced expression of cell cycle-regulatory proteins (p21, p27, and p53) and subsequent G₀/G₁ cell cycle arrest. Moreover, DAS treatment resulted in loss of mitochondrial membrane potential, increased ROS production, and significantly enhanced levels of cytosolic calcium ions in Ca Ski cells. Importantly, DAS treatment was associated with increased GADD153 levels, suggesting enhanced ER stress in DAS-treated Ca Ski cells [23].

DAS-induced caspase-mediated apoptosis was reported in human malignant neuroblastoma SH-SY5Y cells [11]. DAS, as opposed to another garlic derived organosulfur compound DADS, was non-toxic to primary neurons, and it decreased cell viability only in neuroblastoma SH-SY5Y cells at the tested concentrations. DAS-induced molecular changes, including enhanced bax/bcl-2 ratio, release of mitochondrial cytochrome c, and intracellular calcium levels were also observed in DAS treated SH-SY5Y cells. In addition, compared to control cells, DAS treatment significantly increased expression and activity of Ca^{2+} -dependent protease calpain, a known mediator of apoptosis [24]. Moreover, in SH-SY5Y cells, DAS-mediated increase in levels of pro-apoptotic protein Smac/Diablo and reduced expression of inhibitor of apoptosis proteins (c-IAP1 and c-IAP2) were observed. These cellular changes were accompanied by activation of intrinsic caspase cascade in DAS treated cells. Increased activity of caspase-9 and caspase-3 was accompanied by enhanced levels of active fragments for caspase-9 and caspase-3 in DAS treated neuroblastoma SH-SY5Y cells.

DAS treatment was also found effective in treating murine WEHI-3 leukemia cells [25]. DAS treatment decreased cell viability of WEHI-3 cells in a dose-dependent manner. Following administration of WEHI-3 cells in mice, compared to control animals, an increase in tissue weight of liver and spleen was observed along with significant changes in cell markers of white blood cells (Mac-3, CD11b, CD3, and CD19). Importantly, these changes were reversed following DAS treatment. In addition to decreasing the WEHI-3-mediated increased tissue weight of liver and spleen, histopathology of spleen showed a reduced occurrence of neoplastic cells in DAS+WEHI-3 mice in comparison to WEHI-3 treated animals.

In human colon cancer colo 205 cells, exposure to DAS inhibited migration and invasion of these cancer cells [26]. Interestingly, in addition to decreasing the expression of signaling molecules like PI3K, ERK1/2, JNK1/2, and p38, DAS treatment exhibited attenuated levels of matrix metalloproteinases (MMPs). MMPs are extracellular matrix remodeling endopeptidases that are known to play a critical role in tumor growth, invasion, and metastasis [27]. DAS treatment demonstrated a dose dependent inhibition towards expression of MMP-2 and -7 in colo 205 cells indicating its potential anti-metastatic properties. In animal model for familial adenomatous polyposis and sporadic colorectal cancer, DAS significantly reduced the incidence of colonic polyps in a dose-dependent manner [28]. Furthermore, results from a meta-analysis demonstrated a clinical association between high garlic intake and decreased risk for stomach and colorectal cancers [29].

In a study that examined early molecular events following exposure of SKH-1 hairless mice to ultraviolet B radiation (UVB), topical application of DAS demonstrated cancer preventive effects [30]. As reported in other models of cancers, protective effect of DAS against photocarcinogenesis by UVB radiation was marked by decreased level of key mediators of inflammation including nuclear factor κB (NF κB), cyclooxygenase-2 (COX-2), prostaglandin E2, and nitric oxide compared to non-treated UVB-exposed animals. In addition, DAS pretreatment was marked by suppressed cell proliferation following exposure to UVB.

In addition to the above mentioned mechanisms, altered expression and/or activity of CYP2E1 has also been implicated in carcinogenesis. In particular, genetic predisposition *via* inheritance of specific CYP2E1 polymorphism or overexpression of CYP2E1 mRNA have been observed in clinical samples [31–34]. CYP2E1-mediated metabolism has also been implicated in generating carcinogenic DNA adducts, further underscoring the importance of this metabolic enzyme in carcinogenicity [35]. Based on these observations, DAS-mediated inhibition of CYP2E1 (discussed in section 5) can be postulated as an additional mechanisms regulating its anticancer effects.

3. PROTECTIVE EFFECTS OF DAS

In addition to studies reporting anti-cancer properties of DAS, several studies have indicated enhanced survival and protective effects following DAS treatment (Fig. 3B). For instance, protective effects of DAS treatment were observed in N-nitrosodiethylamine (NDEA)-induced liver tumorigenesis [36]. While NDEA treatment compromised several indices of liver function, DAS treatment normalized all non-enzymatic and enzymatic liver functions affected by NDEA. Importantly, DAS blocked the formation of free radicals in liver and restored Glutathione-S-transferase (GST) activity thereby reestablishing the redox homeostasis. In Wistar rats, DAS was found to be protective against gentamicin induced-nephrotoxicity [37]. While gentamicin treatment inhibited activity of major antioxidant enzymes (AOEs) in kidney of treated rats, DAS treatment (in both presence and absence of gentamicin) was marked by increased activity for AOEs. Moreover, DAS-treated animals exhibited decreased immunohistochemical staining for tumor necrosis factor (TNF)- α and NF κ B in renal tissues. These protective antioxidant effects of DAS were attributed to enhanced expression of transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in DAS-treated Wistar rats.

Nrf-2-mediated antioxidants effects of DAS were also observed in rat lung and MRC-5 lung cells [38]. Through modulation of Nrf2 expression and subsequent nuclear translocation in rat lung, DAS treatment was associated with significant upregulation in activity and transcription of several antioxidant enzymes compared to untreated animals. Increased enzyme activity was observed for GST, glutathione reductase, and catalase, while increased transcription of superoxide dismutase (SOD), glutathione peroxidase, and catalase were reported in DAS-treated animals. In addition, DAS-treated rats exhibited increased GSH/GSSG ratio suggesting increased pulmonary antioxidant capacity or reduced oxidative stress. Interestingly, DAS treatment was also associated with enhanced protein levels of heme oxygenase-1 (HO-1), an enzyme responsible for cellular heme metabolism, in lungs. Furthermore, investigations employing human embryonic MRC-5 cells confirmed that DAS causes nuclear translocation of Nrf2, which is regulated by enhanced phosphorylation of signaling molecules p38 MAPK and ERK.

Anti-inflammatory effects of DAS were further highlighted in a study conducted with rat aortic smooth muscle A7r5 cells [39]. Pretreatment with DAS was shown to block TNF- α - and histamine-mediated inflammatory responses. Specifically, DAS pretreatment attenuated TNF- α -induced enhanced expression of TNF- α and in-terleukin (IL)-1 β transcription in A7r5 cells. In addition, DAS treatment inhibited TNF- α -mediated nuclear translocation of

p65, a subunit of NF κ B, along with decreased expression of TNF-receptor-associated death domain (TRADD) and TNF receptor-associated factor 2 (TRAF2). Inhibition of TRADD and TRAF2 by DAS concurrent with blocked NF κ B signaling contributed to an anti-inflammatory response. Histamine-induced inflammation, on the other hand, was inhibited by DAS *via* modulation of ROS production. In addition, DAS was found to inhibit histamine-induced upregulation of PI3K and Akt expressions and their downstream signaling proteins NF κ B and activator protein-1 (AP-1). Importantly, DAS pretreatment induced upregulation of Nrf2 expression, which was reported to be the critical molecular change responsible for the antioxidant effects observed in A7r5 cells.

DAS-mediated anti-inflammatory effects were also found to be effective in animal model studying bleomycin-induced pulmonary fibrosis [40]. In rats exposed to bleomycin, DAS treatment normalized the activity of several AOE and restored glutathione levels in rat lungs. In addition, DAS blocked the bleomycin-induced increase in lipid peroxidation and myeloperoxidase activity thereby functioning as an effective anti-oxidant. Importantly, compared to bleomycin control, concurrent administration of DAS significantly reduced the bleomycin-mediated pulmonary collagen accumulation and fibrotic changes. These pulmonary changes were associated with significant reduction in expressions of iNOS, NF κ B, TNF- α , and IL-1 β in DAS+bleomycin animals compared to bleomycin control animals.

The beneficial anti-inflammatory effects of DAS were also observed in chondrocytes and synovial cells of osteoarthritis patients as reported by Lee, *et al.* [41]. Monosodium urate crystals and IL-1 β treatments were found to upregulate COX-2 expression in cultured human chondrocytes and synovial cells, a characteristic feature of joint inflammatory condition. DAS treatment, however, blocked the induction of COX-2 expression caused by monosodium urate crystals or IL-1 β in chondrocytes and synovial cells. The inhibition of COX-2 upregulation by DAS was via attenuation of NF κ B activation. Furthermore, DAS was effective in blocking Cox-2 upregulation, acute synovitis, and infiltration of polymorphonuclear leukocytes in rat knee joint following direct knee joint cavity injection of monosodium urate crystals.

Antioxidant properties of DAS were found to play a protective role in the rat model of hepatic ischemia-reperfusion injury [42]. While warm ischemia-reperfusion was associated with elevated plasma levels of liver enzymes (ALT, AST, LDH), markers for liver injury, DAS pretreatment, on the other hand, inhibited the increase in plasma concentrations of these liver enzymes. In addition, DAS pretreatment abolished the enhanced levels of lipid peroxidation markers (lipid hydroperoxides and 15-isoprostane-F_{2t}) in plasma and liver samples following ischemia-reperfusion injury in rats. To compensate for the ischemia-reperfusion-induced enhanced oxidative stress, DAS-pretreatment was marked by enhanced hepatic total glutathione content and heme oxygenase-1 expression as compared to vehicle-treated rats. Finally, histological analysis confirmed the protective effects of DAS against hepatic ischemiareperfusion injury marked by improved lobular and cellular structures.

DAS appears to function as a protective agent through multiple mechanisms. This is evident from a report, in which, a different mechanism for DAS-mediated protective effects has

been reported by Green, *et al.* [43]. In this study, DAS treatment was associated with increased transcription of genes regulating nucleotide excision repair in diethylstilbestrol-induced breast tissues cancer. The mRNA levels of Gadd45a, proliferating-cell nuclear antigen (PCNA), and DNA polymerase delta were significantly upregulated in breast tissues collected from DAS and DAS+diethylstilbestrol-treated ACI rats as compared to control animals. By stimulating the nucleotide repair machinery modulated by these genes [44–46], DAS was postulated to reduce the occurrence of DNA adduct formation (anti-carcinogenic). In later studies, protective effects of DAS against chemical induced-DNA damage were confirmed in human breast epithelial cells [47, 48].

In mice exposed to whole-body irradiation, DAS treatment was found to significantly enhance animal survival from 0% to 37% [49]. Furthermore, the protective effect of DAS against sub-lethal dose of irradiation was marked by significant improvement in hematopoietic function as indicated by increased spleen colonyforming units and bone marrow cell survival compared to non-treated animals. Improved lymphocyte counts following DAS treatment validated the enhanced functioning of hematopoietic system.

Neuroprotective effects of DAS against transient cerebral ischemia were observed in an *in vivo* study by Lin, *et al.* [50]. In rats subjected to focal cerebral ischemia for 2h followed by reperfusion for 24h, DAS pretreatment for 7 days before ischemia/reperfusion significantly lowered the infarct volume compared to non-treated rats. Furthermore, decrease in number of TUNEL (terminal dUTP nick end labeling)-positive cells was observed in DAS pretreated rats suggesting reduced apoptosis following DAS treatment. Immunohistochemistry and Western blot analysis revealed a decrease in caspase-3 expression, a hallmark for ischemic cell apoptosis, and increase in anti-apoptotic bcl-2 expression in DAS-pretreated ischemia/reperfusion-induced rats compared to control animals.

CYP2E1 inhibition, on the other hand, was found to be responsible for the protective effects of DAS against chemical-induced hepato- and immune-toxicities [51–53]. While exposure to various chemicals in these studies was associated with signs of liver damage, DAS treatment was observed to block the chemical induced-hepatotoxicity. Similarly, suppression of antibody response associated with chemical-induced toxicity was reversed in animals pretreated with DAS. Inhibition of CYP2E1 expression and associated bioactivation/metabolism of hepatotoxic agents was identified as the underlying mechanism for DAS-mediated protective effects. The beneficial effects of DAS via inhibition of CYP2E1-mediated metabolism have been further discussed in section 5.

4. DAS TRANSPORT AND METABOLISM

As described in the above sections, anticancer, antioxidant, and anti-inflammatory effects of DAS are mainly attributed to signal transduction and regulation of gene expression. However, recent studies have reported a significant interaction of DAS with drug transporters and metabolic enzymes as the underlying mechanism for its beneficial effects. DAS-induced changes in pharmacokinetic profile of drugs are mediated through changes in expression/activity of efflux transporters and phase I/phase II drug metabolizing enzymes.

4.1. Efflux Transporter

Efflux transporters are membrane bound proteins responsible for active transport of drugs from inside the cells to extracellular space. These transporters are implicated in suboptimal therapeutic efficacy and development of drug resistance over time [54]. Increased expression of multidrug resistance proteins (MRP) and P-glycoproteins (P-gp) has been reported in several types of cancers. Modulation of activity/expression of these transporters by DAS can be expected to facilitate altered intracellular drug concentration, which would eventually alter drug efficacy and cellular toxicity. For example, DAS-mediated decreased activity/expression of these transporters would significantly enhance the drug efficacy as well as cellular toxicity due to increased plasma concentration of drugs.

Although only few studies have examined the effects of DAS on transcription and expression of efflux drug transporters, it is postulated that DAS-mediated changes in expression of these transporters could play a significant role in conferring drug resistance. For instance, a study conducted on colo 205 cells reported moderate effect of DAS treatment on transcription of drug efflux transporters [55]. Transcription of MRP1, MRP3, and MRP4 genes were significantly altered in DAS-treated cells compared to untreated cells. *In vivo* study, however, demonstrated no change in expression of MRPs in subcutaneously implanted colo 205 cells but a significant upregulation in Mdr1 gene expression in DAS treated animals.

Importantly, effects of DAS treatment on changes in the expression of multidrug resistance protein-3 (MRP-3) have received particular attention. DAS mediated enhanced transcription of MRP-3 was observed in rodent models [56]. Specifically, multifold increase at mRNA and protein levels of MRP-3 were reported in liver samples from DAS-treated rats in comparison to untreated animals [57]. In addition, DAS treatment was associated with enhanced transcription of MRP-3 gene in the kidney. Overall, these results suggested the possible role of DAS-induced MRP-3 mediated enhanced hepatovascular excretion of compounds in clinical settings. A subsequent study examining *in vivo* effects of DAS in mice reported enhanced transcription of Mrp-3, Mrp-5, and Mrp-7 in liver [58].

Interestingly, Arora, *et al.* [59] have reported DAS mediated reversal of P-gp-induced multidrug resistance. In human leukemia K562 cells, exposure to DAS sensitized the otherwise resistant cells to the effects of vinblastine treatment. Although vinblastine resistant K562 cells demonstrated higher expression of P-gp compared to vinblastine sensitive cells, DAS treatment of vinblastine resistant cells attenuated the expression of P-gp thereby enhancing the cytotoxic effects of vinblastine. Further, immunohistochemistry and Western blot analysis of liver samples from vinblastine-treated mice confirmed the effects of DAS. DAS treatment blocked the vinblastine-induced enhanced expression of P-gp expression in the liver. In summary, this study demonstrated the beneficial effects of DAS in preventing drug resistance to anticancer drugs. Thus, attenuation of P-gp expression by DAS would significantly enhance the drug concentration in tumors thereby maximizing the treatment outcomes.

4.2. Drug Metabolizing Enzymes

Cytochrome P450s (CYPs) are the major subset of enzymes that constitute the phase I drug metabolizing system and catalyze formation of polar metabolites from parent drug compounds [60]. Phase II enzymes, on the other hand, catalyze conjugation of drug metabolites with endogenous substrates to facilitate drug excretion [61]. Modulation of expression of either phase I or phase II enzymes by DAS can therefore be expected to drastically change the drug efficacy and toxicity. In addition, DAS-mediated altered catalytic activity of these enzymes is expected to manifest a variety of drug specific effects through the formation of an active metabolite or toxic intermediates.

Organosulfur compounds found in garlic are known inducers of phase I and phase II drug metabolizing enzymes [62]. In comparison to other tested preventive agents, microarray analysis has revealed that DAS treatment alters the maximum number of genes in rat liver [62]. Specifically, DAS has been shown to selectively induce specific CYP enzymes including CYP3A1/2 and CYP2B1/2 [63]. In addition, DAS-mediated enhanced transcription of CYP2B1/2 and CYP2B10 has been reported in rodents [56, 58]. Subsequent *in vivo* studies have demonstrated the involvement of constitutive androstane receptor (CAR) and Nrf2 in induction of these CYPs [64].

CAR-mediated enhanced transcription of SULT1E1 gene, which encodes for phase II metabolizing enzyme estrogen sulfo-transferase, was observed in DAS-treated mice [65]. In another study, activity of phase II enzymes UDP-Glucuronosyltransferase (liver and kidney), microsomal epoxide hydrolase (liver), and glutathione-S-transferase (liver, kidney, and intestine) were significantly enhanced in tissue samples from DAS-treated rats compared to control animals [66]. Furthermore, tissue specific enhanced expression and activity of glutathione-S-transferase enzyme subunits (intestine, lung, and kidney) were observed in DAS treated rats.

The most important interaction of DAS with drug metabolizing enzymes, however, is with CYP2E1. Studies have shown that protective effects of DAS against cellular toxicity is mediated via inhibition of CYP2E1 activity/expression. The following two sections (5 and 6) discuss the role of CYP2E1-mediated protective effects of DAS.

5. DAS: PROTECTION AGAINST ALCOHOL, ANALGESIC DRUGS, AND OTHER XENOBIOTICS MEDIATED CELLULAR TOXICITY

CYP2E1, a key monooxygenase enzyme, is involved in the metabolism of several endogenous substrates and more than 85 xenobiotics [67]. CYP2E1 isoform contributes to the hepatotoxicity and cancers by metabolic activation of xenobiotics. A well-documented role of CYP2E1 is its involvement in ethanol metabolism, which subsequently leads to oxidative stress [68] (Fig. 4). The induction of CYP2E1 by ethanol that further increases free radical generation through alcohol metabolism causes liver injury and cirrhosis. Moreover, CYP2E1 metabolizes many low molecular industrial solvents and tobacco-specific carcinogens. This bio-activation leads to pronounced formation of toxic secondary metabolites and reactive oxygen species (ROS), which stimulate lipid peroxidation, protein inactivation, and DNA damage ultimately promoting cellular damage. This cellular toxicity

could be alleviated by developing agents that can confer protection against substances or drugs metabolism/activation by CYP2E1.

DAS has attracted a particular interest as a potential therapeutic or prophylactic agent because of its inhibitory actions on CYP2E1-mediated metabolic activation of various chemicals and carcinogens [69]. Organosulfur compounds present in garlic, including DAS, are capable of inhibiting CYP2E1 activity and expression [70]. However, the efficacy of these compounds has not been adequately assessed. In addition, other organosulfur such as DADS and DATS are relatively more toxic than DAS. As discussed in previous sections, most of the studies examining the effects of DAS are primarily focused on its ability to counter tumor growth or chemically-induced tumorigenesis. Even though, several studies suggest the obvious role of DAS on CYP2E1, extensive investigations are necessary to further pursue these compounds as adjunctive therapies or dietary supplement. In this section, protective effects of DAS against CYP2E1-dependent alcohol liver diseases, acetaminophen hepatotoxicity and other xenobiotic-induced cellular toxicity have been reviewed (Fig. 4).

5.1. Alcohol

Alcohol is a principal etiological agent to cause chronic liver diseases. Alcohol dehydrogenase (ADH), aldehyde dehydrogenase, catalase, and CYP2E1 are involved in metabolizing low doses of ethanol. However, CYP2E1 plays more important role in alcohol oxidation at higher doses or in chronic users [71]. CYP2E1-mediated oxidizing process drives the formation of metabolites such as acetaldehyde and ROS, which significantly contribute to the clinical and pathological spectrum of ethanol-associated hepatotoxicity. When alcohol is consumed while taking medication, the generated reactive metabolites can activate or induce CYP2E1 metabolism of drugs to further enhance the production of ROS such as O_2^- , $OH^\cdot$, H_2O_2 and hydroxyl ethyl radicals, which in turn contribute to the adverse biological responses through oxidative stress and lipid peroxidation [72–74]. These reactions subsequently lead to alcohol induced liver damage that including alcoholic fatty liver (steatosis), alcoholic fibrosis, steatohepatitis, cirrhosis and hepatocellular carcinoma [75]. CYP2E1 has been indicated to play a major role in all these conditions although the mechanism is complex and still remain unclear. For example, CYP2E1 regulates the liver triglyceride metabolism by influencing three independent pathways: peroxisome proliferator-activated receptor α (PPAR- α)-mediated fatty acid oxidation, sterol regulatory element-binding protein-1c (SREBP-1c)-regulated hepatic fatty acid synthesis and autophagy-mediated lipid decomposition [76–78]. PPAR- α and SREBP-1c are two important nuclear transcription factors that maintain fatty acid homeostasis in the liver. Moreover, recent studies suggest that CYP2E1 contributes to the ethanol-induced hypoxia and liver fibrosis through activating hypoxia-inducible factor-1 α (HIF-1 α) mediated apoptosis, a key regulator of oxygen homeostasis in mammalian cells [79, 80]. Collectively, these investigations indicate that ethanol oxidative metabolism through CYP2E1 dependent pathway and associated oxidative stress manipulates transcriptional regulation of several genes that are necessary to increase hepatic injuries.

Some studies suggested that in *in vitro* conditions the microsomal ethanol oxidizing system (MEOS), which contains CYP2E1, oxidizes only a small amount of ethanol which can be correspond to less than 20 % of the *in vivo* rate of ethanol metabolism in the rats [77, 81]. On the other hand, Matsumoto, *et al.* [82] using perfused rat liver demonstrated that ADH and CYP2E1 are responsible for 60% and 40% of metabolism of intravenously administered low doses of ethanol in the liver, respectively. This suggests that CYP2E1 plays a predominant role in alcohol metabolism irrespective of the concentration of ethanol in the system. This study also found that perfusion of DAS to ethanol administered animals significantly reduces the hepatic elimination of the drug, indicating the importance of DAS as CYP2E1-led metabolic pathway inhibitor. When rats were fed ethanol with DAS for one month, DAS decreased the CYP2E1 mRNA levels as well as distribution of CYP2E1 protein levels in the liver [83], indicating the effect of DAS on the rate of CYP2E1 transcription and its localization. Another study using the similar intragastric model of ethanol feeding revealed that administration of fresh garlic juice for 8 days significantly attenuates the concentrations of metabolites and activities of enzymes of MEOS [84]. Moreover, alcohol ingestion also altered the CYP2E1-dependent fatty acid metabolism and lipid peroxidation, and feeding these animals with DAS partially restored the ethanol-induced changes in fatty acid composition and fractions in liver [83, 85]. Consistent with this report, an *in vitro* study using hepatocytes from pyrazole-treated rats demonstrated that DAS alone diminishes the arachidonic acid, a representative of polyunsaturated fatty acids, toxicity [86]. This report also showed that DAS can lower the synergistic toxicity of arachidonic acid and salicylate, a substrate of CYP2E1 that is used as an antiinflammatory and analgesic agent, further confirming the ameliorating ability of DAS on CYP2E1-associated ROS generation and lipid peroxidation. Furthermore, exposing ethanol-treated human primary hepatocytes to DAS partially protected the cells from cellular damage by inhibiting CYP2E1 [87].

The current pharmacologic treatments of alcoholic steatosis or steatohepatitis have their limitations with regard to therapeutic efficacies or associated adverse reactions. Progress in the development of drugs to treat alcoholic steatosis is very sluggish due to poor understanding of the disease pathogenesis. The antioxidants potential for treatment strategy has generated considerable attention in the researchers because oxidative stress is a critical underlying mechanism for alcohol induced liver diseases. Two randomized double blinded and placebo controlled clinical studies compared corticosteroids with an antioxidant mixture (vitamins C and E, biotin, selenium, zinc, manganese, copper, magnesium, folic acid, methionine, allopurinol, desferrioxamine, N-acetylcysteine, and β -carotene) to determine whether the antioxidant therapy improves the survival of the patients with alcoholic hepatitis [88, 89]. The broad antioxidant cocktail alone or in combination with corticosteroids did not show any beneficial effect to the patients. In contrary, some other investigations showed that antioxidants such as allopurinol, diphenyleneiodonium sulfate, ebselen, S-adenosylmethionine, or metadoxine prevents alcohol induced injury or improves the recovery of fatty liver conditions [90–94]. Even though further studies are needed to confirm the benefits of antioxidants to treat alcoholic liver diseases, these animal and clinical studies substantiate the importance of targeting oxidative stress pathways to treat alcohol induced liver injuries.

Importantly, a recent study revealed that garlic oil in combination with metadoxine dramatically eliminated the accumulation of fat and induction of CYP2E1 in the liver of ethanol-fed rats [95], indicating promising prospects of garlic organosulfur compounds such as DAS in developing combination therapies for alcoholic steatosis. Likewise, chronic ethanol treated human hepatoma VL-17A cells that over express CYP2E1 were rescued from oxidative stress, toxicity, and aldehyde protein adduct formation upon exposure to 10 μ M DAS [96]. Interestingly, DAS alone showed a substantial recovery from the ethanol-induced cell injury than pyrazole – an ADH inhibitor or 4-methyl pyrazole – a combined inhibitor for CYP2E1 and ADH. Consistently, this study also reported a maximal reduction in ROS production and aldehyde adduct formation with DAS treatment alone compared with pyrazole or 4-methyl pyrazole. This further supports the pivotal role played by CYP2E1 in ethanol metabolism and the importance of DAS to alleviate associated oxidative stress and lipid peroxidation. In contrary to these findings, Ronis, *et al.* [97] showed that ethanol-induced increases in oxidative and ER stress were aggravated by ethanol and DAS cotreatment even though this treatment inhibited ethanol mediated CYP2E1 activity and apoprotein expression levels. In addition, ethanol and DAS co-exposures decreased the hepatic triglyceride content, however, there were no changes in the liver pathology scores and serum alanine amino transferase values. These observations indicate the involvement of other factors in ethanol-induced cellular stress. Nonetheless, the discrepancies in the reported outcomes can be solved by characterizing CYP2E1-dependent ethanol metabolism in great detail and portraying the complete specificity of DAS as a CYP2E1 inhibitor.

Current investigations are mainly focused on identifying molecular mechanistic link between alcoholic fatty liver and CYP2E1 [73, 98]. Fatty liver has mainly been attributed to the imbalances in the liver fatty acid metabolism and redox conditions. A latest study demonstrated that *in vitro* and *in vivo* garlic oil treatment salvages ethanol-induced alterations in transcriptional regulators such as PPAR- α and SREBP-1c, and diminishes CYP2E1 protein levels [99], suggesting the protective effects of garlic constituents such as DAS. However, whether the ameliorating effect of garlic oil on alcoholic steatosis is due to inhibition of CYP2E1 alone and/or directly inhibiting PPAR- α and SREBP-1c proteins remain unclear. This report also showed a significant inhibition of ethanol-induced mitochondrial dysfunction by garlic oil via decreasing mitochondrial malondialdehyde and increasing glutathione levels. Similarly, another report using cells that express CYP2E1 carrying 18R/L11R/L17R mutation, a mutant predominantly targeted to mitochondrion, showed that CYP2E1 is vulnerable to alcohol-stimulated toxicity, and this CYP2E1-mediated metabolism causes the dysfunction of cytochrome c oxidase [100]. In this report authors also demonstrated the effective reversal of alcohol-induced toxicity by treating these cells with DAS and MitoQ, a mitochondrion targeted antioxidant. This report further supports the pronounced role of CYP2E1 in eliciting alcohol-induced cell injury and possibility of DAS as a treatment option for ethanol cell and tissue damage. In addition, accumulation of 4-hydroxynonenal (4-HNE) adducts inhibits proteasome activity in alcoholic liver hepatitis, and CYP2E1-induced oxidative stress and lipid peroxidation have been shown to be associated with 4-HNE adduct formation [101]. Along these lines, one study revealed that alcohol treatment stimulates the formation of Mallory bodies, damaged

intermediate filaments within the hepatocytes, by inhibiting CYP2E1 activity and this inhibitory effect is significantly attenuated by DAS treatment [102].

The ethanol intake-induced cellular toxicity appears to happen through alterations of regulation of CYP2E1 at various levels that includes transcriptional, translational, and post-translational modifications [103]. Accordingly, we reported that the protein kinase C/c-Jun N-terminal kinase/specificity protein1 (PKC/JNK/SP1) pathway is involved in ethanol-mediated CYP2E1 induction in U937 monocytic and SVGA astrocytic cells [104]. In this study we incubated the cells with 100 mM ethanol, which were pretreated with 100 μ M DAS, and observed the effects of DAS on various markers of oxidative stress and cellular toxicity. Alcohol upregulated CYP2E1 expression and production of ROS, and DAS inhibited/abolished ethanol-mediated ROS and cleavage of caspase-3 activity. In addition, DAS inhibited ethanol-induced DNA damage (TUNEL) and cellular toxicity (MTT). Overall, the study suggested a potential role of DAS in inhibiting ethanol-mediated toxicity in extra-hepatic cells by inhibiting CYP2E1-mediated alcohol metabolism and subsequent oxidative stress. The critical role of CYP2E1 in alcohol oxidation and the preventive role of DAS, especially in non-hepatic cells, has further been investigated in neuronal cell lines and animal brain homogenates [105–107]. In this study, ethanol pretreated rat or brain homogenates when exposed to 2 mM DAS significantly diminishes alcohol-derived acetaldehyde and acetate formation [107]. This study further supports the importance of DAS to counteract CYP2E1-mediated metabolic toxicity. The distinct role of alcohol in contributing towards neurological/neurocognitive diseases, such as neuroAIDS [74, 108], concurrent with the role of CYP2E1 in brain alcohol metabolism further emphasizes the potential use of DAS as adjuvant therapy in treating these diseases effectively.

A recent study employing isocaloric pair-feeding ethanol model administered 100 mg/kg/day DAS to mice and examined its effects on ethanol-stimulated alcoholic cardiomyopathy with emphasis on apoptotic stress signaling [109]. Cardiomyocytes isolated from DAS injected animals showed a significant decrease in CYP2E1 protein levels as well as activity, validating the beneficial effects of DAS to inhibit or inactivate CYP2E1. In addition, DAS prevented or reduced the chronic ethanol-induced myocardial contractile dysfunction, intracellular calcium alterations, and diminished free radical production and apoptotic proteins [109], indicating the promising role of DAS as a potential CYP2E1 inhibitor to manage alcoholic cardiomyopathy. Taken together, these investigations provide evidence that favors DAS as an effective natural molecule against alcoholism and to alleviate ethanol-elicited oxidative stress and cellular damage.

5.2. Analgesic Drugs

Acetaminophen (APAP), a popular analgesic remains a leading cause of acute liver injury in the United States. APAP combination therapies and overdose are known causes for this hepatotoxicity [110, 111]. APAP toxicity in children is a major concern because of its heavy usage as an antipyretic agent and also due to its inadvertent overdosing. According to the data from American Association of Poison Control Centers and adverse reactions reported to the Food and Drug Administration, APAP-associated deaths have been increasing rapidly than other over-the-counter drugs like aspirin and ibuprofen [112]. Usually, physicians

recommend APAP to the pregnant women and to the newborn children assuming that this drug is effective and less toxic than other analgesics. For example, some selected studies compared the adverse effects of APAP with ibuprofen and showed that APAP increases the incidences of asthma morbidity [113] and adversely effects the neurodevelopment in children [114]. Moreover, a recent cross-sectional veterans aging cohort study using HIV+ positive patients revealed that this population is at risk of APAP-induced hepatotoxicity [115]. All these observations point out that the APAP-linked toxicity may be due to the metabolites of APAP.

Although phase II reactions glucuronidation and sulfation account for predominant elimination of the APAP from the system, bioactivation of APAP to toxic metabolite is generated by CYP2E1 [116]. Oxidation of APAP by CYP2E1 produces N-acetyl-P-benzoquinone Imine (NAPQI), a highly reactive electrophile, which causes liver injury [117]. At appropriate dose of APAP, NAPQI is generally eliminated through the conjugation with GSH. However, in overdosing conditions it depletes the systemic GSH levels causing hepato- and renal- toxicity [118]. Interestingly, a recent report demonstrated that even normal dose of APAP is linked to APAP-associated acute liver failure [119]. APAP-induced toxicity can be exacerbated by other factors including alcohol abuse, concurrent drug usage or fasting [119–121]. Thus, there a need to explore CYP2E1-mediated APAP metabolism and develop antidotes that can counteract hepatotoxicity by inhibiting CYP2E1.

Several investigations have showed the protective effects of DAS and other organosulfur compounds against APAP-induced toxicity [122, 123]. DAS (50 mg/kg) administration prior to or shortly after the treatment of APAP significantly protected liver injury in Fisher 344 rats [124], primarily due to the inhibition of CYP2E1. The same group also demonstrated that diallyl sulfone (DASO₂), a metabolite of DAS, also acts as shielding agent from the APAP hepatotoxicity through inhibition or inactivation of CYP2E1 with IC₅₀ of 0.11 mmol/L [125]. Furthermore, *in vitro* studies using CYP2E1 transfected cells also revealed the beneficiary effects of DAS against the cytotoxicity of APAP by inhibiting CYP2E1-mediated oxidation [126]. Thus, these results suggest the protective effects of DAS against APAP induced hepatotoxicity when it was given before, during, or soon after the drug treatment.

5.3. Other Xenobiotics

In addition to its pivotal role in ethanol and acetaminophen metabolism, CYP2E1 is known to play a significant role in the metabolism of other xenobiotics [127]. Alteration in CYP2E1 activity, therefore, can produce marked changes in efficacy and toxicity of its substrates. Anesthetic halothane, for instance, is primarily metabolized by CYP2E1 and responsible for production of metabolites that can acetylate hepatic proteins. Inhibition of CYP2E1 activity, as hypothesized, by disulfiram was demonstrated to block the production of halothane-derived reactive intermediates suggesting a protective role of CYP2E1 inhibition [128]. Likewise, ethylene dichloride, one of the highest manufactured volatile organic intermediate compound, was reported to be primarily metabolized by CYP2E1 [129]. Exposure of mice to ethylene dichloride has been shown to enhance expression and activity of hepatic CYP2E1.

Similarly, carbon tetrachloride (CCl₄)-induced hepatotoxicity is mediated by CYP2E1. CCl₄ undergoes bioactivation by CYP2E1 to produce the trichloromethyl free radical, which subsequently reacts with the fatty acids present in membrane lipid to initiate lipid peroxidation. A study conducted in CYP2E1 knockout mice conclusively demonstrated the beneficial effects associated with absence of the CYP2E1 [130]. Likewise, the role of CYP2E1 in mediating chemical carcinogenicity has been delineated in studies with CYP2E1-null mice [131]. A significant reduction in the levels of reactive metabolites of known carcinogens suggest that anti-carcinogenic effects are associated with absence of CYP2E1 activity.

These observations underscore the importance of selective CYP2E1 inhibitors in blocking the production of reactive metabolites. Inhibition of enzyme activity using CYP2E1 inhibitors can provide a perfect clinical solution for containing cellular toxicity from hazardous chemicals like carbon tetrachloride and chemical carcinogens. At the same time, optimal modulation of CYP2E1 activity can significantly reduce toxicity associated with known drugs like halothane without compromising bioactivation of drug molecule. In fact, the protective effects of DAS by inhibiting CYP2E1-mediated biotransformation of N-nitrosodimethylamine (NDMA) and CCl₄ have long been reported [132, 133]. In these studies, pretreatment of rats with DAS (1.75 mmol/kg) before administering NDMA or CCl₄ decreased the elevation of serum transaminases by perhaps inactivating or inhibiting CYP2E1 [134]. Subsequently, the authors investigated the mode of action of DAS on CYP2E1 and demonstrated that DAS follow a competitive inhibitory mechanism to inhibit substrate metabolism followed by suicide inhibition to inactivate the CYP2E1 enzyme [135]. However, these studies were not followed further in *in vivo* as well as with regard to their molecular mechanism responsible for DAS-mediated inhibition of NDMA and CCl₄ toxicities. In another study, pre-treatment with DAS was found to play a protective role against thioacetamide-induced toxicity in rats via inhibition of CYP2E1 expression in liver microsomes [51].

In addition to the xenobiotics that are environmental contaminants, other drugs are actively metabolized by CYP2E1. Thus inhibition of CYP2E1 activity can lead to enhanced therapeutic effects of these drugs. Theophylline, for instance, is metabolized by various CYP isoforms and amongst them CYP2E1 was identified as a low-affinity high-capacity isoform mainly responsible for 8-hydroxylation [136]. Induction of CYP2E1 expression in rats subjected to chemical induced diabetes was associated with significant alteration in the plasma concentrations of theophylline and its metabolite [137]. Therefore, inhibition of CYP2E1 activity can be expected to enhance the efficacy of theophylline.

6. DAS: POTENTIAL PROTECTION AGAINST DISEASES

6.1. HIV

HIV viral genes encode for structural (Gag, Pol, and Env), regulatory (Tat and Rev), and accessory (Vpr, Vpu, Vif and Nef) proteins that are required to complete viral life cycle in the host cells. Accumulating evidence clearly suggests that these viral proteins induce the generation of various free radicals, which subsequently lead to chronic oxidative stress in HIV positive population [138, 139]. Studies using samples obtained from the HIV positive

patients revealed that this persistent oxidative stress is due to disturbances in the antioxidant defense system especially in glutathione, superoxide dismutase (SOD), catalase, S-methyl transferase levels [138, 140]. The altered redox state makes the human body more vulnerable to the HIV disease progression and other opportunistic infections. It mainly contributes to the exacerbation of the HIV pathogenesis through increased viral replication and inflammatory responses, alterations in immune cell functional properties, cell apoptosis, and cognitive dysfunction [141]. Among the HIV viral proteins, transactivator of transcription (Tat) protein has been well characterized in HIV-associated oxidative stress [138, 142]. Tat has been shown to cause oxidative stress in enterocytes, lung fibroblasts, astrocytes, neuronal, and epithelial cells of the blood brain barrier [139, 142–145]. It appears that Tat mainly manipulates the glutathione system, a major cellular thiol molecule involved in the maintenance of cellular redox state. Similarly other viral proteins such as gp120, Vpr, Nef, and HIV reverse transcriptase [146–150] have been implicated in the alterations of oxidative stress indices in HIV infection and neuropathogenesis. Taken together these studies point out that HIV viral proteins have a propensity to induce oxidative stress in various tissue types through generation of ROS, inflammatory cytokines, and other secondary metabolites.

Alcohol dependency is very common in HIV positive population. Nearly half the infected people consume alcohol and it not only effects the adherence to therapy but also increases the antiretroviral drugs-induced hepatotoxicity [74, 151]. These alcohol-mediated liver injuries are often seen as hepatic fibrosis or cirrhosis. Alcoholic HIV patients who have hepatitis experience greater side effects due to ineffective metabolism of HIV drugs such as protease inhibitors [152]. Furthermore, opioid and non-opioid analgesics used for pain management in HIV positive individuals appear to induce cellular toxicity [115, 153]. Even though the underlying mechanism for these damaging effects is not well understood, it has been proposed that abnormal metabolism by CYP enzymes and activation of multitude stress responses mitigate the toxic effects of secondary metabolites leading to hepatotoxicity [154].

The deleterious effects of oxidative stress and hepatotoxicity in the HIV patients may be alleviated by including adjunctive agents in the therapy that can scavenge free radicals or specifically target oxidative stress signaling pathways. In support of this view a recent study using astrocytes demonstrated that CYP2E1 is involved in gp120- and gp120+methamphetamine (MA)-mediated oxidative stress [146]. In this study, the authors observed significant induction of CYP2E1 by gp120 and MA followed by protective effects of DAS against not only gp120/MA-induced ROS production but also against apoptosis induced by these insults. Moreover, since other HIV viral proteins Nef and Vpr also increased oxidative stress and were cytotoxic to astrocytic cell lines (unpublished observation), we anticipate that DAS may be able to rescue Nef- and Vpr-mediated toxicities. More recent studies indicated that the HIV viral proteins and HIV reverse transcriptase enzyme in host cells induce the production of ROS through Nrf2 pathway [150, 155]. Since DAS is known to mediate its antioxidant properties through Nrf2 pathway, we speculate that Nrf2 pathway may be involved in DAS-mediated protection against viral protein-induced toxicity [152–154].

6.2. Diabetes

Recent studies have implicated enhanced expression and activity of CYP2E1 in diabetes, which appears to be responsible for the cellular toxicity observed in this pathological condition. For example, direct evidence for CYP2E1-mediated development of diabetic vascular dysfunction was reported in a rodent model of chemical-induced diabetes [156]. Diabetes associated enhanced free radical production, increased oxidative stress, and endothelial dysfunction are implicated in accelerated development of cardiovascular complications in patients. Importantly, in rat subjected to streptozotocin-induced, insulin-deficient type I diabetes, significantly enhanced expression of CYP2E1 was observed in aortic tissue. Importantly, reduced expression of CYP2E1 was associated with decreased ROS production, enhanced nitric oxide availability, and overall improved endothelial function. Furthermore, an *in vitro* study that examined the effects of hyperglycemia on hepatic cells further highlighted the putative role of CYP2E1 in mediating diabetes-induced cellular toxicity [157]. Hepatic VL-17A cells cultured in a hyperglycemic culture medium (50 mM glucose) demonstrated decreased cell viability, increased ROS, and enhanced production of advanced glycated end products in association with upregulated CYP2E1 levels. Importantly, treatment with DAS attenuated hyperglycemia mediated cellular toxicity in VL-17A cells suggesting the importance of CYP2E1 in liver injury associated with diabetes.

Given the strong evidence supporting insulin-induced post-translational modifications of CYP2E1 mRNA [158, 159], the recent reports of a close association between CYP2E1 induction and diabetes-mediated cellular toxicity in animal model and cell culture is intuitive. Lack of insulin-mediated control of CYP2E1 expression and increased free radical production by CYP2E1 can directly contribute towards the toxicity observed in diabetes. Inhibition of CYP2E1 activity can therefore be rationalized as a suitable approach for therapeutic intervention. By blocking the effects of CYP2E1, attenuation of diabetes associated complications including hepatic toxicity and endothelial dysfunction is expected to improve the lifestyle and better clinical management of diabetic patients.

6.3. Parkinson's Disease (PD)

CYP2E1 is also known to be expressed in the dopaminergic neurons of substantia nigra. A recent genomic analysis of PD patients' brains revealed a possible role of CYP2E1 in determining PD susceptibility [160]. This study reported decreased methylation of CYP2E1 gene in association with enhanced CYP2E1 mRNA levels in brains samples collected from PD patients. Although preliminary, this finding suggests a possible role of CYP2E1 in progression towards PD. Furthermore, an earlier report had revealed significant association between a single nucleotide polymorphism of CYP2E1 and occurrence of PD in a Swedish population [161], further suggesting the putative role of CYP2E1 in PD. Therefore, a novel selective CYP2E1 inhibitors such as DAS would be helpful in examining the role of CYP2E1 in development of PD, as well as in serving as potential drug candidates for PD patients.

6.4. High-Fat Diet Induced Obesity and Non-Alcoholic Steatohepatitis

Significant interaction between high-fat diet and CYP2E1 expression has previously been documented [162]. In a more recent study using CYP2E1 knockout (CYP2E1KO) mice model, the role of CYP2E1 expression in modulating high-fat diet induced obesity was reported [163]. In this study, absence of CYP2E1 expression was associated with a marked attenuation in increase in body weight following long-term maintenance on high-fat diet. Importantly, CYP2E1KO mice did not exhibit a change in total food intake during the course of the study. In addition to curbing obesity associated with high-fat diet, knocking out the CYP2E1 expression resulted in improved lipid profile in animals as indicated by decreased plasma triglyceride and free fatty acid levels compared to wild type control animals. In a different study, induction of hepatic CYP2E1 activity has also been reported in pigs, which were subjected to two-months of continuous high-fat/high-cholesterol diet compared to the control group of animals [164]. In another study, CYP2E1 has been shown to play a central role in the development of high-fat mediated non-alcoholic steatohepatitis, via induction of oxidative stress, further underscoring the involvement of CYP2E1 in high-fat diet mediated metabolic abnormalities [165]. Therefore, the existing reports support the potential role of novel DAS analogs in mitigating the adverse effects of high-fat diet through efficient inhibition of CYP2E1 activity/expression.

7. CYP2E1-MEDIATED METABOLISM OF DIALLYL SULFIDE AND CELLULAR TOXICITY

DAS is rapidly metabolized into many products; diallyl sulfoxide (DASO), diallyl sulfone (DASO₂), and allyl mercaptan [69, 135] (Fig. 1). While allyl mercaptan is a breakdown product of DAS, DASO and DASO₂ are formed as a result of S-oxidation of DAS. DAS, DASO, DASO₂, and allyl mercaptan are further converted into epoxides followed by GSH conjugated compounds prior to their elimination. Among DAS metabolites, DASO and DASO₂ are the major metabolic products, formation of which are mediated through CYP2E1 enzyme. DASO and DASO₂ are further activated into their respective epoxides, which are toxic to hepatic as well as extra-hepatic cells. In addition, this oxidation reaction causes autocatalytic destruction of the enzyme [69, 135]. In addition to DASO and DASO₂, DAS can also go through direct epoxidation, which is also toxic to the cells. Therefore, designing and developing DAS analogs, which retains or even strengthened the inhibitory characteristics towards CYP2E1, and decreases its ability as a CYP2E1 substrate is highly desirable.

8. DEVELOPMENT OF DAS ANALOGS AS NOVEL THERAPEUTICS

As discussed above, in addition to a competitive inhibitor, DAS is also a substrate of CYP2E1. Since it is much stronger nucleophile than terminal carbon of DAS, sulfur strongly binds to the heme of CYP2E1 and leads to either competitive inhibition of CYP2E1 or metabolism of DAS into DASO. DAS-mediated inhibition of CYP2E1 prevents alcohol/drugs-mediated toxicity, while DAS metabolism by CYP2E1 causes DAS-mediated toxicity. Therefore, DAS can be chemically modified to create a relatively better inhibitor and weaker substrate of CYP2E1.

8.1. Design of Novel DAS Analogs

To achieve its desired effect, DAS can potentially be modified as a sole competitive inhibitor or as a suicide inhibitor of CYP2E1 without being a substrate for the enzyme. The modifications rendering DAS analogs as sole CYP2E1 inhibitors can be achieved by undertaking a structure-activity relationship (SAR) study. Firstly, DAS can be modified at the sulfur position by substituting sulfur with other heteroatoms in a way that DAS retains its ability to interact with heme of CYP2E1 but loses the capacity to get oxidized e.g. addition of oxygen or amine at the sulfur position. Figure 5 shows DAS analogs (diallyl ether and diallyl-amine) as a sole competitive inhibitor of CYP2E1. In addition, DAS can be modified at the carbon atom adjacent to the heteroatom by incorporating relatively stronger nucleophiles. Such substitutions are expected to enhance the binding of DAS analogs to the heme of CYP2E1. These compounds are expected to bind the heme of the enzyme in type II manner with relatively higher affinity than DAS. Type II binding occurs as a result of replacement of water molecule from the heme with the ligand in irreversible manner. Thus, these inhibitors do not act as substrates. We have earlier shown the differential binding characteristics (type I and type II) of eight protease inhibitors with CYP3A4 based on physicochemical properties of these protease inhibitors [166]. The knowledge obtained from this study can therefore be employed to design and characterize these inhibitors for CYP2E1. To achieve suicide inhibition of CYP2E1, in addition to increasing the affinity of central heteroatom to heme of enzyme, oxidation of the terminal double bond in DAS analogs by CYP2E1 has to be blocked. Since terminal alkene oxidation is a known precursor for phase II conjugation of DAS, chemical modifications that decrease the probability of alkene oxidation will help achieve mechanism based inhibition of CYP2E1. Finally, we will also examine DAS analogs with varying lengths of allyl side chains with methyl or other small alkyl groups. Smaller side chain, for instance, allyl methyl sulfide has been reported to increase the binding affinity of the sulfur atom with the heme of CYP2E1 (Fig. 5) [13].

Bulky substitutions at the terminal carbon of DAS analogs may affect the binding to active site of CYP2E1 due to increased steric hindrance. In addition, CYP2E1 active site is relatively smaller than the active site of most other CYP enzymes [167]. It can be noted that CYP enzymes in general contain several substrate recognition/active site amino acid residues (up to 15 amino acids), which interact with ligand and stabilize ligand-enzyme binding [168, 169]. Thus, the predicted DAS analogs upon modification may lose the ability to bind the heme of CYP2E1. However, there is also strong evidence that CYP enzymes alter their conformations based on the ligand present (induce fit model), which gives confidence that these analogs may be able to fit with the active site upon conformational change. Therefore, it is logical to first test the ability of these analogs to bind CYP2E1 effectively using molecular modeling and ligand docking.

8.2. Molecular Modelling, Synthesis, and Biochemical Characterizations of Potential DAS Analogs

Molecular model of CYP2E1 will be prepared using its crystal structure [170, 171]. Since several CYP2E1 structures, alone as well as complex with ligands (inhibitors/substrates), are known [171, 172], we will use the most appropriate structure of CYP2E1 as a molecular model to dock DAS analogs. The most appropriate structure will be selected by comparing

the size, shape, and geometry of DAS with the ligands that have been complexed with CYP2E1. Further, DAS analogs will be docked in to the active site of CYP2E1 using manual and/or auto docking methods [173]. In the case of manual docking, a particular atom of DAS analogs will be fixed with the heme of CYP2E1 and energy minimization will be carried out upon fixing the parameters that allow free movement of enzyme-ligand complex within 10Å of the heme. After energy minimization, DAS analogue with the least energy and lowest number of conformers will be selected for further consideration. In the case of autodocking, DAS analogs will be combined with CYP2E1 model without fixing a particular atom with the heme of the enzyme. Upon energy minimization, these analogs will be compared, and analogs with minimum energy and minimum number of conforms will be selected for further consideration. Furthermore, using modeling studies we will also predict the binding affinity of these compounds with CYP2E1 and compare the data with the parent compound DAS.

Based on the ligand-enzyme docking predictions, novel DAS analogs will be synthesized using standard chemical methods. If standard methods fail to yield the desirable products, we will modify the methods as needed. These novel DAS analogs will then be characterized for its binding, inhibitory, and metabolic activities using pure human CYP2E1 enzyme. The analogs with the highest binding/inhibitory affinity (lowest K_a , IC_{50} , and/or K_i values) and lowest metabolic activity (highest K_m and/or lowest k_{cat} values) compared with its parent compound DAS, will be selected. These analogs will also be tested to determine whether they decrease expression of CYP2E1 mRNA and proteins. As described previously, DAS has been shown to decrease the expression of CYP2E1. A decrease expression of the protein and increased inhibition of the enzyme activity may additively or synergistically decrease CYP2E1-mediated cellular toxicity.

In addition to DAS analogs, we also propose to design similar DADS and DATS analogs because these compounds have been shown to reduce CYP2E1 expression relatively higher than DAS. Although these compounds are known to be more toxic than DAS, it is possible that the analogs of DADS and DATS are relatively less toxic than the parent compounds without compromising their CYP2E1 inhibitory effects. It is also possible that these analogs are even stronger CYP2E1 inhibitors than DADS and DATS. As described above, the analogs will be first studied using molecular docking followed by their synthesis, if not commercially available, and *in vitro* activity.

8.3. Pharmacological Evaluation of Potentially Novel DAS Analogs

Upon characterizations for their physical, chemical, and genetic interactions with CYP2E1, the lead compounds are expected to be useful in treating several conditions (Fig. 2). In addition to their beneficial effects in pathological conditions marked by CYP2E1 overexpression (diabetes, PD), the novel CYP2E1 inhibitors can be rationalized to block CYP2E1 governed metabolic toxicities. For instance, the novel DAS analogs are expected to help prevent CYP2E1-mediated toxicity caused by alcohol and drugs in HIV-infected and uninfected patients in several cell types, including hepatocytes, blood cells, astrocytes, and neurons. Similarly, DAS analogs which retain the antioxidant effects of allyl sulfides along

with potent CYP2E1 inhibitor effects can serve as ideal dietary supplements in cancer therapy.

Although the proposed chemical modifications are expected to decrease the overall lipophilicity and therefore toxicity associated with DAS, the novel DAS analogs will be tested for their inherent cellular toxicity. Preliminary studies will be carried out in primary cells (hepatocytes, monocytes, astrocytes, and neurons) followed by preclinical studies in rodent models and subsequent clinical evaluations. Enhancing the pharmacological/toxicological ratio in these novel DAS analogs is expected to yield promising investigational new drugs. The potential drug candidates may be used as a novel therapy alone or in combination of existing therapies to prevent/treat alcohol, analgesic drugs, and HIV mediated toxicities and other pathological conditions.

8.4. Nanoformulation of DAS Analogs

Although DAS analogs can be used directly as therapeutic agents in treating cancer, HIV, and other ailments, targeted drug delivery might be more beneficial. In the past decade, numerous anticancer drugs have been nanoformulated to reduce drug toxicity associated with the anticancer drugs [174]. For instance, nanoparticle-based delivery of doxorubicin in a rodent model of hepatocellular carcinoma was associated with significant reduction in cardiotoxicity associated with the free drug [175]. Similarly, in the last few years, antiretroviral drugs are also being formulated especially for enhanced drug delivery across blood-brain-barrier to treat HIV infection in the brain [176, 177]. CNS bioavailability of antiretroviral drugs like azidothymidine 5'-triphosphate [178] and saquinavir [179] was reported to be significantly higher following their dosing as nanoformulations. Therefore, in addition to conventional dosing, nanoformulations of DAS analogs will also be prepared for improving efficacy and minimizing the adverse effects associated with these compounds. Nanoformulation strategy for DAS analogs will be based on documented efforts with other allyl sulfide compounds like DADS [180]. In this study, novel niosomal-based formulation of DADS was shown to be more effective than the free form.

9. CONCLUDING REMARKS

DAS and other organosulfur compounds, active ingredients of garlic, have been studied for several decades for their potential to prevent/treat cancer. These compounds are potentially efficacious in preventing/treating a variety of cancer cells as shown using *in vitro* and *in vivo* studies. However, due to a relative lack of specificity to various cancer cells, and toxicity to the healthy cells, these compounds have not been able to make to the market. Various mechanisms have been proposed for their anticancer activity including regulation of its antioxidant property. Therefore, we believe that based on their mechanism of action appropriate modification of these compounds can be prepared, which may be clinically used as an adjuvant therapy to treat a variety of cancers. In the past few years, DAS has also been shown to rescue alcohol, analgesic drugs, and other xenobiotic mediated cellular toxicity by inhibiting CYP2E1. Since CYP2E1 is also induced in HIV, diabetic, and PD patients, who regularly consume alcohol and analgesic drugs, and are exposed to other xenobiotics, DAS has potential application in these diseases. Thus, DAS by virtue of inhibiting CYP2E1, has

the potential to be used as a novel therapeutic in alcohol and analgesic drug users, as well as in HIV, in diabetic, and PD patients who suffer from liver toxicity and toxicity of other extrahepatic cells. However, DAS, as a result of its rapid metabolism to DASO and DASO₂, also causes cellular toxicity. Therefore, there is a need to modify the parent DAS into an analogue, which is a stronger inhibitor, but a weaker substrate of CYP2E1. The study for designing and developing a lead compound of DAS is currently underway.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from the National Institute of Health grant AA022063.

Biography

Santosh Kumar

REFERENCES

1. Block E. The chemistry of garlic and onions. *Sci. Am.* 1985; 252(3):114–119. [PubMed: 3975593]
2. Thomson M, Ali M. Garlic [*Allium sativum*]: A Review of its Potential Use as an Anti-Cancer Agent. *Curr. Cancer Drug Targets.* 2003; 3(1):67–81. [PubMed: 12570662]
3. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. *J. Nutr.* 2001; 131(3s):955S–962S. [PubMed: 11238796]
4. Omar SH, Al-Wabel NA. Organosulfur compounds and possible mechanism of garlic in cancer. *Saudi. Pharm. J.* 2010; 18(1):51–58. [PubMed: 23960721]
5. Iciek M, Kwecien I, Wlodek L. Biological Properties of Garlic and Garlic-Derived Organosulfur Compounds. *Environ. Mol. Mutag.* 2009; 50(3):247–265.
6. Egenschwind C, Eckard R, Kemper FH. Metabolism of Garlic Constituents in the Isolated Perfused-Rat-Liver. *Planta Med.* 1992; 58(4):301–305. [PubMed: 1438588]
7. Freeman F, Koda Y. Garlic Chemistry - Stability of S-(2-Propenyl) 2-Propene-1-Sulfinothioate (Allicin) in Blood, Solvents, and Simulated Physiological Fluids. *J. Agric. Food Chem.* 1995; 43(9): 2332–2338.
8. Rai SK, Sharma M, Tiwari M. Inhibitory effect of novel dial-lyldisulfide analogs on HMG-CoA reductase expression in hyper-cholesterolemic rats: CREB as a potential upstream target. *Life Sci.* 2009; 85(5–6):211–219. [PubMed: 19523964]
9. Yi L, Su Q. Molecular mechanisms for the anti-cancer effects of diallyl disulfide. *Food Chem. Toxicol.* 2013; 57:362–370. [PubMed: 23583486]
10. Wang HC, Pao J, Lin SY, Sheen LY. Molecular mechanisms of garlic-derived allyl sulfides in the inhibition of skin cancer progression. *Ann. N. Y. Acad. Sci.* 2012; 1271:44–52. [PubMed: 23050963]
11. Karmakar S, Banik NL, Patel SJ, Ray SK. Garlic compounds induced calpain and intrinsic caspase cascade for apoptosis in human malignant neuroblastoma SH-SY5Y cells. *Apoptosis.* 2007; 12(4): 671–684. [PubMed: 17219050]
12. Cao HX, Zhu KX, Fan JG, Qiao L. Garlic-derived allyl sulfides in cancer therapy. *Anticancer Agents Med. Chem.* 2014; 14(6):793–799. [PubMed: 24851880]

13. Wargovich MJ, Goldberg MT. Diallyl sulfide. A naturally occurring thioether that inhibits carcinogen-induced nuclear damage to colon epithelial cells *in vivo*. *Mutat. Res.* 1985; 143(3): 127–129. [PubMed: 4010692]
14. Hayes MA, Rushmore TH, Goldberg MT. Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. *Carcinogenesis*. 1987; 8(8):1155–1157. [PubMed: 3608098]
15. Hong JY, Wang ZY, Smith TJ, Zhou S, Shi S, Pan J, Yang CS. Inhibitory effects of diallyl sulfide on the metabolism and tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mouse lung. *Carcinogenesis*. 1992; 13(5):901–904. [PubMed: 1587006]
16. Haber-Mignard D, Suschetet M, Berges R, Astorg P, Siess MH. Inhibition of aflatoxin B1- and N-nitrosodiethylamine-induced liver preneoplastic foci in rats fed naturally occurring allyl sulfides. *Nutr. Cancer*. 1996; 25(1):61–70. [PubMed: 8837862]
17. Wu PP, Chung HW, Liu KC, Wu RSC, Yang JS, Tang NY, Lo C, Hsia TC, Yu CC, Chueh FS, Lin SS, Chung JG. Diallyl sulfide induces cell cycle arrest and apoptosis in HeLa human cervical cancer cells through the p53, caspase- and mitochondria-dependent pathways. *Int. J. Oncol.* 2011; 38(6):1605–1613. [PubMed: 21424116]
18. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell*. 1997; 88(3):323–331. [PubMed: 9039259]
19. Kalra N, Arora A, Shukla Y. Involvement of multiple signaling pathways in diallyl sulfide mediated apoptosis in mouse skin tumors. *Asian Pac. J. Cancer Prev.* 2006; 7(4):556–562. [PubMed: 17250426]
20. George J, Singh M, Srivastava AK, Bhui K, Shukla Y. Syn-ergistic growth inhibition of mouse skin tumors by pomegranate fruit extract and diallyl sulfide: evidence for inhibition of activated MAPKs/NF-kappaB and reduced cell proliferation. *Food Chem. Toxicol.* 2011; 49(7):1511–1520. [PubMed: 21443920]
21. Shin HA, Cha YY, Park MS, Kim JM, Lim YC. Diallyl sulfide induces growth inhibition and apoptosis of anaplastic thyroid cancer cells by mitochondrial signaling pathway. *Oral Oncol.* 2010; 46(4):e15–e18. [PubMed: 20219414]
22. Chiu TH, Lan KY, Yang MD, Lin JJ, Hsia TC, Wu CT, Yang JS, Chueh FS, Chung JG. Diallyl Sulfide Promotes Cell-Cycle Arrest Through the p53 Expression and Triggers Induction of Apoptosis Via Caspase- and Mitochondria-Dependent Signaling Pathways in Human Cervical Cancer Ca Ski Cells. *Nutr. Cancer*. 2013; 65(3):505–514. [PubMed: 23530650]
23. Oyadomari S, Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ.* 2004; 11(4):381–389. [PubMed: 14685163]
24. Storr SJ, Carragher NO, Frame MC, Parr T, Martin SG. The calpain system and cancer. *Nat. Rev. Cancer*. 2011; 11(5):364–374. [PubMed: 21508973]
25. Yu FS, Wu CC, Chen CT, Huang SP, Yang JS, Hsu YM, Wu PP, Ip SW, Lin JP, Lin JG, Chung JG. Diallyl sulfide inhibits murine WEHI-3 leukemia cells in BALB/c mice *in vitro* and *in vivo*. *Hum. Exp. Toxicol.* 2009; 28(12):785–790. [PubMed: 19850653]
26. Lai KC, Hsu SC, Kuo CL, Yang JS, Ma CY, Lu HF, Tang NY, Hsia TC, Ho HC, Chung JG. Diallyl sulfide, diallyl disulfide, and diallyl trisulfide inhibit migration and invasion in human colon cancer colo 205 cells through the inhibition of matrix metalloproteinase-2,-7, and-9 expressions. *Environ. Toxicol.* 2013; 28(9):479–488. [PubMed: 21695758]
27. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J.* 2011; 278(1):16–27. [PubMed: 21087457]
28. Kang JS, Kim TM, Shim TJ, Salim EI, Han BS, Kim DJ. Modifying effect of diallyl sulfide on colon carcinogenesis in C57BL/6J-ApcMin/(+) mice. *Asian Pac. J. Cancer Prev.* 2012; 13(4): 1115–1118. [PubMed: 22799291]
29. Fleischauser AT, Poole C, Arab L. Garlic consumption and cancer prevention: meta-analyses of colorectal and stomach cancers. *Am. J. Clin. Nutr.* 2000; 72(4):1047–1052. [PubMed: 11010950]
30. Cheng JM, Tsai KD, Perng DS, Wang JS, Wei CC, Lin JC. Diallyl sulfide protects against ultraviolet B-induced skin cancers in SKH-1 hairless mouse: analysis of early molecular events in

- carcinogenesis. *Photodermatol. Photoimmunol. Photomed.* 2011; 27(3):138–146. [PubMed: 21535167]
31. Vaclavikova R, Hubackova M, Stribrna-Sarmanova J, Kodet R, Mrhalova M, Novotny J, Gut I, Soucek P. RNA expression of cytochrome P450 in breast cancer patients. *Anticancer Res.* 2007; 27(6C):4443–4450. [PubMed: 18214058]
 32. Danko IM, Chaschin NA. Association of CYP2E1 gene polymorphism with predisposition to cancer development. *Exp. Oncol.* 2005; 27(4):248–256. [PubMed: 16404342]
 33. Xu L, Yang M, Zhao T, Jin H, Xu Z, Li M, Chen H. The polymorphism of CYP2E1 Rsa I/Pst I gene and susceptibility to respiratory system cancer: a systematic review and meta-analysis of 34 studies. *Medicine (Baltimore).* 2014; 93(27):e178. [PubMed: 25501063]
 34. Kiss I, Orsos Z, Gombos K, Bogner B, Csejtei A, Tibold A, Varga Z, Pazsit E, Magda I, Zolyomi A, Ember I. Association between allelic polymorphisms of metabolizing enzymes (CYP 1A1, CYP 1A2, CYP 2E1, mEH) and occurrence of colorectal cancer in Hungary. *Anticancer Res.* 2007; 27(4C):2931–2937. [PubMed: 17695473]
 35. Linhart K, Bartsch H, Seitz HK. The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts. *Redox Biol.* 2014; 3:56–62. [PubMed: 25462066]
 36. Ibrahim SS, Nassar NN. Diallyl sulfide protects against N-nitrosodiethylamine-induced liver tumorigenesis: role of aldose re-ductase. *World J. Gastroenterol.* 2008; 14(40):6145–6153. [PubMed: 18985804]
 37. Kalayarasan S, Prabhu PN, Sriram N, Manikandan R, Aru-mugam M, Sudhandiran G. Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. *Eur. J. Pharmacol.* 2009; 606(1–3):162–171. [PubMed: 19374873]
 38. Ho CY, Cheng YT, Chau CF, Yen GC. Effect of diallyl sulfide on *in vitro* and *in vivo* Nrf2-mediated pulmonary antioxidant enzyme expression via activation ERK/p38 signaling pathway. *J. Agric. Food Chem.* 2012; 60(1):100–107. [PubMed: 22118872]
 39. Ho CY, Weng CJ, Jhang JJ, Cheng YT, Huang SM, Yen GC. Diallyl sulfide as a potential dietary agent to reduce TNF-alpha- and histamine-induced proinflammatory responses in A7r5 cells. *Mol. Nutr. Food Res.* 2014; 58(5):1069–1078. [PubMed: 24415531]
 40. Kalayarasan S, Sriram N, Sudhandiran G. Diallyl sulfide attenuates bleomycin-induced pulmonary fibrosis: critical role of iNOS, NF-kappaB, TNF-alpha and IL-1beta. *Life Sci.* 2008; 82(23–24):1142–1153. [PubMed: 18462759]
 41. Lee HS, Lee CH, Tsai HC, Salter DM. Inhibition of cyclooxygenase 2 expression by diallyl sulfide on joint inflammation induced by urate crystal and IL-1beta. *Osteoarthritis Cartilage.* 2009; 17(1):91–99. [PubMed: 18573668]
 42. Shaik IH, George JM, Thekkumkara TJ, Mehvar R. Protective effects of diallyl sulfide, a garlic constituent, on the warm hepatic ischemia-reperfusion injury in a rat model. *Pharm. Res.* 2008; 25(10):2231–2242. [PubMed: 18626753]
 43. Green M, Newell O, Aboyade-Cole A, Darling-Reed S, Thomas RD. Diallyl sulfide induces the expression of nucleotide excision repair enzymes in the breast of female ACI rats. *Toxicol. Lett.* 2007; 168(1):40–44. [PubMed: 17125941]
 44. Albertson TM, Ogawa M, Bugni JM, Hays LE, Chen Y, Wang YP, Treuting PM, Heddle JA, Goldsby RE, Preston BD. DNA polymerase epsilon and delta proofreading suppress discrete mutator and cancer phenotypes in mice. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106(40):17101–17104. [PubMed: 19805137]
 45. Smith ML, Ford JM, Hollander MC, Bortnick RA, Amundson SA, Seo YR, Deng CX, Hanawalt PC, Fornace AJ. p53-mediated DNA repair responses to UV radiation: Studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol. Cell. Biol.* 2000; 20(10):3705–3714. [PubMed: 10779360]
 46. Stolimenov I, Helleday T. PCNA on the crossroad of cancer. *Biochem. Soc. Trans.* 2009; 37:605–613. [PubMed: 19442257]

47. Aboyade-Cole A, Darling-Reed S, Oriaku E, McCaskill M, Thomas R. Diallyl sulfide inhibits PhIP-induced cell death via the inhibition of DNA strand breaks in normal human breast epithelial cells. *Oncol. Rep.* 2008; 20(2):319–323. [PubMed: 18636192]
48. McCaskill ML, Rogan E, Thomas RD. Diallyl sulfide inhibits diethylstilbestrol induced DNA damage in human breast epithelial cells (MCF-10A). *Steroids.* 2014; 92:96–100. [PubMed: 25278253]
49. Katoch O, Khan GA, Dwarakanath BS, Agrawala PK. Mitigation of hematopoietic radiation injury by diallyl sulphide. *J. Environ. Pathol. Toxicol. Oncol.* 2012; 31(4):357–365. [PubMed: 23394448]
50. Lin XM, Yu SS, Chen YL, Wu JX, Zhao J, Zhao Y. Neuroprotective effects of diallyl sulfide against transient focal cerebral ischemia via anti-apoptosis in rats. *Neurol. Res.* 2012; 34(1):32–37. [PubMed: 22196859]
51. Kim NH, Lee S, Kang MJ, Jeong HG, Kang W, Jeong TC. Protective Effects of Diallyl Sulfide against Thioacetamide-Induced Toxicity: A Possible Role of Cytochrome P450 2E1. *Bio-mol. Ther. (Seoul).* 2014; 22(2):149–154.
52. Yanagiba Y, Suzuki T, Suda M, Hojo R, Gonzalez FJ, Nakajima T, Wang RS. Cytochrome P450 2E1 is responsible for the initiation of 1,2-dichloropropane-induced liver damage. *Toxicol. Ind. Health.* 2015
53. Jeong HG, Lee YW. Protective effects of diallyl sulfide on N-nitrosodimethylamine-induced immunosuppression in mice. *Cancer Lett.* 1998; 134(1):73–79. [PubMed: 10381132]
54. Kathawala RJ, Gupta P, Ashby CR Jr, Chen ZS. The modulation of ABC transporter-mediated multidrug resistance in cancer: A review of the past decade. *Drug Resist. Updat.* 2015; 18C:1–17. [PubMed: 25554624]
55. Lai KC, Kuo CL, Ho HC, Yang JS, Ma CY, Lu HF, Huang HY, Chueh FS, Yu CC, Chung JG. Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*. *Phytomedicine.* 2012; 19(7):625–630. [PubMed: 22397993]
56. Cherrington NJ, Slitt AL, Maher JM, Zhang XX, Zhang J, Huang WD, Wan YJY, Moore DD, Klaassen CD. Induction of multidrug resistance protein 3 (MRP3) *in vivo* is independent of constitutive androstane receptor. *Drug Metab. Dispos.* 2003; 31(11):1315–1319. [PubMed: 14570762]
57. Slitt AL, Cherrington NJ, Maher JM, Klaassen CD. Induction of multidrug resistance protein 3 in rat liver is associated with altered vectorial excretion of acetaminophen metabolites. *Drug Metab. Dispos.* 2003; 31(9):1176–1186. [PubMed: 12920174]
58. Maher JM, Cheng XG, Slitt AL, Dieter MZ, Klaassen CD. Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metab. Dispos.* 2005; 33(7):956–962. [PubMed: 15833929]
59. Arora A, Seth K, Shukla Y. Reversal of P-glycoprotein-mediated multidrug resistance by diallyl sulfide in K562 leukemic cells and in mouse liver. *Carcinogenesis.* 2004; 25(6):941–949. [PubMed: 14729595]
60. Iyanagi T. Molecular mechanism of phase I and phase II drug-metabolizing enzymes: Implications for detoxification. *Int. Rev. Cytol.* 2007; 260:35–112. [PubMed: 17482904]
61. Jancova P, Anzenbacher P, Anzenbacherova E. Phase II Drug Metabolizing Enzymes. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub.* 2010; 154(2):103–116. [PubMed: 20668491]
62. Lubet RA, Yao R, Grubbs CJ, You M, Wang Y. Induced expression of drug metabolizing enzymes by preventive agents: role of the antioxidant response element. *Chem. Biol. Interact.* 2009; 182(1): 22–28. [PubMed: 19695238]
63. Guyonnet D, Belloir C, Suschetet M, Siess MH, Le Bon AM. Mechanisms of protection against aflatoxin B(1) genotoxicity in rats treated by organosulfur compounds from garlic. *Carcinogenesis.* 2002; 23(8):1335–1341. [PubMed: 12151352]
64. Fisher CD, Augustine LM, Maher JM, Nelson DM, Slitt AL, Klaassen CD, Lehman-McKeeman LD, Cherrington NJ. Induction of drug-metabolizing enzymes by garlic and allyl sulfide

- compounds via activation of constitutive androstane receptor and nuclear factor E2-related factor 2. *Drug Metab. Dispos.* 2007; 35(6):995–1000. [PubMed: 17353348]
65. Sueyoshi T, Green WD, Vinal K, Woodrum TS, Moore R, Negishi M. Garlic Extract Diallyl Sulfide (DAS) Activates Nuclear Receptor CAR to Induce the Sult1e1 Gene in Mouse Liver. *Plos One.* 2011; 6(6)
 66. Guyonnet D, Siess MH, Le Bon AM, Suschetet M. Modulation of phase II enzymes by organosulfur compounds from allium vegetables in rat tissues. *Toxicol. Appl. Pharmacol.* 1999; 154(1):50–58. [PubMed: 9882591]
 67. Trafalis DT, Panteli ES, Grivas A, Tsigris C, Karamanakis PN. CYP2E1 and risk of chemically mediated cancers. *Expert Opin. Drug Metab. Toxicol.* 2010; 6(3):307–319. [PubMed: 20073996]
 68. Wu D, Cederbaum AI. Oxidative stress mediated toxicity exerted by ethanol-inducible CYP2E1. *Toxicol. Appl. Pharmacol.* 2005; 207(2 Suppl):70–76. [PubMed: 16019049]
 69. Jin L, Baillie TA. Metabolism of the chemoprotective agent diallyl sulfide to glutathione conjugates in rats. *Chem. Res. Toxicol.* 1997; 10(3):318–327. [PubMed: 9084912]
 70. Davenport DM, Wargovich MJ. Modulation of cytochrome P450 enzymes by organosulfur compounds from garlic. *Food Chem. Toxicol.* 2005; 43(12):1753–1762. [PubMed: 16000231]
 71. Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. *Physiol. Rev.* 1997; 77(2):517–544. [PubMed: 9114822]
 72. Cederbaum AI. Cytochrome P450 2E1-dependent oxidant stress and upregulation of anti-oxidant defense in liver cells. *J. Gastroenterol. Hepatol.* 2006; 21(Suppl 3):S22–S25. [PubMed: 16958665]
 73. Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. *Free Radic. Biol. Med.* 2008; 44(5):723–738. [PubMed: 18078827]
 74. Kumar S, Jin M, Ande A, Sinha N, Silverstein PS, Kumar A. Alcohol consumption effect on antiretroviral therapy and HIV-1 pathogenesis: role of cytochrome P450 isozymes. *Expert Opin. Drug Metab. Toxicol.* 2012; 8(11):1363–1375. [PubMed: 22871069]
 75. Miller AM, Horiguchi N, Jeong W-I, Radaeva S, Gao B. Molecular mechanisms of alcoholic liver disease: innate immunity and cytokines. *Alcohol. Clin. Exp. Res.* 2011; 35(5):787–793. [PubMed: 21284667]
 76. Zeng T, Zhang C-L, Song F-Y, Zhao X-L, Xie K-Q. CMZ reversed chronic ethanol-induced disturbance of PPAR- α possibly by suppressing oxidative stress and PGC-1 α acetylation, and activating the MAPK and GSK3 β pathway. *PLoS One.* 2014; 9(6)
 77. Ceni E, Mello T, Galli A. Pathogenesis of alcoholic liver disease: Role of oxidative metabolism. *World J. Gastroenterol.* 2014; 20(47):17756–17772. [PubMed: 25548474]
 78. Donohue TM Jr, Thomes PG. Ethanol-induced oxidant stress modulates hepatic autophagy and proteasome activity. *Redox Biol.* 2014; 3:29–39. [PubMed: 25462063]
 79. Wang X, Wu D, Yang L, Gan L, Cederbaum AI. Cytochrome P450 2E1 potentiates ethanol induction of hypoxia and HIF-1 α *in vivo*. *Free Radic. Biol. Med.* 2013; 63:175–186. [PubMed: 23669278]
 80. Yun J-W, Son M-J, Abdelmegeed MA, Banerjee A, Morgan TR, Yoo S-H, Song B-J. Binge alcohol promotes hypoxic liver injury through a CYP2E1-HIF-1 α -dependent apoptosis pathway in mice and humans. *Free Radic. Biol. Med.* 2014; 77:183–194. [PubMed: 25236742]
 81. Lieber CS, DeCarli LM. The role of the hepatic microsomal ethanol oxidizing system (MEOS) for ethanol metabolism *in vivo*. *J. Pharmacol. Exp. Ther.* 1972; 181(2):279–287. [PubMed: 4402282]
 82. Matsumoto H, Matsubayashi K, Fukui Y. Evidence that cytochrome P-4502E1 contributes to ethanol elimination at low doses: effects of diallyl sulfide and 4-methyl pyrazole on ethanol elimination in the perfused rat liver. *Alcohol. Clin. Exp. Res.* 1996; 20(1 Suppl):12A–16A.
 83. Morimoto M, Hagbjörk AL, Wan YJ, Fu PC, Clot P, Albano E, Ingelman-Sundberg M, French SW. Modulation of experimental alcohol-induced liver disease by cytochrome P450 2E1 inhibitors. *Hepatology.* 1995; 21(6):1610–1617. [PubMed: 7768506]
 84. Kishimoto R, Ueda M, Yoshinaga H, Goda K, Park SS. Combined effects of ethanol and garlic on hepatic ethanol metabolism in mice. *J. Nutr. Sci. Vitaminol.* 1999; 45(3):275–286. [PubMed: 10524347]
 85. Morimoto M, Reitz RC, Morin RJ, Nguyen K, Ingelman-Sundberg M, French SW. CYP-2E1 inhibitors partially ameliorate the changes in hepatic fatty acid composition induced in rats by

- chronic administration of ethanol and a high fat diet. *J. Nutr.* 1995; 125(12):2953–2964. [PubMed: 7500173]
86. Wu D, Cederbaum AI. Cyclosporine A protects against arachi-donic acid toxicity in rat hepatocytes: role of CYP2E1 and mitochondria. *Hepatology.* 2002; 35(6):1420–1430. [PubMed: 12029627]
 87. Liu L-G, Yan H, Yao P, Zhang W, Zou L-J, Song F-F, Li K, Sun X-F. CYP2E1-dependent hepatotoxicity and oxidative damage after ethanol administration in human primary hepatocytes. *World J. Gastroenterol.* 2005; 11(29):4530–4535. [PubMed: 16052683]
 88. Phillips M, Curtis H, Portmann B, Donaldson N, Bomford A, O'Grady J. Antioxidants versus corticosteroids in the treatment of severe alcoholic hepatitis--a randomised clinical trial. *J. Hepatol.* 2006; 44(4):784–790. [PubMed: 16469404]
 89. Stewart S, Prince M, Bassendine M, Hudson M, James O, Jones D, Record C, Day CP. A randomized trial of antioxidant therapy alone or with corticosteroids in acute alcoholic hepatitis. *J. Hepatol.* 2007; 47(2):277–283. [PubMed: 17532088]
 90. Kono H, Rusyn I, Bradford BU, Connor HD, Mason RP, Thurman RG. Allopurinol prevents early alcohol-induced liver injury in rats. *J. Pharmacol. Exp. Ther.* 2000; 293(1):296–303. [PubMed: 10734182]
 91. Kono H, Arteel GE, Rusyn I, Sies H, Thurman RG. Ebse-len prevents early alcohol-induced liver injury in rats. *Free Radic. Biol. Med.* 2001; 30(4):403–411. [PubMed: 11182296]
 92. Kono H, Rusyn I, Uesugi T, Yamashina S, Connor HD, Dikalova A, Mason RP, Thurman RG. Diphenyleneiodonium sulfate, an NADPH oxidase inhibitor, prevents early alcohol-induced liver injury in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2001; 280(5):G1005–G1012. [PubMed: 11292610]
 93. Mato JM, Cámara J, Fernández de Paz J, Caballería L, Coll S, Caballero A, García-Buey L, Beltrán J, Benita V, Caballería J, Solà R, Moreno-Otero R, Barroa F, Martín-Duce A, Correa JA, Parés A, Barroa E, García-Magaz I, Puerta JL, Moreno J, Boissard G, Ortiz P, Rodés J. S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J. Hepatol.* 1999; 30(6):1081–1089. [PubMed: 10406187]
 94. Addolorato G, Ancona C, Capristo E, Gasbarrini G. Metadox-ine in the treatment of acute and chronic alcoholism: a review. *Int. J. Immunopathol. Pharmacol.* 2003; 16(3):207–214. [PubMed: 14611722]
 95. Ki SH, Choi JH, Kim CW, Kim SG. Combined metadox-ine and garlic oil treatment efficaciously abrogates alcoholic steatosis and CYP2E1 induction in rat liver with restoration of AMPK activity. *Chem.-Biol. Interact.* 2007; 169(2):80–90. [PubMed: 17606256]
 96. Swaminathan K, Clemens DL, Dey A. Inhibition of CYP2E1 leads to decreased malondialdehyde-acetaldehyde adduct formation in VL-17A cells under chronic alcohol exposure. *Life Sci.* 2013; 92(6–7):325–336. [PubMed: 23352969]
 97. Ronis MJ, Korourian S, Blackburn ML, Badeaux J, Badger TM. The role of ethanol metabolism in development of alcoholic steatohepatitis in the rat. *Alcohol.* 2010; 44(2):157–169. [PubMed: 20116195]
 98. Lu Y, Wu D, Wang X, Ward SC, Cederbaum AI. Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. *Free Radic. Biol. Med.* 2010; 49(9):1406–1416. [PubMed: 20692331]
 99. Zeng T, Zhang C-L, Song F-Y, Zhao X-L, Xie K-Q. Garlic oil alleviated ethanol-induced fat accumulation via modulation of SREBP-1, PPAR- α , and CYP2E1. *Food Chem. Toxicol.* 2012; 50(3–4):485–491. [PubMed: 22138249]
 100. Bansal S, Srinivasan S, Anandasadagopan S, Chowdhury AR, Selvaraj V, Kalyanaraman B, Joseph J, Avadhani NG. Additive effects of mitochondrion-targeted cytochrome CYP2E1 and alcohol toxicity on cytochrome c oxidase function and stability of respirasome complexes. *J. Biol. Chem.* 2012; 287(19):15284–15297. [PubMed: 22396533]
 101. Raza H, John A. 4-hydroxynonenal induces mitochondrial oxidative stress, apoptosis and expression of glutathione S-transferase A4-4 and cytochrome P450 2E1 in PC12 cells. *Toxicol. Appl. Pharmacol.* 2006; 216(2):309–318. [PubMed: 16843508]

102. Bardag-Gorce F, French BA, Nan L, Song H, Nguyen SK, Yong H, Dede J, French SW. CYP2E1 induced by ethanol causes oxidative stress, proteasome inhibition and cytokeratin ag-gresome (Mallory body-like) formation. *Exp. Mol. Pathol.* 2006; 81(3):191–201. [PubMed: 17034788]
103. Novak RF, Woodcroft KJ. The alcohol-inducible form of cytochrome P450 (CYP 2E1): role in toxicology and regulation of expression. *Arch. Pharm. Res.* 2000; 23(4):267–282. [PubMed: 10976571]
104. Jin M, Ande A, Kumar A, Kumar S. Regulation of cytochrome P450 2e1 expression by ethanol: role of oxidative stress-mediated pkc/jnk/sp1 pathway. *Cell Death Dis.* 2013;4.
105. Wickramasinghe SN. Neuroglial and neuroblastoma cell lines are capable of metabolizing ethanol via an alcohol-dehydrogenase-independent pathway. *Alcohol. Clin. Exp. Res.* 1987; 11(3):234–237. [PubMed: 3307484]
106. Wickramasinghe SN. Observations on the biochemical basis of ethanol metabolism by human macrophages. *Alcohol Alcohol.* 1986; 21(1):57–63. [PubMed: 3954830]
107. Zimatkin SM, Pronko SP, Vasiliou V, Gonzalez FJ, Dei-trich RA. Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 2006; 30(9):1500–1505. [PubMed: 16930212]
108. Silverstein PS, Kumar S, Kumar A. HIV-1, HCV and alcohol in the CNS: potential interactions and effects on neuroinflammation. *Curr. HIV Res.* 2014; 12(4):282–292. [PubMed: 25053363]
109. Zhang R-H, Gao J-Y, Guo H-T, Scott GI, Eason AR, Wang X-M, Ren J. Inhibition of CYP2E1 attenuates chronic alcohol intake-induced myocardial contractile dysfunction and apoptosis. *Biochim. Biophys. Acta.* 2013; 1832(1):128–141. [PubMed: 22967841]
110. Watkins PB, Kaplowitz N, Slattery JT, Colanese CR, Colucci SV, Stewart PW, Harris SC. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. *JAMA.* 2006; 296(1):87–93. [PubMed: 16820551]
111. Lancaster EM, Hiatt JR, Zarrinpar A. Acetaminophen hepato-toxicity: an updated review. *Arch. Toxicol.* 2014
112. Nourjah P, Ahmad SR, Karwoski C, Willy M. Estimates of acetaminophen (Paracetamol)-associated overdoses in the United States. *Pharmacoepidemiol. Drug Saf.* 2006; 15(6):398–405. [PubMed: 16294364]
113. Lesko SM, Louik C, Vezina RM, Mitchell AA. Asthma morbidity after the short-term use of ibuprofen in children. *Pediatrics.* 2002; 109(2):E20. [PubMed: 11826230]
114. Brandlistuen RE, Ystrom E, Nulman I, Koren G, Nordeng H. Prenatal paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study. *Int. J. Epidemiol.* 2013; 42(6):1702–1713. [PubMed: 24163279]
115. Edelman EJ, Gordon KS, Lo Re V, Skanderson M, Fiellin DA, Justice AC, Team VP. Acetaminophen receipt among HIV-infected patients with advanced hepatic fibrosis. *Pharmacoepidemiol. Drug Saf.* 2013; 22(12):1352–1356.
116. Hazai E, Vereczkey L, Monostory K. Reduction of toxic metabolite formation of acetaminophen. *Biochem. Biophys. Res. Commun.* 2002; 291(4):1089–1094. [PubMed: 11866476]
117. Jan Y-H, Heck DE, Dragomir A-C, Gardner CR, Laskin DL, Laskin JD. Acetaminophen reactive intermediates target hepatic thioredoxin reductase. *Chem. Res. Toxicol.* 2014; 27(5):882–894. [PubMed: 24661219]
118. Brune K, Renner B, Tiegs G. Acetaminophen/paracetamol: A history of errors, failures and false decisions. *Eur. J. Pain.* 2014
119. Moore ND, Gulmez E, Blin P. Is paracetamol hepatotoxic at normal doses? *BMJ.* 2013;346.
120. Suzuki A, Yuen N, Walsh J, Papay J, Hunt CM, Diehl AM. Co-medications that modulate liver injury and repair influence clinical outcome of acetaminophen-associated liver injury. *Clin. Gastroenterol. Hepatol.* 2009; 7(8):882–888. [PubMed: 19362607]
121. Johnson BP, Walisser JA, Liu Y, Shen AL, McDearmon EL, Moran SM, McIntosh BE, Vollrath AL, Schook AC, Takahashi JS, Bradfield CA. Hepatocyte circadian clock controls acetaminophen bioactivation through NADPH-cytochrome P450 oxidoreductase. *Proc. Natl. Acad. Sci. U. S. A.* 2014; 111(52):18757–18762. [PubMed: 25512522]
122. Yang CS. Inhibition of carcinogenesis and toxicity by dietary constituents. *Adv. Exp. Med. Biol.* 2001; 500:541–550. [PubMed: 11764996]

123. Yang CS, Chhabra SK, Hong JY, Smith TJ. Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sul-fide (DAS) and related compounds from garlic. *J. Nutr.* 2001; 131(3s):1041S–1045S. [PubMed: 11238812]
124. Hu JJ, Yoo JS, Lin M, Wang EJ, Yang CS. Protective effects of diallyl sulfide on acetaminophen-induced toxicities. *Food Chem. Toxicol.* 1996; 34(10):963–969. [PubMed: 9012771]
125. Lin MC, Wang EJ, Patten C, Lee MJ, Xiao F, Reuhl KR, Yang CS. Protective effect of diallyl sulfone against aceta-minophen-induced hepatotoxicity in mice. *J. Biochem. Toxicol.* 1996; 11(1):11–20. [PubMed: 8806047]
126. Dai Y, Cederbaum AI. Cytotoxicity of acetaminophen in human cytochrome P4502E1-transfected HepG2 cells. *J. Pharmacol. Exp. Ther.* 1995; 273(3):1497–1505. [PubMed: 7791125]
127. Tanaka E, Terada M, Misawa S. Cytochrome P450 2E1: its clinical and toxicological role. *J. Clin. Phar. Ther.* 2000; 25(3):165–175.
128. Spracklin DK, Emery ME, Thummel KE, Kharasch ED. Concordance between trifluoroacetic acid and hepatic protein trifluoroacetylation after disulfiram inhibition of halothane metabolism in rats. *Acta Anaesthesiol. Scand.* 2003; 47(6):765–770. [PubMed: 12803597]
129. Sun Q, Wang G, Gao L, Shi L, Qi Y, Lv X, Jin Y. Roles of CYP2e1 in 1,2-dichloroethane-induced liver damage in mice. *Environ. Toxicol.* 2015
130. Wong FW, Chan WY, Lee SS. Resistance to carbon tetra-chloride-induced hepatotoxicity in mice which lack CYP2E1 expression. *Toxicol. Appl. Pharmacol.* 1998; 153(1):109–118. [PubMed: 9875305]
131. Sohn OS, Fiala ES, Requeijo SP, Weisburger JH, Gonzalez FJ. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. *Cancer Res.* 2001; 61(23):8435–8440. [PubMed: 11731424]
132. Brady JF, Li D, Ishizaki H, Yang CS. Effect of Diallyl Sul-fide on Rat Liver Microsomal Nitrosamine Metabolism and Other Monooxygenase Activities. *Cancer Res.* 1988; 48(21):5937–5940. [PubMed: 3167846]
133. Fanelli SL, Castro GD, de Toranzo EG, Castro JA. Mechanisms of the preventive properties of some garlic components in the carbon tetrachloride-promoted oxidative stress. Diallyl sulfide; diallyl disulfide; allyl mercaptan and allyl methyl sulfide. *Res. Commun. Mol. Pathol. Pharmacol.* 1998; 102(2):163–174. [PubMed: 10100508]
134. Brady JF, Wang MH, Hong JY, Xiao F, Li Y, Yoo JS, Ning SM, Lee MJ, Fukuto JM, Gapac JM. Modulation of rat hepatic microsomal monooxygenase enzymes and cytotoxicity by diallyl sulfide. *Toxicol. Appl. Pharmacol.* 1991; 108(2):342–354. [PubMed: 2017758]
135. Brady JF, Ishizaki H, Fukuto JM, Lin MC, Fadel A, Gapac JM, Yang CS. Inhibition of cytochrome P-450 2E1 by diallyl sulfide and its metabolites. *Chem. Res. Toxicol.* 1991; 4(6):642–647. [PubMed: 1807447]
136. Ha HR, Chen J, Freiburghaus AU, Follath F. Metabolism of theophylline by cDNA-expressed human cytochromes P-450. *Br. J. Clin. Pharmacol.* 1995; 39(3):321–326. [PubMed: 7619675]
137. Kim YC, Lee AK, Lee JH, Lee I, Lee DC, Kim SH, Kim SG, Lee MG. Pharmacokinetics of theophylline in diabetes mellitus rats: induction of CYP1A2 and CYP2E1 on 1,3-dimethyluric acid formation. *Eur. J. Pharm. Sci.* 2005; 26(1):114–123. [PubMed: 15985363]
138. Pocernich CB, Sultana R, Mohmmad-Abdul H, Nath A, Butterfield DA. HIV-dementia, Tat-induced oxidative stress, and antioxidant therapeutic considerations. *Brain Res. Brain Res. Rev.* 2005; 50(1):14–26. [PubMed: 15890409]
139. Price TO, Ercal N, Nakaoke R, Banks WA. HIV-1 viral proteins gp120 and Tat induce oxidative stress in brain endothelial cells. *Brain Res.* 2005; 1045(1–2):57–63. [PubMed: 15910762]
140. Gil L, Martínez G, González I, Tarinas A, Alvarez A, Giuliani A, Molina R, Tápanes R, Pérez J, León OS. Contribution to characterization of oxidative stress in HIV/AIDS patients. *Pharmacol. Res.* 2003; 47(3):217–224. [PubMed: 12591017]
141. Steiner J, Haughey N, Li W, Venkatesan A, Anderson C, Reid R, Malpica T, Pocernich C, Butterfield DA, Nath A. Oxidative stress and therapeutic approaches in HIV dementia. *An-tioxid. Redox Signal.* 2006; 8(11–12):2089–2100.
142. Li W, Li G, Steiner J, Nath A. Role of Tat protein in HIV neuropathogenesis. *Neurotox. Res.* 2009; 16(3):205–220. [PubMed: 19526283]

143. Buccigrossi V, Laudiero G, Nicastro E, Miele E, Esposito F, Guarino A. The HIV-1 transactivator factor (Tat) induces entero-cyte apoptosis through a redox-mediated mechanism. *PLoS One*. 2011; 6(12)
144. Cota-Gomez A, Flores AC, Ling X-F, Varella-Garcia M, Flores SC. HIV-1 Tat increases oxidant burden in the lungs of transgenic mice. *Free Radic. Biol. Med.* 2011
145. Aksenov MY, Hasselrot U, Bansal AK, Wu G, Nath A, Anderson C, Mactutus CF, Booze RM. Oxidative damage induced by the injection of HIV-1 Tat protein in the rat striatum. *Neurosci. Lett.* 2001; 305(1):5–8. [PubMed: 11356294]
146. Shah A, Kumar S, Simon SD, Singh DP, Kumar A. HIV gp120- and methamphetamine-mediated oxidative stress induces astrocyte apoptosis via cytochrome P450 2E1. *Cell Death Dis.* 2013; 4:e850. [PubMed: 24113184]
147. Louboutin J-P, Agrawal L, Reyes BAS, Van Bockstaele EJ, Strayer DS. HIV-1 gp120-induced injury to the blood-brain barrier: role of metalloproteinases 2 and 9 and relationship to oxidative stress. *J. Neuropathol. Exp. Neurol.* 2010; 69(8):801–816. [PubMed: 20613638]
148. Ronaldson PT, Bendayan R. HIV-1 viral envelope glycoprotein gp120 produces oxidative stress and regulates the functional expression of multidrug resistance protein-1 (Mrp1) in glial cells. *J. Neurochem.* 2008; 106(3):1298–1313. [PubMed: 18485102]
149. Deshmane SL, Mukerjee R, Fan S, Del Valle L, Michiels C, Sweet T, Rom I, Khalili K, Rappaport J, Amini S, Sa-way BE. Activation of the oxidative stress pathway by HIV-1 Vpr leads to induction of hypoxia-inducible factor 1 α expression. *J. Biol. Chem.* 2009; 284(17):11364–11373. [PubMed: 19204000]
150. Isaguliantis M, Smirnova O, Ivanov AV, Kilpelainen A, Kuzmenko Y, Petkov S, Latanova A, Krotova O, Engström G, Karpov V, Kochetkov S, Wahren B, Starodubova E. Oxidative stress induced by HIV-1 reverse transcriptase modulates the enzyme's performance in gene immunization. *Hum. Vaccin. Immunother.* 2013; 9(10):2111–2119. [PubMed: 23881028]
151. Kumar S, Rao P, Earla R, Kumar A. Drug-drug interactions between anti-retroviral therapies and drugs of abuse in HIV systems. *Expert Opin. Drug Metab. Toxicol.* 2015; 11(3):343–355. [PubMed: 25539046]
152. Sherman KE, Thomas D, Chung RT. Human immunodeficiency virus and liver disease forum 2012. *Hepatology.* 2014; 59(1):307–317. [PubMed: 23904401]
153. Krashin DL, Merrill JO, Trescot AM. Opioids in the management of HIV-related pain. *Pain Physician.* 2012; 15(3 Suppl):ES157–ES168. [PubMed: 22786454]
154. Kumar S. Role of Cytochrome P450 Systems in Substance of Abuse Mediated HIV-1 Pathogenesis and NeuroAIDS. *J. Drug Metab. Toxicol.* 2012; 03(01)
155. Fan X, Staitieh BS, Jensen JS, Mould KJ, Greenberg JA, Joshi PC, Koval M, Guidot DM. Activating the Nrf2-mediated antioxidant response element restores barrier function in the alveolar epithelium of HIV-1 transgenic rats. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2013; 305(3):L267–L277. [PubMed: 23748533]
156. Schafer A, Galuppo P, Fraccarollo D, Vogt C, Widder JD, Pfrang J, Tas P, Barbosa-Sicard E, Ruetten H, Ertl G, Fleming I, Bauersachs J. Increased cytochrome P4502E1 expression and altered hydroxyeicosatetraenoic acid formation mediate diabetic vascular dysfunction: rescue by guanylyl-cyclase activation. *Diabetes.* 2010; 59(8):2001–2009. [PubMed: 20522591]
157. Swaminathan K, Kumar SM, Clemens DL, Dey A. Inhibition of CYP2E1 leads to decreased advanced glycosylated end product formation in high glucose treated ADH and CYP2E1 over-expressing VL-17A cells. *Biochim. Biophys. Acta.* 2013; 1830(10):4407–4416. [PubMed: 23707663]
158. Woodcroft KJ, Hafner MS, Novak RF. Insulin signaling in the transcriptional and posttranscriptional regulation of CYP2E1 expression. *Hepatology.* 2002; 35(2):263–273. [PubMed: 11826398]
159. Moncion A, Truong NT, Garrone A, Beaune P, Barouki R, De Waziers I. Identification of a 16-nucleotide sequence that mediates post-transcriptional regulation of rat CYP2E1 by insulin. *J. Biol. Chem.* 2002; 277(48):45904–45910. [PubMed: 12270935]

160. Kaut O, Schmitt I, Wullner U. Genome-scale methylation analysis of Parkinson's disease patients' brains reveals DNA hypomethylation and increased mRNA expression of cytochrome P450 2E1. *Neurogenetics*. 2012; 13(1):87–91. [PubMed: 22238121]
161. Shahabi HN, Westberg L, Melke J, Hakansson A, Belin AC, Sydow O, Olson L, Holmberg B, Nissbrandt H. Cytochrome P450 2E1 gene polymorphisms/haplotypes and Parkinson's disease in a Swedish population. *J. Neural Transm.* 2009; 116(5):567–573. [PubMed: 19381774]
162. Yun YP, Casazza JP, Sohn DH, Veech RL, Song BJ. Pretranslational activation of cytochrome P450IIE during ketosis induced by a high fat diet. *Mol. Pharmacol.* 1992; 41(3):474–479. [PubMed: 1545775]
163. Zong H, Armoni M, Harel C, Karnieli E, Pessin JE. Cytochrome P-450 CYP2E1 knockout mice are protected against high-fat diet-induced obesity and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* 2012; 302(5):E532–E539. [PubMed: 22185839]
164. Puccinelli E, Gervasi PG, Pelosi G, Puntoni M, Longo V. Modulation of cytochrome P450 enzymes in response to continuous or intermittent high-fat diet in pigs. *Xenobiotica*. 2013; 43(8):686–698. [PubMed: 23360109]
165. Abdelmegeed MA, Banerjee A, Yoo SH, Jang S, Gonzalez FJ, Song BJ. Critical role of cytochrome P450 2E1 (CYP2E1) in the development of high fat-induced non-alcoholic steatohepatitis. *J. Hepatol.* 2012; 57(4):860–866. [PubMed: 22668639]
166. Kumar S, Kumar A. Differential effects of ethanol on spectral binding and inhibition of cytochrome P450 3A4 with eight protease inhibitors antiretroviral drugs. *Alcohol Clin. Exp. Res.* 2011; 35(12):2121–2127. [PubMed: 21682753]
167. Li J, Wei DQ, Wang JF, Yu ZT, Chou KC. Molecular dynamics simulations of CYP2E1. *Med. Chem.* 2012; 8(2):208–221. [PubMed: 22385180]
168. Johnson EF, Connick JP, Reed JR, Backes WL, Desai MC, Xu L, Estrada DF, Laurence JS, Scott EE. Correlating structure and function of drug-metabolizing enzymes: progress and ongoing challenges. *Drug Metab. Dispos.* 2014; 42(1):9–22. [PubMed: 24130370]
169. Orr ST, Ripp SL, Ballard TE, Henderson JL, Scott DO, Obach RS, Sun H, Kalgutkar AS. Mechanism-based inactivation (MBI) of cytochrome P450 enzymes: structure-activity relationships and discovery strategies to mitigate drug-drug interaction risks. *J. Med. Chem.* 2012; 55(11):4896–933. [PubMed: 22409598]
170. Porubsky PR, Battaile KP, Scott EE. Human cytochrome P450 2E1 structures with fatty acid analogs reveal a previously unobserved binding mode. *J. Biol. Chem.* 2010; 285(29):22282–22290. [PubMed: 20463018]
171. Porubsky PR, Meneely KM, Scott EE. Structures of human cytochrome P-450 2E1. Insights into the binding of inhibitors and both small molecular weight and fatty acid substrates. *J. Biol. Chem.* 2008; 283(48):33698–33707. [PubMed: 18818195]
172. Lewis DF, Lake BG, Bird MG, Loizou GD, Dickins M, Goldfarb PS. Homology modelling of human CYP2E1 based on the CYP2C5 crystal structure: investigation of enzyme-substrate and enzyme-inhibitor interactions. *Toxicol. In vitro*. 2003; 17(1):93–105. [PubMed: 12537967]
173. Earla R, Kumar S, Wang L, Bosinger S, Li J, Shah A, Gangwani M, Nookala A, Liu X, Cao L, Jackson A, Silverstein PS, Fox HS, Li W, Kumar A. Enhanced methamphetamine metabolism in rhesus macaque as compared with human: an analysis using a novel method of liquid chromatography with tandem mass spectrometry, kinetic study, and substrate docking. *Drug Metab. Dispos.* 2014; 42(12):2097–2108. [PubMed: 25301936]
174. Ruiz ME, Gantner ME, Talevi A. Applications of nanosystems to anticancer drug therapy (Part II. Dendrimers, micelles, lipid-based nanosystems). *Recent Pat. Anticancer Drug Discov.* 2014; 9(1):99–128. [PubMed: 23578193]
175. Golla K, Cherukuvada B, Ahmed F, Kondapi AK. Efficacy, safety and anticancer activity of protein nanoparticle-based delivery of doxorubicin through intravenous administration in rats. *PLoS One*. 2012; 7(12):e51960. [PubMed: 23284832]
176. Mahajan SD, Aalinkhel R, Law WC, Reynolds JL, Nair BB, Sykes DE, Yong KT, Roy I, Prasad PN, Schwartz SA. Anti-HIV-1 nanotherapeutics: promises and challenges for the future. *Int. J. Nanomedicine*. 2012; 7:5301–5314. [PubMed: 23055735]

177. Gendelman HE, Gelbard HA. Adjunctive and long-acting nanoformulated antiretroviral therapies for HIV-associated neuro-cognitive disorders. *Curr. Opin. HIV AIDS*. 2014; 9(6):585–590. [PubMed: 25226025]
178. Saiyed ZM, Gandhi NH, Nair MP. Magnetic nanoformulation of azidothymidine 5'-triphosphate for targeted delivery across the blood-brain barrier. *Int. J. Nanomedicine*. 2010; 5:157–166. [PubMed: 20463931]
179. Mahajan SD, Roy I, Xu G, Yong KT, Ding H, Aalinkeel R, Reynolds J, Sykes D, Nair BB, Lin EY, Prasad PN, Schwartz SA. Enhancing the delivery of anti retroviral drug "Saquinavir" across the blood brain barrier using nanoparticles. *Curr. HIV Res*. 2010; 8(5):396–404. [PubMed: 20426757]
180. Alam M, Zubair S, Farazuddin M, Ahmad E, Khan A, Zia Q, Malik A, Mohammad O. Development, characterization and efficacy of niosomal diallyl disulfide in treatment of disseminated murine candidiasis. *Nanomedicine*. 2013; 9(2):247–256. [PubMed: 22858760]

Fig. (1).

Enzymatic transformation of alliin and proposed scheme for the metabolism of DAS.

Alliinase converts alliin to allicin with pyruvate and ammonium ion as bi-products of this reaction. Self-condensation of sulfenic acid molecules formed in this reaction yields allicin. This unstable allicin is transformed into lipophilic polysulfides such as DAS, DADS and DATS. DAS further undergoes S-oxidation by CYP2E1 to generate its metabolites DASO and DASO₂. Additional oxidation of DAS and its metabolites at the terminal double bonds directs them to GSH conjugation. The structures of these compounds were made using ChemDraw Ultra 7.0.1 (CambridgeSoft Corporation, Cambridge, MA).

Fig. (2).

Schematic representation of potential uses for novel diallyl sulfide (DAS) analogs. Inhibition of CYP2E1 activity by DAS analogs is expected to reduce cellular toxicity associated with alcohol-, analgesics-, and xenobiotic-metabolism. In pathological conditions associated with CYP2E1 over expression or CYP2E1-mediated adverse effects including Diabetes/hyperlipidemia, Parkinson's, HIV, and Cancer, DAS attenuates disease pathogenesis and toxicity. In addition, antioxidant/anti-inflammatory effects of DAS further support its use as dietary supplement.

Fig. (3).

Schematic representation of molecular changes associated with DAS. A. Anti-cancer effects of DAS treatment in tumor cells is attributed to enhanced apoptosis. The key cellular event mediating this increase in apoptosis includes ROS-induced enhanced DNA damage. Increased ROS production also activates downstream pathways like ER stress and intracellular calcium release. Enhanced ER stress in turn activates the release of mitochondrial cytochrome c, which increases caspase-mediated apoptosis. DAS-mediated significant increase in the levels of proapoptotic protein (bax) and p53-mediated cell cycle arrest have been identified as alternate pathways activating apoptosis. B. Protective effects of DAS have been summarized. The major cellular changes associated with protective effects of DAS include increased expression of AOE, glutathione levels, and reduced activation of proinflammatory signaling molecules like NFκB and COX-2. Enhanced nuclear translocation of transcription factor NRF2 has been identified as an important event responsible for the antioxidant effects observed with DAS. DAS: diallyl sulfide, ROS: reactive oxygen species, ER: endoplasmic reticulum, AOE: anti-oxidant enzymes, GSH: glutathione, GSSG: oxidized glutathione, Nrf2: nuclear factor (erythroid-derived 2)-like 2, HO-1: heme oxygenase-1, NFκB: nuclear factor κB, p38 MAPK: p38 mitogen activated protein kinase, COX-2: cyclooxygenase-2, PI3K: phosphoinositide 3-kinase.

Fig. (4).

Mechanism of CYP2E1 activation by substrates and inhibition by diallyl sulfide (DAS). Metabolic processing of various substrates by CYP2E1 induces the formation of toxic secondary metabolites and reactive oxygen species (ROS). Accumulation of these reactants damages the cellular structures by injuring cell membranes, causing DNA and proteins adducts formation, and ultimately causes imbalances in redox state and promotes apoptosis and cell death. DAS or its analog can rescue the system from these deleterious effects by inhibiting CYP2E1 metabolism most likely using competitive and/or suicide inhibitory mechanism.

Fig. (5).
Structures of known diallyl sulfide analogs. The structures of these compounds were made using ChemDraw Ultra 7.0.1 (CambridgeSoft Corporation, Cambridge, MA).