To carry out pharmacological evaluation of Glutamine against isoniazid-rifampin induced hepatotoxicity in laboratory rat

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Abstract : Isoniazid and rifampin, widely used antitubercular drugs, often cause liver damage. This study examined whether glutamine can protect the liver from INH–RIF–induced toxicity in rats. Wistar rats were treated with INH–RIF to produce hepatotoxicity, while separate groups received glutamine along with the drugs. Liver function markers (ALT, AST, ALP, bilirubin) and oxidative stress indicators (MDA, GSH, SOD, CAT) were measured, and liver tissues were examined microscopically. INH–RIF administration led to significant biochemical and structural liver injury. Glutamine supplementation reduced these harmful effects, improved antioxidant activity, and restored liver architecture. Overall, glutamine showed notable hepatoprotective effects, suggesting its potential as a supportive therapy to reduce antitubercular drug–induced liver damage.

Index Terms – hepatotoxicity, bilirubin, SOD, ALT, AST

I. INTRODUCTION

Hepatotoxicity-

Hepatotoxicity refers to liver damage or injury caused by exposure to various toxic substances. These substances can include medications, chemicals, herbal supplements, environmental toxins, and even some natural compounds. Hepatotoxicity can manifest as acute liver injury, chronic liver diseases, or even fulminant hepatic failure, depending on the severity and duration of exposure. (Kaplowitz N et al., 2005).

There are several mechanisms through which hepatotoxicity can occur:

Direct Cellular Injury: Some substances can directly damage liver cells (hepatocytes), leading to cell death and inflammation. This can occur through chemical reactions or the generation of toxic metabolites (Jaeschke H et al., 2013).

Oxidative Stress: Certain substances can induce oxidative stress in the liver, leading to the production of reactive oxygen species (ROS) and free radicals. Prolonged oxidative stress can damage cellular components and impair liver function.(Jaeschke H et al., 2013).

1. **Immune-Mediated Reactions:** In some cases, the immune system may mistakenly target liver cells, leading to autoimmune hepatitis or other immune-mediated liver diseases. This can result in chronic inflammation and tissue damage.(Krawitt E. L. et al., 2006)
2. **Metabolic Disturbances:** Disruption of normal metabolic pathways in the liver can also contribute to hepatotoxicity. For example, some medications may interfere with the synthesis or metabolism of essential molecules, leading to liver dysfunction.(James et al., 2005)

Hepatotoxicity can present with a range of symptoms, including jaundice (yellowing of the skin and eyes), abdominal pain, nausea, vomiting, fatigue, and changes in urine color. In severe cases, it can lead to liver failure, which is a life-threatening condition requiring urgent medical attention.

- **Causes of Hepatotoxicity:**

1. **Medications**: Certain prescription drugs, over-the-counter medications, and herbal supplements can cause hepatotoxicity. Examples include acetaminophen (Tylenol), nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, statins, certain antibiotics, and antifungal medications.
2. **Alcohol**: Chronic alcohol abuse can lead to alcoholic liver disease, a common cause of hepatotoxicity characterized by liver inflammation, fatty liver, fibrosis, and cirrhosis.
3. **Viral Hepatitis**: Hepatitis viruses, particularly hepatitis B and C, can cause inflammation and damage to liver cells, leading to hepatotoxicity.
4. **Environmental Toxins**: Exposure to certain environmental toxins, such as industrial chemicals, pesticides, and heavy metals like lead and mercury, can damage the liver.
5. **Herbal Supplements**: Some herbal supplements, particularly those containing kava, comfrey, and green tea extract, have been associated with hepatotoxicity.

- **Symptoms of Hepatotoxicity:**

1. **Jaundice**: Yellowing of the skin and eyes due to the buildup of bilirubin, a substance produced during the breakdown of red blood cells.
2. **Fatigue**: Feeling tired or weak, which can be a result of decreased liver function and impaired energy metabolism.
3. **Abdominal Pain**: Pain or discomfort in the upper right side of the abdomen, where the liver is located, may occur due to liver inflammation or enlargement.
4. **Nausea and Vomiting**: Digestive symptoms such as nausea, vomiting, and loss of appetite may occur as a result of liver dysfunction.
5. **Dark Urine**: Urine may appear dark or tea-colored due to the presence of bilirubin, which is excreted by the kidneys when the liver is unable to process it properly.
6. **Pale Stools**: Stools may become pale or clay-colored due to reduced bile production by the liver.
7. **Swelling**: Swelling of the abdomen (ascites) or legs (edema) may occur due to fluid retention caused by liver dysfunction.
8. **Mental Confusion**: Hepatic encephalopathy, a condition characterized by impaired brain function due to liver failure, can cause confusion, forgetfulness, and changes in behavior or mood.(Navarro V. J. et al., 2006).

- **Diagnosis of Hepatotoxicity:**

1. **Medical History**: A thorough medical history, including information about medication use, alcohol consumption, exposure to toxins, and risk factors for viral hepatitis, helps identify potential causes of hepatotoxicity.
2. **Physical Examination**: Physical examination may reveal signs of liver disease, such as jaundice (yellowing of the skin and eyes), abdominal tenderness, and hepatomegaly (enlargement of the liver).
3. **Laboratory Tests**:
   - Liver Function Tests: Blood tests measure levels of liver enzymes (AST, ALT, ALP), bilirubin, and albumin to assess liver function and detect signs of liver injury.
   - Viral Hepatitis Serology: Testing for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), and hepatitis C antibody (anti-HCV) helps diagnose viral hepatitis.
   - Imaging Studies: Imaging tests such as ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI) may be performed to evaluate liver structure and detect abnormalities.
4. **Liver Biopsy**: In some cases, a liver biopsy may be necessary to assess the extent of liver damage and determine the underlying cause of hepatotoxicity.(McGill et al., 2019).

- **Management and Treatment of Hepatotoxicity:**

1. Discontinuation of Causative Agent: If hepatotoxicity is medication-induced, discontinuing the offending drug or substance is essential to prevent further liver damage.
2. Supportive Care:
   - Rest and Hydration: Adequate rest and hydration help support liver function and promote recovery.
   - Nutrition: A balanced diet rich in nutrients supports liver health and may aid in liver regeneration.
   - Monitoring: Close monitoring of liver function tests and clinical status is necessary to track disease progression and response to treatment.

3. Specific Treatments:

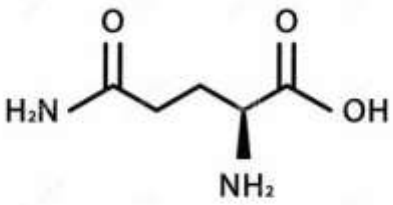
- Antidotes: In cases of acute drug overdose or poisoning (e.g., acetaminophen overdose), specific antidotes such as N-acetylcysteine (NAC) may be administered to counteract toxicity.
- Antiviral Therapy: For viral hepatitis (e.g., hepatitis B, hepatitis C), antiviral medications may be prescribed to suppress viral replication and reduce liver inflammation.
- Immunomodulatory Therapy: In autoimmune liver diseases (e.g., autoimmune hepatitis, primary biliary cholangitis), immunosuppressive medications may be used to suppress the immune response and reduce liver inflammation.
- Liver Transplantation: In severe cases of acute liver failure or end-stage liver disease, liver transplantation may be necessary to replace the damaged liver with a healthy donor liver.(Fontana R et al., 2013).

- **Importance of finding hepatoprotective agents:**

1. **Mitigation of Drug-Induced Liver Injury (DILI):** Many medications, including those used in the treatment of infectious diseases, chronic conditions, and cancer, can cause liver injury as an adverse effect. Hepatoprotective agents offer the potential to mitigate or prevent such drug-induced liver injury, thereby preserving liver function and minimizing treatment-associated morbidity and mortality.

2. **Enhanced Medication Safety:** Hepatotoxicity represents a significant safety concern in clinical practice and can lead to treatment interruptions, dose reductions, or discontinuation of potentially life-saving medications. Identifying effective hepatoprotective agents allows for safer medication use by reducing the risk of liver injury and enabling the continued use of essential therapies.
3. **Supportive Care in Liver Diseases:** Hepatoprotective agents play a crucial role in the supportive management of various liver diseases, including acute and chronic liver conditions such as hepatitis, cirrhosis, and non-alcoholic fatty liver disease (NAFLD). These agents can help alleviate liver inflammation, promote hepatocyte regeneration, and prevent disease progression, thus improving patient outcomes and quality of life.
4. **Prevention of Liver Damage from Environmental Toxins:** Hepatoprotective agents may offer protection against liver damage caused by environmental toxins, pollutants, and dietary factors. By enhancing liver detoxification pathways, antioxidant defenses, and cellular repair mechanisms, these agents can help safeguard the liver from the harmful effects of environmental exposures.
5. **Potential in Hepatitis and Viral Infections:** Hepatoprotective agents hold promise in the management of viral hepatitis, including hepatitis B and C infections, by reducing liver inflammation, fibrosis, and progression to cirrhosis or hepatocellular carcinoma (HCC). Additionally, they may complement antiviral therapies by supporting liver function and enhancing treatment response rates.
6. **Promotion of Liver Health and Longevity:** Beyond disease management, hepatoprotective agents contribute to overall liver health and longevity by preserving liver function, reducing the risk of liver-related complications, and promoting longevity. Incorporating hepatoprotective strategies into preventive healthcare practices can help maintain liver wellness and prevent the onset of liver diseases in at-risk populations.(Loguercio et al., 2003).

- **Glutamine**



Glutamine
$C_5H_{10}N_2O_3$

Glutamine is a vital amino acid with diverse physiological functions in the human body. Here's an overview of its key characteristics and roles:

1. Amino Acid Structure:

Glutamine is one of the 20 amino acids commonly found in proteins, encoded by the codons CAA and CAG.

It has a side chain containing an amino group and an amide group, making it unique among the standard amino acids.

2. Metabolic Functions:

Glutamine serves as a key substrate for various metabolic pathways, including protein synthesis, nucleotide synthesis, and energy production.

It plays a crucial role in maintaining the integrity of the intestinal mucosa, serving as a primary fuel source for enterocytes and supporting gut barrier function.

Glutamine also serves as a precursor for the synthesis of glutathione, an essential antioxidant molecule that helps protect cells from oxidative stress.

3. Clinical Applications:

Glutamine supplementation has been studied for its potential therapeutic benefits in various medical conditions, including critical illness, cancer, gastrointestinal disorders, and liver disease.

In clinical practice, glutamine is often used as a nutritional supplement in patients with critical illness or undergoing intensive care to support immune function, maintain gut integrity, and prevent muscle wasting.(Newsholme, P et al., 2018).

- **Introduction of glutamine in heapatotoxicity:**

1. Hepatoprotective Effects of Glutamine:

Preclinical studies have provided compelling evidence supporting the hepatoprotective effects of glutamine in various models of liver injury.

Glutamine supplementation has been shown to attenuate oxidative stress, reduce inflammation, preserve mitochondrial function, and promote hepatocyte regeneration in animal models of hepatotoxicity.

The mechanisms underlying the hepatoprotective effects of glutamine include its role as a precursor for glutathione synthesis, modulation of inflammatory cytokines, and preservation of mitochondrial bioenergetics.

2. Clinical Evidence and Applications:

While preclinical studies have demonstrated promising results, clinical evidence regarding the efficacy of glutamine in preventing or ameliorating hepatotoxicity is still emerging.

Clinical studies evaluating the effects of glutamine supplementation in patients with liver disease, including alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), and drug-induced liver injury, have shown mixed results.

Further research is needed to elucidate the optimal dosing regimens, timing of administration, and patient populations that may benefit most from glutamine supplementation in the context of hepatotoxicity.(Yao K et al., 2018).

- **Glutamine: Mechanisms and Potential Hepatoprotective Effects**

Glutamine, a non-essential amino acid, plays crucial roles in various physiological processes, including cellular metabolism, energy production, immune function, and antioxidant defense mechanisms:

1. **Role in Liver Function:**
    - Glutamine is a key substrate for hepatic gluconeogenesis, a process vital for maintaining blood glucose levels during fasting states.
    - It serves as a precursor for the synthesis of glutathione, the most abundant intracellular antioxidant in the liver, which helps protect hepatocytes from oxidative damage.
    - Glutamine also participates in the urea cycle, ammonia detoxification, and protein synthesis in the liver, contributing to overall liver function and homeostasis.
2. **Anti-inflammatory and Immunomodulatory Properties:**
    - Glutamine exhibits anti-inflammatory properties by modulating the production of pro-inflammatory cytokines and inhibiting the nuclear factor-kappa B (NF-κB) signaling pathway.
    - It promotes the production of anti-inflammatory cytokines and enhances the function of regulatory T cells, contributing to immune balance and attenuating liver inflammation.
3. **Protection Against Oxidative Stress:**
    - Glutamine acts as a precursor for the synthesis of glutathione, a potent antioxidant that helps neutralize reactive oxygen species (ROS) and protect hepatocytes from oxidative damage.
    - It enhances the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, further enhancing cellular antioxidant defenses in the liver.
4. **Maintenance of Gut Barrier Function:**
    - Glutamine plays a crucial role in maintaining the integrity and function of the intestinal mucosal barrier, thereby preventing bacterial translocation and endotoxemia.
    - By preserving gut barrier function, glutamine helps reduce systemic inflammation and liver injury associated with gut-derived toxins and microbial products.
5. **Promotion of Hepatocyte Regeneration:**
    - Glutamine supports hepatocyte proliferation and regeneration by providing energy and substrates for cell growth and repair processes.
    - It stimulates the expression of growth factors such as hepatocyte growth factor (HGF) and insulin-like growth factor 1 (IGF-1), which promote hepatocyte survival and proliferation.
6. **Modulation of Cell Signaling Pathways:**
    - Glutamine influences various cell signaling pathways involved in cell survival, apoptosis, and inflammation, including the mammalian target of rapamycin (mTOR) pathway and the mitogen-activated protein kinase (MAPK) pathway.
    - By modulating these pathways, glutamine exerts protective effects against liver injury and promotes hepatocyte viability and function.(Curi M et al., 2005).

- **Analysis Of Potential Adverse Effect & Drug Interaction Associated with Glutamine Use-**

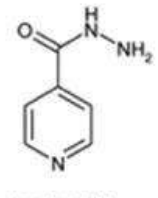
1. **Adverse Effects:**

- Gastrointestinal Effects: Some individuals may experience mild gastrointestinal symptoms such as bloating, flatulence, or diarrhea with high-dose glutamine supplementation.

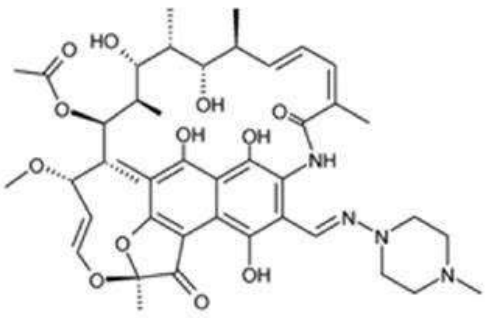
- Allergic Reactions: Rare cases of allergic reactions, including rash, itching, and swelling, have been reported with glutamine supplementation, particularly in individuals with pre-existing allergies or sensitivities.
- Neurological Effects: Excessive glutamine intake may lead to excitotoxicity and neurological symptoms such as headache, dizziness, or confusion in susceptible individuals.
- Metabolic Effects: Glutamine supplementation may affect glucose metabolism and insulin sensitivity in some individuals, potentially leading to hyperglycemia or hypoglycemia in diabetic patients or those with metabolic disorders.
- Renal Effects: Prolonged use of high-dose glutamine supplementation may exacerbate renal dysfunction or increase the risk of kidney stones in individuals with pre-existing renal impairment.

2. **Drug Interactions:**
   - Anticoagulants: Glutamine supplementation may potentiate the effects of anticoagulant medications such as warfarin, leading to an increased risk of bleeding.
   - Chemotherapy Agents: Glutamine supplementation may interact with certain chemotherapy agents, affecting their efficacy or increasing the risk of adverse effects. Close monitoring is recommended when using glutamine supplementation concomitantly with chemotherapy.
   - Anti-seizure Medications: Glutamine supplementation may interfere with the efficacy of anti-seizure medications such as phenytoin or valproic acid by affecting their metabolism or pharmacokinetics. Adjustments to medication dosing may be necessary.
   - Immunosuppressants: Glutamine supplementation may modulate immune function and interfere with the efficacy of immunosuppressive medications such as corticosteroids or cyclosporine. Close monitoring of immune function and medication levels is advised.
3. **Monitoring and Management:**
   - Healthcare providers should monitor patients receiving glutamine supplementation for signs of adverse effects or drug interactions, particularly in vulnerable populations such as those with underlying medical conditions or taking multiple medications.
   - Patient education regarding potential adverse effects and drug interactions associated with glutamine use is essential to ensure safe and appropriate use of supplementation.

- **Isoniazid-Rifampin :**



Isoniazid (1)



Rifampicin (2)

The pharmacokinetics of isoniazid and rifampin, two key components of tuberculosis (TB) treatment regimens, are complex and involve absorption, distribution, metabolism, and excretion processes. Here's an overview of the pharmacokinetics of isoniazid and rifampin:

1. **Absorption:**
   - **Isoniazid:** Isoniazid is well absorbed after oral administration, with peak plasma concentrations typically reached within 1-2 hours. Absorption can be affected by factors such as food intake and gastric pH.
   - **Rifampin:** Rifampin is also well absorbed after oral administration, with peak plasma concentrations achieved within 2-4 hours. Food intake can decrease the rate but not the extent of rifampin absorption.
2. **Distribution:**
   - Both isoniazid and rifampin have good tissue penetration, including penetration into the lungs, liver, and cerebrospinal fluid (in the case of rifampin). They also exhibit high protein binding, with approximately 70-90% of isoniazid and 80-95% of rifampin bound to plasma proteins.
3. **Metabolism:**
   - **Isoniazid:** Isoniazid undergoes hepatic metabolism primarily via acetylation by the enzyme N-acetyltransferase 2 (NAT2). The acetylated metabolite, acetylisoniazid, is then further metabolized to inactive metabolites.

- **Rifampin:** Rifampin is metabolized mainly in the liver by cytochrome P450 enzymes, primarily CYP3A4, to form a range of metabolites, including desacetylrifampin, 25-O-desacetylrifampin, and 3-formylrifamycin SV.

4. **Excretion:**
    - Both isoniazid and rifampin are primarily eliminated via hepatic metabolism, with the metabolites excreted in the urine. Renal excretion contributes to the elimination of approximately 75-95% of the administered dose of isoniazid and rifampin.
5. **Drug Interactions:**
    - Both isoniazid and rifampin are potent inducers of hepatic drug-metabolizing enzymes, including cytochrome P450 enzymes. As a result, they can significantly alter the pharmacokinetics of co-administered drugs by increasing their metabolism and reducing their plasma concentrations.(Peloquin, C. A. 1999)

- **Pharmacokinetic of Isoniazid-Rifampin related to Hepatotoxicity :**

The pharmacokinetics of isoniazid and rifampin are closely related to hepatotoxicity, as both drugs undergo hepatic metabolism and have the potential to cause liver injury. Here's how the pharmacokinetic properties of isoniazid and rifampin are relevant to hepatotoxicity:

1. **Metabolism:**
    - **Isoniazid:** Isoniazid is primarily metabolized in the liver via acetylation by the enzyme N-acetyltransferase 2 (NAT2). The acetylated metabolite, acetylisoniazid, undergoes further metabolism to inactive metabolites. Genetic polymorphisms in NAT2 can influence the rate of isoniazid acetylation and the risk of hepatotoxicity.
    - **Rifampin:** Rifampin is metabolized mainly by hepatic cytochrome P450 enzymes, primarily CYP3A4, to form various metabolites. These metabolites, including desacetylrifampin, 25-O-desacetylrifampin, and 3-formylrifamycin SV, may contribute to hepatotoxicity through oxidative stress and other mechanisms.
2. **Formation of Reactive Metabolites:**
    - Both isoniazid and rifampin can undergo metabolic activation to form reactive intermediates that can lead to hepatocellular injury. For example, isoniazid can be metabolized to acetylhydrazine and other toxic intermediates, while rifampin can produce reactive metabolites through its metabolism by cytochrome P450 enzymes.
3. **Induction of Enzymes:**
    - Both isoniazid and rifampin are known to induce hepatic drug-metabolizing enzymes, such as cytochrome P450 enzymes. This induction can lead to increased metabolism of other drugs and endogenous substances, potentially exacerbating hepatotoxicity by increasing the formation of reactive metabolites or altering the balance of pro-inflammatory and anti-inflammatory mediators in the liver.
4. **Cumulative Effects:**
    - The risk of hepatotoxicity with isoniazid and rifampin may be influenced by factors such as cumulative drug exposure, duration of treatment, and patient-specific factors (e.g., age, comorbidities, genetic polymorphisms). Prolonged or high-dose therapy with isoniazid and rifampin may increase the risk of hepatotoxicity, especially in patients with pre-existing liver disease or impaired hepatic function.(Ramachandran, G. 2013).

- **Molecular Mechanisms of Isoniazid-Rifampin induced Hepatotoxicity-**

1. **Metabolic Activation:** Both isoniazid and rifampin undergo biotransformation in the liver. Isoniazid is metabolized by cytochrome P450 enzymes, primarily CYP2E1, to form acetylhydrazine, which is further metabolized to acetylating intermediates. These reactive metabolites can lead to hepatocellular injury by covalently binding to hepatic proteins and causing cellular damage. Rifampin is metabolized by various cytochrome P450 enzymes, including CYP3A4, and its metabolites may also contribute to hepatotoxicity.(Hand W. L et al., 1999).
2. **Oxidative Stress:** Reactive metabolites generated during the metabolism of isoniazid and rifampin can induce oxidative stress in hepatocytes. This leads to the production of reactive oxygen species (ROS) and oxidative damage to cellular components such as lipids, proteins, and DNA. Oxidative stress plays a significant role in hepatocellular injury and inflammation observed in isoniazid-rifampin induced hepatotoxicity.(Yew, W. W et al., 2017).
3. **Mitochondrial Dysfunction:** Isoniazid has been shown to impair mitochondrial function by inhibiting enzymes involved in electron transport chain (ETC) function, such as cytochrome c oxidase (complex IV). Rifampin may also contribute to mitochondrial dysfunction through mechanisms that are not fully understood. Impaired mitochondrial function leads to ATP depletion, oxidative stress, and mitochondrial damage, contributing to hepatocyte injury and cell death.(Lauterburg, B. H et al., 1985).
4. **Immune-Mediated Mechanisms:** Hepatotoxicity induced by isoniazid and rifampin may involve immune-mediated pathways. Activation of immune cells, such as Kupffer cells and infiltrating lymphocytes, leads to the release of pro-inflammatory cytokines and chemokines, promoting inflammation and tissue damage in the liver. Autoimmune-mediated liver injury, such as drug-induced autoimmune hepatitis, may also occur in susceptible individuals.(Shuster, D. L et al., 2008).

5. **Genetic Susceptibility:** Genetic factors play a role in determining individual susceptibility to isoniazid-rifampin induced hepatotoxicity. Polymorphisms in genes encoding drug-metabolizing enzymes (e.g., CYP2E1, NAT2) and drug transporters (e.g., ABCB1) can influence the ADME of isoniazid and rifampin, affecting the risk of hepatotoxicity.(Sutradhar et al., 2006)

- **Current treatment approaches of Isoniazid-Rifampin induced Hepatotoxicity-**

The treatment of isoniazid-rifampin induced hepatotoxicity primarily involves prompt recognition, discontinuation of the offending agents, supportive care, and management of liver injury. Here are some current treatment approaches and considerations:

1. **Discontinuation of Isoniazid and Rifampin:** The first step in managing hepatotoxicity induced by isoniazid and rifampin is to discontinue these medications promptly. This helps prevent further liver damage and allows for the recovery of liver function.
2. **Monitoring and Supportive Care:** Close monitoring of liver function tests (LFTs) is essential to assess the extent of liver injury and guide management decisions. Supportive care measures, including adequate hydration, nutritional support, and rest, may be beneficial in supporting liver function and overall patient well-being.
3. **Management of Symptoms:** Symptomatic management may be necessary to alleviate symptoms such as abdominal pain, nausea, and fatigue. Analgesics, antiemetics, and other supportive medications may be prescribed as needed.
4. **Liver Function Monitoring:** Serial monitoring of liver function tests (LFTs), including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase levels, is crucial to assess the progression of liver injury and guide treatment decisions.
5. **Corticosteroids:** In cases of severe or fulminant hepatotoxicity, corticosteroids may be considered as adjunctive therapy to reduce inflammation and mitigate liver damage. However, their use remains controversial, and decisions regarding steroid therapy should be individualized based on clinical judgment and the severity of liver injury.
6. **Liver Transplantation:** In rare cases of acute liver failure or fulminant hepatic failure refractory to medical management, liver transplantation may be considered as a life-saving intervention. However, transplantation is typically reserved for patients with irreversible liver damage and a poor prognosis despite maximal supportive care.
7. **Avoidance of Hepatotoxic Agents:** After recovery from hepatotoxicity, patients should be counseled to avoid hepatotoxic agents, including isoniazid and rifampin, in the future. Alternative medications or treatment regimens may be considered for tuberculosis management in individuals with a history of drug-induced hepatotoxicity. (Dekhuijzen R et al., 2008).

- **Experimental study in isoniazid-rifampin induced hepatotoxic rat model-**

1. Study Design:

- The study utilized a rat model of isoniazid-rifampin-induced hepatotoxicity, where rats were administered isoniazid and rifampin to induce liver injury.
- Rats were divided into different treatment groups, including a control group receiving isoniazid and rifampin only and experimental groups receiving additional interventions such as glutamine supplementation.
- The interventions were administered either concurrently with isoniazid and rifampin or as pre-treatment before drug administration to assess their potential preventive or protective effects against hepatotoxicity.

2. Intervention and Outcome Measures:

- Glutamine supplementation was administered orally or intravenously at varying doses, and its effects on liver injury were evaluated through biochemical, histological, and molecular analyses.
- Liver function tests, including serum levels of liver enzymes (ALT, AST), markers of oxidative stress, and histopathological examination of liver tissue, were performed to assess liver injury and inflammation.
- Additional outcome measures may have included markers of apoptosis, mitochondrial function, and inflammatory cytokines to elucidate the underlying mechanisms of hepatoprotection.

3. Findings:

- The study findings demonstrated that glutamine supplementation attenuated isoniazid-rifampin-induced liver injury in the rat model.
- Rats receiving glutamine supplementation showed reduced levels of liver enzymes (ALT, AST), indicating preservation of liver function and decreased hepatocellular damage.
- Histological examination of liver tissue revealed reduced hepatic necrosis, inflammation, and oxidative stress in rats treated with glutamine compared to controls.
- Molecular analyses may have shown modulation of apoptotic pathways, preservation of mitochondrial function, and downregulation of pro-inflammatory mediators in glutamine-treated rats, further supporting its hepatoprotective effects.

The need for effective strategies to prevent or mitigate drug-induced liver injury, given the widespread use of isoniazid and rifampin in TB treatment and the potentially life-threatening consequences of hepatotoxicity:

1. **Essential Medications in TB Treatment:** Isoniazid and rifampin are cornerstone medications in the treatment of TB, forming the backbone of standard anti-tuberculosis therapy. These drugs are highly effective in killing Mycobacterium tuberculosis bacteria and are essential for successful TB treatment outcomes.
2. **High Incidence of Hepatotoxicity:** Despite their efficacy, isoniazid and rifampin are associated with a significant risk of hepatotoxicity, which can range from mild liver enzyme elevations to severe liver injury and acute liver failure. The incidence of hepatotoxicity with these medications underscores the need for proactive measures to minimize the risk of liver injury in patients receiving TB treatment.
3. **Potential Life-Threatening Consequences:** Hepatotoxicity associated with isoniazid and rifampin therapy can have serious and potentially life-threatening consequences, including acute liver failure, liver transplantation, and death. Severe hepatotoxicity can necessitate hospitalization, intensive medical management, and discontinuation of TB treatment, compromising the effectiveness of therapy and increasing the risk of disease progression and transmission.
4. **Impact on Treatment Adherence and Outcomes:** Hepatotoxicity can lead to interruptions or discontinuations of TB treatment, which can negatively impact treatment adherence, increase the risk of treatment failure, and contribute to the emergence of drug-resistant TB strains. Managing hepatotoxicity effectively is crucial for maintaining treatment continuity and achieving favorable treatment outcomes in patients with TB.
5. **Risk Factors and Variability in Susceptibility:** Individual patients may have varying susceptibility to hepatotoxicity based on factors such as age, sex, genetics, concomitant medical conditions, and concurrent use of other medications. Identifying patients at higher risk of hepatotoxicity and implementing tailored monitoring and management strategies is essential for optimizing treatment safety and efficacy.
6. **Need for Comprehensive Risk Management:** Effective strategies to prevent or mitigate drug-induced hepatotoxicity in TB treatment require a multi-faceted approach, including pre-treatment screening for liver disease and risk factors, close monitoring of liver function during therapy, early detection of hepatotoxicity, prompt intervention and management of liver injury, and consideration of alternative treatment regimens or hepatoprotective interventions when appropriate.(Menzies D et al., 2003).

Various parameters related to liver histology, liver enzyme levels, oxidative stress markers, and other relevant outcomes:

1. **Histological Changes:**
   - Histological analysis of liver tissue in animal models has revealed that isoniazid and rifampin administration leads to hepatocellular injury, characterized by hepatocyte necrosis, inflammation, and fibrosis.
   - Glutamine supplementation has been shown to mitigate these histological changes, resulting in reduced hepatocellular damage, inflammation, and fibrosis.
   - Specifically, glutamine-treated animals exhibit preservation of hepatic architecture, reduced hepatocyte necrosis, and decreased infiltration of inflammatory cells into the liver tissue compared to untreated animals.(Vashi C et al., 2020).
2. **Liver Enzyme Levels:**
   - Isoniazid-rifampin-induced hepatotoxicity is associated with elevated levels of liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum, indicative of liver injury.
   - Glutamine supplementation has been shown to attenuate the elevation of liver enzyme levels induced by isoniazid and rifampin, indicating a protective effect against liver injury.
   - Animals receiving glutamine exhibit lower serum levels of ALT and AST compared to untreated animals, suggesting reduced hepatocellular damage and improved liver function.(Vashi C et al., 2020).
3. **Oxidative Stress Markers:**
   - Oxidative stress is a key mechanism underlying isoniazid-rifampin-induced hepatotoxicity, characterized by increased production of reactive oxygen species (ROS) and lipid peroxidation.
   - Glutamine supplementation has been found to reduce oxidative stress markers in the liver, including decreased levels of ROS, lipid peroxidation products, and oxidized proteins.
   - Glutamine-treated animals show enhanced antioxidant capacity, as evidenced by increased levels of glutathione and elevated activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase.(Sharma S et al., 2018).
4. **Other Relevant Outcomes:**
   - In addition to histological changes, liver enzyme levels, and oxidative stress markers, other relevant outcomes in preclinical studies include assessments of inflammatory cytokines, apoptotic markers, and liver function parameters.
   - Glutamine supplementation has been shown to decrease the expression of pro-inflammatory cytokines, such as TNF-α and IL-6, and reduce apoptotic cell death in the liver.

- Furthermore, glutamine-treated animals exhibit improvements in liver function tests, such as albumin levels, bilirubin levels, and coagulation parameters, indicative of enhanced liver function and recovery from hepatotoxicity.

- Assessment Of Behavioural Outcome Using Standardized Test Such as SOD, GSH, MDA, Nitric oxide and Total Protein

1. Superoxide Dismutase (SOD):

- SOD is an enzyme that plays a crucial role in antioxidant defense by catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide.
- Decreased SOD activity is indicative of increased oxidative stress and impaired antioxidant capacity in hepatotoxicity.
- Assessment of SOD levels provides insights into the extent of oxidative damage and the efficacy of antioxidant defenses in the liver.

2. Glutathione (GSH):

- GSH is a major intracellular antioxidant that plays a critical role in protecting cells from oxidative damage and maintaining redox homeostasis.
- Depletion of GSH levels is a marker of oxidative stress and impaired antioxidant capacity in hepatotoxicity.
- Measurement of GSH levels provides information on the cellular antioxidant status and the ability to counteract oxidative damage in the liver.

3.Malondialdehyde (MDA):

- MDA is a byproduct of lipid peroxidation and serves as a biomarker of oxidative stress and lipid damage in hepatotoxicity.
- Elevated MDA levels reflect increased oxidative damage to cell membranes and lipids in the liver.
- Quantification of MDA levels allows for the assessment of lipid peroxidation and the degree of oxidative injury in hepatocytes.

4. Nitric Oxide (NO):

- NO is a signaling molecule involved in various physiological processes, including inflammation, vasodilation, and oxidative stress.
- Dysregulated production of NO can contribute to oxidative stress, inflammation, and tissue damage in hepatotoxicity.
- Measurement of NO levels provides insights into the inflammatory response and nitrosative stress in the liver.

5. Total Protein:

- Total protein levels serve as a general marker of liver function and integrity.
- Decreased total protein levels may indicate impaired protein synthesis and liver dysfunction in hepatotoxicity.

The world health organization estimates that 10.6 million new cases and 1.3 million deaths from tuberculosis (tb) would occur globally in 2023, making it a significant global health concern (who, 2024). Isoniazid (inh), rifampicin (rif), pyrazinamide (pza), and ethambutol (emb) are the mainstays of tb chemotherapy. Since they have a strong bactericidal activity against mycobacterium tuberculosis, inh and rif are the most effective and essential first-line drugs among them.

WISTER RAT :-

Rattus norvegicus is the Wister rat's scientific name. The common Norway rat, which also goes by the scientific name Rattus norvegicus, is a member of the same species as Wistar rats, a particular strain of laboratory rats created at The Wister Institute .

- 1.Scientific Name: Rattus norvegicus
- 2.What it is: A specific, standardized strain of albino laboratory rat
- 3. Origin: Developed at The Wister Institute in Philadelphia, beginning around 1906 .

Because of their docile disposition, constant genetic and physiological characteristics, and simplicity of handling, Wister Rats are employed in research as good, standardized animal models for illness research and therapeutic testing. Their accessibility, brief lifetime, and quick breeding cycle also help to make research more productive and economical, especially in areas like neuroscience, pharmacology, and toxicity.

GLUTAMINE :-

The most prevalent free amino acid in the human body, glutamine is essential for antioxidant defense, nitrogen transport, and acid-base balance. Since glutamine syntheses can produce glutamine endogenously from glutamate and ammonia, it is regarded as non-essential under normal physiological conditions. However, because glutamine is used more than it is synthesized, it becomes conditionally required during stressful conditions including illness, trauma, burns, or exposure to hepatotoxic drugs (Cruzat et al., 2018). Through a variety of biochemical and cellular processes, glutamine supplementation has shown protective, restorative, and antioxidant benefits in hepatic injury, especially in drug-induced liver damage (such as that caused by isoniazid and rifampicin).

Isoniazid :-

N-acetyltransferase-2 (NAT2) is the primary enzyme in the liver that converts isoniazid INH to acetyl isoniazid. Further hydrolysis of this metabolite produces acetyl hydrazine, which cytochrome P450 2E1 (CYP2E1) oxidizes to produce reactive intermediates including acetyl radicals and hydrazine (Metushi et al., 2016). The highly reactive chemicals hydrazine and acetyl hydrazine cause

hepatocellular necrosis by covalently attaching to cellular macromolecules such as proteins, lipids, and nucleic acids. Reactive oxygen species (ROS) produced by these metabolites cause mitochondrial damage, glutathione depletion, and lipid peroxidation.

Rifampicin :-

Rifampicin By activating hepatic enzyme systems, rifampicin increases INH-induced liver damage while having a low intrinsic hepatotoxicity. Through the pregnane X receptor (PXR) route, RIF is a strong inducer of CYP2E1, CYP3A4, and UDP-glucuronyl transferases (Chen et al., 2015). CYP2E1 activation intensifies oxidative stress and GSH depletion by increasing the oxidation of acetyl hydrazine to hepatotoxic intermediates. further encourages cholestasis by disrupting the multidrug resistance-associated protein (MRP2) and bile salt export pump (BSEP), which results in bile acid buildup and subsequent hepatocyte damage (Jin et al., 2013)

Mechanism of Isoniazid and rifampicin :-

Isoniazid Metabolism and Toxic Intermediates Mechanism of Isoniazid and Rifampicin Following oral treatment, N-acetyltransferase-2 (NAT2) in the liver mostly converts INH to acetyl isoniazid. Further hydrolysis of this metabolite produces acetylhydrazine, which cytochrome P450 2E1 (CYP2E1) oxidizes to produce reactive intermediates including acetyl radicals and hydrazine (Metushi et al., 2016). Highly reactive hydrazine and acetylhydrazine cause hepatocellular necrosis by covalently attaching to cellular macromolecules such as proteins, lipids, and nucleic acids. Reactive oxygen species (ROS) produced by these metabolites cause mitochondrial damage, glutathione depletion, and lipid peroxidation. Because acetylhydrazine builds up in their hepatocytes and intensifies oxidative damage, people who are slow acetylators—caused by genetic variations in the NAT2 gene—are more vulnerable (Tostmann et al., 2008).

Biochemical Consequence :-

↑ Malondialdehyde (MDA) (marker of lipid peroxidation)
↓ Reduced glutathione (GSH)
↓ Catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) activity.

Rifampicin and Cytochrome P450 :-

Rifampicin itself has low intrinsic hepatotoxicity but potentiates INH-induced liver damage by activating hepatic enzyme systems. RIF is a potentinducofCYP2E1, CYP3A4, and UDP-glucuronyl transferase through the pregnane X receptor (PXR)pathway (Chen et al., 2015). CYP2E1 activation intensifies oxidative stress and GSH depletion by increasing the oxidation of acetylhydrazine to hepatotoxic intermediates. By disrupting the bile salt export pump (BSEP) and multidrug resistance-associated protein (MRP2), rifampicin also contributes to cholestasis by causing bile acid buildup and subsequent hepatocyte damage (Jin et al., 2013).

Oxidative Stress and Mitochondrial Dysfunction :-

The electron transport chain (ETC) is hampered by excessive ROS produced by INH and RIF metabolism, which damages mitochondrial membranes. When the mitochondrial permeability transition pore (mPTP) opens, cytochrome-c is released into the cytosol and the mitochondrial membrane potential (Δψm) is lost. Apoptosis is triggered by this event, which activates caspase-9 and caspase-3 (Sharma et al., 2011).

Mitochondrial injury leads to:

Decreased ATP production → energy depletion. Increased intracellular calcium → enzymatic degradation of cell structures release of apoptotic factors → programmed cell death. Inflammatory Response and Immune-Mediated InjuryOxidative stress and necrotic hepatocytes release damage-associated molecular patterns (DAMPs) that activate Kupffer cells (liver macrophages). Tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) are pro-inflammatory cytokines produced by activated Kupffer cells that exacerbate hepatocellular inflammation and necrosis (Remapped & Aithal, 2013). The development of drug–protein adducts that function as neoantigens and trigger cytotoxic T-cell responses are examples of immune-mediated hypersensitivity in action (Metushi et al., 2016).

| Isoniazid (INH) | ↓
(NAT2) Acetylation ↓
| Acetyl isoniazid | Hydrolysis ↓ (RIF induces CYP2E1 ↑) ↓
| Acetylhydrazine | | (CYP2E1 oxidation) | ↓
Reactive metabolites (hydrazine, free radicals) ↓
| Oxidative Stress: | | ↑ ROS, ↑ MDA, ↓ GSH, ↓ SOD | ↓
Mitochondrial dysfunction ↓ Apoptosis / Necrosis of hepatocytes ↓ ↑
TNF-α, IL-6, IL-1β → Inflammation
↓ Hepotoxicity .

Pharmacological Role of Glutamine :-

Glutamine as a Precursor for Glutathione (GSH) Being a precursor of glutathione (GSH), the main intracellular antioxidant in the liver, is one of glutamine's most important hepatoprotective functions.

Pathway summary:

Glutamine → Improved gut barrier → ↓ LPS translocation → ↓ hepatic inflammation

Role in Heat Shock Protein (HSP)-

Heat shock proteins (HSPs), particularly HSP70, are cytoprotective molecular chaperones that preserve protein homeostasis in stressful situations. Glutamine increases the expression of HSPs.

***Mechanism:**

Heat shock factor-1 (HSF-1) is activated by glutamine, which increases HSP70 transcription.

***Function:** By suppressing NF-κB activation, HSP70 binds denatured proteins, suppresses pro-inflammatory signaling, and stops apoptosis (Singleton & Wischmeyer ,2006)

***Immunomodulatory and Anti-Inflammatory Effects :-**

Anti-inflammatory and Immunomodulatory Impacts The function of immune cells, such as neutrophils, lymphocytes, and macrophages, is significantly influenced by glutamine. Glutamate reduces inflammation during hepatic damage via a number of mechanisms: inhibition of NF-κB activation, which lowers TNF-α, IL-1β, and IL-6 transcription (Wang et al., 2015). IL-10, an anti-inflammatory cytokine that encourages hepatocyte regeneration, is enhanced. preservation of redox-sensitive signaling, which stops necrosis and an overabundance of inflammation.

***Experimental Evidence:**

Glutamate supplementation in rats with INH-RIF-induced hepatotoxicity resulted in t↓ Serum ALT, AST, and bilirubin↓ TNF-α, IL-6, and IL-1β levels↑ in a research by Liu et al. (2015). Expression of hepatic GSH and HSP70 attests to its immunomodulatory and antioxidant defenses.

***Experimental Evaluation in Rats:-**

Study Design (common in published works) Animal model: Wistar or Sprague-Dawley rats. Hepatotoxicity induction: INH (50 mg/kg) + RIF (50 mg/kg), oral, daily for 28 days.

Treatment groups:

1.Control (normal saline).

2.INH–RIF (toxic control).

3.INH–RIF + glutamine (varied doses, 200–1000 mg/kg).

4.Standard hepatoprotective agent (e.g., silymarin).

***Parameters Evaluated :-**

Biochemical markers: ALT, AST, ALP, total bilirubin, albumin.

Oxidative stress markers: MDA, GSH, SOD, catalase.

Histopathology: necrosis, inflammation, fatty degeneration.

Molecular assays: expression of TNF-α, IL-6, caspases (apoptosis markers).

***Antioxidant Restoration-**

(↑ GSH) Glutathione (GSH), the main intracellular antioxidant defense in hepatocytes, is synthesized from glutamine, a crucial precursor. It enhances the detoxification of reactive oxygen species (ROS) and reactive metabolites generated during INH–RIF metabolism by donating glutamate for the synthesis of GSH (Curi et al., 2005). By shielding cellular proteins and lipids from peroxidation, GSH restoration helps to avoid oxidative damage.

***Protection of Mitochondria :-**

One of the main targets of oxidative stress caused by drugs is the mitochondria. By preserving the redox balance and serving as an anaplerotic substrate for the tricarboxylic acid cycle, glutamine promotes mitochondrial integrity by guaranteeing sufficient ATP synthesis and averting the switch to mitochondrial permeability (Hong et al., 2015). Glutamate-supplemented hepatocytes exhibit protection against apoptosis, as evidenced by their retained mitochondrial membrane potential (Δψm) and reduced cytochrome-c release.

***Inhibition of Inflammation :-**

Due to its strong immunomodulatory effects, glutamine reduces the inflammatory reactions that come with hepatotoxicity. It increases the production of the anti-inflammatory cytokine IL-10 while inhibiting the activation of nuclear factor-κB (NF-κB), a crucial transcription factor that controls pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6 (Wang et al., 2015). This modification lessens cytokine-mediated hepatocyte damage and Kupffer cell activation.

Stabilization of Membranes :-

By restoring phospholipids and preserving osmotic balance, glutamine helps to stabilize the membranes of cells and organelles. It preserves the structural integrity of hepatocyte plasma and mitochondrial membranes and shields membrane lipids from peroxidation through its antioxidant and energy-restoring qualities (Yousefipour et al., 2017). The hallmark of hepatocellular damage, the release of enzymes (ALT, AST) into the bloodstream, is prevented by this membrane-stabilizing activity.

***Experimental Procedure:-**

The effect of glutamine and silymarin shall evaluate into following groups containing six rat in each group viz ;

Group 1 : Normal group : The rat received only vehicle (Distilled water)

Group 2 : Vehicle control : The rat received isoniazid (100 mg/kg) + rifampicin (100 mg/ kg) and only vehicle (Distilled water ,10 mg/ kg)

Group 3: Silymarin (100) group The rat will receive isoniazid (100 mg/ kg)+Rifampicin (100mg/ kg). They will be pre - treated with Silymarin at a dose of 100mg/ kg, p.o., for 15 days.

Group 4: Glutamine (50) group : The rat has received isoniazid (100 mg/ kg) + Rifampicin (100mg/ kg).They will be pre - treated with Glutamine with low dose of 50 mg/kg p.o., for 15 days.

Group 5: Glutamine (100) group:The rat has received isoniazid (100 mg/ kg)+Rifampicin (100mg/ kg).They will be pre - treated with at a medium dose 100 mg/kg p.o., for 15 days.Group

Group 6 : Glutamine (200) group : The rat has received isoniazid (100 mg/ kg) They will be pre - treated with Glutamine at a high dose of 200 mg/kg p.o., for 15 days

NEED OF THE STUDY.

Isoniazid (INH) remains a cornerstone drug in tuberculosis (TB) therapy; however, the growing incidence of INH resistance significantly threatens treatment success and global TB control efforts. Resistance primarily arises from genetic mutations in **katG, inhA, and ahpC**, which interfere with INH activation and its therapeutic action. Additionally, emerging evidence highlights the contribution of **efflux pump mechanisms**, further complicating resistance patterns. These challenges emphasize the urgent requirement for alternative strategies to protect patients from INH-induced complications and improve treatment outcomes.

Glutamine has shown promising hepatoprotective effects against INH-induced liver injury by reducing inflammation, maintaining mitochondrial integrity, and restoring antioxidant balance. Its ability to modulate immune responses and decrease oxidative stress suggests it may serve as a potential supportive therapy to minimize hepatotoxicity during anti-TB treatment. Therefore, investigating the protective role of glutamine in INH-induced hepatotoxicity is essential to develop safer treatment regimens and enhance therapeutic tolerance in patients receiving INH.

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