ejpmr, 2021,8(12), 01-24.

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

LAST FIVE YEARS' LABORATORY BASED STUDIES TO ADDRESS ACETAMINOPHEN INDUCED HEPATOTOXICITY

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Article Received on 25/09/2021 **Article Revised on 15/10/2021** **Article Accepted on 05/11/2021**

ABSTRACT

Acetaminophen is a commonly used analgesic drug that is considered an over-the-counter drug. Chronic use of this analgesic drug, as well as overdose, can cause hepatotoxicity. Acetaminophen metabolism takes place within liver. Hepatotoxicity from this drug is caused by the development of the poisonous NAPQI metabolite, as well as glutathione (GSH) depletion, oxidative stress, and mitochondrial dysfunction, all of which lead to a decrease in adenosine triphosphate (ATP) storage. There are so many other factors that are responsible for acetaminophen-induced hepatotoxicity. Many studies have recently been conducted in order to establish therapeutic options by designing a new medicine to improve acetaminophen toxicity. Many investigations have been conducted in the laboratory to determine the hemodynamic effects and in several studies drugs were given intraperitoneal. The herbal medicine was also used by the researchers to significantly reduce acetaminophen toxicity. They derived a variety of natural compounds from various plants and used these items in their research. There have been numerous studies conducted in order to discover a better alternative antidote. These experiments focused on a variety of molecules, including Nrf2, c-Met, thrombospondin-1, CYP2E1, and others. These were proved only in the lab-based animal trial. In future, preclinical trials are required to better determine the safety and efficacy of these compounds.

KEYWORDS: Hepatotoxicity, Acetaminophen, Intraperitoneal, herbal compound, antidote, NAPQI, Glutathione hormone.

INTRODUCTION

Acetaminophen is most often known as an over-the-counter pain reliever. However, it is the most widely used painkiller in the United States, and it can induce serious liver damage. When administered therapeutically, it is generally a safe medicine. This is a medicine that has been around for more than a century. It was not deemed an over-the-counter medicine before to 1955(Hussain et al. 2020). This is a crucial medication for all three stages of pain. It can be used in conjunction with a non-steroidal analgesic medication to relieve moderate pain. This medicine can be used in conjunction with opioids when the pain is severe. Patients with stomach ulcers who are unable to take nonsteroidal anti-inflammatory drugs due to contraindications or who have a reaction to aspirin should consider acetaminophen for pain relief. It can also be utilized as a treatment option in patients who are pregnant, have a blood coagulation problem, or have a fever(Pergolizzi et al. 2008). Acetaminophen overdose is a common cause of hepatotoxicity. Hepatotoxicity can be caused by a variety of circumstances, including the environment, fasting, persistent alcohol consumption, age, sex, and severe drug reactions. Overdosing can be deliberate or unintentional. Hepatotoxicity can be caused by either of these circumstances. (Myers et al. 2008). Hepatotoxicity can occur with therapeutic doses, and several studies have shown that it is the cause of persistent alcohol use. However, in the vast majority of situations, therapeutic doses are both safe and effective. When a man consumes 40 grams of alcohol per day and a woman consumes 20 grams of alcohol per day, it is considered alcohol abuse(Larson et al. 2005). Acute or chronic alcohol use is possible. Our bodies are more harmed by chronic alcohol usage. It has the potential to trigger microsomal enzyme induction as well as paracetamol metabolic activation. As a result, hepatotoxicity rises(Ghosh et al. 2020). Hepatotoxicity can sometimes cause by mitochondrial malfunction. Acetaminophen can covalently bind to mitochondrial proteins, causing mitochondrial malfunction(Nelson 1990). The maximum daily dose of acetaminophen is 4g, and it should not be exceeded. In general, an adult's dose should be 325 to 650 mg every 4 to 6 hours, or 1g every 6 hours if necessary. However, for children, the normal dose is 10 to 15 mg/kg every 4 to 6 hours, with a maximum dose of 50 to 70 mg/kg if necessary(Lancaster, Hiatt, and Zarrinpar 2015). According to the American National

Poison Data System, acetaminophen is one of the 25 medications responsible for a substantial number of deaths (Mowry et al. 2015). Even when the dose is used medically, acetaminophen-induced hepatotoxicity is caused by a variety of variables, including alcohol addiction, starvation, and many more(Tittarelli et al. n.d.). currently several studies have been conducted for developing treatment strategies by designing a new drug for the betterment of N-acetyl-p-aminophenol (APAP) toxicity. In these experiments the researchers used different kinds of natural compounds which were ingested or administered intraperitoneal or orally in several types of animals which were affected with APAP hepatotoxicity. Intraperitoneal administration is important for determining hemodynamic effect.

Here the literatures selected on acetaminophen-induced hepatotoxicity were reviewed with special attention to reach an aspect of shedding light on laboratory based several current research on some new molecules which can be a target for developing a new antidote drug for the remedies of APAP hepatotoxicity

MATERIAL AND METHOD

The scientific research articles were selected from the different search engines which include Medline, Science Direct, PubMed, Google Scholar, nature.com, and the collected research paper was up to January 2021. The following keywords were used to find out the research articles. The keywords included acetaminophen, hepatotoxicity, paracetamol, acetaminophen poisoning, liver injury, fatalities, factor, side effects. The papers which were appropriate were selected for this review and excluded those which were not suitable for this review writing.

Epidemiology

In the United States of America, the most common analgesic is acetaminophen. It is reported that in the USA acetaminophen is the major cause of hepatotoxicity(Herndon and Dankenbring 2014) (Clark et al. 2012) (Michna et al. 2010). Around thirty thousand patients are admitted to a hospital in the USA every year due to the cause of acetaminophen-induced hepatotoxicity(Blieden et al. 2014). It is a concerning issue that the intentional and unintentional overdose ratio is almost similar an which is approximately 52 percent vs 48 percent. Both of these intentional and unintentional groups are responsible for liver failure and sometimes liver transplantation must be needed(Herndon and Dankenbring 2014) (Blieden et al. 2014). Recent study showed that unintentional overdose is greatly responsible for hepatotoxicity which is approximately 63 percent due to the combination therapy with opioids as well as 17 percent of patients were affected with liver injury(Michna et al. 2010) (Blieden et al. 2014). Another epidemiologic study reported that approximately 6 percent prescriptions are filled with acetaminophen alone or with combination therapy with opioids exceeded the dose greater than 4g per day(Clark et al. 2012) (Michna et al. 2010). Overdose of acetaminophen is the major cause of liver injury as well as death in united states of America and other counties and this overdose situation is occurring approximately 50 percent cases. But these overdose cases are mostly seen in the United States and which is approximately more than 100000 per year and annual emergency room visits are recorded more than 56000. The annual number of hospitalized patients is approximately 2600 and approximately 450 patients are dead due to acetaminophen-induced hepatotoxicity(Lee 2004). Several studies proved that approximately 29 percent of patients with acute liver injury secondary to acetaminophen-induced hepatotoxicity undergo a liver transplant, as well as mortality rate, which is approximately 28 percent(Bunchorntavakul and Reddy 2013).

Physiopathology and Metabolism

Acetaminophen contains weak acid. So weak acid is a property of acetaminophen. For this reason, acetaminophen absorbs in the duodenum. If acetaminophen ingestion and food consumption is happening concurrently in that situation the absorption time may be delayed(McGill and Jaeschke 2013). Delayed absorption time of acetaminophen harmful for those patients who are affected with chronic liver disease because in this case drug serum half-life is increased or prolonged. A patient with chronic liver illness is at risk of a prolonged medication serum half-life (by 2.0 to 2.5 hours on average and as well as up to more than 4 hours) if extended-release APAP formulations are consumed, similar to how current meal consumption causes APAP absorption to be delayed. While an overdose of APAP results in peak serum concentrations (10–20 mg/mL) in 4 hours, a patient who takes the medicine carefully achieves peak concentrations in 1.5 hours, with such a half-life of 1.5–3 hours. At the microscopic level, APAP metabolism takes place within liver microsomes. While the routes are well understood, and the technical details of their molecular mechanisms are beyond the scope of this paper, it is worth emphasizing that not all patients with APAP consumption and hepatotoxicity have the same outcome. This metabolism has three phases and within these three phases, the major one is the phase two pathway where 90 percent acetaminophen is funneled. In this phase II metabolic pathway, the conjugation reaction of acetaminophen is catabolized by UDP-glucuronosyl transferases and sulfotransferase and thus converted into glucuronidated and sulfated metabolites and these are eliminated through urine from the body. Approximately only 2 percent acetaminophen is excreted from the body without metabolism. Approximately 10 percent acetaminophen will go through phase I oxidation where hepatic CYP2E1 is involved in this process. This phase is more harmful to the body because where more harmful N-acetyl-para-benzo-quinone imine is produced. Phase III entails metabolite transfer via biliary excretion, which necessitates the use of transporters(McGill and Jaeschke 2013). Hepatotoxicity from APAP is caused by the development of the poisonous NAPQI metabolite, which

is present in large amounts, as well as glutathione (GSH) depletion, oxidative stress, and mitochondrial dysfunction, all of which lead to a decrease in adenosine triphosphate (ATP) storage(Clark et al. 2012) (Jaeschke, McGill, and Ramachandran 2012) (Jaeschke and McGill 2015). Other hepatotoxic pathways include the creation of toxic free radicals like peroxynitrite from the interaction of superoxide and nitric oxide, which then produce nitro tyrosine adducts inside the mitochondria. GSH, which is depleted during APAP toxicity, neutralizes reactive oxygen species and ONOO-, enhancing oxidative damage in this way. The disruption of the mitochondrial membrane permeability transition pore by ROS and reactive nitrogen species (RNS) causes mitochondrial membrane malfunction(Jaeschke et al. 2012) (Jaeschke and McGill 2015). In the case of non-toxic APAP consumption, NAPQI is rapidly conjugated by hepatic GSH to create harmless mercaptate and cysteine molecules, which are eliminated in urine(McGill and Jaeschke 2013).

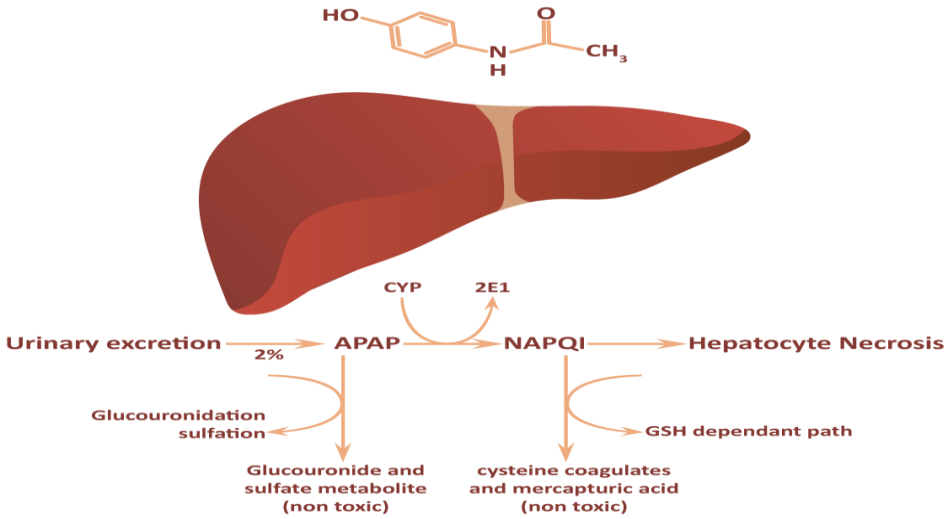

Fig. 1: This is the metabolic pathway of acetaminophen. It has three phases. Where approximately 2 percent acetaminophen is excreted in urine without any metabolism. And in phase II 90 percent acetaminophen involves in a conjugation reaction which is catabolized by UDP-glucuronosyl transferases and sulfotransferase and thus converted into glucuronidated and sulfated metabolites. These metabolites are easily excreted in the urine. 5 to 9 percent is involved in oxidation reaction which is catabolized by the CYP2E1 resulting in the formation of harmful NAPQI. Where phase III involves metabolic reaction.(McGill and Jaeschke 2013).

It is scientifically proved that NAPQI tends to bind with mitochondrial protein to deplete glutathione as well as depletes the function of antioxidants. This binding is also responsible for the alteration in ATP-synthase alpha-subunit of mitochondria and resulting in less effective or ineffective ATP production occurs (Jaeschke et al. 2012) (Jaeschke and McGill 2015). Acetaminophen can bind to mitochondrial proteins covalently which cause mitochondrial dysfunction. Increased levels of systolic calcium and modified mitochondrial protein are responsible for the suppression of mitochondrial respiration as well as the production of adenosine triphosphate resulting in mitochondrial oxidative stress is introduced. Peroxy nitrite production is greatly increased which is a potent oxidant and nitrating agent. This peroxynitrite can cause further mitochondrial dysfunction by binding covalently with the cellular proteins. As a result, the mitochondrial membrane potentially collapses due to an alteration in permeability of the mitochondrial membrane. Cell cytoplasm is loaded with mitochondrial proteins as well as adenosine phosphate production is disrupted. Thus, hepatocyte necrosis is occurred(Jaeschke and Bajt 2006) (Nelson 1990). In the case of non-toxic ingestion of acetaminophen there is a rapid conjugation occurs during the processing of NAPQI to form mercaptate and cysteine compounds and this is controlled by the hepatic glutathione hormone. mercaptate and cysteine compounds can easily excrete in urine(McGill and Jaeschke 2013).

Sometimes a large amount of paracetamol is ingested either as intentional attempts or unintentional overdose. So, conjugation is unable to safely process the paracetamol molecule. As a result, a large amount of paracetamol is oxidized by the cyp450 isozyme system and produces a large amount of N-acetyl-p-benzoquinone imine. So, glutathione gives the best effort to detoxify the N-acetyl-p-benzoquinone imine molecule but unfortunately, glutathione fails to detoxify it because our body has limited storage of glutathione. As a result, N-acetyl-p-benzoquinone imine accumulates in our liver and induces acute hepatic necrosis.

Related factors with apap induced hepatotoxicity

An ingested dose of the drug and the time length from ingestion of acetaminophen to the therapy especially N acetylcysteine is considered as a determining factor

which is the most important factor in developing and severe acetaminophen-induced hepatotoxicity(Liu, Govindarajan, and Kaplowitz 2004). There are so many other factors that are responsible for acetaminophen-induced hepatotoxicity. These factors included Dose as well as their using pattern, Chronic and acute use of alcohol, Age and gender, Medication-related factor, Current nutritional status and Chronic liver disease.

Doses and Their using pattern

The generally recommended dose for acetaminophen is 4g per day. It was not thought before the 1980's that acetaminophen is responsible for liver injury. But in the US the researchers found from a retrospective study that 20 percent of patients were affected with acetaminophen-induced hepatotoxicity(Larson et al. 2005).

Overdose of acetaminophen is a common cause of hepatotoxicity. There are many risk factors for developing hepatotoxicity which include environment, fasting situation, chronic alcohol use, age, sex, adverse drug reaction. Overdose can be an intentional or unintentional case. Both situations can be responsible for the development of hepatotoxicity(Myers et al. 2008).

In a recent study, the researcher collected data of 662 patients and the duration of time was 6 years long. From which they found that 275 patients were affected with acetaminophen-induced liver toxicity. This study expressed that the annual percentage of acetaminophen-induced hepatotoxicity patients is increased from 28 percent to 51 percent and it means that day by day the number of acetaminophen-related liver injuries is increased. Of 275 patient's 48 percent were affected due to the use of unintentional overdose and 44 percent were affected due to intentional overdose. But this finding was really surprising because their unintentional percentage is greater than the intentional. It was found that 81 percent of patients who have been affected with acetaminophen related liver injury because of taking acetaminophen and other analgesics due to the prevention of acute or chronic pain. Of 275 patient's 65 percent survived and 23 percent patient died and these patients died without liver transplantation(Larson et al. 2005).

This study expressed that unintentional overdose is more dangerous than intentional overdose as well as it showed that the number of patients is increasing day by day and the number of patients was doubled in their 6 years experimental time (Larson et al. 2005).

Nowadays it is a concerning issue that the mortality rate is higher in case of unintentional overdose of acetaminophen than an intentional overdose. A study on 663 patients showed that unintentional overdose causes a higher rate of organ dysfunction as well as the higher rate of mortality. According to the study from 110 unintentional patients, 42 were affected with acetaminophen-induced hepatotoxicity and the percentage is 38.2 percent while from 500 intentional patients 128 were affected with this type of injury and this percentage is 25.6 percent. It is found that most of the unintentional overdose patients are alcohol user or older age(Craig et al. 2011).

Chronic use of alcohol

Alcohol consumption may be acute or chronic. Chronic alcohol consumption is more harmful to our bodies. It can be the cause of microsomal enzyme induction as well as can responsible for metabolic activation of paracetamol. Thus, hepatotoxicity is increased. So, there is a complex relationship between ethanol and paracetamol. Overdose of acetaminophen is one of the main causes of acetaminophen-induced hepatotoxicity in the USA and UK. Acetaminophen toxicity is potentiated and increased due to chronic alcoholism when acetaminophen is ingested and this can also be increased even after the alcohol clearance from the body(Ghosh et al. 2020).

It is clinically proved that acute alcoholism has a protective activity that can inhibit the microsomal acetaminophen oxidation and thus it reduces the production of N-acetyl-p-benzoquinone imine also known as NAPQI. So this is the dual role of alcohol. This analysis also suggested that in the case of chronic alcohol users if acetaminophen is ingested shortly after clearance of alcohol from the body there is a high risk of increasing acetaminophen-induced hepatotoxicity(Ghosh et al. 2020).

A recent study suggested that after an acute overdose of acetaminophen an experimental report over 362 patients within the period of 24 hours, 49 percent of them were habitual to acute alcoholism. This experiment exhibits an important thing which is those people who ingested alcohol acutely from them only 5.1 percent of people are affected with acetaminophen-induced hepatotoxicity. And those who did not ingest alcohol from them 15.2 percent are affected with acetaminophen-induced hepatotoxicity. If we compare both types of patients, we can say acute alcoholism exhibit a lower risk of acetaminophen-induced hepatotoxicity(Waring et al. 2008).

Chronic alcoholism exhibits different characteristics from acute alcoholics. It increases CYP2E1 as well as decreases the production of glutathione. CYP2E1 is increased twofold due to the stabilization of enzymes by the chronic ingestion of alcohol(Anon n.d.). Due to chronic alcoholism malnourishment can occur. Malnourishment is responsible for the depletion of hepatic glutathione. Thus, hepatotoxicity is induced. Most of the data suggested that therapeutic misadventure or a repeated dose of acetaminophen increases acetaminophen hepatotoxicity in the case of chronic alcoholic patients(Anon n.d.) (Hodgman and Garrard 2012).

The recommended dose of acetaminophen is 4g per day. A recent experiment has shown that hepatotoxicity is more common in recent fasting than in recent alcohol use for the patient who took the dose of 4g to 10g acetaminophen per day. Recent alcohol users are more commonly affected with liver toxicity when they use more than 10g of acetaminophen per day. Generally, liver toxicity is shown for those patients who take greater than 15g of acetaminophen. They found from their case that the chronic alcohol user who took less than 10g of acetaminophen were affected with liver toxicity. Chronic alcohol use can easily induce the expression of CYPIIE1 which involves the production of a large amount of N-acetyl-p-benzoquinone imine. As a result, glutathione generally unable to detoxify this large amount of N-acetyl-p-benzoquinone imine because there is limited storage of glutathione in our body(Whitcomb and Block 1994).

They found two people in their study who were not recent or chronic alcohol users but were affected with acetaminophen-induced hepatotoxicity. It was noticed that these two patients were fasting before taking paracetamol(Whitcomb and Block 1994).

Nowadays regular alcohol users must be more common everywhere in the world. Recently a study was done and which was about acetaminophen-induced hepatotoxicity in regular users of alcohol. 67 patients were affected with liver injury after the ingestion of acetaminophen and the dose of acetaminophen was therapeutic. From which 64% patients took alcohol greater than 80g per day and 35% patients took 60g or less per day. Other patient reports were unclear. The nontoxic range of acetaminophen dose is >6g per day and 60% of patients taking this range of acetaminophen. Within the 40 percent received recommended dose which is less than 4g per day and remaining 20 percent received 4.1g to 6g per day.20 percent of patients have died even after the use of a therapeutic dose of acetaminophen. This was only because of regular use of alcohol which means a chronic alcoholic. Here ethanol plays a major role to induce cytochrome P-4502EI as well glutathione can be depleted by the effect of ethanol. So malnutrition is very common in this case. Liver injury generally marked by the elevated level of aspartate transaminase or aspartate aminotransferase(Anon n.d.).

A study reported that excessive drinkers showed a higher suicide rate which is approximately 80 times higher than the normal population.

Age and Gender

A retrospective study was performed in Royal North Shore Hospital (RNSH) to differentiate the characteristics of the older patient and younger patient who were supposed to be affected with acetaminophen toxicity. This study showed that a greater number of older patients were faced with accidental and chronic exposure compared to younger patients. There were a smaller number of older patients who took acetaminophen overdose intentionally compared to the younger patient. For the older patient, it was a concerning issue that they were affected with acetaminophen-induced liver toxicity even at a therapeutic dose but they had a higher ALT concentration level. Another reason was they took more than 4g of acetaminophen per day for a particular day due to increased pain but when pain decreased, they used to take a therapeutic dose. The serum paracetamol level of the older patient is detectable because of distribution and clearance of paracetamol volume is decreased in the older patient. So, in most of the case older patient cannot take NAC to compare to the younger patient because of NAC generally used to treat acute acetaminophen overdose exposure and this is not acceptable for the chronic paracetamol overdose. So old patients are in more risky condition compare to younger patients. But it is a concerning issue that a greater percentage of the younger adult is affected with this toxicity due to suicidal acetaminophen overdose(Kane et al. 2012).

This study also showed that there is a greater percentage of women are affected with acetaminophen-induced liver toxicity compared to men as well as this percentage is acceptable for both old and young(Kane et al. 2012).

A study shows that women are at high risk of acetaminophen-induced hepatotoxicity than men. High-grade encephalopathy is very common in women with acetaminophen-induced hepatotoxicity because of co ingestions with a sedating agent. For this reason, women are at high risk of hepatic encephalopathy. Another reason is the combination of opioids acetaminophen drugs which is more common in women. Hepatic encephalopathy is tow fold higher in women with co-ingestion compare to women without co-ingestion(Rubin et al. 2018). The combination therapy of acetaminophen and opioid drugs can cause a higher risk of high-grade hepatic encephalopathy than in the combination of acetaminophen and diphenhydramine.

Acetaminophen clearance is less in women and generally is 22 percent greater in men than in women(Miners, Attwood, and Birkett 1983). Because glucuronidation activity is less in women than in men. This is the key enzyme for acetaminophen metabolism(Court 2010). A study reported that women are more likely to use acetaminophen in case of pain but men are more likely to use aspirin for pain. The study also proved that the regular use of over-the-counter analgesic is more common in women than in men(Paulose-Ram et al. 2003).

Psychiatric illness is more common in women than in men. Scientific data showed that psychiatric illness is more common in the case of acetaminophen-induced hepatotoxic patients than in general people. This is applicable for both intentional and unintentional overdoses(Pezzia et al. 2017).

The female gender is at high risk of acetaminophen-induced hepatotoxicity than men. Fulminant hepatic failure is greatly occurring in women than men. So severe reaction is more common in women.

Medication-related factor

There are some OTC drugs as well as some prescribed drugs which can interact with liver metabolism. It means that some drugs increase the toxicity level. For example, epileptic drugs which include phenytoin, phenobarbital, etc. are responsible for increasing hepatotoxicity. Some antituberculosis drugs which include rifampin, isoniazid, etc. are responsible are increasing hepatotoxicity. There are some herbs which include garlic, St. John's wort as well as germander are responsible for the mechanical enhancement of the CYP system(Bunchorntavakul and Reddy 2013).

Anticonvulsant and antituberculosis are used concomitantly may involve the induction of the CYP system. Thus, increase the synthesis of NAPQI which can cause acetaminophen-induced hepatotoxicity(Larson 2007) (Bray et al. 1992). Combination therapy of paracetamol with codeine can cause more harm when patients take overdose accidentally. A recent study showed that 440 patients are died due to the use of this combination therapy when an accidental overdose occurs. Combination therapy with antihistamine also can cause acute liver failure when it is not used therapeutically(Hopkins, Dobbin, and Pilgrim 2018).

Nutritional status

Malnutrition is more dangerous for hepatotoxicity because it can cause glutathione depletion. If the patient's nutritional status is poor at that condition glutathione hormone reserve can be beaten easily and hepatic glucuronidation can cause a reduction of the level of glutathione hormone. If the poor nutritional status is a cause of chronic alcoholism as the result CYP activity of the patient is increased. Thus, glutathione hormone level is decreased(Whitcomb and Block 1994).

Chronic liver disease

In cirrhotic liver metabolism of acetaminophen is less than a normal liver. The patient who has chronic liver disease and take alcohol frequently they are free from an elevated risk of APAP hepatotoxicity. For these patients less than 4000mg per day is but recommended dose is 2000mg per day especially where mark hepatic decomposition or active alcohol use(Dart et al. 2006).

Clinical manifestations

It is critical to find out about APAP overdose because early therapy can prevent significant morbidity and mortality. Some so many patients show only minimal or sometimes non-specific symptoms. The symptoms are nausea, vomiting, malaise, as well as abdominal pains. APAP-induced hepatotoxicity is divided into four sequential stages. Table 1 indicate sequential stage of acetaminophen-induced hepatotoxicity.(Yoon et al. 2016)

Table 1: Sequential stage of acetaminophen-induced hepatotoxicity (Yoon et al. 2016).

Stage	Duration	Description
Stage I	0-24 hours post-intake	In this stage, the patient may be asymptomatic or sometimes nausea, vomiting, or pain in right hypochondrium. Severity depends on the dose ingested. AST and ALT values generally normal.
Stage II	24-72 hours post-intake	Improvement of stage I symptoms. It is also known as a latent period. The patient may be asymptomatic, pain in right hypochondrium, acute renal failure (some degree), acute pancreatitis (rare), enhancement of transaminases, Significant reduction in prothrombin time, and antithrombin III (Mazer and Perrone 2008).
Stage III	72-96 hours post-intake	Established hepatocellular failure, hepatic encephalopathy, renal failure, coma, metabolic acidosis, pancreatitis, maximum elevation of transaminases, hypophosphatemia, hypoglycemia, coagulation disorder (Anon n.d.).
Stage IV	96 hours to 2 weeks post-intake	Recovery of general deterioration, organ failure, death. If clinical manifestation is observed delay which means if it is observed 2 or 3 months after toxification. As a result, the histologic recovery period is longer. And it will be longer than the clinical recovery (Bunchorntavakul and Reddy 2013).

There are so many poor prognostic signs which include renal failure, lactic acidosis, hypoglycemia, cerebral edema as well as any signs of which cause immediate liver transplantation.(Harrison et al. 1990) APAP hepatotoxicity can cause aminotransferases > 10,000 IU/L as well as it is one of the major causes of liver injury.

Diagnosis of APAP overdose

Physical examination and patient history are the main things for the diagnosis as well as the time course is an

important thing for the diagnosis. Generally, APAP level is measured when the patients are admitted to the emergency department of the hospital after the toxic ingestion of acute substances or if the mental status is altered or self-harm case. 4-hour APAP level is critical to find or close to it also critical to find. This value is important because it guides the therapy as well as the patient outcome. Laboratory studies are necessary for finding the clinical parameters. These parameters include arterial blood gas, coagulation profile, hepatic function tests as well as a urine drug screen, etc.(Rumack 2002). It is important that in patients with direct hyperbilirubinemia where hyperbilirubinemia is greater than 10 mg/dL, during the interpretation of APAP level the clinicians should be cautious. This is most important because a drug-induced liver injury can grow up hyperbilirubinemia significantly.

After a single acute overdose of acetaminophen (within 24 hours of intake), Romack-Matthew nomogram should be accepted in APAP hepatotoxicity management(Rumack 2002). This type of nomogram can plot time which is independent versus concentration of APAP. Here time determines in an hour. Possible hepatotoxicity is determined when the level of APAP is 200 µg/mL at 4 hours or after 16 hours of acute ingestion the APAP level is 25 µg/mL. If a patient shows this serum level of APAP he or she is at a higher risk of severe acetaminophen-induced hepatotoxicity(Mcgovern et al. 2015). In this case NAC therapy should be recommended.

It is an important thing that A high toxicity line is parallel to the probable toxicity line which begins at 4 hours and the APAP level is 300 µg/mL. As a result, the severe hepatotoxicity rate is 90 percent well as the data rate is 24 percent(Prescott et al. 1979). Currently conservative measurement is established in Australia, the USA and New Zealand which is begins at 4 hours and the APAP level is 150 mg/mL. Which is more conservative. This is also known as the treatment line. This treatment line is intentionally set lower because which proper history of APAP ingestion is not revealed as well as laboratory error(Smilkstein et al. 1988). This treatment line is sited 25 percent below the toxicity line, especially the probable toxicity line. A recent study suggested that 150 lines very well for identification as well as it helps to protects the high-risk patients(Smilkstein et al. 1988). Here NAC therapy should be initiated because it shows less adverse effect.

Another important point is an evaluation after repeated overdose. The symptoms which include nausea, right upper quadrant pain, hepatomegaly, vomiting as well as encephalopathy is responsible for the elevation of APAP level. If any patient shows this type of symptoms, he or she should be checked the APAP level(Rumack 2004). In this case NAC therapy should be administered if the patient's ALT level is elevated. If the patient's APAP level is not detected at that time NAC therapy does not give any benefit.

Serum biomarkers have great importance when APAP diagnosis presents an unclear scenario. Other multiple biomarkers can act as an indicator as well as it can identify liver injury. Most importantly these markers can predict the patient outcome(Xie et al. 2015). Protein adduction is higher in patient's serum who is affected with acetaminophen-induced hepatotoxicity due to overdose of APAP(Xie et al. 2014). Human microRNAs are considered as a potential biomarker and indicate the APAP-induced hepatotoxicity on the molecular level. These human microRNAs mainly indicate APAP hepatotoxicity at the point which comes before the elevation of aminotransferases.

Treatment of APAP overdose

The patient who comes to early medical attention to track down the problem within four hours of single acute ingestion should be treated with activated charcoal because it can limit the drug absorption. But before using it, must be noticed about contraindication. If there is not any contraindication at that time charcoal treatment should be activated. These contraindications include unprotected airways as well as the problem with the GI tract. This is considered as a fortunate situation for the patients(Underhill, Greene, and Dove 1990). Gastric lavages is another important treatment and it is generally done within two hours of post intake or when toxicity is unknown as well as the time of ingestion is not cleared(Underhill et al. 1990).

Generally, NAC is a prodrug specifically it is a cysteine prodrug. It is used as a precursor of glutathione hormone. It is used in a patient with APAP-induced hepatotoxicity as an antidote or it is used for those patients who are at a higher risk of developing APAP hepatotoxicity. NAC can restore the glutathione hormone store by providing cysteine which can neutralize the harmful metabolite of APAP which is known as NAPQI. By increasing the sulfonation pathway NAC therapy can decrease the NAPQI molecule(Heard 2008). Mortality rate is decreased by the use of NAC therapy and the percentage of mortality rate which is reduced from 5% to 0.7%(Chun et al. 2009).

There is some general clinical indications for NAC therapy which include

- NAC therapy should be administered within 24 hours of ingestion.
- At four hours serum APAP level is 140 mg/L and at 10 hours serum APAP level is 50 mg/L
- When APAP-induced hepatotoxicity is severe which means that AST: ALT greater than 1000.
- Another indication is acute poisoning generally within 1 hour of ingestion as well as in the past twenty-four hours there is not any other product containing acetaminophen.

- Without ingestion of sustain release formulation if there is acute poisoning occurs.
- Baseline normal AST (aspartate aminotransferase) ALT (alanine aminotransferase) as well as INR
- The best time for using the NAC therapy is the first eight to ten hours when the risk of hepatotoxicity is less than 5 percent. Generally, when the treatment line is below the APAP level on the Rumack-Matthew nomogram.
- Within 8 hours of ingestion if the APAP level is unknown at that time NAC is given experimentally.(Yoon et al. 2016)

There are two types of NAC treatment which are oral and intravenous. Table 2 indicate oral administration of NAC therapy(Heard 2008).

Table 2: Oral administration of NAC therapy(Heard 2008).

Initial dose	Generally, the initial dose 140 mg/kg which is diluted in 5 percent of liquid especially in fruit juice.
Posterior dose	The posterior dose is 70 mg/kg and it should be given 17 times.
Total administered dose	The total administered dose is 1330 mg/kg
Treatment duration	The duration of treatment is 72 hours.

Intravenous administration is given for those patients who are affected with acute liver failure or when oral administration is contraindicated. There is some contraindication which includes pancreatitis, coma as well as gastrointestinal tract insufficiency. There is also some risk of an anaphylactic reaction and which is approximately 10 to 20 percent(Kerr et al. 2005). Table 3 indicate intravenous administration of NAC therapy(Heard 2008).

Table 3: Intravenous administration of NAC therapy (Heard 2008).

Initial dose	The initial dose is 150 mg/kg and which is in 150 ml of 5 percent serum glucose within an hour.
Posterior dose	the posterior dose is 50 mg/kg and which is in 500 ml of 5 percent serum glucose within four hours as well as the posterior dose is 100 mg/kg and which is in 500 ml of 5 percent serum glucose within 16 hours.
Total administered dose	The Total administered dose is 300 mg/kg
Treatment duration	The total treatment duration is 21 hours

The life-saving procedure is liver transplantation. There are some prognostic factors which include KCH criteria, MELD as well as APACHE II scores can determine the liver transplant candidates. These all are considered objective indicators(Mcgovern et al. 2015).

The role of NAC

- By increasing the level of Nrf2 and HO-1 mRNA it can inhibit reactive oxygen species.
- It protects mitochondria from dysfunction.
- It involves the elimination of GDH release as well as JNK activation.
- It involves the scavenging of harmful NAPI metabolites during the early metabolism phase.
- It can reduce the protein binding during the earl phase by the enhancement of glutathione hormone levels.(Yoon et al. 2016)

Nowadays different studies provided more knowledge which helps our health sector for developing the new drug. Another important study presented the protective role of glutathione in man. In this study, they indicated that acetaminophen is generally converted into arylating agents in the liver and this arylating agent is reactive. Glutathione only can detoxify these arylating agents. depletion of glutathione may have occurred when the patient takes a large dose of acetaminophen. As a result, hepatic necrosis is occurred by the arylation of the hepatic macromolecule. This paper also reported cysteamine which is glutathione-like nucleophiles. Mice were protected from arylation using these nucleophiles as well as it can prevent hepatic necrosis, cell death which is caused by reactive metabolite. This glutathione also plays a protective role in man. If the mercapturic acid is identified in our urine which indicates that acetaminophen is converted into an electrophilic reactant. 4mM3 is the normal concentration of glutathione in our liver. As well as it is the normal concentration in various animals. During hepatic necrosis due to the cause of ingestion of acetaminophen at that time, more than 70 percent of hepatic glutathione are depleted. If the amount of toxic metabolite is at least 4mmol and this amount is well enough to cause liver necrosis(Mitchell et al. 1974).

Current research on APAP hepatotoxicity

Effect of green synthesized herbal gold nanoparticles (AuNPs)

Recently many studies have been done for developing treatment strategies. In a recent laboratory-based research, Mitra et al showed that the dose of herbal gold nanoparticles has a protective role against acetaminophen-induced hepatotoxicity. In their study, they used male albino rats and divided them into 6 groups. The first group was considered as a control group and the second group was given only

acetaminophen 500 mg/kg/day. This was given for 14 days. Other groups such as group 3, group 4, group 5, group 6, were given acetaminophen and AuNPs concurrently. In this case, the dose of acetaminophen was 500 mg/kg/day and the dose of AuNPs was 55, 175, 550, 2000 µg/kg/day. This was given for 14 days. Within these doses, 175 µg/kg/day showed more beneficial activity. As a result, the parameter such as serum glutamate oxaloacetate transaminase or SGOT, serum glutamic pyruvic transaminase or SGPT, alkaline phosphatase or ALP, bilirubin, and malondialdehyde (MDA) concentrations were significantly increased. And inversely the parameter which includes CAT or catalase activity, CAT or catalase activity, and glutathione hormone activity were significantly decreased in a hepatotoxic group compared to the control group. This study proved that co-administration of acetaminophen with AuNPs dose 175 µg/kg/day can restore all the wellness parameters. Thus, this study proved that 175 µg/kg/day of AuNPs have a better protective profile against acetaminophen-induced hepatotoxicity(Mitra et al. 2020).

Effect of the ligand-activated transcription factor aryl hydrocarbon receptor

Schuran et al. found that aryl hydrocarbon receptor (Ahr) can potentiate acetaminophen-induced hepatotoxicity. aryl hydrocarbon receptor (Ahr) is a transcription factor. Aryl hydrocarbon receptor can integrate dietary, environmental, microbial well as a metabolic signal into complex cellular transcriptional programs which can potentiate hepatotoxicity. In this experiment researchers taken mice especially wild-type mice in which lacking Ahr in hepatocyte or myeloid cells. This was treated with a specific ligand which is known as ITE or Ahr ligand 2-(1'H-indole-3'- carbonyl)-thiazole-4-carboxylic acid methyl ester. This ligand was co-administered with acetaminophen. ITE ligand involves the activation of the aryl hydrocarbon receptor. This ligand is generally nontoxic when using alone but it increases the acetaminophen-induced hepatotoxicity. In this situation after administration of acetaminophen overdose the mortality rate will be high and which is approximately 80 percent vs 0 percent in comparison with the control group. This study also revealed that when co-administration of acetaminophen with this ligand can damage the hepatocyte even at the therapeutic dose of acetaminophen. Mainly the activation of the aryl hydrocarbon receptor is responsible for the accumulation of APAP toxic metabolite which is known as N-acetyl-para-benzo-quinone imine(Schuran et al. 2021).

Effect of Tanshinone IIA

Currently, Zhang et al. revealed that tanshinone IIA has a protective effect against acetaminophen-induced hepatotoxicity. Tan IIA is an active component of *S. miltiorrhiza*. It is an antioxidant and shows better detoxifying activity. Generally, in comparison with acetaminophen treated mice, pretreatment of tanshinone IIA is responsible for the reduction of the plasma level of APAP toxic metabolite such as N-acetyl-p-benzoquinone imine (NAPQI) as well as involved with the enhancement of its bile level. After pretreatment of tanshinone IIA, the experiment showed that nuclear factor E2-related factor 2 such as Nrf2 and multidrug resistance-associated protein 2 such as Mrp2 as well as multidrug resistance-associated protein 4 such as Mrp4 mRNA as well as protein expression were significantly induced and which was normally seen in Nrf2+/+ mouse liver. The enhancement of Mrp2 as well as Mrp4 mRNA and protein expression in Nrf2−/− mouse liver is much lower. It means that Nrf2 deficient in mice liver. Generally, Nrf2 tends to bind with responsive elements of the antioxidant of Mrp2 as well as Mrp4. The experiment also revealed that Tan IIA is responsible for the enhancement of the expression of Nrf2, MRP2, and MRP4 through the inhibition of the expression of HOTAIR which full form is HOX transcript antisense RNA. Collectively this study suggested that Tan IIA plays an important role in reducing acetaminophen-induced hepatotoxicity. Tan IIA can change the pharmacokinetic profile of the acetaminophen as well as its toxic metabolite by using the HOTAIR-Nrf2-MRP2/4 signaling pathway(Zhang et al. 2020).

Effect of co-administration of NAC and Acetaminophen

Nakhaee et al. suggested that N-acetylcysteine dose-dependently develops the analgesic effect of acetaminophen. Acetaminophen is responsible for hepatotoxicity which is a concerning issue in recent years. NAC is considered a gold standard treatment for acetaminophen overdose-induced hepatotoxicity. For their study, they used male Sprague-Dawley rats. There was a control saline group, APAP generally 300 mg/kg, NAC 600 mg/kg, and APAP in combination with NAC groups that received 300 mg/kg APAP as well as 200–600 mg/kg NAC which includes AN200, AN400, AN600. These were administered orally. The antinociceptive effect of these drugs was measured in 30, 60, and 90 minutes later the administration of these rugs. Generally, NAC increases the analgesic effect of acetaminophen in a dose-dependent manner(Nakhaee et al. 2021). Acetaminophen showed the highest antinociceptive activity at the dose of 300 mg/kg in comparison with the control group. This effect is shown at 30,60 and 90min later after administration. NAC gave the only analgesic at the dose of 600 mg/kg. This effect is shown 60 and 90min later after administration. Generally, APAP 300 mg/kg in combination with NAC 600 mg/kg showed a very good analgesic effect which is better than the APAP group after 60 or 90 minutes of administration. At all the time points AN600 showed greater analgesia than the AN200. But AN400 is greater than AN200 at the time point 60 and 90 minutes. But AN400 and AN600 did not show any statistical difference(Nakhaee et al. 2021).

Overexpression of intestinal epithelial chemokine (C-C Motif) Ligand 7

Niu et al. proved that disruption of the gut barrier is a responsible factor for acetaminophen-induced hepatotoxicity. A recent study suggested that administration of acetaminophen can cause leakiness of the gut as well as acetaminophen is also responsible for the upregulation of colonic epithelial chemokine (C-C motif) ligand 7 such as CCL7. Myosin light chain kinase phosphorylation was significantly increased in Intestinal epithelial cell-specific CCL7 transgenic mice. As well as where gut permeability was elevated and bacterial translocation was increased in comparison with wild-type mice. It was revealed by Global transcriptome analysis that in Intestinal epithelial cell (IEC)-specific CCL7 transgenic mice the expression of proinflammatory genes is significantly increased in comparison with wildtype mice. Thus, in intestinal epithelial cells, overexpression of CCL7 can excite the acetaminophen-induced hepatotoxicity. In this study, the researcher used tissue-specific genetically modified mice, as well as this, is the first study that reported that IEC CCL7 disrupts intestinal epithelial barrier function. This study also discovered a piece of new evidence which is the breakdown of colonic integrity is associated with acetaminophen-induced hepatotoxicity. So, intestinal integrity should be maintained for reducing the acetaminophen-induced hepatotoxicity(Niu et al. 2020).

Effect of hypothermia

Another study revealed that hypothermia has a hepatoprotective effect against acetaminophen-induced hepatotoxicity. Hypothermia can alleviate oxidative stress as well as it can advocate the function of the mitochondria.

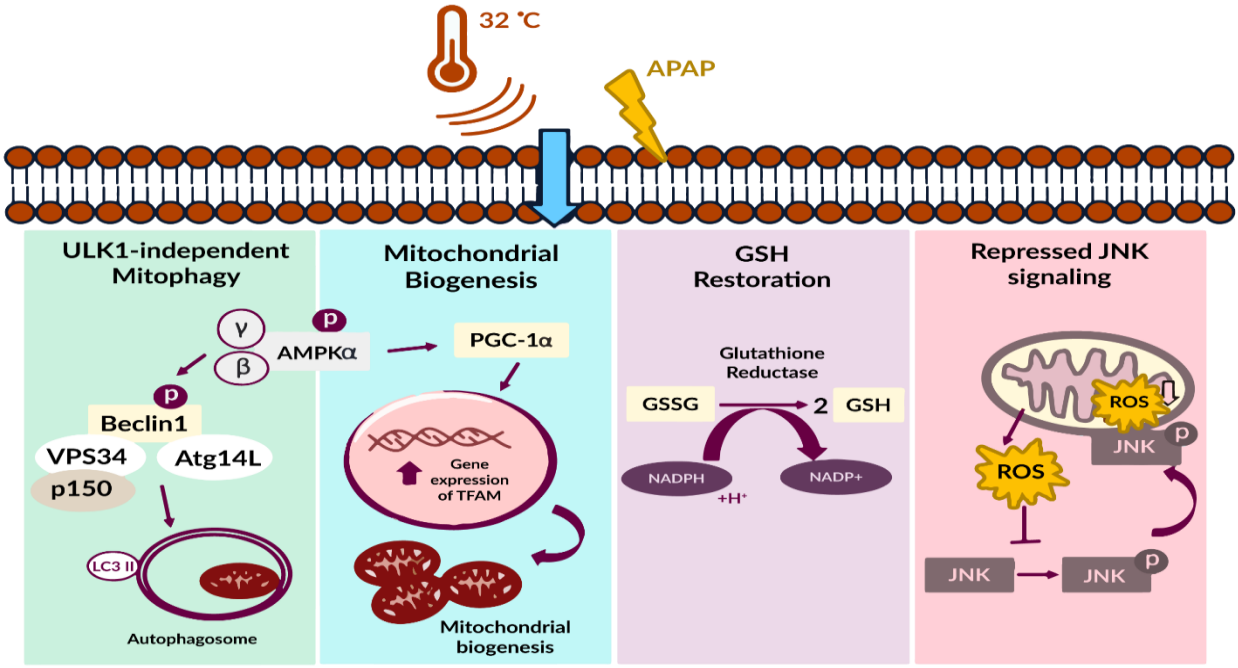

Fig. 2: Moderate hypothermia which is at 32 C can diminishing the acetaminophen-induced hepatic injury. With the help of AMPK mediated mitophagy as well as the biogenesis of the mitochondria, hypothermia can save the pool of functional mitochondria. Damaged mitochondria can be removed by the ULK1-independent mitophagy. This has happened during acetaminophen-induced hepatic injury when mitochondrial biogenesis is involved with the production of new functional mitochondria. Hypothermia can also promote the recycling of the glutathione hormone for restoring glutathione hormone. It also prevents the JNK signaling pathway from inhibiting oxidative stress. Thus, hepatic necrosis is reduced.(Tan and Ho 2020).

Involvement of autophagy

Zhao et al. reported that impairment of autophagy is an important factor for inducing APAP hepatotoxicity. The self-digestion pathway shows the cytoprotective effect under stress conditions. Here autophagy mainly conserves this important self-digestion pathway. This study aimed to investigate the mechanism of autophagy at the time of the acetaminophen-induced cell necrosis in an auditory cell line of a mouse (HEI-OC1) as well as the cochlear explant culture of a mouse. Their study reported that in APAP-treated HEI-OC1 cells, the expression of LC3-II protein as well as the expression autophagic structures was significantly enhanced. They also reported that in APAP-treated HEI-OC1 cells, the expression of lysosomal enzymes was significantly reduced as well as the yellow puncta of mRFP-GFP-LC3 fluorescence and the degradation of p62 protein were also significantly decreased. So, the data reported that acetaminophen treatment is responsible for the degradation of autophagy as well as induces lysosomal dysfunction. It is also reported that lysosomal dysfunction is directly involved with the impairment of autophagy. NAC treatment can

partially reduce the impairment of autophagy. Inhibition of autophagy is more dangerous which is happened by knocking down Atg5 and Atg7. As a result, apoptosis and oxidative stress is significantly increased(Zhao et al. 2021).

The role of ferroptosis in a murine model of APAP-induced acute liver failure

A newly discovered cell type is known as ferroptosis. It is caused by the significant loss of cellular homeostasis which is mainly cellular redox homeostasis. An experimental study was done to investigate the role of ferroptosis in the murine model as glutathione depletion can cause APAP-induced hepatotoxicity. During acetaminophen-induced hepatotoxicity, ferroptosis is considered as an initial as well as the predominant event of this hepatotoxicity. This study also reported that hepatic lipid peroxidation of n-6 PUFA generally occurred through the autooxidation process. High dose APAP lethality can be significantly prevented by the ferroptosis-specific inhibitor Fer-1. APAP lipid peroxidation, as well as APAP hepatotoxicity, were significantly prevented by the DFO. lipid peroxides derived from n6 PUFA and which is elevated by APAP. In APAP-induced lipid oxidation, the predominant mechanism is known as autooxidation. The major finding of this study was ferroptosis was considered as a therapeutic target for acute liver failure which is induced by acetaminophen(Yamada et al. 2020).

Effects of 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl

Tempol is a metal-independent superoxide dismutase-mimetic as well as a membrane-permeable radical scavenger. So, it can easily pass through a biological membrane. Ge et al. have shown that tempol is capable of inhibiting oxidative stress in the patient of acetaminophen-induced hepatotoxicity. Excessive production of superoxide is the main cause of acute hepatotoxicity. In the case of this type of liver toxicity superoxide dismutase, catalase, as well as glutathione, were reduced crucially but hydrogen peroxide and the concentrations of malondialdehyde (MDA) were increased. This study proved that superoxide dismutase was decreased with acetaminophen-induced acute liver injury and this condition was reversibly increased when treated with tempol. Thus, glutathione and catalase were increased when treated with tempol. But tempol decreased the concentration of malondialdehyde (MDA) and hydrogen peroxide(Ge et al. 2019).

Effect of mangiferin

Chowdhury et al. have found that mangiferin is an important agent which increases the production of glutathione hormone as well as involves the reduction of APAP-CYS formation. After the overdose of acetaminophen, mangiferin reduces the formation of APAP-CYS by inhibiting the JNK activation. Phosphorylated-c-Jun N-terminal kinase (p-JNK) is responsible for the reactivation of oxidative stress which can initiate inflammation and thus lead to hepatotoxicity. Mangiferin is used as an anti-inflammatory, anti-cancer, antiapoptotic well as and anti-diabetic agent(Chowdhury et al. 2019).

Inhibition of JNK related apoptosis and necroptosis by oroxyloside

Liao et al. proved that oroxyloside is an important agent which involves the protection of acetaminophen-induced hepatotoxicity. It is a natural flavonoid which is isolated from Scutellaria baicalensis Georgi. It is proved that this agent is capable of preventing liver apoptosis in mice liver. Oxidative radical is another main cause of liver injury when it is produced excessively. Oroxyloside can prevent oxidative stress in mice as well as it can prevent endoplasmic reticulum stress in mice. Thus, this agent can prevent acetaminophen-induced hepatotoxicity in mice. This agent can protection of hepatocytes by the inhibition of oxidative stress mitochondrial dysfunction(Liao et al. 2020).

Effects of exogenous thymosinβ4 (Tβ4) treatment

Wang, Li, and Chen proved that exogenous thymosinβ4 (Tβ4) plays an important role in the treatment of acetaminophen-induced hepatotoxicity. This study showed that after six hours of administration of acetaminophen there is a significant increase in the level of Serum alanine aminotransferase as well as aspartate aminotransferase. But the level of Serum alanine aminotransferase, as well as aspartate aminotransferase, is decreased significantly by the co-administration of exogenous thymosinβ4. This also decreases necrosis as well as inflammation which is induced by APAP. Thus, this agent can prevent hepatic glutathione depletion as well as malondialdehyde formation and it also helps in the reduction of superoxide dismutase (SOD) activities(Wang, Li, and Chen 2018).

The association of M1-/M2-macrophage polarization with DAMPs and Autophagy

Tsuji et al. suggested that the organization of damage-associated molecular patterns with M1-/M2-macrophage polarization and autophagy can be responsible for the pathogenesis. This pathogenesis was mainly hepatic pathogenesis as well as which was the pathogenesis of acetaminophen-induced rat liver injury. This study was the first study about the analysis of M1-/M2-macrophage polarization with acetaminophen-induced rat liver injury(Tsuji et al. 2020).

Effect of capmatinib

Saad et al reported that lapatinib which is also known as a c-Met inhibitor can reduce APAP hepatotoxicity. For their experiment, they injected mice with capmatinib before 2 hours and after 2 hours, and after 4 hours of APAP administration. But the use of capmatinib before 2 hours and after 2 hours of administration showed better results against APAP hepatotoxicity. And this capmatinib is mediated by lowering the level of lipid peroxidation as well as nitrosative stress. Cap also

decreased the release of TNF-α, IL-1β, IL-17A, IL-6, and MCP-1 which are mainly known as proinflammatory mediators. This cap also able to reduce hepatic IL-22 and PCNA expressions which are elevated APAP hepatotoxicity. It shows more potentiality at the earl phase of intoxication(Saad et al. 2020).

Effect of Thrombospondin-1

Frampton et al. reported that the activity of glutathione was reduced when thrombospondin-1 was inhibited. Thrombospondin-1 is known as homotrimeric protein. Thrombospondin-1 can induce antioxidant signaling by the activation of latent TGFβ1 as well as interaction with Nrf2 protein. In APAP treated mice the expression of TGFβ1, TSP1 was increased when it was compared with the control group. So, if there is the elimination of TSP1 protein, as a result, TGFβ1 signaling is reduced in APAP treated mice. As well as it can cause a reduction of Nrf2 expression and GSH activity. Thus, cell death occurred.(Frampton et al. 2020)

Effects of hesperetin

Wan et al. reported that hesperetin showed anti-apoptosis, anti-oxidation, and anti-inflammatory activity. The findings revealed that pretreatment with hesperetin reduced APAP-induced acute liver injury in mice in a dose-dependent manner, as indicated by reduced blood enzyme activity, hepatic pathological damage, and apoptosis. Thus, it can reduce APAP hepatotoxicity. This was mainly involved with the upregulation of heme oxygenase-1 expression as well as blocking the activation of Toll-like receptor (TLR)-4 signal. The results showed that hesperetin protected against APAP-induced acute liver injury by reducing hepatocyte necrosis and apoptosis, oxidative stress, and the inflammatory response by up-regulating HO-1 expression. It is a dihydrogen flavonoid compound that shows different pharmacological activities(Wan et al. 2020).

Effect of the deficiency of NADP+-dependent IDH2

Hepatotoxicity caused by acetaminophen (APAP) is a primary cause of liver failure, and it is linked to the production of reactive oxygen species (ROS), decreased levels of reduced glutathione (GSH), and overall oxidative stress. Kim, Lee, and Park suggested that Mitochondrial NADP+-dependent isocitrate dehydrogenase (IDH2) is an important enzyme for the maintenance of the antioxidant system and which is possible by the generation of NADPH. So, deficiency of IDH2 can lead to mitochondrial dysfunction which is an excess level as well as induction of ER stress. This study expressed that IDH2 showed beneficial effect against APAP hepatotoxicity(Kim, Lee, and Park 2019).

Effect of Exogenous activation of toll-like receptor 5 signaling

Z. Zhou et al. suggested that TLR5 signaling activity can reduce APAP hepatotoxicity. They looked into the role of TLR5 signaling in the aftermath of an APAP overdose. Mice were given an intraperitoneal injection of APAP to induce ALI, followed by an injection of flagellin one hour later. Flagellin reduced histopathologic lesions, serum biochemicals, oxidative stress, and inflammation in APAP-induced ALI. Flagellin treatment involved with translocation of Nrf2 as well as activation of Nrf2. This experiment demonstrated that the TLR5 signal is responsible for the induction of Nrf2 activation. Thus, flagellin treatment showed protective activity against APAP hepatotoxicity(Z. Zhou et al. 2021).

Effect of SIRT6 through alleviating oxidative stress

Overdosing on acetaminophen (APAP) is the most common cause of drug-induced liver injury, and the prognosis is determined by the balance of hepatocyte death and regeneration. Sirtuin 6 (SIRT6) has been shown to protect against DNA damage caused by oxidative stress. Y. Zhou et al. reported that SIRT6 has a key role in protecting APAP-induced hepatotoxicity because it involves the reduction of oxidative stress as well as promotion of hepatocyte proliferation. Sirt6 knockdown in AML12 cells exacerbated APAP-induced hepatocyte mortality and oxidative stress, hindered cell viability and proliferation, and decreased the levels of the proteins CCNA1, CCND1, and CKD4. Sirt6 knockdown significantly reduced APAP-induced NRF2 activation, GSTμ and NQO1 transcriptional activity, and Nrf2, Ho-1, Gstμ and Gstα mRNA levels. Furthermore, a co-immunoprecipitation (Co-IP) test revealed a possible protein interaction between SIRT6 and NRF2. Generally, P53 is responsible for the activation of NRF2. SIRT6 event is an important event for linking p53 and NRF2 inpatient with APAP hepatotoxicity(Y. Zhou et al. 2021).

Effect of fibroblast growth factor 1

The metabolic regulator fibroblast growth factor 1 (FGF 1) has various physiological functions. Wang et al. showed that fibroblast growth factor 1 is a key protector against APAP hepatotoxicity. When compared to littermates, PAP significantly elevated circulating levels of ALT and AST, but FGF1 considerably reduced increases in blood levels of ALT and AST. FGF1 also reduced APAP-induced centrilobular necrosis, according to histological analysis of the livers. Here the main function of fibroblast growth factor 1 is the inhibition of oxidative stress as well as the inhibition of endoplasmic reticulum stress. It can also inhibit inflammation and thus it inhibits apoptosis. Through inhibition of inflammation, apoptosis, and oxidative and endoplasmic reticulum stress, FGF1 protects mice from APAP-induced hepatotoxicity. As a result, FGF1 could be a viable treatment for APAP-induced acute liver damage (Wang et al. 2019).

Effect of an ethanol extract

LI et al. proved that Schisandra Chinensis stems have a protective role against APAP hepatotoxicity. Before a single hepatotoxic dose of APAP (250 mgkg-1) was injected into mice, they were given SCE extract for seven days. Significant decreases in aspartate

transaminase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA) contents, as well as elevations in reduced glutathione (GSH) and superoxide dismutase (SOD) levels indicated that SCE administration improved liver dysfunction and oxidative stress. These findings were linked to the observation that SCE pretreatment dramatically reduced 4-hydroxynonenal (4-HNE) and 3-nitrotyrosine expression levels (3-NT). By exposing cells to APAP, SCE dramatically reduced the expression of Bax, mitogen-activated protein kinase (MAPK), and cleaved caspase-3. Thus, it can act by the regulation of different pathways which include MAPK and caspase-3 signaling pathways. It can reduce the expression of 4-hydroxynonenal as well as also reduces 3-nitrotyrosine and cleaved caspase-3(LI et al. 2018).

Effect of the blockade of IL-17 as potential therapeutic target

Overdosing on acetaminophen (APAP) causes reactive oxygen species (ROS), hepatocyte necrosis, and cell death, as well as abrupt liver failure. The pro-inflammatory cytokine interleukin-17 (IL-17) is involved in the recruitment of neutrophils to sites of inflammation and subsequent damage during liver ischemia-reperfusion injury. Lee et al. experimented that deficiency of IL-17 can cause reduced APAP liver injury. The deficiency of IL-17 also reduced ERK phosphorylation at the time of the APAP-induced liver injury. IL-17 deficiency reduces APAP-induced liver injury, MPO activity, pro-inflammatory cytokines which includes tumor necrosis factor-α, IL-6, and interferon -γ levels, and inflammatory cell infiltration in the liver which includes neutrophils, macrophages. So, IL-17 blocking may be a potential therapeutic target(Lee et al. 2018).

Effect of CORM A-1

Oxidative stress is usually linked to acute liver damage. Upadhyay et al. suggested that the A-1 molecule which is the releasing molecule of carbon monoxide can reduce APAP-induced hepatotoxicity. They looked into the therapeutic potential of CORM A-1 (carbon monoxide releasing molecule A-1) in the treatment of oxidative stress-induced liver damage. This molecule can induce Nrf2 as well as related genes and thus it can improve the survival rate of mice. It can facilitate nuclear translocation of Nrf 2 in HepG2 cells as well as upregulation of ARE genes. It also involved the Inhibition of the interaction between Nrf2 and Keep-1. CORM A-1 decreases oxidative stress by upregulating Nrf2 and associated genes and restoring hepatic GSH, which reduces hepatocyte necrosis and hence liver injury, contributing to a better overall survival rate(Upadhyay et al. 2018).

Effect of Betaine as a methyl Donor and S-adenosylmethionine precursor

Betaine act as a methyl donor as well as it also acts as an S-adenosylmethionine precursor which has a protective role against acetaminophen-induced hepatotoxicity. This is also able to restore mitochondrial complex II activity as well as restore the level of glutathione. It also increases mitochondrial membrane potential as well as SOD level. It also restores glutathione peroxidase and catalase activity. Betaine can reduce mitochondrial lipid peroxidation and reactive oxygen species or ROS. According to Khodayar et al. the most effective dose for rescuing APAP hepatotoxicity in mice was betaine (500 mg/kg). Betaine protects against APAP hepatotoxicity by protecting mitochondrial complex II and regenerating mitochondrial GSH levels via boosting GCLC expression. Betaine exerted antioxidant effects distinct from other antioxidants via altering cysteine availability in the liver's transsulfuration pathway. Thus it can play a protective role against APAP hepatotoxicity(Khodayar et al. 2018).

Effect of berberine

Due to a lack of safe and effective treatment medicines, acetaminophen (APAP) hepatotoxicity remains the most common cause of drug-induced liver injury. Berberine (BBR) is another important natural agent which is derived from Rhizoma Coptidis which has a protective role against hepatotoxicity. Zhao et al. suggested that the effects of BBR pretreatment on APAP-induced hepatic pathological abnormalities, as well as serum aminotransferases and the liver/body weight ratio, were dramatically reduced. This agent can increase the levels of hepatic UDP-glucuronosyltransferases as well as Increases the level of sulfotransferases and reduces the level of MDA and MPO. BBR pretreatment decreased hepatic MDA and MPO levels, blocked JNK phosphorylation, and increased nuclear Nrf-2 and its downstream gene Mn-SOD expression. Furthermore, BBR clearly inhibited DNA fragmentation caused by APAP. Pretreatment with BBR significantly reduced the production of pro-inflammatory cytokines, HMGB1, p-p65, and cleaved caspase-1, and prevented macrophage and neutrophil infiltration. It is also responsible for the inhibition of oxidative stress resulting it can inhibit hepatocyte necrosis and inflammatory response(Zhao et al. 2018).

Effect of medical ozone therapy

Tezcan et al. reported that ozone therapy showed a potential effect against APAP hepatotoxicity. At a statistically significant level, ozone had a protective effect on APAP hepatotoxicity. Because of its antioxidant properties, ozone is recognized to have therapeutic effects in a variety of ailments. This ozone therapy plays a protective role in reducing oxidative stress as it can increase the antioxidant defense system. According to the findings, ozone could be used as a routine additional therapy in the treatment of acute APAP hepatotoxicity(Tezcan et al. 2018).

Effect of dexamethasone (DEX)-activated GR on Fgf21 expression

Signaling through the glucocorticoid receptor (GR) is critical for cell growth and development, as well as drug

metabolism. It acts as a cytoprotector by reducing the harmful effects of substances like dioxins, acetaminophen (APAP), and alcohols. Vispute et al. looked at how dexamethasone (DEX)-activated GR impacts Fgf21 expression and how it influences APAP-induced hepatotoxicity development. Their results revealed that DEX boosted Fgf21 mRNA and protein expression in mouse liver, as well as cultured mouse and human hepatoma cells, in a dose/concentration and time-dependent manner. fibroblast growth factor 21 involves the regulation of glucose, energy metabolism, and lipid. It also shows the cytoprotective role. In wild-type mice, pretreatment with 2 mg/kg DEX reduced APAP-induced liver injury, but not in Fgf21-null animals. Dexamethasone involves the induction of Fgf21 expression in mouse liver as well as human hepatoma cells. Fgf21 is mainly a target gene of GR(Vispute et al. 2017).

Effect of selenoprotein MsrB1

Overdosing on acetaminophen (APAP) causes immediate liver damage and failure due to the formation of reactive oxygen species and the depletion of glutathione (GSH). After an APAP challenge, MsrB1 deficiency exacerbated hepatic oxidative stress, including hydrogen peroxide generation, lipid peroxidation, and protein oxidation. Methionine sulfoxide reductase B1 acts as an antioxidant that is responsible for the reduction of methionine R-sulfoxide. The deficiency of Methionine sulfoxide reductase B1 can elevate oxidative stress which includes enhancement of lipid peroxidation, hydrogen peroxide production as well as increase the level of protein oxidation. Also, this type of deficiency can involve the reduction of glutathione hormone stores(Kim et al. 2017).

Effect of Celastrol and BBG combination

In APAP-hepatotoxicity in mice, Abdelaziz et al. explored the pharmacological suppression of these upstream signaling molecules by celastrol and brilliant blue G (BBG) (separately or simultaneously). Combination therapy of celastrol and brilliant blue G has a key role in inhibiting the consumption of hepatic antioxidants. Lipid peroxidation aldehydes were also limited by the use of this combination therapy. This combination also showed different beneficial effects which include: Decreased accumulation of inflammatory cells, promoting the regulation of pro-inflammatory and anti-inflammatory cytokines. The reparative capacity of injured cells was increased(Abdelaziz et al. 2017).

Intraperitoneal administration of APAP with other protective agents

Recently there are so many studies have been done for developing treatment strategies by designing a new drug for the betterment of APAP toxicity. Nrf2 activator involves the reduction of ROS as well as it can restore GSH. Gadd45β agonist involve with the prevention of JNK activation. TLR4 antagonist has also shown a protective role against inflammation. In the laboratory, there are so many experiments have been done to find out the hemodynamic effect. In some experiments, drugs were administered intraperitoneally or orally. The following table showed the hemodynamic effect of intraperitoneal administration.

Table 4: Here, we can see the intraperitoneally administered acetaminophen-induced hepatotoxicity as well as administration of some beneficial agent which can reduce the severity of APAP-induced hepatotoxicity.

Study	Drugs	Duration	Grouping	Hemodynamic effect of intraperitoneal administration	Limitation
(Mitra et al. 2020)	Acetaminophen (500 mg/kg/day), AuNPs (55,175, 550,2000µg/kg/day)	14 days	There were 6 groups. **Group1:** control group; **Group2:** given acetaminophen; **groups 3, 4,5,6:** co-administration of acetaminophen with AuNPs	SGOT, SGPT, ALP, bilirubin, and MDA levels were significantly increased as well as SOD, CAT, and GSH levels were decreased in the hepatotoxic group in comparison with the control group. co-administration of acetaminophen with AuNPs dose 175 µg/kg/day can restore all the wellness parameters.	There were a small group of animals. This is only a lab-based study. further study should be needed to find out the detailed mechanism for the management of hepatotoxicity by using AuNPs.
(Niu et al. 2020)	Acetaminophen (300 mg/kg), phosphate-buffered saline	3 or 24 hours	the control group was treated with phosphate-buffered saline as well as C57BL/6J mice were treated with APAP for 3 and 24 hours	In the APAP group, the FD-4 (Fluorescein Isothiocyanate-dextran) concentration is higher compare to the controlled group and which was observed at 3 or 24 hours after APAP administration. At 3 hours and 24 hours after APAP administration, fecal albumin was increased significantly. tight junction occludin and claudin-4 protein levels were significantly decreased	Further research should be needed to understand the mechanism by which hepatic inflammation at baseline in CCL7tgIEC mice sensitizes the liver to APAP toxicity. There were a small group of animals. This is only a lab-based study.

				in the APAP group. immunofluorescence showed prominent disruption of colonic epithelium barrier.	
(Yamada et al. 2020)	Acetaminophen (200 mg/kg), Fer-1 (10 mg/kg)	3 hours	Control group, disease group, treatment group	Serum ALT and AST levels were increased significantly at 3 hours of APAP intake. When mice are treated with Fer-1 (10 mg/kg) 1 h before APAP injection resulting in ALT and AST level is significantly decreased.	This is only a lab-based study. subcellular locations in which lipid peroxidation occurs during ferroptosis remain unclear.
(Wang et al. 2018)	APAP (500 mg/kg).	6 hours for APAP and Tβ4 was administered at 0 h, 2 h, and 4 h after APAP injection.	For the investigation of Tβ4 treatment: control group, APAP group, APAP + T100 group as well as APAP + T200 group. To investigate the role of autophagy: APAP+T200 group and APAP+T20+CQ group.	ALT, AST was significantly increased after 6 hours of APAP intake. ALT, AST levels were decreased when co-administration of Tβ4.	Further investigation should be needed to explain the basic mechanism of Tβ4 in autophagy regulation. This is only a lab-based study.
(Saad et al. 2020)	APAP-dose (500 mg/kg/20 mL) And Cap-dose (10 mg/kg/10 mL)	24 hours	There were 5 groups. 1.Normal Control 2.APAP 3. Cap 2 h prior APAP 4. Cap 2 h post-APAP 5. Cap 4 h post-APAP 6.NAC 2 h post-APAP	A wide area of cellular necrosis occurred, proinflammatory mediators were increased as well as lipid peroxidation and nitrosative stress were also reduced when only administration of APAP. When the cap is administered before 2 hours and after 2 hours of APAP administration were able to solve those problems.	There were a small group of animals. This is only a lab-based study. further study should be needed to find out the detailed mechanism for the management of hepatotoxicity by using capmatinib (Cap)
(Frampton et al. 2020)	300 mg/kg as well as 600 mg/kg of APAP	6 hours	They divided mice into 3 groups.	Inhibition of TSP1 protein results; Decrease GSH activity Decrease Nrf2 expression Reduce TGFβ1 signaling	This is only a lab-based study. Their small group of mice was performed for the experiment.
(Wan et al. 2020)	APAP 350 mg/kg; hesperetin (5, 10, 30 mg/kg,	24 hours	All mice were divided into 4 groups.	When APAP was administered alone as a result; The rate of LDH cytotoxicity was high. Cell viability was less. Higher rate of apoptosis. Hesperetin reduced reactive oxygen species (ROS). Upregulation of heme oxygenase-1 expression.	Their small group of mice was performed for the experiment. It was a lab-based study.
(Kim et al. 2019)	APAP (250, 500 mg/kg) Mito-TEMPO 2 mg/kg	24 h	several groups each group contains five mice.	Role of APAP in IDH2 deficient mice: generation of ROS GSH level was reduced Mitochondrial damage induced ER stress Role of mito tempo: Increased mitochondrial	There were a small group of animals. This is only a lab-based study. further study should be needed to find out the detailed mechanism for the management of hepatotoxicity.

				superoxide anion.	
(Y. Zhou et al. 2021)	APAP 400 mg/kg, APAP solution was made fresh in 0.9% saline at 40 mg/mL	0, 0, 6, 12, 24, 48 and 72 h	Male C57BL/6 mice n=6	Elevation of ALT and AST. Oxidative stress Centrilobular necrosis at 6 hours 6 h (40 to50%) and between 12 and 24 hours 60 to 70%. At 48 hours less hepatocellular necrosis was observed. At 72h it was observed 10 to 20% necrosis. ALT and AST level was significantly increased after 12 hours of APAP administration.	This study reported that SIRT6 has a key role in protecting APAP-induced hepatotoxicity. But this is only a lab-based study. Small groups of animals.
(Wang et al. 2019)	APAP dissolved in PBS (500 mg/kg) FGF1 solution (1.0 mg/kg)	6 hours	C57BL/6 mice 3 groups: 1.Control 2. APAP 3.APAP+ FGF1	Role of APAP: Reduced the levels of T-AOC, GSH-Px, and T-SOD. Increased MDA. Increased the level of ALT as well as AST. centrilobular necrosis. endoplasmic reticulum stress oxidative stress severe inflammation Role of fibroblast growth factor 1 (FGF 1): Suppression of oxidative stress Suppression of inflammation. Suppression of endoplasmic reticulum stress Suppression of apoptosis	Lab-based study. Small number animals.
(LI et al. 2018)	APAP (250 mg·kg−1); APAP + SCE (200, 300, 400 mg·kg−1)	24 hours	Five groups Per group: 8 mice	Role of APAP: Increased ALT and AST Increased malondialdehyde (MDA) reduced glutathione (GSH) Reduced superoxide dismutase (SOD) Role of SCE: Decreased expression levels of 4-hydroxynonenal (4-HNE) Reduced 3-nitrotyrosine (3-NT) Reduced cleaved caspase-3	The molecular mechanism of Schisandra Chinensis (SCE) should be clarified. And it should clarify as a hepatoprotective agent in clinical application.
(Lee et al. 2018)	APAP (300 mg/kg)	16 hours	IL-17 wide type mice IL-17 KO mice	Increased activity of hepatic myeloperoxidase (MPO) Increased serum alanine transferase (ALT) In this experiment, the researchers found that the deficiency of IL 17 can cause a reduction in infiltration of the inflammatory cell as well as proinflammatory cytokines.	IL-17 blocking may be a target therapeutic potential.
(Upadhyay et al. 2018)	APAP (300mg/kg, 600 mg/kg) CORM A-1 (20 mg/kg)	4 and 12 h	There were 7 groups (mice) n=6	Role of APAP: Increased serum transaminase Increased depletion of GSH Increased hepatocyte necrosis Role of CORM A-1: It facilitates the nuclear translocation of Nrf 2 in HepG2 cells. Upregulates ARE genes Decreased serum transaminase Decreased depletion of GSH	Further study should be needed to observe the therapeutic potential in APAP hepatotoxicity in humans.

				Decreased hepatocyte necrosis Reduced APAP-induced mortality Inhibited the interaction between Nrf2 and Keep-1	
(Khodayar et al. 2018)	Different doses of betaine (125, 250, 500, 1000 mg/kg) APAP (300mg/kg)	24 hours	All mice are divided into 6 groups	Role of APAP: Increased ALT and AST activity Decreased mitochondrial GSH depletion Increased ROS Decreased SOD, GPX, and CAT activities Inhibited mitochondrial respiration Role of betaine: Protection of mitochondrial complex II Regeneration of mitochondrial GSH Enhancement of GCLC expression. Enhanced superoxide dismutase Increased glutathione peroxidase Increased glutamate-cysteine ligase catalytic protein expression	Not specified
(Zhao et al. 2018)	APAP (500 mg/kg); BBR (2 and 5 mg/kg)	6 hours	Mice were divided into 4 groups	Role of APAP: Increased ALT and AST level Increased oxidative stress Hepatic necrosis Increased MDA level Increased MPO level Role of Berberine: Enhance the levels of hepatic UDP-glucuronosyltransferases. Increased the level of sulfotransferases Decreased the level of MDA and MPO Inhibition of JNK phosphorylation Increased the expression of nuclear Nrf-2 Prevention of APAP-induced DNA fragmentation Decreased expression of the pro-inflammatory cytokine, p-p65, HMGB1, cleaved caspase-1. Prevented infiltration of macrophages and neutrophils Prevention of oxidative stress.	A hospital study should be designed to observe the therapeutic efficacy of Berberine.
(Visputte et al. 2017)	APAP (300mg/kg), DEX (2 mg/kg)	24 hours	Mice are divided into 4 groups	Role of APAP Increased ALT and AST level Depletion of glutathione level Role of Dexamethasone pretreatment: Induced GR activation Induced Fgf21 expression. Reduced acute acetaminophen-induced hepatotoxicity.	Not specified
(Tezcan et al. 2018)	APAP (300 mg/kg) Ozone (20 mcg/0.5 mL)	Not specified	Mice were divided into 3 groups. There were 24 mice	Role of APAP: Elevation of AST, ALT, ALP levels Necrosis and inflammation Role of APAP+ozone:	It should require further study to examine the effect of ozone on mitochondria which is damaged as well as the effect of ozone on the

				Reduced oxidative stress The increased antioxidant defense system	impaired antioxidant defense system. Also, the human study should be designed to examine the effect of ozone on human liver injury. So, the further study include: molecular or gene expression studies
(Kim et al. 2017)	APAP (300 mg/kg)	6 hours	MsrB1–/– and MsrB1+/+ mice	Role of APAP: ALT and AST levels are higher in MsrB1–/– mice. Also, higher serum LDH levels in MsrB1–/– than in MsrB1+/+ mice Increased hydrogen peroxide significantly in MsrB1–/– than in MsrB1+/+ mice. Basal HNE levels were significantly increased in MsrB1–/– mice. Protein-carbonyl levels were significantly increased in MsrB1–/– mice.	Not specified
(Abdel aziz et al. 2017)	APAP (500 mg/kg; 0.5-0.6 ml/25-30 g); Celastrol 2 mg/kg; BBG 50 mg/kg	24 hours	Mice were divided into 5 groups	Role of APAP: Increased ALT, AST, LDH level Apoptosis alongside necrosis Increased oxidative stress. Decreased GSH and SOD concentrations Increased hepatic MDA concentration Increased 4-HNE concentration Role of celastrol and brilliant blue G combination: Decreased ALT, AST, and LDH level Significantly decreased APAP-hepatic oxidative stress Significantly decreased APAP-hepatic inflammation. Prevented consumption of hepatic antioxidants. Decreased overproduction of lipid peroxidation aldehyde. Reduced accumulation of inflammatory cells The increased reparative capacity of injured hepatocytes	Not specified

Effect of herbal extracted natural compound

The researchers also experimented with the herbal product for the betterment of APAP toxicity. They extracted different natural products from different plants. Thus, they used these products for their research. The following table showed some research on natural herbal products which are extracted from different plants.

Table 5: Some herbal extracted natural compounds which showed beneficial activity against APAP hepatotoxicity on current preclinical studies.

Study	Natural herbal compound	Beneficial effect
(Mitra et al. 2020)	Herbal Gold Nanoparticles (AuNPs)	1.No cellular necrosis 2.Less lipid accumulation 3. Less lipid infiltration

		4. portal inflammations 5. no hepatocyte degeneration
(Zhang et al. 2020)	Tanshinone IIA	It inhibits the expression of HOX transcript antisense RNA (HOTAIR). As a result, 1.Significant induction of nuclear factor E2-related factor 2 (Nrf2) 2. Significant induction of multidrug resistance-associated protein 2 (Mrp2) 3. Significant induction of multidrug resistance-associated protein 4 (Mrp4).
(Ginting et al. 2021)	The red betel leaves extract (RBLE)	1.Increases CYP2E1 and GPX gene expression. 2.Increases live cells percentage. 3.Decreases ROS level and TNF-α concentration.
(Chowdhury et al. 2019)	Mangiferin	1.Inhibition of sustained JNK activation after APAP overdose 2.It involves improving the metabolism of acetaminophen 3.APAP-Cys adduct formation is followed by JNK-mediated oxidative stress and inflammation.
(Liao et al. 2020)	Oroxyloside (a natural flavonoid)	1.It involves the inhibition of APAP-induced JNK related apoptosis 2.Both in vivo and in vitro it increases antioxidant defenses, reversing ER stress as well as maintain the mito-balance of the hepatocyte.
(LI et al. 2018)	SCE (Schisandra chinensis stems)	1.Decreased expression levels of 4-hydroxynonenal (4-HNE) 2.Reduced 3-nitrotyrosine (3-NT) 3.Reduced cleaved caspase 3
(Zhao et al. 2018)	BBR (Berberine)	1.Enhance the levels of hepatic UDP-glucuronosyltransferases. 2.Increased the level of sulfotransferases 3.Decreased the level of MDA and MPO 4.Inhibition of JNK phosphorylation 5.Increased the expression of nuclear Nrf-2 6.Prevention of APAP-induced DNA fragmentation

Different molecules for designing a new antidote for the remedies of APAP hepatotoxicity

There are so many researches that have been done to find out the better alternative antidote. These experiments were targeted on the different molecule which includes Nrf2, c-Met, thrombospondin-1, CYP2E1, etc. These were proved only in the lab-based animal trial. So proper clinical trials should be needed to find out the effectiveness of these molecules. So, for the betterment of APAP hepatotoxicity, a proper clinical trial should be conducted.

Table 6: Here is the list of different molecules which might be the target for designing a new drug.

Category	Direction
c-Met inhibitor(Saad et al. 2020)	Decreased hepatic IL-22 and PCNA expressions.
Inhibition of thrombospondin-1(Frampton et al. 2020)	Decrease Nrf2 expression Reduce TGFβ1 signaling
Hesperetin (dihydrogen flavonoid compound)(Wan et al. 2020)	Upregulation of heme oxygenase-1 expression. Blocking the activation of Toll-like receptor (TLR)-4 signal
(TLR)-4 antagonist	Reduced inflammation
The activation of aryl hydrocarbon receptor(Schuran et al. 2021)	Upregulation of Cyp1a2 expression

Nrf2 activator	Reduction of ROS and restore GSH.
CCL7 overexpression(Niu et al. 2020)	Enhanced APAP
CYP2E1 inhibitor	Involve with the Lowering of NAPQI production.

CONCLUSION

Acetaminophen poisoning is ongoing in the world especially in the United States, United Kingdom and many other developed countries. Intentional or unintentional overdose of this drug can cause hepatotoxicity and sometimes it can cause liver injury. Many cases of acetaminophen hepatotoxicity, liver transplantation is necessary. Sometimes combination therapy such as acetaminophen with antihistamine drugs can cause acute liver failure. Acute acetaminophen-induced hepatotoxicity shows a higher mortality rate approximately which is 30 percent when liver transplantation is not sufficient for it. Nowadays it is a concerning issue that unintentional overdose causes toxicity, as well as women and alcoholics, are at higher risk of acetaminophen-induced hepatotoxicity. Despite extensive investigations into the mechanisms of cell death, only a single antidote is available in clinical use. However, there have recently been more efforts made to discover potential new antidote drugs for this indication. In future, preclinical trials are required to better determine the safety and efficacy of the compounds reviewed in this article.

ACKNOWLEDGMENT

This work was done without funding.

AUTHORS' CONTRIBUTION

Conceptualization: Mohammad Borhan Uddin, Writing and editing: Abdullah Al Mahmud Ashik and Mohammad Borhan Uddin. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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