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Recent Updates on Acetaminophen Hepatotoxicity: The Role of Nrf2 in Hepatoprotection

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Acetaminophen (APAP) known as paracetamol is the main ingredient in Tylenol, which has analgesic and anti-pyretic properties. Inappropriate use of APAP causes major morbidity and mortality secondary to hepatic failure. Overdose of APAP depletes the hepatic glutathione (GSH) rapidly, and the metabolic intermediate leads to hepatocellular death. This article reviews the mechanisms of hepatotoxicity and provides an overview of current research studies. Pharmacokinetics including metabolism (activation and detoxification), subsequent transport (efflux)-facilitating excretion, and some other aspects related to toxicity are discussed. Nuclear factor erythroid 2-related factor 2 (Nrf2)-regulated gene battery plays a critical role in the multiple steps associated with the mitigation of APAP toxicity. The role of Nrf2 as a protective target is described, and potential natural products inhibiting APAP toxicity are outlined. This review provides an update on the mechanism of APAP toxicity and highlights the beneficial role of Nrf2 and specific natural products in hepatoprotection.

***Key words*:** Acetaminophen, Hepatotoxicity, Nrf2, Natural product

INTRODUCTION

Acetaminophen (APAP) is a commonly used non-narcotic analgesic producing reduction of fever and relief of pain. APAP was first approved as OTC preparation by the FDA in the 1950s and has been available for years with extensive safety and effective history. However, overdose of APAP has recently been estimated a high rank as a major cause of fulminant hepatic failure and severe hepatotoxicity in the US and many European countries (1,2). The toxicity of APAP may be attributed to an acute overdose, repeated excessive dosing or mixed medications containing APAP.

APAP is detoxified mainly via formation of sulfate- and glucuronide-conjugates. When the enzymes saturated, APAP is increasingly metabolized into a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P450 (CYP). The NAPQI is subsequently detoxified by glutathione (GSH) and the conjugated metabolite is excreted. When GSH is depleted, NAPQI is accumulated in the hepatocyte and interacts with thiol-containing proteins leading to hepatic necrosis (3).

Covalent bindings between NAPQI and cellular proteins have been shown to be a high correlation with toxicity. Therefore, the control of NAPQI conjugation and its efflux determining excretion is a promising strategy to manage hepatotoxicity. The transcription factor nuclear factor-erythroid 2 related factor 2 (Nrf2) exerts influence on the several steps including GSH synthesis, antioxidative stress system, conjugation, transport and excretion of the metabolites via binding to the antioxidant response element (ARE) for hepatoprotection. The Nrf2-mediated gene battery serves as a pleiotropic target resistant to hepatic injury.

Clinically, N-acetylcysteine (NAC), a precursor of GSH, is the primary antidote for an APAP overdose for several decades (4). NAC replenishes the glutathione store and enhances hepatic recovery. However, the NAC therapy has a limit to protect liver from an APAP insult because of a narrow therapeutic window or limited timing of NAC administration. In addition, the reversal of GSH level is not enough to arrest progress of APAP-induced hepatotoxicity (5-7).

Previous studies monitoring beneficial natural products against APAP have been performed to determine serum index (e.g. aspartate aminotransferase (AST), alanine aminotransferase (ALT)), lipid peroxidation and pathological examination. In this review, current researches based on the mechanistic

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target against APAP hepatotoxicity are investigated, which can consider applications of new management.

APAP-induced hepatotoxicity. APAP toxicity remains the most common cause of drug-induced hepatic failure. Most deaths from hepatic failure by APAP overdose occur within the first week after ingestion. The border dose of APAP to cause hepatotoxicity is believed to be between 125~150 mg/kg (8). Diseases including alcoholism, malnutrition, HIV infection and cancer which are associated with metabolic disturbances decrease detoxification of NAPQI supporting that the variable sensitivity to APAP depending on the patient's circumstance may be another risk factor. APAP-induced hepatotoxicity may progress to acute hepatic failure requiring immediate medical care. Even though 90% of patients are recovered from hepatic damage by proper treatment and management, liver transplantation is now the only therapy for patients that are not recovered.

The mechanism of APAP toxicity. In human, greater than 80% of APAP is metabolized to APAP-glucuronide (APAP-Glu) and APAP-sulfate in the liver by direct conjugation of glucuronyltransferases and sulfotransferases, respectively and these non-toxic metabolites are excreted into the urine. APAP-cysteine conjugate, APAP-NAC and unchanged APAP also are found in the urine. When glucuronyltrasferases and sulfotransferases become saturated, a small fraction of APAP (about 5% to 10%) is metabolized by CYP (predominantly CYP2E1) to NAPQI, the main electrophilic reactive metabolite. Under normal conditions, NAPQI is detoxified by either reduction back to APAP or conjugation with GSH. GSH storage is easily depleted under high dose of APAP resulting in covalent bonds between NAPQI and other cellular macromolecules. This subsequent event of APAP metabolic activation is a main initiating event of hepatocellular injury under hepatic GSH depletion especially in mitochondria (9). The metabolism of APAP mainly occurring in hepatocyte is defined (Fig. 1).

The differential sensitivity to APAP toxicity exists in the species. Mice are highly sensitive to APAP toxicity (10). There are very close similarities to the mechanism of APAP toxicity between human and mice (11). The proportion of the glutathione conjugate pathway (toxication pathway) to the sulfate/glucuronide conjugate pathway (inactivation pathway) in the APAP metabolism in mice highly implies that the predominance of the toxication pathway of APAP (APAP-

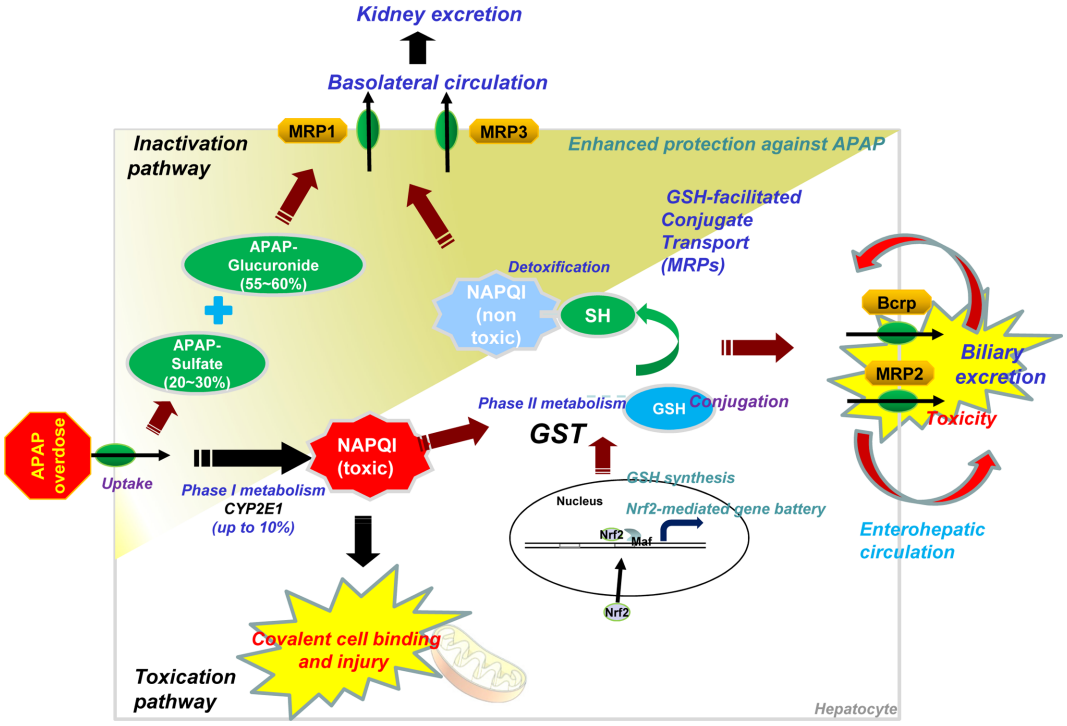

Fig. 1. The metabolic pathways of APAP accompanied with efflux into the bile and urine in hepatocyte. More than 80~90% of APAP is conjugated with glucuronic acid or sulfate following renal excretion. A small fraction is metabolized by CYP to form the reactive metabolite NAPQI, which can be conjugated with GSH. The excretion of GSH conjugated metabolite into the bile and urine is depicted. The transport of the metabolite into the bile can initiate enterohepatic recirculation, which affects the development of hepatotoxicity. Unconjugated NAPQI covalently binds to a cellular protein under GSH depletion, which causes hepatocyte damage. acetaminophen, APAP; breast cancer resistance protein, Bcrp; cytochrome P450 2E1, CYP2E1; glutathione, GSH; glutathione S-transferase, GST; multidrug resistance-associated protein transporters, MRP; N-acetyl-p-benzoquinone imine, NAPQI; NF-E2 related factor2, Nrf2.

glutathione conjugate) results in increased sensitivity to hepatotoxicity.

New discoveries on the molecular mechanisms of APAP hepatotoxicity continue to be made. The liver and the bile duct in human play a more important role in the breakdown of GSH conjugates (12). Bile duct ligation or a decrease in enterohepatic recirculation of APAP result in reduced sensitivity to APAP by an increase in urinary excretion of GSH conjugated APAP (13,14). The recovery of APAP-Glu in the perfusate and bile increased more than threefold upon increase of the dose of APAP. The basolateral secretion of APAP-Glu into blood decreased enterohepatic recirculation and increased urinary excretion (13). The pattern of metabolite formation, distribution and excretion seems to be determinant of APAP hepatotoxicity.

The mechanisms of APAP toxicity are still complicate even though there is a lot of information in the literature. Interestingly, the newly recruited monocyte-derived tissue macrophages after APAP-induced injury mainly contributed to removal of dead cells and activation of liver regeneration (15). It is generally accepted that hepatic damage by APAP is necrosis, even though the involvement of apoptotic mechanism in the APAP hepatotoxicity is proposed (16). An oxidative phase which may be generated after metabolic phases of APAP (GSH depletion and adduct formation) occurs with increased oxidative stress, lipid peroxidation, disturbance of calcium homeostasis, loss of mitochondrial membrane potential, and hepatotoxicity (9). There are some reports on the nuclear effect of APAP including impaired DNA repair and DNA adduct formation (17).

Nrf2 as a potential therapeutic target against APAP-induced hepatotoxicity. There are several evidences to support the fact that Nrf2-mediated gene regulation is efficacious in the protection of APAP-induced hepatotoxicity. The electrophilic stress leads to disrupted interaction between Nrf2 and kelch-like ECH associating protein 1 (keap1), Nrf2 inhibitor protein, which permits the suppression of Nrf2 degradation following transcription of a large battery of cytoprotective genes via binding to the antioxidant response element (ARE) (18). These Nrf2-medicated genes are required for the regulation of the hepatic function related to GSH synthesis, conjugation, detoxification and transport (Fig. 2). The higher susceptibility of Nrf2-null mice and extreme resistance of hepatocyte keap1-null mice to APAP toxicity supported that Nrf2-mediated gene battery serves as targets of hepatoprotection (18,19).

Nrf2 has a profound effect on hepatic metabolism. The Nrf2-dependent antioxidant defense system composed of GSH synthesis, phase II detoxifying enzyme, and reactive oxygen species inactivating enzymes plays a key role in protecting cells upon oxidative damage by NAPQI (20). Nrf2-

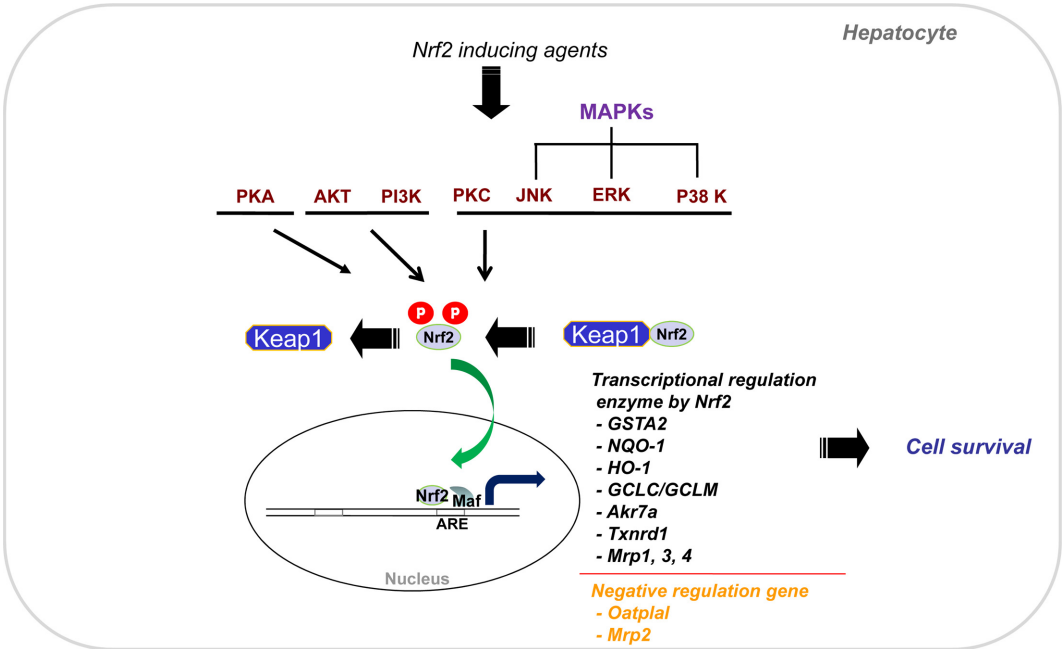

Fig. 2. The Nrf2-dependent gene regulation. The Nrf2 released from Keap1 translocates to the nucleus through multiple upstream cell signaling pathways. Once in the nucleus, Nrf2 causes heterodimerization with a small Maf protein. This complex interacts with ARE in the promoter of target genes and activates gene transcription. The Nrf2-mediated gene battery in hepatocytes is shown. Some genes are negatively regulated by Nrf2. aldo-keto reductase 7A, Akr7a; glutamate cysteine ligase, GCL; glutathione S-transferase alpha2, GSTA2; heme oxygenase-1, HO-1; NAD(P)H:quinone oxidoreductase 1, NQO1; multidrug resistance-associated protein transporters, Mrp; orgarnic anion-transporting polypeptides, Oatp1al; protein tyrosine phosphatase 1B, PTP1B; thioredoxin reductase 1, Txnrd1.

mediated gene expression of glutamate cysteine ligase (GCL) facilitating GSH synthesis ameliorates NAPQI-induced hepatotoxicity (21). Phase II drug-metabolizing enzymes, such as glutathione S-transferase (GST) and NAD(P)H: quinone oxidoreductase 1 (NQO1), which serve to detoxify the reactive intermediates, fail to function in Nrf2 knockdown or knockout system. The GST catalyzes the conjugation of GSH with elctrophilic NAPQI following elimination concomitant with a decrease of hepatic NAPQI content.

In addition to the role of Nrf2 on the hepatic detoxification of NAPQI, Nrf2 stimulates hepatic gene expression of multidrug resistance-associated protein (Mrp) transport, which exerts the efflux of xenobiotic and its conjugates into the bile or urine instead of their accumulation in the liver (22). Mrp2 transports conjugates of glucuronate, sulfate and GSH into the bile (from hepatocyte) or urine (from renal proximal tubular cells), whereas Mrp3 and Mrp4 in the basolateral membrane transport these into the bloodstream towards renal excretion (22,23). High levels of APAP-glu in the plasma by hepatic Mrp3 induction lead to excretion into the kidney, which suppresses APAP-toxicity (13,14,20). Breast cancer resistance protein (Bcrp) in the canalicular membrane also contributes efflux of GSH conjugate to the bile (24). The dysregulation of ARE in the protective antioxidant defense system and transporters (especially efflux) is attributed to high sensitivity to APAP hepatotoxicity in the Nrf2-knockout mice (20,25). The coordination of detoxification and transport pathways by Nrf2 may enhance action in the mitigation of cellular injury.

Liver-specific loss of Atg5 related to autophagy causes persistent activation of Nrf2 and suppresses APAP-induced liver injury (26). The disruption of liver-specific selenoprotein thioredoxin reductase 1, attenuating the activation of the apoptosis signaling-regulating kinase 1 (ASK1) and the c-jun N-terminal kinase pathway, mitigates APAP hepatotoxicity via Nrf2-mediated genes including GSTs, GCLC, Mrp3/4 (27). Protein tyrosine phosphatase 1B, a negative regulator of tyrosine kinase growth factor signaling was increased after APAP overdosing and loss of the gene mitigates APAP hepatotoxicity via Nrf2-mediated antioxidant response signaling through glycogen synthase kinase 3b/Src kinases axis (28). There are ongoing studies on new Nrf2-regulated genes associated with APAP hepatotoxicity.

Modulators of APAP toxicity. Excessive APAP beyond the capacity of conjugation pathways results in an increase of NAPQI formation by CYP pathway leading to GSH depletion and ultimately hepatic injury. As mentioned above, GSH plays a key role in the detoxification of NAPQI. The relationship between GSH content and APAP toxicity is reciprocal. By far, NAC (Gluimmicil^{TM}) is the only choice to treat the APAP hepatic failure just in case of early presenter (less than 15 h after a single APAP overdose) because NAC replenishes GSH depletion by excessive APAP (29). However, patients of chronic ingestion of high dose of APAP or delayed administration of NAC are at risk of hepatic failure and death. And NAC has adverse events including gastrointestinal disturbances and anaphylatoid reaction (8). Therefore, subsequent investigation based on mechanistic targets of APAP toxicity is required to present alternative management for acute hepatic failure in clinic. APAP in combination of methionine, a GSH precursor was formulated based on the mechanism of hepatoprotection. Furthermore, S-adenosylmethionine has been proposed as an alternative to NAC in patients who develop symptom late after an APAP overdose (30).

Because NAPQI production by the CYP system especially CYP2E1 is the risk factor, any agents inhibiting the enzyme activity suppress the APAP hepatotoxicity. Clinical researches supported this notion of metabolic target for the treatment of APAP hepatotoxicity. Chronic alcoholics with low level of plasma GSH and high CYP2E1 showed a fivefold increase in the risk of hepatic encephalopathy (31). Recently, the combination with NAC and cimetidine (a CYP inhibitor) enhanced the protective effect of NAC implying that multiple targeting is useful to protect APAP-mediated hepatotoxicity and minimizes the adverse effect of NAC (32). In the previous research, we reported that Korean red ginseng suppressing CYP2E1 expression altered the APAP profile with a high parent APAP (33). Purinergic receptor antagonist protects APAP-induced hepatotoxicity by CYP inhibition (34).

There is also increasing evidence showing that modulators of transcription factor can be good candidates protecting APAP hepatotoxicity. Clofibrate, a peroxisome proliferating agent resulted in protection against APAP-induced hepatotoxicity via facilitating both elimination of APAP from liver and plasma and urinary excretion of parent APAP (35). An Nrf2 activator butyrate hydroxylanisole protected against APAP hepatotoxicity (36). Oleanolic acid, a natural triterpenoid compound found in many plants attenuated APAP hepatotoxicity via Nrf2 activation (37). An oleanolic acid derivative, CDDO-Me is currently in late phase II stages of clinical development (38). Ginsenoside Rg3, a major component of Korean red ginseng is efficacious in protecting APAP insult, due to GSH repletion and coordinated gene regulations of GSH synthesis and Mrp family genes by Nrf2 (21).

The strategy screening natural products from traditional medicines increasingly has been applied to investigate novel therapeutic. We reviewed literatures with searching keywords: acetaminophen and extract using the databases Medline. Based upon the results, the scientific name of natural products with protective potential and parameter studied were summarized in Fig. 3 and Table 1. *Azadirachta indica* also known as Neem is reported to be effective in the APAP-induced hepatotoxicity via regulation of NaK-ATPase activity and GSH content (39). Some candidates influence on the metabolism including an inhibition of CYP expres-

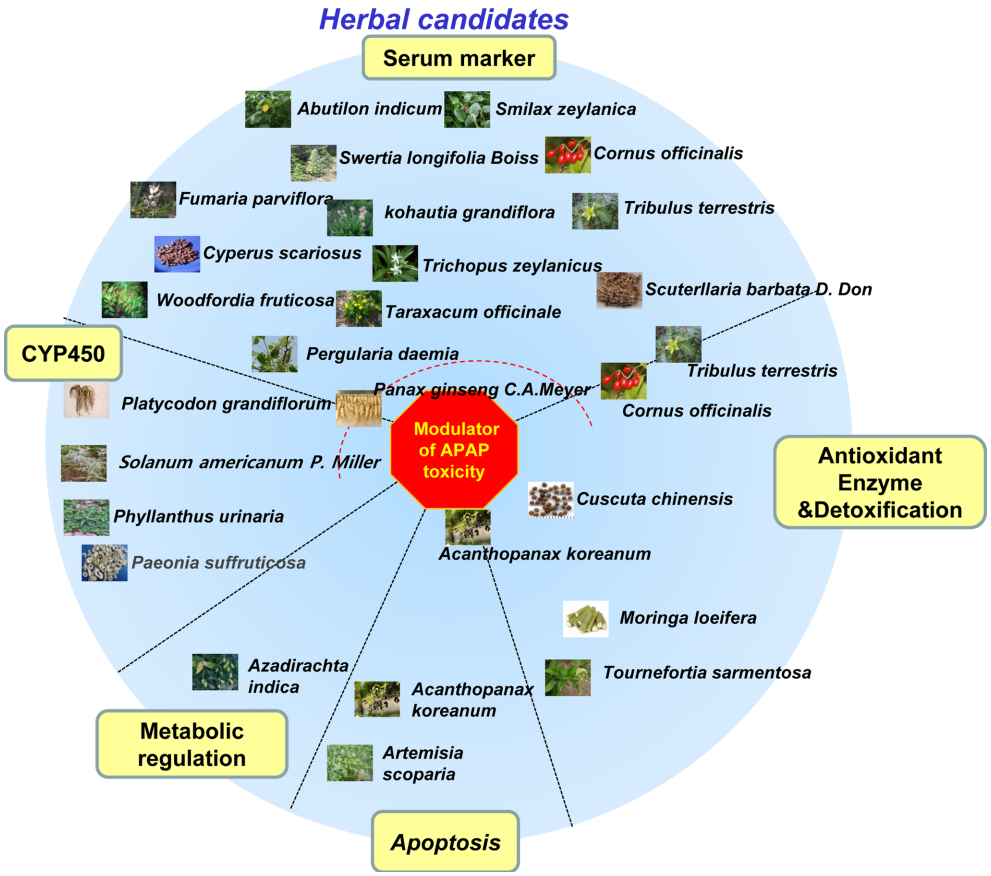

Fig. 3. The potential candidates from natural products against APAP hepatotoxicity. The Pie chart shows the percentage distribution of the target, the scientific name of plants and action targets studied. The natural products presented on the dotted line indicate the effect on both sides. A red dotted line in the chart displays the target of *Panax ginseng C.A.Meyer*. The original source of images for each plant is from the google site.

Table 1. The pharmacological effects of the natural products in the APAP-overdose model

Scientific name	Effects	Reference
Abutilon indicum	AST↓, ALT↓, LPO↓	(44)
Artemisia scoparia	AST↓, ALT↓	(45)
Azadirachta indica	AST↓, ALT↓, NaK-ATPase activity, GSH↑, Thiobarbutiric acid	(39)
Scuterllaria barbata D. Don	AST↓, ALT↓	(46)
Cuscuta chinensis	GSH↑, SOD↑, MDA↓	(47)
Cyperus scariosus	AST↓, ALT↓, LPO↓	(48)
Fumaria parviflora	AST↓, ALT↓, LPO↓	(49)
Kohautia grandiflora	AST↓, ALT↓, ALP↓	(50)
Moringa oleifera	AST↓, ALT↓, MDA↓, GSH↑, SOD↑, CAT↑	(40)
Paeonia suffruticosa	CYP2E1↓, DNA fragmentation	(41)
Panax ginseng C.A.Meyer	AST↓, ALT↓, GSTA2↑, CYP2E1↓, GSH↑	(33)
Pergularia daemia	AST↓, ALT↓	(51)
Phyllanthus urinaria	CYP2E1↓	(52)
Platycodon grandiflorum	CYP2E1↓, CYP1A2↓	(42)
Solanum americanum P. Miller	AST↓, ALT↓, GSH↑, CYP↓	(53)
Swertia longifolia Boiss	AST↓, ALT↓,	(54)
Taraxacum officinale	ROS↓, Thiobarbituric acid, GSH↑	(55)
Tribulus terrestris	AST↓, ALT↓, ALP↓, SOD↑, GSH↑	(43)
Trichopus zeylanicus	AST↓, ALT↓, LPO↓	(56)
Woodfordia fruticosa	AST↓, ALT↓, Albumin↓, GSH↑	(57)

sion and/or an increase in detoxifying genes such as GST, superoxide dismutase (SOD) and catalase (CAT) (40-43). Korean red ginseng obtained from steamed *Panax ginseng C.A.Meyer* and its active component Rg3 showed pleiotropic protective effect that is mediated by Nrf2 (21,33). The advantageous effect of Korean red ginseng seems to modulate multiple steps related to APAP hepatotoxicity. Taken together, the multiple action of Nrf2 modulating distribution, metabolism and excretion of the electrophilic metabolite is a promising strategy to manage APAP hepatotoxicity.

CONCLUSIONS

APAP is widely used antipyretic drug all over the world but may cause significant morbidity and mortality in case of toxic-dose ingestion and improper use. Hepatic GSH depletion, NAPQI accumulation and subsequent adduct formation are believed as the main causes of APAP toxicity. We discussed current issues on other events including dysfunction of mitochondria, oxidant stress, nuclear DNA fragmentation and alteration of innate immunity. NAC is the most effective antidote of acute APAP poisoning. In case of chronic ingestion and late presentation of symptom, new regimen based on the therapeutic target is required to overcome NAC limitation. In this review, we elucidated the pivotal role of Nrf2 for hepatic protection on the multiple steps related with APAP hepatotoxicity. Modulators to increase GSH conjugation, suppress NAPQI production or alter efflux of the intermediate metabolite into the bile and urine can be considered as alternative strategies against APAP overdose. This review gives information on the current mechanistic studies of APAP toxicity and reveals potential candidates to make progress in new therapeutic management against APAP poisoning.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to disclose for any of the authors.

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