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Current etiological comprehension and therapeutic targets of acetaminophen-induced hepatotoxicity

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ABSTRACT

Acetaminophen (APAP) is the most popular mild analgesic and antipyretic drug used worldwide. APAP overdose leads to drug-induced hepatotoxicity and can cause hepatic failure if treatment delayed. It is adequately comprehended that the metabolism of high-dose APAP by cytochrome P450 enzymes generates *N*-acetyl-*p*-benzoquinone imine (NAPQI), a toxic metabolite, which leads to glutathione (GSH) depletion, oxidative stress, and activation of various complex molecular pathways that initiate liver injury and downstream hepatic necrosis. Administration of activated charcoal followed by N-acetylcysteine (NAC) is considered the mainstay therapy; however, including side effects and limitation of rescuing for the delayed patients where liver transplantation may be a lifesaving procedure. Many complex signal transduction pathways such as c-Jun NH_2-terminal kinase (JNK), mammalian target of rapamycin (mTOR), nuclear factor (NF)-κB, and NF (erythroid-derived 2)- like 2 (Nrf2) are involved in the development of APAP hepatotoxicity, but yet hasn't been comprehensively studied; thus, the search for effective antidotes and better management strategies continues. Here, we reviewed the most current advances to elucidate the etiological factors and therapeutic targets that could provide better strategies for the management of APAP-induced hepatotoxicity.

1. Introduction

Acetaminophen, also known as N-acetyl para-aminophenol, APAP, or paracetamol, is one of the most widely used, over the counter analgesic and antipyretic agents. Although its exact mechanism of action remains unknown and not categorized as a non-steroidal anti-inflammatory drug (NSAID) it selectively inhibits cyclooxygenase-2 (COX2) [1]. Similar to other NSAIDs, acetaminophen has analgesic and antipyretic properties but doesn't reduce inflammation [2]. Paracetamol was first marketed in the US in 1950 under the name Triagesic, as a combination of paracetamol, aspirin, and caffeine [3]. Since then, it has been one of the most commonly used analgesics worldwide.

APAP is believed to be safe at therapeutic doses, but when taken at single doses > 125 mg/kg, it can cause liver damage [4]. However, smaller doses may also cause liver damage, particularly in people with chronic alcohol abuse or anorexia. Some of the patients may have distinct APAP metabolism disorders at the mitochondria and molecular levels. Acetaminophen is the most common drug in the USA and is consumed by 500 million people weekly for various diseases, such as muscle pain, tooth pain, headache, arthritis, common cold, fever, and menstrual pain. When taken in amounts higher than the therapeutic dose (4000 g/per day for an adult), it leads to elevation of serum ALT and AST, a clear indication of liver injury [5].

Approximately 90–95 % of APAP is conjugated with glutathione (GSH) and 5–10 % is metabolized by the cytochrome P 450 (CYP) enzyme system, as shown in Fig. 1. The major phase 2 metabolism of APAP starts with sulfation and glucuronidation of its phenolic group. At therapeutic or safe doses, approximately 50 % of APAP is conjugated with glucuronic acid [6], and approximately 30 % is sulfated by phenol sulfotransferases [7]. The glucuronic acid conjugates are rapidly removed from hepatocytes, mainly by excretion (> 75 %) into the bile [8]. A smaller fraction (< 25 %) of the APAP-glucuronide is excreted into the plasma through the basolateral transporter multidrug resistance-associated protein 3 (mrp3) [9]. The APAP-sulfate conjugate is excreted into the bile via mrp2 and to a lesser extent by human breast cancer resistance protein 1 [10].

A relatively small fraction (5–10 %) of a therapeutic dose of APAP is metabolized by the CYP system to form *N*-acetyl-*p*-benzoquinone imine

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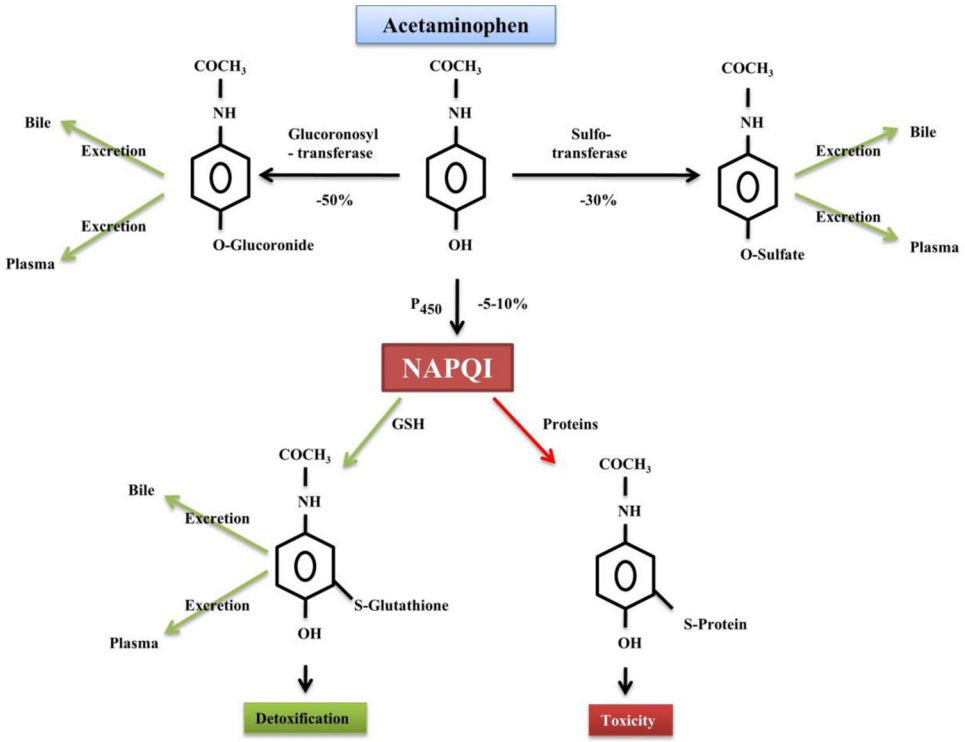

Fig. 1. The way APAP metabolizes. APAP is mainly metabolized through glucuronidation and sulfation. In the event of APAP overdose, APAP glucuronidation pathway is saturated due to limit amount of available glutathione (GSH). It leads to mass production of *N*-acetyl-*p*-benzoquinone imine (NAPQI) through extensive cytochrome P_{450} (CYP_{450}) metabolism which caused hepatotoxicity.

(NAPQI), the main toxic metabolite [11] (Fig. 1). GSH, which is found abundant in the liver (≈10mM) detoxifies this electrophile NAPQI by conjugating them with mercapturic acid and cysteine [12]. But in the case of APAP overdose GSH store becomes limited and the excess amount of NAPQI then binds with numerous cysteine residues [13]. Protein binding of NAPQI occurs mainly in centrilobular hepatocytes, which eventually undergo oxidative stress, mitochondrial dysfunctions and necrotic cell death [14].

N-acetyl cysteine (NAC), the precursor of GSH, has been widely used to treat APAP overdoses since 1980 [15]. After extensive first-pass metabolism, NAC (only FDA approved antidote for APAP toxicity) is deacetylated to cysteine and conjugated with glutamate, which is further converted into glutamylcysteine by the action of glutamate-cysteine ligase within hepatocytes. Glutamylcysteine is then combined with glycine by the action of GSH synthase to produce GSH [16]. Covalent bonding of acetaminophen is lessened by NAC which reduces hepatic necrosis [17]. NAC has also been reported for decreasing inflammatory response induced in the liver [18]. Besides scavenging ROS and peroxynitrite NAC also improve mitochondrial energy metabolism through ATP maintenance [19]. Currently, NAC is administered as the treatment for APAP-induced liver toxicity (AIHT), but transplantation could be the only treatment option for some patients [20] (Figs. 2 and 3).

2. Involvement of different potential targets for hepatotoxicity treatment

2.1. Role of cytochrome P450 (CYP) enzymes

Numerous CYP isoforms like CYP1A2, CYP2A6, CYP2D6, CYP2E1, and CYP3A4 can influence metabolize APAP, but the potential of each isoform to cause liver toxicity varies considerably [21]. CYP2E1 is the major CYP enzyme, an effective catalyst for NAPQI formation which makes it a selective target for APAP overdose management, though there is no report of NAC and CYP2E1 direct interaction. Benzyl alcohol has been studied in AIHT for its inhibition of the enzyme activities of Cyp1A2, Cyp2E1, and Cyp3A4 by 20 %–50 % but has a narrow therapeutic window [22]. Hau et al. (2009) demonstrated that *Phyllanthus urinaria* extract attenuated AIHT by decreasing CYP2E1 expression. Similarly, CYP2E1-targeted treatment with 4-methylpyrazole (4 M P), wuzhi, and boswellic acid improved hepatotoxicity [23–25]. Chiew et al. (2017) reported that increased NAC administration can significantly reduce hepatotoxicity within 21 h. Cimetidine, a Cytochrome P450 enzyme inhibitor [26] was applied combined with NAC for AIHT treatment [27]. But clinical trial with this combination showed that there was no significant improvement from the NAC treatment alone [28].

2.2. Activation of c-Jun N-terminal kinase (JNK)

The c-Jun NH_2-terminal kinase (JNK) belongs to a subgroup of mitogen-activated kinases (MAPKs) and is activated predominantly by cytokines and exposure to environmental stress [29]. Phosphorylated-JNK (p-JNK) translocation to the mitochondria increases the mitochondrial permeability pore transition and induces ROS accumulation inside mitochondria which is the main cause of JNK-dependent cell death in AIHT [30]. Normal and excessive APAP doses cause sustained JNK activation and translocation to the mitochondria, increasing mitochondrial permeability and pore transition. But at nontoxic dose ALT increment or necrosis doesn't occur which is opposite to the lethal dose [31]. 4 M P inhibits the translocation of p-JNK into the mitochondria and prevents liver injury [32]. The growth arrest and DNA damage-inducible 45 (*Gadd45*) family of genes, including *Gadd45a*, *Gadd45b*, and *Gadd45c*, are stress sensors that modulate responses of mammalian cells to different genotoxic and physiological stresses [33,34]. Kim et al. (2015) found that metformin acts on Gadd45β to inhibit JNK activation [35]. The absence of metformin leads to sustained JNK activation and increased liver injury after partial hepatectomy [36]. In our previous study, MAN also showed that JNK deactivation leads to hepatic protection [37]. Cubero et al. (2016) finding suggested that JNK1 and JNK2 both have importance for cell survival. They also demonstrated that SP600125 (a classical JNK inhibitor) can improve hepatic injury with the JNK1 and JNK2 knockdown mice which indicates that SP600125 mechanism works off-target [38].

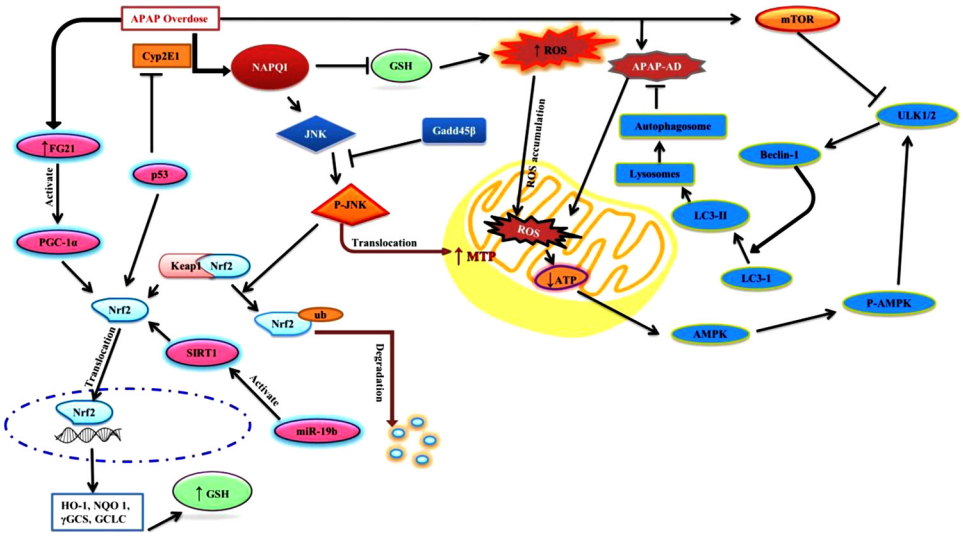

Fig. 2. Illustration of CYP, JNK, Nrf2, and Autophagy pathway in AIHT. APAP overdose promote excess NAPQ1 with the help of CYP2E1 which limits GSH volume and increases ROS production. Mitochondrial ROS accumulation decrease ATP is sensed by AMPK and phosphorylate to activate ULK1/2 mediated autophagy. ULK1/2 recruits Beclin-1 and LC-1 converts to LC-II followed by lysosomes to autophagosomes which engulf APAP-AD. NAPQI also phosphorylate JNK and translocate to mitochondria to increase MTP and ROS production; which can be inhibited by Gadd45β. Nrf2-Keap-1 complex followed ubiquitination to degrade Nrf2 in the homeostasis condition but in stress conditions like AIHT; Nrf2 translocates to the nucleus and transcript HO-1, NQO1, γGCS, and GCLC which contribute to produce GSH. Nrf2 nuclear translocation also influenced by FG21 mediated PGC-1α activation, miR-19b mediated SIRT1 activation and p53. P53 also resists CYP2E1 side by side p-JNK might influence Nrf2 ubiquitination.

2.3. Nrf2-antioxidant responsive element (ARE) pathway participation

NF (erythroid-derived 2)-like 2 (Nrf2, also known as NFE2L2), is a transcription factor encoded by the *NFE2L2* gene and a basic leucine zipper (bZIP) protein. Nrf2 activation facilitate quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), and the synthesis of the antioxidant molecule GSH [39,40] which become limited in AIHT. Chan et al. and Enamoto et al. showed that $Nrf2^{-/-}$ mice were highly susceptible to APAP treatment and the cause of death was accelerated GSH depletion compared to the wild-type [41,42]. APAP itself can induce Nrf2 activation when GSH storage become saturated but not sufficient for inverting the injury [43]. Aside according to Chen et al. (2020) p-JNK targets Nrf2 to degrade in AIHT which broadens the pathophysiology of AIHT [44]. There are several reports where Nrf2 was activated in some other ways. Fibroblast growth factor 21(FGF21) is overexpressed by APAP overdose and can activate peroxisome proliferator-activated receptor co-activator protein-1α (PGC-1α) which increases Nrf2 abundance in the liver [45]. Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of tyrosine kinase growth factor signaling, was found elevated in the case of APAP toxicity. By knocking down PTP1B, liver injury was markedly reduced by glycogen synthase kinase 3 (GSK3)β/Nrf2 activation [46]. Tumor suppressor p53 activation can attenuate hepatic toxicity. Doxorubicin, a p53 activator can improve liver injury by activating Nrf2 and reducing CYP2e1 expression [47]. Another recent study suggested that inhibition of microRNA miR-19b leads to the activation of NAD-dependent deacetylase sirtuin-1 (SIRT1) and promotes antioxidant protection via Nrf2 [48]. In AIHT excess free NAPQI covalently binds with protein thiols which expose mitochondria and cytoplasm to ROS [49]. So, the molecules that have antioxidant properties and can uplift the GSH level would be a good choice. Noh et al. (2015) showed that activation of Nrf2 and its downstream proteins protected against APAP toxicity by introducing sulforaphane that releases Nrf2 from its inhibitory complex with Keap1 [50]. Rescuing liver injury by Nrf2 activation mode has been reported vastly. Antioxidant molecules like quercetin, Shikonin, Corilagin, Licochalcone, Ginsenoside-Rg1 are tested for their protection against APAP toxicity but these molecules were introduced as pretreatment which is clinically irrelevant [51–55]

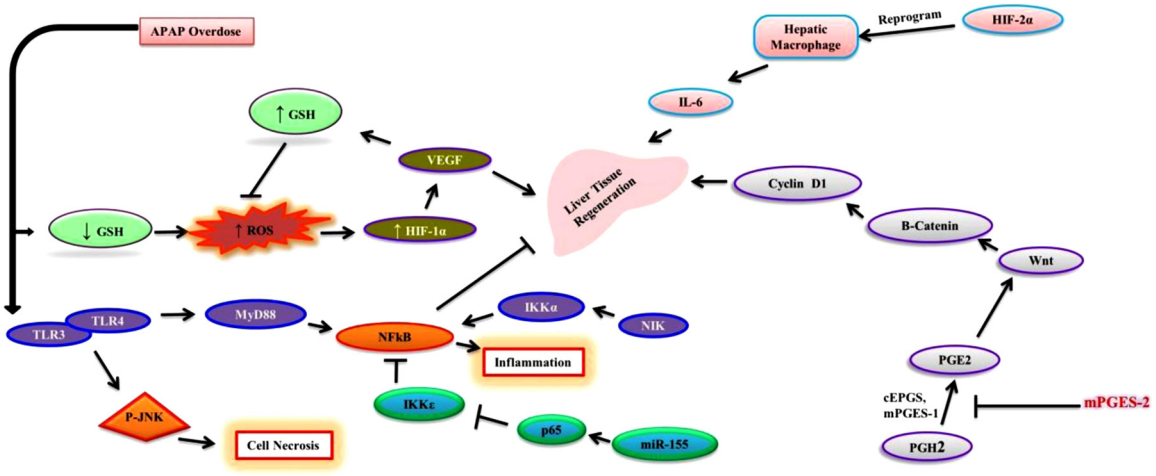

Fig. 3. Schematic representation of liver regeneration and prostaglandin involvement in AIHT. APAP supratherapeutic dose influences ROS increment leads to increase HIF-1α and VGEF to improve GSH and LTR, aside HIF-2α also reprograms hepatic macrophages to influence IL-6 LTR. In AIHT condition TLR3/TLR4 activate to produce MyD88 followed by NFkB which further fabricates inflammation and resist LTR. NIK influence IKKα to produce more NFkB which can be inhibited by miR-155 mediated p65/IKKε. PGH2 converts into PGE2 and recruits Wnt/β-catenin to improve LTR which can be disturbed by mPGES-2.

2.4. Activation of autophagy

APAP overdose affects different cellular components causing effects such as mitochondrial membrane disruption and nuclear DNA damage, leading to cell death. APAP inhibited the mechanistic target of rapamycin (mTOR) activity in primary hepatocytes by increased generation of mitochondrial ROS and decreased cellular ATP levels, leading to AMP-activated protein kinase (AMPK) - uncoordinated 51-like kinase-1 (Ulk1) activation and autophagy. The pharmacological induction of autophagy by rapamycin or torin1 protects against necrosis, whereas inhibition of autophagy by 3-methyladenine (3-MA), chloroquine (CQ), or leupeptin further exacerbates APAP-induced necrosis and liver injury [56,57].

APAP can form APAP-AD (APAP protein adducts) in murine and human hepatocytes [58]. APAP-AD has also been detected in the mitochondria and potentially contributes to APAP-induced mitochondrial dysfunction and subsequent oxidative stress [59,60]. Therefore, timely removal of APAP-AD could contribute to improving APAP-induced mitochondrial damage and provide sufficient ATP. These are critical processes necessary for the recovery from APAP intoxication in mice and humans [61,62]. Ni et al. (2016) showed that the removal of APAP protein adducts by autophagy protects against APAP-induced liver injury in mice. They found that APAP-AD showed a punctuated pattern and was colonized with green fluorescent protein-light chain 3 (GFP-LC3)-positive autophagosomes and Lamp1 positive lysosomes, which were decreased by knockdown of P62 (sequestosome 1), a classical autophagy receptor [57]. NAC is widely used for APAP-induced hepatoxicity by suppressing autophagy but improving APAP-induced necrosis [56]. Paradoxically, Sun et al. (2018) reported that Ulk1 and 2 deficiencies protect mice from APAP-induced liver injury. They showed that mice with liver-specific double knockout of Ulk1 and Ulk2 (Ulk1/2 LDKO) displayed normal autophagic activity after overnight fasting but were strongly resistant to APAP-induced liver injury. Another interesting finding was that Ulk1 and Ulk2 activated MAPK kinase 4 and 7 (MKK4/7), the upstream kinases and activators of JNK, that mediate APAP-induced liver injury [63]. Saberi et al. (2014) showed that Ro-31-8245 and Go6983, protein kinase C (PKC) inhibitors inhibited JNK activation and upregulated p-AMPK-mediated apoptosis [64].

2.5. Involvement of the liver regeneration pathways

Liver tissue regeneration (LTR) in the late phase of AIHT where many complex mechanisms involved. Epidermal growth factor receptor (EGFR) involves in hepatocyte proliferation. EGFR inhibition (after 12 H of overdose) reduces survivability but when inhibited at the early stage (2 H) it can improve injury and mitochondrial dysfunction induced necrosis. Apart from the dual role of EGFR, it's a key regulator of cyclin-D1 which is important for cell proliferation [65]. APAP overdose activates early GSK-3β and reduce cyclin-D1 activity which can be inverted by inhibition of GSK3 and improve LTR [66]. Wnt/β-catenin pathway in liver regeneration is very significant by expressing cell proliferation initiator cyclin-D1 [67].

Interleukin-6 (IL-6) is a very important promoter for liver revival in the case of AIHF [68]. A recent study indicates that hypoxia-inducible factors-2α (HIF-2α) can reprogram hepatic macrophages to produce IL-6 to protect from APAP overdose [69]. Vascular endothelial growth factor (VEGF) has an important role in angiogenesis and liver regeneration and its inhibition decrease cell proliferation [70]. Hypoxia-inducible factor 1α (HIF-1α) is increased by the oxidative stress but when inhibited it shows cytoprotection but reduces hepatocyte regeneration [71]. HIF-1α binds with DNA which subsequently increases VEGF expression [72] and exogenous human recombinant VEGF administration improves tissue regeneration, toxicity even GSH level [73].

Nuclear factor-κB is a protein complex that controls the transcription of DNA, cytokine production, cell survival, and different cellular responses to stimuli. Toll-like receptors (TLRs) are well recognized to respond to immunomodulation. The activation of TLR-3 and TLR-4 was reported to induce the production of adaptor proteins such as myeloid differentiation factor 88 (MyD88), NF-κB, p-JNK, tumor necrosis factor-α (TNF-α) and generation of oxidants inflammatory cytokines and chemokines, all of which facilitate APAP-induced liver injury [74]. The TLR-4 inhibitor TAK-242 protects against APAP toxicity by down-regulating TLR-4 [75]. In these signaling networks, receptor-interacting proteins (RIPs) also play a crucial role in activating NF- κB and driving cell necrosis [76].

Horng et al. (2017) found that Mulberry leaf extract (MLE) showed hepatoprotection by improving the hepatic oxidative status and suppressing protein expression of NF-kB, JNK, MyD88, receptor-interacting protein 1 (RIP1), and RIP3 [77]. N HU et al. (2017) showed that TLR4 knockout (TLR KO) mice exhibited high NF-κB activation. Notch signaling regulates innate NLRP3 inflammasome activation by regulating high mobility group box 1 (HMGB1)/TLR4/NF-κB activation in APAP-induced liver injury, where the Notch1-Hes1 signaling cascade is involved in the regulation of innate immunity in APAP-triggered liver inflammation [78]. The introduction of antioxidants such as diallyl disulfide (DADS), paeonol, and resveratrol can decrease NF-κB expression [79–81]. Micro RNA 155 (miR-155) showed considerable potential as a therapeutic target, that provides hepatoprotection by regulating NF-κB expression through p65 and inhibitor of NF-κB kinase ε (IKKε) expression [82].

NF-κB not only linked with the inflammatory response but also a critical step to initiate liver regeneration [83]. Yang et al. (2009) showed that the prolonged administration of NAC for 72 h in mice causes delayed hepatic regeneration in the mice model due to reduced NF-κB-DNA binding [84]. Another recent study shows that NF-kB-inducing kinase (NIK) can suppress LTR in acute liver disease [85] which could be a potential target for AIHT.

2.6. Involvement of the prostaglandins

APAP is believed to act as a cyclooxygenase (COX) inhibitor that prevents prostaglandin production to achieve analgesic and antipyretic effects. However, APAP does not bind to the active sites of COX, instead, it diminishes COX activity by reducing its oxidized form [86]. During APAP overdose, increased levels of COX-1, COX-2, prostaglandin D2 (PGD2), and prostaglandin E2 (PGE2) were found [87] leading to inflammation. PGE2 is responsible for liver cancer cell growth [88] but it also can show hepatic-protection during APAP toxicity [89] and liver regeneration through Wnt signaling [90]. Three prostaglandin E synthases (PGES) known to catalyze prostaglandin H2 (PGH2) to PGE2 are PGES1 (mPGES1), mPGES2, and cytosolic PGES (cPGES) [91]. APAP does not inhibit mPGES1 [92], but there is not sufficient evidence regarding its relationship with mPGES2 and cPGES where mPGES-2 is a GSH-dependent PGES that forms a complex with GSH and heme and that only heme-free mPGES-2 with GSH binding exhibits PGE2 synthetic activity [93]. Hu Wang et.al (2019) showed that mPGES-2 knockout mice can resist AIHT side by side improve GSH amount, PGE2 level, inflammation and APAP-cystine adduct formation [94]. Considering the lack of GSH during APAP overdose, mPGES-2 could be worthy of further research.

2.7. Advancement in early APAP toxicity detection

Serum ALT and AST are the most popular for liver function test and measurement for APAP toxicity. But the core problem with ALT is that it is less specific and unable to measure early toxicity. miRNA-122-5p (miR-122) increases significantly at high APAP concentration in the body. It can be used to measure early toxicity but the major problem involves its assay technique. Currently, the PCR technique is used to measure miR-122 which is an overlong process and not suitable at the emergency. A new method is currently under development to trace early miR-122 concentration (clinical trial number NCT03497104) with

the help of electrochemical impedance spectroscopy. Side by side, JNK activation can specifically reduce glutathione S-transferases A1 (GSTA1) content and expression in the liver but increased in serum which could be another new biomarker to detect early liver injury [95].

2.8. Present therapeutic approach

APAP was first introduced for human consumption in 1887 [96] and the first case of AIHT was reported in the 1960s [97]. APAP overdose poisoning can be classified into four stages: preclinical toxic effects with normal serum ALT and AST, Hepatic injury with elevated serum ALT and AST, Hepatic failure and recovery. Each category is important for proper management because each stage involves different managements [98]. Patient's survival rates are very high until they hepatic failure where the mortality rate of 20–40 % [99].

Within 1–2 h of supratherapeutic ingestion of APAP activated charcoal can be introduced to limit gastrointestinal absorption of paracetamol [100]. But if ingestion crosses more than 2 h then NAC is given orally or intravenously (i.v) with a loading dose of 140 mg/kg and 150 mg/kg respectively [101]. After the loading dose, the treatment with NAC carries out for the next 20–24 h according to the protocol. The major problem with an oral dose of NAC is the unpleasant taste and odor leads to vomiting and delays the procedure but can be overturned if given i.v [102]. Hepatic failure is the last stage where the only way to survive is to liver transplantation [103].

Besides awful odor and taste of NAC for oral ingestion, it can present various adverse effects like rash, anaphylactoid reactions, bronchospasm, hypotension even death [104]. Mahmoudi et al. case report mentioned a 23 years old female APAP overdose patient died due to accidental NAC overdose [105]. Due to low bioavailability, NAC has to be given in high doses which increases the medical cost from $72,000 to $650,000 [106]. Besides all, NAC treatment after 8 h of the overdose still unable to rescue from mortality [107]. For the last five decades, numerous molecules are introduced for APAP overdose management which works around various mechanisms for APAP overdose management.

2.9. Potential drugs for AIHT management

Different molecular interventions at diverse pathways showed rescue where most of them are presented as pre-treatment and it's clinically not appropriate in the case of AIHT. Some of them are having good potentiality to go for a clinical trial (Table 1). Resolvin D2, a lipid mediator blocks neutrophil entry to reduce inflammation and does a very good job in case of a broad therapeutic window (3–12 h) than NAC [108]. Beta-Adrenoceptor agonist Isoproterenol rescues AIHT mice by promoting Wnt, even in broaden therapeutic window (3 h) where NAC failed to reduce toxicity [109]. Ψ-Glutathione (ψ-GSH) is an alternative GSH mimetic that can reduce oxidative stress and rise APAP-GSH adduct even after 4 h of post APAP overdose where NAC failed its effectiveness. With age, GSH synthesis becomes altered which makes ψ-GSH more advantageous than NAC [110]. N-acetylcysteine Amide (NACA) has shown more effective to lessen up oxidative stress then NAC [111].

Table 1
Potential drugs list for acetaminophen overdose management. Av = available; N/Av = not available of clinical trial or application data.

Drug	Clinical application	Therapeutic window
Resolvin D2	Av	3 − 12 h
Isoproterenol	Av	3 h
ψ-GSH	N/Av	1 − 4 h
NACA	N/Av	1.5 h
4 M P	N/Av	1.5 h
Mangiferin	Av	1 h
Metformin	Av	1 h

Table 2
List of several molecules that might be a target to develop and design a new drug for AIHT betterment.

Category	Direction
Gadd45β agonist	Prevent JNK activation.
Nrf2 activator	Reduction of ROS and restore GSH.
VEGF agonist	Recover LTR.
TLR4 antagonist	Lessen inflammation.
NIK inhibitor	Decrease inflammation and improve LTR.
mPGES-2 inhibitor	Enhance LTR, restore GSH, and decrease APAP-AD.
CYP2E1 inhibitor	Lower NAPQI production.
Autophagy activator	Decrease APAP-AD.

4 M P is also a potent drug, reduces CYP2E1 activity, and lessens up NAPQI production [23,32]. Mangiferin is a natural occurring xanthone, also can improve AIHT by JNK activation and dipping APAP-Cys adduct [37] which has also applied to human orally [112]. Metformin, a popular drug of choice for type-2 diabetic treatment also showed promise to treat AIHT [35]. These are some very potent drugs that might be considerable for AIHT management where some of these drugs have clinically applied for a different medical condition but not in AIHT.

2.10. Prospects of new drug discovery

AIHT mediated oxidative stress, and hepatic necrosis has been regulated by several complex pathways. The most common clinical treatment for APAP overdose condition is to administer N-acetylcysteine (NAC), a very powerful anti-oxidant that works by enhancing the level of GSH [113]. In a clinical trial by high-volume plasma exchange in patient's combination of NAC with acute liver failure reduced the liver transplantation to rescue from mortality [114].

By discussing the pathways involved in AIHT, we would like to suggest some molecules that might present potentiality as a target for future drug discovery (Table 2). Gadd45β agonist can inhibit APAP-induced MKK4 phosphorylation which leads to reduce JNK activation and AIHT improvement [35]. Nrf2 is a master antioxidant and regulates GSH synthesis which made this specific molecule is a very interesting target and its activators showed promising results in AIHT [50]. Liver regeneration after APAP overdose is vital for survival and angiogenesis is a key action for LTR where VEGF comes to play its role. Exogenous VEGF not only helps to regeneration but also improve the GSH level [73]. TLRs are responsible for the induction of MyD88, NF-κB, p-JNK, and TNF-α liable for inflammation and impaired LTR in AIHT. Along with the activation of the Notch pathway might improve inflammation [75,78]. So, a TLR antagonist or a Notch activator might be a good target. Side by side, NIK can reduce LTR in another disease which is still unexplored in the case of AIHT [85]. mPGES-2 inhibition leads to improve inflammation, LTR, GSH level, and APAP-AD in AIHT which makes it a suitable candidate for drug target but an mPGES-2 inhibitor yet to be discovered [94]. CYP2E1 is a key molecule that initiates the NAPQI metabolite production and its pharmacological deactivation leads to AIHT amelioration [23]. Autophagy activation can reduce necrosis and APAP-AD formation [56]. Besides, a recent study shows that the posttranslational modification of proteins called O-GlcNAcylation can accelerate GSH synthesis and decrease the formation of acetaminophen-protein adduct [115]. So, the molecules that can activate autophagy might serve a good therapeutic purpose for AIHT. Molecules that can restore GSH and remove APAP-AD might present more advantages in AIHT, cause in practical clinical scenarios APAP overdosed patients admit into the hospital in the delayed phase. So, at that particular time point molecules that can increase GSH level and eradicate APAP-protein adducts can come handy.

But targeting complex signal transduction molecules might not a proper choice because some molecules have a negative impact in the early phase of the toxicity but are found to be very important for the

late recovery. For example, as discussed before JNK was thought to be a positive regulator for developing toxicity through its downstream molecular cascade but later it's found that it is also important for cell survival. The same case is also applied for NF-κB. So, as our perspective to develop new intervention three major points should be considered **A.** ability to restore hepatic GSH, **B.** scavenging ROS, and **C.** long post-treatment therapeutic window. Before exploring it's very important to confirm that the new molecule should not hepatotoxic by itself. For example, Temsirolimus (CCI-779) is an mTOR inhibitor and influence autophagy but also increases the liver AST level itself alone [116].

3. Conclusion

During the last decade, APAP overdose has become a common threat causing the development of liver failure. Subsequently, numerous attempts have been made to improve the understanding of the molecular and sub-molecular interactions underlying this pathology. The formation of NAPQI and counteracting depletion of GSH is the basis of this complication. The standard NAC therapy acts by regenerating GSH level which has a limited therapeutic window and with great expanse. Many pieces of researches have been conducted on the search for an alternate antidote. Most of them are targeted in different molecules, such as NF-κB, mTOR, Nrf2, etc. for the protection but most of them failed to recover sufficiently. Moreover, less evidence from clinical trials questions the effectiveness of those molecules. Therefore, careful clinical trials should be conducted to establish better AIHT management.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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