CHAPTER 1

INTRODUCTION

The safety and efficacy of the drugs used in the treatment of various clinical conditions in any individual remains complex and multifactorial and difficult to analyse or identify the suspected drug that causes the Adverse Drug Reaction (ADR).

1.1 Role of liver in drug-induced hepatotoxicity:

Liver being a principle organ for playing several vital roles in the body, is involved in several biochemical pathways, metabolism of nutritional factors, metabolising the administered drugs or any substance that is ingested, which could be either herbal or even natural chemicals. Thus, making it important to observe, for the drug-induced hepatotoxicity, at all phases of drug development that includes the pre-clinical toxicity studies, the different phases of clinical trial including the post-marketing surveillance.

The Drug Induced Liver Injury (DILI) is defined as the injury caused by exposure to a drug or non-infectious toxic agent and is associated with different levels of organ dysfunction [1]. Despite the advancement in research at molecular level, understanding and characterizing the mechanisms involved in causing the Drug induced Liver Injury, it is still difficult to diagnose and identify the suspected drug.

1.2 Types of drug induced liver injury:

The drug induced liver injury are mainly of two types:(1) Dose-dependent, which is also called as predictable, direct toxicity, reproducible and occurs after the consumption of the drug that exceeds a known toxic threshold level. In such cases, the liver injury that occurs is proportional to the administered dose [2], example
Paracetamol; (2) while the **Dose-independent** Drug induced Liver Injury is also called as unpredictable and idiosyncratic that occurs even at the therapeutic doses, and the **liver injury** caused is **not always proportional** to the **administered dose**, further, the time of damage, onset can also vary example Diclofenac, Sulindac, Trovafloxacin $^{[3,4]}$.

**1.3 An overview of drug induced liver injury:**

Paracelsus stated that, “all substances (drugs) are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.” Any drug, therefore, despite of its trivial therapeutic action has a potential to harm. With the limitations on toxicity studies and clinical trials, in the process of a new drug development, the adverse drug effects that occur may not be in total are detected, before introduced into the market for the patient’s use. Therefore, it becomes imperative to detect the infrequent yet significant adverse drug reactions that occur when the drug has entered the market. This can be achieved by the post-marketing surveillance.

The liver injury caused by the drug may vary with the extent of the damage, ranging from mild fatty liver to necrosis. Though uncommon and rare, it is contributing to the morbidity and mortality in the general population and remains as a potential complication for most of the prescribed drugs $^{[5,6]}$. Despite of the relative frequency, little information is available on the long-term outcome of drug induced liver injury. The reasons could include missed diagnosis, difficulty in establishing definite diagnosis, particularly in cases where the hepatotoxicity is reversible following the drug withdrawal with limited long term follow up (Dantrolene-induced chronic hepatitis or Flucloxacillin-induced cholestasis $^{[7,8]}$).
1.4 Incidence of drug induced liver injury:

Although, the incidence of drug induced liver injury is found to be low, the probability of it should always be considered in any case of the liver injury. According to the literature study, the incidence was between 1 in 10,000 and 1 in 100,000 which was found to be increased from the evidences of the recent study. The information from the recent registries show an annual incidence of 19.1 cases per 10,000 inhabitants in Iceland, 13.9 cases per 100,000 inhabitants in France, with hospitalization of 5% and mortality 6% [4].

A prospective study conducted in US have shown that, 13% of the total cases were diagnosed as idiosyncratic hepatotoxicity; while 39% with acetaminophen-induced hepatotoxicity, however, it was interesting to know with the recent prevalence rates in south-east Asian registries, which revealed that 70% of the drug induced liver injury cases occurred due to Herbal And Dietary Supplements (HDS), which is surprisingly found to be increased in its prevalence; even through the Western registers, attributing to 16% of the total drug induced liver injury to be due to Herbal And Dietary Supplements [9]. The drug induced liver injury has been found as an important cause of hospital admissions, which are increased over the decades and is 45% in Spain [10].

In India, the drug induced liver injury contributes to 1.4% of the gastrointestinal admissions and 2.5% of hepatobiliary admissions, with gradual increase in the numbers over a period of years, of which 0.7% were found to be Idiosyncratic Drug induced Liver Injury (IDILI) [11, 12]. Although, there occurs geographical difference in the common drugs causing Drug induced Liver Injury, worldwide antimicrobials are considered the most common particularly in Europe, Amoxicillin and flucloxacillin are found to be the common drugs in the Europe, while in India, Antituberculosis drugs
are contributing more to the drug induced liver injury\textsuperscript{[11,13]}. As compared to the Western world, where Paracetamol or Acetaminophen was found to be the leading cause of Acute Liver Failure (ALF), followed by the antimicrobials. In India, both in adults and children, the antituberculosis drugs have been the leading cause of for drug induced liver injury, followed by the Non-Steroidal Anti Inflammatory Drugs (NSAIDs) 10\% \textsuperscript{[14]}. The incidence of liver injury caused by the Non-Steroidal Anti Inflammatory Drugs is ranging from 1 – 9 cases per 100,000 persons exposed, indicating an increased risk of these preparations which remains as a common drug used in the treatment of the most painful conditions. Diclofenac sodium, widely used among the Non-Steroidal Anti Inflammatory Drugs, across the world is known for its hepatotoxicity, where more than 60 cases were reported by Bank and co-workers in 1995 \textsuperscript{[15]}, indicating that small number of hospitalisation 0.023\% is the strongest evidence for it to bear hepatotoxic effect.

1.5 Mechanisms of drug induced liver injury:

The exact mechanisms of the drug induced liver injury remains unclear and depends on the hepatotoxicity that could be either predictable (Paracetamol) or unpredictable (Diclofenac, Sulindac, and Flucloxicillin). The mechanism involved, in causing hepatic injury-induced hypersensitivity and metabolic aberration, in case of predictable hepatotoxicity, massive hepatocellular necrosis, when the Paracetamol is consumed in large doses. It is known to release a toxic metabolite \textbf{N-acetyl-p-benzoquinone imine} (NAPQI), which depletes the hepatoprotective glutathione, which in turns results in mitochondrial dysfunction, oxidative stress, that culminates into cellular damage, causing necrosis and death \textsuperscript{[16]}, while in case of idiosyncratic; the \textbf{inflammatory stress} hypothesis is considered, which results to conjugate with the drug
metabolite, that has a potential to precipitate Drug induced Liver Injury, with an evidence of important role of the innate and adaptive immune system through; involved in the pathogenesis of Drug induced Liver Injury \(^{[17]}\).

1.6 Risk factors of drug induced liver injury:

With a wide range of drugs, including Antimicrobials, NSAIDs, Antiepileptic, Antipsychiatric drugs etc., causing the drug induced liver injury, several factors are known to influence the drug induced liver injury, and are hence considered as the **risk factors** these includes; the age, gender, alcohol, concomitant use of drugs, nutrition, HIV, genetic factors, the dose and the body mass of the individual.

1.7 Evaluation of drug induced liver injury:

Apart from the clinical evaluation, the diagnosis includes the causality assessment to identify the suspected drug; evaluation of the biochemical parameters which indicate the liver functioning status, and further; the histopathological studies to reveal and confirm the clinical diagnosis. Liver imaging can also remain the infiltrative hepatic diseases and fatty live diseases. The histopathological information could be drug-specific and would indicate the severity and latency of the biochemical pattern.

Although, 90% of recoveries have been registered on discontinuation of the drug, some may progress with the outcome as chronic liver disease \(^{[18]}\). The prognosis has been poor in women, elderly, individuals with pre-existing liver disease; those habituated to alcohol and individuals with genetic defect. Hence, it is always important to monitor the liver enzymes which are indicative of the hepatotoxicity.
1.8 Treatment of drug induced liver injury:

The treatment for Drug induced Liver Injury mainly consists of discontinuation of the involved drug, followed by treatment with specific drugs. The specific drugs for the treatment of Drug induced Liver Injury are very scarce. However, N-Acetylcysteine (NAC) remains as a specific antidote for Paracetamol or Acetaminophen-induced toxicity, where it is known to benefit by replenishing the Glutathione stores. Similarly, as symptomatic treatment, drugs like Corticosteroids, Antihistamines, Cholestyramine, L-Carnitine, Folic acid, Methionine and Ursodeoxycholic acid have been used in the treatment of Drug induced Liver Injury [19, 20].
1.9 Aim and Objectives of the Study:

1.9.1 AIM:

The present research was conducted to explore the hepatoprotective action of DL-Methionine and N-Acetylcysteine on the albino rats on dose-related hepatotoxicity of the hepatotoxic drug Diclofenac sodium.
1.9.2 OBJECTIVES:

1) To evaluate dose-dependent hepatic injury by orally administered Diclofenac sodium.

2) To evaluate the hepatic changes due to the dose dependent hepatic injury caused by Diclofenac sodium.

3) To demonstrate the hepatoprotective effect of DL-Methionine against the hepatotoxic drug Diclofenac sodium by oral route of administration in small animals.

4) To demonstrate the hepatoprotective effect of N-Acetylcysteine against the hepatotoxic drug Diclofenac sodium by oral route of administration in small animals.

5) To compare the hepatoprotective effect of DL-Methionine with N-Acetylcysteine.

6) To demonstrate the hepatoprotective effect of N-Acetylcysteine on hepatotoxic drug other than Paracetamol.