CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Effect of Rosuvastatin on Inflammation in Paracetamol-Induced Hepatotoxicity in Mice

by

Tuba Naseer

A thesis submitted in partial fulfillment for the degree of Master of Philosophy

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Dedicated to my family who dedicated their lives to teach me how to step forward...!

CERTIFICATE OF APPROVAL

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Tuba Naseer

(Registration No: MPH233012)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Muhammad Faheem	RIU, Islamabad
(b)	Internal Examiner	Dr. Mahboob Alam	CUST, Islamabad
(c)	Supervisor	Dr. Fazlullah khan	CUST, Islamabad

Dr. Fazlullah Khan
Thesis Supervisor
October, 2025

Dr. Fazlullah Khan
Head
Dept. of Pharmacology
October, 2025

Dr. Muzaffar Abbas
Dean
Faculty of Pharmacy
October, 2025

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Abstract

Paracetamol (PCM)-induced hepatotoxicity remains a significant clinical challenge, primarily mediated through oxidative stress and inflammatory pathways. This study evaluated the hepatoprotective efficacy of rosuvastatin against PCM-induced liver injury in mice. Animals were divided into four groups: control (normal saline + 1% DMSO), PCM-only (300 mg/kg), and two treatment groups receiving PCM with rosuvastatin (3 mg/kg or 6 mg/kg, orally). Liver function was assessed by measuring serum ALT and AST levels, while oxidative stress and inflammation were evaluated through hepatic glutathione (GSH), BCA protein assay, and TNF- ELISA. PCM administration significantly elevated liver enzymes (ALT: 220.0 ± 6.5 U/L, AST: 190.0 ± 6.00 U/L vs. control ALT: 56.3 ± 10.4 U/L, AST: 45.3 ± 2.02 U/L; p ¡ 0.05), indicating severe hepatocellular damage. Rosuvastatin co-treatment dose-dependently reduced these elevations, with the 6 mg/kg dose showing superior protection (ALT: 78.0 ± 2.5 U/L, AST: 62.0 ± 2.00 U/L; $p < 0.001$ vs. PCM group). The BCA assay revealed increased protein concentration in PCM-treated mice (12.86 ± 0.58 μg/μl vs. control 6.42 ± 0.31 μg/μl; p <0.05), reflecting cellular injury, which was mitigated by rosuvastatin (8.33 ± 0.39 μg/μl at 6 mg/kg; $p < 0.05$). TNF- levels, a marker of inflammation, were significantly higher in the PCM group (5.78 ± 0.43 μg/μl vs. control 1.94 ± 0.11 μg/μl; p <0.001) but normalized with rosuvastatin (2.89 ± 0.22 μg/μl at 6 mg/kg; $p < 0.01$). These findings demonstrate that rosuvastatin attenuates PCM-induced hepatotoxicity by restoring liver enzyme levels, reducing oxidative stress, and suppressing pro-inflammatory cytokines. The dose-dependent hepatoprotection suggests rosuvastatin's potential as an adjunct therapy for drug-induced liver injury, likely through its antioxidative (GSH preservation) and anti-inflammatory (TNF-α inhibition) mechanisms. Further studies are warranted to explore its clinical applicability.

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Abbreviations

ADH	Alcohol Dehydrogenase
ALDH	Aldehyde Dehydrogenase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AVMA	American Veterinary Medical Association
BCA	Bicinchoninic Acid (protein assay)
cAMP	Cyclic Adenosine Monophosphate
CAT	Catalase
COX	Cyclooxygenase
COX-2	Cyclooxygenase-2
DALY	Disability-Adjusted Life Year
DAMP	Damage-Associated Molecular Patterns
DMSO	Dimethyl Sulfoxide
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
EP2/EP4	Prostaglandin E2 Receptors
HSCs	Hepatic Stellate Cells
IACUC	Institutional Animal Care and Use Committee
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IP	Intraperitoneal
IV	Intravenous
LPS	Lipopolysaccharide

MEOS	Microsomal Ethanol Oxidizing System
NF-B	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NSAID	Non-Steroidal Anti-Inflammatory Drug
PBS	Phosphate Buffered Saline
PDGF-β	Platelet-Derived Growth Factor-beta
PGE	Prostaglandin E2
PGT	Prostaglandin Transporter
PPE	Personal Protective Equipment
PRR	Pattern Recognition Receptors
ROS	Reactive Oxygen Species
SC	Subcutaneous
SD	Standard Deviation
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumor Necrosis Factor Alpha
α-SMA	Alpha-Smooth Muscle Actin

Symbols

C_2H_5OH	Chemical formula for ethanol
CH_3CHOH	1-hydroxyethyl radical
$CHCl_3$	Chemical formula for chloroform
H_2O_2	Hydrogen peroxide
$HO\cdot$	Hydroxyl radical
mg/kg	Milligrams per kilogram (dosage)
g/kg	Grams per kilogram (dosage)
μL	Microliter
μg/μL	Microgram per microliter
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
$O_2^{\cdot -}$	Superoxide anion
U/L	Units per liter (enzyme concentration)
Zn^{2+}	Zinc ion (used in enzyme catalysis)
$^\circ$C	Degree Celsius (temperature)

Chapter 1

Introduction

1.1 Liver

The liver is formed like a wedge or cone, with an apex projecting to the left and a base to the right. At around 1500 grams, or roughly 2.5% of an average adult's total weight, this organ is the largest internal organ in the body. In terms of anatomy, the liver is mostly found in the right hypochondrium and epigastric area, although it also reaches the left lateral line and the left hypochondrium [1]. The falciform ligament, which connects the liver to the diaphragm and the anterior abdominal wall, mainly divides the liver into two lobes, the right and left lobes. At around six times the size of the left lobe, the right lobe is the largest of these. The quadrate and caudate lobes are the liver's two minor lobes in addition to these big lobes. Peritoneal reflection supports the liver's location, while the rib cage protects it. Blood is provided to the liver from two main sources: the hepatic artery, which transports oxygenated blood from the heart, and the hepatic portal vein, which transports blood rich in nutrients from the digestive tract [2].

1.2 Functions of the Liver

Numerous vital biological processes, including as vascular, immunological, metabolic, secretory, and excretory functions, are carried out by the liver. By

producing bile, a fluid that includes water, bile salts, cholesterol, medications, hormones, and the pigment bilirubin, it plays a vital role in digestion. The liver's ability to temporarily store extra nutrients is one of its primary functions. These nutrients are restored during times of plenty and released when needed, such as during periods of fasting. The liver catalyzes the metabolism of lipids, proteins, and carbohydrates. Additionally, it preserves minerals including iron and copper, water-soluble vitamin C, glycogen, and fat-soluble vitamins A, D, E, and K [3].

The liver also acts as a blood reservoir, eliminates waste materials like urea and bile, and detoxifies narcotics and other dangerous compounds. Additionally, it breaks down lactate, endotoxins, and germs. The production of immunoglobulins and the phagocytic activity of Kupffer cells are two significant immunological processes carried out by the liver. It is also in charge of hematopoiesis, or the production of blood cells, in the fetus [4].

The liver has a unique ability to regenerate, which is surprising. The liver can return to its normal size and shape if a portion of it is removed.

Through the portal vein and hepatic artery branches, blood enters the liver lobules and travels to the central vein via the liver sinusoids. Imaging, liver biopsy, and blood testing are among the diagnostic procedures that can be used to assess liver function [2].

1.3 Microscopic Structure of the Liver

The liver's primary functional cells, hepatocytes, are involved in bile synthesis, detoxification, and metabolism. In order to facilitate the effective transmission of chemicals, sinusoidal capillaries allow direct contact between the blood and hepatocytes. Kupffer cells are specialist macrophages that eliminate pathogens, aged red blood cells, and cell debris, which is a crucial part of the immune system. cells, also referred to as hepatic stellate cells, store vitamin A and aid in tissue repair and liver regeneration [3].

1.4 Metabolic Roles of the Liver

The liver is the center of metabolism in the human body, regulating glucose, protein, and fat metabolism. In carbohydrate metabolism, the liver stores excess glucose as glycogen and releases it when blood glucose levels are low. It also performs gluconeogenesis, or the creation of glucose from sources other than carbs such as amino acids and lipids.

The liver produces a variety of important proteins, including albumin, globulins, prothrombin, and fibrinogen. These proteins aid in maintaining blood osmotic pressure, promoting immunological responses, and facilitating blood coagulation. The liver regulates the storage and breakdown of triglycerides and other lipids, as well as the production of cholesterol [4].

1.5 Detoxification role of the liver

Detoxification is a major function of the liver. It detoxifies toxic compounds that enter the body or are generated during metabolism. Liver enzymes digest medicines and chemicals, transforming them into less hazardous forms and eliminating them from the body.

The liver oxidizes alcohol, rendering it less harmful. The liver also transforms ammonia, a hazardous byproduct of protein synthesis, to urea, which the kidneys safely eliminate [5].

1.6 Digestive Function of the Liver

The liver aids digestion by secreting bile, a liquid containing bile acids, phospholipids, and cholesterol. Bile is in charge of both emulsification and fat digestion. It is stored in the gallbladder and flows into the small intestine when digesting fatty foods, aiding in the absorption of fats through the gut lining [5].

1.7 Liver Diseases

The liver is prone to a variety of disorders, including viral hepatitis, fatty liver disease, cirrhosis, and liver cancer. Viral hepatitis is a virus-induced liver inflammation. Hepatitis A is often contracted by contaminated food and water and resolves without causing long-term damage. Hepatitis B is shared through blood, intercourse, or breastfeeding and can proceed to a chronic infection that predisposes to cirrhosis and organ Cirrhosis is caused by persistent liver damage, in which normal liver tissue is replaced with fibrotic scar tissue, significantly impairing liver function. It is primarily caused by chronic viral hepatitis, prolonged alcohol usage, and severe fatty liver disease. In late stages, liver failure may occur, necessitating a liver transplant [6].

Liver cancer arises as a result of uncontrolled cell growth within the liver. Chronic infection with hepatitis B and C, cirrhosis, and exposure to carcinogens such as aflatoxins are risk factors. Liver cancer is frequently identified late in its development, thus treatments can be limited and the consequences more severe [7].

1.7.1 Drug-Induced Liver Injury

Drug-induced liver injury is an adverse liver reaction precipitated by the use of medications, herbal products, or other foreign material. It is usually classified into three types according to the mechanism of harm: intrinsic, idiosyncratic, and indirect [6].

1.8 Types of DILI

Intrinsic DILI a drug's or its metabolites' harmful character is linked to intrinsic DILI, which is dose-related and predictable. Similar things tend to happen, and experimental models can replicate it. Acetaminophen overdose serves as one example.

Idiosyncratic DILI is mediated by specific genetic or environmental variables, is dose-independent, and is typically unpredictable. The illness might differ significantly from person to person and has delayed symptoms.

Indirect DILI is when a medication affects the liver or immune system in an indirect way, which can lead to the development of new liver disorders or make existing ones worse, such as chronic viral hepatitis. It is still difficult to stratify DILI because several medications that are thought to cause one category also show signs of another. Distinguishing these classes is often complicated by host characteristics and varying dosage limits [8].

DILI is difficult to diagnose since it can mimic most liver diseases and does not have specific markers. It is usually diagnosed by ruling out other potential causes. Because of its unpredictable nature, DILI continues to be a major issue in clinical practice [9].

1.9 Epidemiology

DILI is uncommon but most probably under reported. Incidence depends on the region and study design. Its increased prevalence is due to factors like greater medication use, aging populations, and increasing use of herbal and dietary supplements. In Western nations, prescription medications are predominantly responsible for DILI, whereas in most Asian nations, herbal preparations are most frequently implicated [10].

1.10 Paracetamol

1.11 Introduction to Paracetamol

Paracetamol, also known as acetaminophen, is a widely used analgesic (pain reliever) and antipyretic (fever reducer). It is one of the most commonly consumed

medications worldwide, both as a prescription drug and over-the-counter remedy.

The drug is often preferred due to its favorable safety profile at therapeutic doses and its lack of anti-inflammatory action, which distinguishes it from non-steroidal anti-inflammatory drugs (NSAIDs) [11]. IUPAC Name of paracetamol is N-(4-hydroxyphenyl) acetamide and Chemical Formula is $C_8H_9NO_2$. Paracetamol is composed of. A hydroxyl group (-OH) attached to a benzene ring and an acetamide group ($-NHCOCH_3$).

This structure contributes to its high solubility in water and mild polarity. Molecular weight is 151.16 g/mol. Appearance is White crystalline powder taste is Slightly bitter and Soluble in hot water, slightly soluble in cold water and paracetamol. Classified in different classes like, Pharmacological Class contain Analgesic and antipyretic. Biopharmaceutical Classification contain Class I (High solubility, high permeability) [12].

1.12 Pharmacokinetics of Paracetamol

TABLE 1.1: Pharmacokinetic profile of paracetamol following oral administration

Parameter	**Description**
Absorption	Rapidly absorbed in the small intestine
Peak Plasma	30 minutes to 2 hours after oral administration
Distribution	Uniformly distributed across body fluids, low plasma protein binding (10–25%)
Metabolism	Primarily metabolized in the liver via glucuronidation and sulfation
Elimination	Renal excretion, primarily as non-toxic conjugates
Half-life	Approximately 2–3 hours at therapeutic doses

1.13 Mechanism of Action and Toxicological Pathways of Paracetamol with Hepatoprotective Role of Rosuvastatin

Paracetamol (acetaminophen) is a commonly used antipyretic and analgesic drug. Despite its clinical effectiveness, the entire mechanism of action has yet to be clarified. Paracetamol is considered to have its pharmacological activity mainly through inhibition of the cyclooxygenase (COX) enzyme system with a selective effect on the COX-2 isoform in the central nervous system (CNS). This inhibition decreases the synthesis of prostaglandins, which are mediators of pain and fever, particularly prostaglandin E2 (PGE2). Unlike classical non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol does not significantly inhibit COX enzymes in peripheral tissues due to the presence of high levels of peroxides, which limit its efficacy outside the CNS. As a result, its anti-inflammatory activity is negligible [7].

Although its tolerability profile is generally favorable at therapeutic levels, paracetamol overdose is a predominant cause of drug-induced liver injury (DILI) and acute liver failure globally. Upon ingestion, about 85–90% of paracetamol is metabolized by hepatic conjugation via phase II metabolic processes—viz. glucuronidation and sulfation—aided by UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs), respectively. A minor fraction (5–10%) is metabolized by cytochrome P450 enzymes, especially CYP2E1, CYP1A2, and CYP3A4, into a highly reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI).

Under normal conditions, NAPQI is rapidly detoxified through conjugation with reduced glutathione (GSH), forming non-toxic mercapturic acid derivatives excreted in urine. Nevertheless, in overdose situations, the major metabolic pathways are saturated, leading to elevated NAPQI production. At the same time, hepatic reserves of GSH are consumed, with free NAPQI building up. The metabolite binds covalently to cellular macromolecules such as mitochondrial proteins, leading to oxidative stress, mitochondrial damage, lipid peroxidation, and eventually

hepatocyte necrosis or apoptosis. Oxidative damage is further exacerbated by the generation of reactive oxygen species (ROS) and inflammatory cytokines such as tumor necrosis factor-alpha ($TNF-\alpha$) and interleukin-1 beta ($IL-1\beta$) [8].

Given the complex pathogenesis of paracetamol-induced hepatotoxicity, therapeutic agents that can attenuate oxidative stress and inflammatory responses are of significant interest. Rosuvastatin, a synthetic statin mainly employed in lipid-lowering therapy, has been found to be a potential hepatoprotective drug because of its pleiotropic effects. In addition to its action of inhibiting HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, rosuvastatin also shows antioxidant, anti-inflammatory, and anti-apoptotic actions. It has been reported to increase endogenous antioxidant protective mechanisms like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), thus lowering oxidative stress. Rosuvastatin inhibits pro-inflammatory cytokine (e.g., $TNF-\alpha, IL-6$) gene expression and nuclear factor-kappa B (NF-B) activation, a transcription factor integral to inflammation. It is also capable of increasing the activity of endothelial nitric oxide synthase (eNOS), enhancing hepatic microcirculation and cellular resistance. These protective mechanisms together counteract paracetamol-induced liver damage by restoring redox balance, stabilizing mitochondrial function, and suppressing cytokine-dependent cell death [9].

1.14 Central vs Peripheral Action

Central: Analgesic, Antipyretic Peripheral: Minimal Anti-inflammatory Activity

1.15 Therapeutic Uses

Paracetamol is clinically used to manage Mild to moderate pain (headache, dental pain, musculoskeletal pain), Fever associated with infections and Postoperative pain Osteoarthritis-related discomfort. It is often used in combination with opioids

or NSAIDs for enhanced pain relief. Toxic Dose in Adults is >7.5–10 g/day and in Children is >150–200 mg/kg.

1.16 Metabolism and Toxicity

Under normal conditions, paracetamol is conjugated in the liver and excreted safely. However, in overdose, Excess APAP is metabolized by CYP2E1 into NAPQI (N-acetyl-p-benzoquinone imine), a highly reactive toxic metabolite, NAPQI depletes glutathione (GSH) and binds to liver proteins, causing oxidative stress and hepatic necrosis [13].

1.17 Stages of Paracetamol-Induced Hepatotoxicity

TABLE 1.2: Clinical Stages of Paracetamol Toxicity with Characteristic Symptoms and Timeline

Stage	**Time Post-Ingestion**	**Clinical Features**
Stage I	0–24 hours	Nausea, vomiting, malaise, anorexia
Stage II	24–72 hours	Abdominal pain, elevated liver enzymes
Stage III	72–96 hours	Peak hepatotoxicity, liver failure, encephalopathy
Stage IV	4–14 days	Recovery or death due to liver failure

1.18 Liver Function Tests

AST, ALT Increase within 24–48 hours INR, PT, Bilirubin Elevate in severe toxicity NAPQI-protein adducts and glutathione depletion are mechanistic indicators.

1.19 Antidote and Management

N-Acetylcysteine (NAC) replenishes glutathione stores, Enhances detoxification of NAPQI and most effective when administered within 8–10 hours of overdose. Other Treatments include Activated charcoal (if given early) and Supportive care for hepatic failure (fluids, electrolytes, monitoring) [14].

1.20 Paracetamol Metabolism and Toxicity Pathway

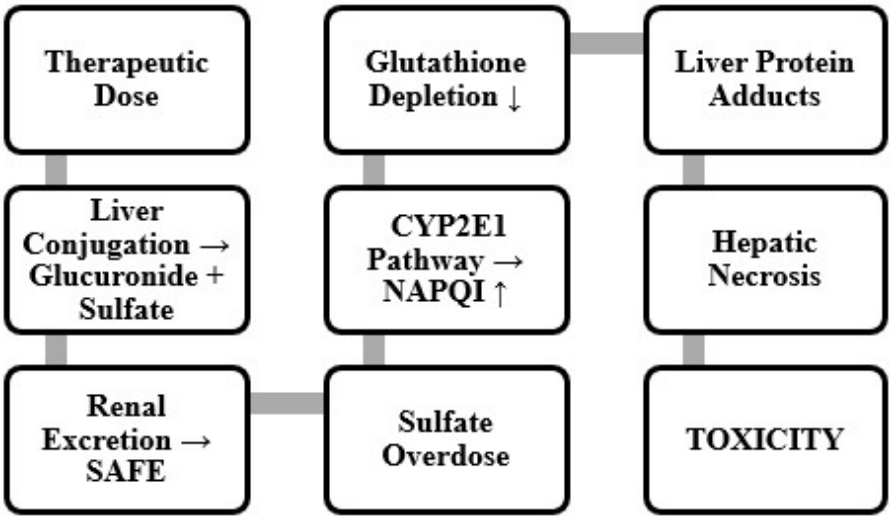


FIGURE 1.1: Flowchart showing the metabolic pathways of paracetamol and the shift to toxic NAPQI formation during overdose.

This flowchart illustrates the biotransformation of paracetamol at therapeutic and toxic doses. Under normal conditions, paracetamol is primarily metabolized via

glucuronidation and sulfation pathways, resulting in safe, water-soluble conjugates. In overdose situations, the metabolic pathway shifts towards cytochrome P450 (mainly CYP2E1) activation, producing the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Excessive NAPQI depletes hepatic glutathione reserves, triggers oxidative stress, and causes hepatocellular injury.

1.21 Oxidative Stress

Oxidative stress arises when ROS exceed antioxidant capacity, resulting in hepatocellular injury. Drug-induced ROS generation depletes glutathione (GSH), allowing mitochondrial HO accumulation and dysfunction. Lipid peroxidation (LPO) damages membranes, disrupting ion gradients and leading to necrosis. Superoxide reacts with nitric oxide to form peroxynitrite, which damages DNA, proteins, and mitochondria.

The Keap1/Nrf2 pathway regulates antioxidant defense; under stress, Nrf2 translocates to the nucleus and induces genes like HO-1, GCLC, and NQO1. Several hepatotoxic drugs (e.g., diclofenac, troglitazone) increase oxidative stress. N-acetylcysteine (NAC) replenishes GSH and is clinically effective. Genetic variability in redox regulation contributes to DILI susceptibility [15].

1.22 Mitochondrial Dysfunction in DILI

Mitochondrial impairment is a central event in DILI. Drugs disrupt the ETC, reducing ATP and elevating ROS, triggering apoptosis or necrosis. Some agents (e.g., valproic acid) impair fatty acid oxidation or damage mtDNA. Others (e.g., diclofenac) inhibit mtDNA replication. Mitochondrial damage releases DAMPs, activating immune responses. In contrast, mitophagy removes damaged mitochondria, mitigating injury and promoting regeneration. Genetic mutations (e.g., POLG) increase vulnerability to mitochondrial DILI [16].

1.23 Endoplasmic Reticulum Stress in DILI

ER stress occurs from ROS over production and Ca^{2} imbalance, leading to protein misfolding and activation of the unfolded protein response (UPR). UPR sensors (PERK, IRE1, ATF6), normally bound by BiP, trigger stress signaling when released. Prolonged ER stress leads to apoptosis via caspases, Ca^{2} release, and mitochondrial damage [17].

1.24 Apoptosis Signaling in Liver Injury

Apoptosis is a regulated process that removes damaged liver cells without causing inflammation. It involves two main pathways:

1.25 Extrinsic Pathway

Triggered by external signals like TNF, FasL, and TRAIL binding to death receptors (e.g., Fas, TNFR), leading to formation of the death-inducing signaling complex (DISC), which activates caspase-8. This in turn activates effector caspases (e.g., caspase-3), executing cell death. FLIP can inhibit caspase-8 and also aids in liver regeneration.

1.26 Intrinsic Pathway

Activated by internal stress, it involves mitochondrial disruption via Bax/Bak activation (mediated by tBid), leading to cytochrome c release and apoptosome formation. This activates caspase-9, followed by caspase-3, leading to apoptosis. Hepatocytes mainly rely on this pathway. Anti-apoptotic proteins (e.g., Bcl-2) can block this process. Lysosomal enzyme cathepsin B can amplify apoptosis by promoting mitochondrial cytochrome c release. Both pathways intersect and are

tightly regulated in the liver; imbalance can result in excessive hepatocyte death, contributing to liver injury [18].

1.27 Mechanism by Which Rosuvastatin Decreases Liver Toxicity

Rosuvastatin, a potent HMG-CoA reductase inhibitor (statin), exhibits multiple protective mechanisms in the liver beyond its cholesterol-lowering action. These mechanisms can be grouped into antioxidant, anti-inflammatory, and mitochondrial protective effects.

1.28 Mechanistic Flow of Rosuvastatin in Reducing Paracetamol-Induced Hepatotoxicity

TABLE 1.3: Pathophysiological sequence of paracetamol-induced hepatotoxicity with associated cellular consequences.

Sr	Key points	Consequences
1	Paracetamol overdose → Excessive NAPQI formation	NAPQI (toxic metabolite) accumulates due to CYP450 metabolism
2	NAPQI depletes glutathione (GSH) → Oxidative stress	Mitochondrial dysfunction, lipid peroxidation, and ROS surge
3	Kupffer cell activation → Pro-inflammatory cytokines (TNF-, IL-6)	Amplification of inflammation and recruitment of immune cells
4	Hepatocyte apoptosis/necrosis	Liver injury (elevated ALT/AST, centrilobular necrosis)

1.29 Antioxidant Mechanism

Increases expression and activity of endogenous antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Reduces malondialdehyde (MDA) levels, a marker of lipid peroxidation. Inhibits NF-B pathway, reducing transcription of pro-inflammatory cytokines ₋ Tumor necrosis factor-alpha ($TNF-\alpha$), Interleukin-1 beta ($IL-1\beta$) and ↓ Interleukin-6 (IL-6). Stabilizes mitochondrial membrane potential, Prevents cytochrome c release, reducing apoptotic signaling and Enhances ATP production and reduces cell death. It improves nitric oxide bioavailability and vascular flow and reduces leukocyte adhesion and hepatic infiltration [19].

1.30 Overall Effects on the Liver

Decrease in serum markers of liver damage (ALT, AST)

Preservation of hepatic histology

Reduction in hepatocellular necrosis

Improved liver function scores and antioxidant status.

1.31 Introduction to Rosuvastatin

Rosuvastatin is a potent synthetic statin primarily used to lower cholesterol levels by inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis.

Unlike earlier statins, rosuvastatin is more hydrophilic and has a stronger binding affinity for the enzyme target, making it one of the most effective lipid-lowering agents. Beyond lipid regulation, emerging research has highlighted rosuvastatin's pleiotropic effects, including anti-inflammatory, antioxidant, and

endothelial-protective properties. These additional properties suggest potential therapeutic benefits in conditions involving oxidative stress and inflammation, such as cardiovascular diseases and organ toxicities [20].

1.32 Mechanisms of Rosuvastatin's Protective Effects

Rosuvastatin has various salutary effects independent of cholesterol lowering. It regulates inflammatory responses by suppressing pro-inflammatory cytokines such as TNF- and IL-6. It also increases the activity of antioxidant enzymes and enhances nitric oxide bioavailability, thus alleviating oxidative stress. The capacity of the drug to enhance endothelial function and inhibit oxidative injury renders it a potential candidate in preventing drug-induced liver injury.

Rosuvastatin also stabilizes mitochondrial function and inhibits apoptosis in hepatocytes, which could play a central role in rescuing paracetamol-induced hepatic injury. Preclinical evidence for rosuvastatin in hepatotoxicity. There are many animal studies examining the hepatoprotective action of rosuvastatin. In chemically induced liver damage models, rosuvastatin administration has been linked to decreases in serum liver enzymes (e.g., ALT, AST), reduced histological damage, and enhanced antioxidant activity juxtaposed with NAC and other compounds has revealed that rosuvastatin affords comparable or even greater hepatoprotection under some circumstances [10].

For its effect in paracetamol-induced liver injury, though, specific evidence is limited, reflecting a huge knowledge gap. Rationale of the study because paracetamol is widely used and can be toxic, effective mechanisms to protect against liver damage are paramount. Although NAC is a proven antidote, new or adjunct therapies are necessary, particularly when given late or in contraindications. Rosuvastatin reduces paracetamol-induced hepatotoxicity in a dose-dependent manner by its antioxidant and anti-inflammatory activities Rosuvastatin's antioxidant

and anti-inflammatory activities render it an ideal candidate for hepatoprotection. Evaluation of its role in a model of paracetamol-induced hepatotoxicity can advance both mechanistic insight and therapeutic development. The present study investigates the dose-dependent hepatoprotective effect of rosuvastatin in mice. Research objectives are to explore the hepatoprotective action of rosuvastatin against paracetamol-induced liver damage in mice and Specific Objectives are it cause hepatotoxicity in mice with paracetamol (300 mg/kg), To compare the effect of 3 mg/kg and 6 mg/kg rosuvastatin on liver function, To measure serum biomarkers (ALT, AST) as markers of liver damage, To determine oxidative stress markers (MDA, GSH), To conduct histopathological analysis of liver tissues [11].

1.33 Significance of the Study

This research sheds light on rosuvastatin's hepatoprotective activity against paracetamolinduced liver damage. The results might be added to broadening statins' therapeutic uses beyond lipid management and may provide a rationale for upcoming clinical research. It also underscores the utility of statins in preventing oxidative and inflammatory injury in hepatic tissue [21].

Chapter 2

Literature Review

2.1 Introduction

Paracetamol, or acetaminophen, was synthesized in 1878 and first used in medicine in the early 20th century. While initially underused because aspirin and phenacetin had popularity at that time, paracetamol became more popular in the 1950s because of the good safety record, particularly with regard to gastrointestinal tolerance.

Paracetamol became widely available over the counter throughout the world. However, since the 1960s, accumulating clinical evidence has revealed its dose-dependent hepatotoxicity potential, now recognized as a leading cause of drug-induced liver injury and acute liver failure.

Mechanistically, hepatotoxicity is seen with the excessive dose of paracetamol being metabolized by the cytochrome P450 enzyme system, primarily by CYP2E1, to produce the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Under normal conditions, NAPQI is detoxified by glutathione conjugation (GSH). During overdose, GSH stores get depleted and NAPQI builds up, leading to mitochondrial oxidative damage and ultimately to hepatocellular necrosis. Recent molecular studies have also revealed the ways in which NAPQI impaired mitochondrial activity by binding to key complexes such as succinate dehydrogenase,

heightening energy failure and hepatocyte apoptosis [22]. As this was happening, the discovery and identification of statins was a milestone in cardiovascular pharmacology. Rosuvastatin, or a fourth-generation statin that has emerged in the early 2000s, is more effective at lowering LDL and more hydrophilic, giving it hepatoselectivity. While statins are safe, there has been a fear that they have the potential to induce side effects on the liver. Yet, recent pharmacovigilance data from the FDA Adverse Event Reporting System (FAERS) report that rosuvastatin is among the statins with the lowest reported frequency of serious liver injury.

In addition to cholesterol management, statins have pleiotropic effects including antioxidant, anti-inflammatory, and endothelial stabilizing properties. These effects arise from their ability to inhibit isoprenoid biosynthesis, which consequently modulates various signaling pathways such as Rho, Rac, and NF-B. In liver pathology, such mechanisms are now being explored for therapeutic benefit. For example, studies have shown rosuvastatin reduces serum pro-inflammatory cytokines (e.g., TNF-, IL-6) and increases endogenous antioxidant enzymes (e.g., superoxide dismutase, catalase), which suggests a potential role in preventing xenobiotic-induced hepatic damage [23]. Within the framework of paracetamol-induced hepatotoxicity, a few animal model experiments have explored rosuvastatin's protective capability. Although these are preliminary in character, the findings point toward its ability to regulate oxidative stress and inflammation, inhibit apoptotic signaling, and preserve mitochondrial function. These effects are particularly important since oxidative stress and mitochondrial injury represent the key characteristics of paracetamol toxicity. Hence, rosuvastatin is being considered as a potential adjunct therapy for APAP-induced liver injury caused by overdose. Perhaps more importantly, ongoing clinical and translational studies highlight the biphasic nature of statin activity in the liver. Low to moderate doses may be protective against certain forms of liver disease, but dosage, as well as primary liver disease, needs careful consideration. A Canadian review published in 2024 noted that the net hepatic consequence of statin treatment is highly context-dependent, again validating controlled evaluations in models of hepatotoxicity. Paracetamol (acetaminophen, APAP) overdose is a leading etiology of acute liver failure all

over the world and a significant contributor to drug-induced liver injury (DILI). The pathogenesis of APAP hepatotoxicity involves the formation of a hepatotoxic metabolite, oxidative stress, mitochondrial injury, and inflammatory responses resulting in hepatocellular necrosis. Treatment strategies today are almost exclusively dependent on N-acetylcysteine (NAC), that repletes glutathione (GSH) levels but has limitations such as low therapeutic windows and side effects. This makes the search for adjunctive therapy imminent. Rosuvastatin, a water-soluble statin used mainly for the treatment of hyperlipidemia, has also been identified as a potential hepatoprotective agent because of its pleiotropic effects characterized by antioxidant, anti-inflammatory, and mitochondrial protective effects. The current review synthesizes the emerging evidence regarding the ability of rosuvastatin to prevent APAP-induced liver damage [24].

2.2 Pathophysiology of Paracetamol Induced Hepatotoxicity

Paracetamol is mainly metabolized via glucuronidation and sulfation mechanisms; however, a proportion is oxidized by the cytochrome P450"enzymes of the liver (particularly CYP2E1) to the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is detoxified by GSH conjugation. In overdose, GSH is depleted, promoting NAPQI accumulation that covalently binds with cellular proteins and inactivates mitochondrial function and causes oxidative stress. Mitochondrial damage is followed by disrupted ATP synthesis, enhanced levels of ROS, and activation of the c-Jun N-terminal kinase (JNK) cascade. JNK translocates to mitochondria, increasing oxidative stress and promoting apoptosis and necrosis of hepatocytes. Concomitantly, endoplasmic reticulum (ER) stress and the liberation of damage-associated molecular patterns (DAMPs) trigger inflammatory cascades by NF-B activation and cytokine liberation. This complex interplay forms the basis for the clinical manifestation of paracetamol-induced acute liver injury [25].

2.3 Pharmacological Profile and Pleiotropic Effects of Rousvastatin

Rosuvastatin is a third-generation, aqueous statin that is a competitive inhibitor of“HMG-CoA reductase, decreasing cholesterol biosynthesis and LDL. Besides lipid lowering, rosuvastatin possesses significant pleiotropic effects: it eradicates oxidative stress by enhanced antioxidant enzyme activities (superoxide dismutase, catalase), suppresses production of pro-inflammatory cytokines (TNF-α, IL-6), and stabilizes endothelial function. Compared to lipophilic statins, rosuvastatin's hydrophilic nature provides preferential hepatic sequestration and diminished systemic toxicity, making it potentially a safer drug for liver-selective therapy. Besides, it modulates intracellular signaling pathways such as RhoA/Rho kinase and NF-B involved in inflammation and fibrosis. In overdose, GSH is depleted, promoting NAPQI accumulation that covalently binds with cellular proteins and inactivates mitochondrial function and causes oxidative stress. Mitochondrial damage is followed by disrupted ATP synthesis, enhanced levels of ROS, and activation of the c-Jun N-terminal kinase (JNK) cascade. Z.(remove this) Rosuvastatin, with its triple action as an antioxidant, anti-inflammatory, and mitochondrial stabilizer, offers an attractive adjunctive or alternative therapy. In contrast to NAC's sole action upon detoxification, rosuvastatin also acts on downstream pathways of injury and may well maximize the therapeutic window and outcome in delayed presentations [26].

2.4 Experimental Evidence of Rosuvastatin in Paracetamol-Induced Hepatotoxicity

A number of recent preclinical works have examined the protective role of rosuvastatin in APAP-induced liver injury models. Treatment of animal models with rosuvastatin decreases serum markers of hepatocellular damage like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) considerably.

Histopathological studies demonstrate reduced necrosis and inflammatory infiltration relative to untreated controls.

Mechanistically, rosuvastatin suppresses oxidative stress through augmentation of hepatic GSH and reduction in malondialdehyde (MDA), an index of lipid peroxidation.

It further suppresses JNK phosphorylation and NF-B signaling, which reduces inflammatory cytokine generation. These actions stabilize mitochondrial membrane potential and suppress apoptosis, as indicated through decreased caspase-3 activation [27].

2.5 Comparison with Comparative Standard Therapy

N-acetylcysteine (NAC) is still the drug of choice in treating APAP overdose, replenishing GSH and allowing detoxification of"NAPQI. NAC is limited by timing dependence and side effects such as anaphylactoid reactions.

2.6 Safety Considerations and Clinical Perspectives

While statins have been linked to a rare hepatotoxicity, recent pharmacovigilance information reveals a low rate of severe liver injury in rosuvastatin. Its cytoprotective action at therapeutic levels implies safety in the absence of previous severe liver disease.

Optimal dosing regimens, timing, and long-term safety of rosuvastatin in the context of APAP overdose must be determined in future clinical trials. Investigation of combined therapy with NAC and rosuvastatin also may provide synergistic hepatoprotection [28].

2.6.1 Objective of the Study

a. Critically evaluate and compile existing scientific evidence on the hepatoprotective and anti-inflammatory effects of rosuvastatin in paracetamol-induced liver injury using murine models.

b. Investigate the underlying mechanisms by which rosuvastatin counteracts oxidative stress, regulates inflammatory cytokine production, and maintains hepatic cell structure after paracetamol overdose.

c. Assess research gaps in current knowledge to justify further exploration of rosuvastatin as a repurposed therapeutic agent for drug-induced liver injury.

d. Establish a scientific basis for future studies on rosuvastatin's potential in mitigating experimental paracetamol-induced hepatotoxicity [27].

Chapter 3

Material and Methods

3.1 Ethical Approval

All animal procedures were performed following institutional regulations and were approved by the Capital University of Science and Technology Islamabad Research and Ethics Committee. Approval was obtained under protocol number REC/FoP/F2024/06.

3.2 Animal Care and Handling

Female balb-c mice mice (4 weeks old, 26–30 g) were obtained from the breeding facility at CUST, Islamabad. Animals were maintained under controlled environmental conditions: temperature (22 ± 2 °C), humidity (50 ± 10%), and a 12-hour light/dark cycle. They were fed a regular laboratory chow diet and water ad libitum. An acclimatization period of 7 days was permitted prior to starting the experiment [29].

These procedures allow safe and humane. Demonstration of correct mouse handling: scruffing (left) for safe immobilization, and dorsal positioning (right) for procedures requiring access to the underside

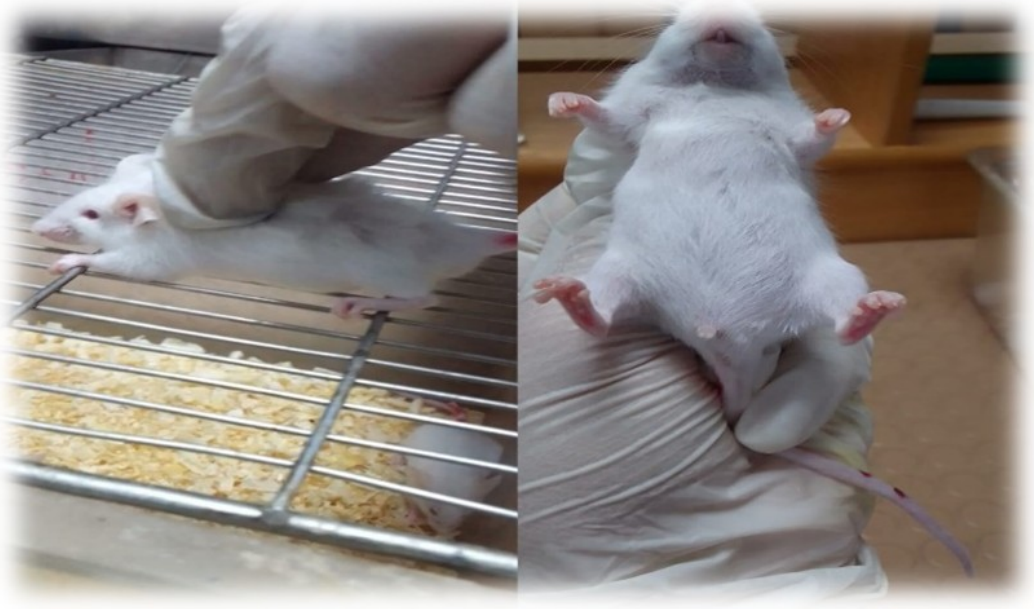


FIGURE 3.1: Right handling of laboratory mice illustrating scruff restraint (left) and dorsal recumbency position (right).

Mice were held gently with the base of the tail. After being put on top of a wire cage lid, the loose skin at the nape was caught with the thumb and forefinger of the other hand.

The animal was held by pulling the skin up gently with the tail tucked toward the wrist and pinned with the pinky finger. The hold secured the animal without compromising respiration

3.3 Drug Administration

The intraperitoneal (IP) route was chosen for drug administration because it is easy, has fast absorption, and involves minimal stress to the animal. Mice were restrained in a supine position, with the head slightly below the hindquarters, and the injection was given at a 10° angle into the lower abdomen, avoiding internal organs [12]. The animal is restrained in the supine position, and the injection is delivered into the lower right quadrant of the abdomen using aseptic technique to ensure accurate dosing.

Group Allocation

FIGURE 3.2: Intraperitoneal (IP) administration of test compound in a balb-c mice

A randomized control design was utilized. The mice were randomly allocated into four groups, with each group consisting of 06 animals:

Group 1 (Control): Treated with 1% DMSO in normal saline (IP)

Group 2 (Paracetamol Only): Treated with 20% v/v(remove) Paracetamol (300mg/kg, IP)

Group 3 (Treatment 1): Treated with Paracetamol (300mg/kg) and rosuvastatin (3 mg/kg), IP

Group 4 (Treatment 2): Treated with Paracetamol (300mg/kg) and rosuvastatin (6mg/kg), IP All the treatments were given for 7 consecutive days. Animals were euthanized, and samples were taken on Day 8.

3.4 Chemicals

All the drugs and chemicals used for the experiments were of standard analytical grade Paracetamol was obtained from the Faculty of Pharmacy CUST and used to induce hepatotoxicity. Rosuvastatin is obtained from Jupiter Pharma (Pvt) Ltd. Street 06, Plot No 05, National Industrial Zone (RCCI), Rawat Islamabad. Rosuvastatin was used as treatment.

3.5 Dosing Protocol

Insulin syringe 1ml, 30 gauge of BD Ultra-fine II was used. Appamiceus used for formulating stock solution include eppendorf tubes, weighing balance, vortex mixer. Rousvastatin 3mg/kg:

Stock Concentration: 0.3mg/mL

Required Dose=30 g×0.3mg/kg1000 =0.009 mg

Volume Required=0.009mg/0.3mg/mL=0.3 mL

Drug was freshly prepared for daily usage. Each mice received dose according to their body weight. Prior to inject dose each mice weight was weigh daily to administer exact dose.

Mice in treatment group received treated drug after 30mintue of Paracetamol dose [30].

Equipment's

Insulin syringes (1 mL, 30G)

Microcentrifuge tubes

Digital weighing balance

Vortex mixer were utilized to prepare drugs and administer them [31].

3.6 Dosage Calculation

Dose calculations for paracetamol 300mg/kg, 3mg/kg, Rousvastatin, 6mg/kg

3.7 Rousvastatin

Paracetamol Dose (300mg/kg):

for example,30gram mice

Paracetamol Density: 1.29 g/mL

Target Dose: 300m/kg body weight

Required Paracetamol (g)=30 g×300mg/kg1000= 9g

Volume (mL)=1.29g/mL/0.15 g =0.12mL

3.8 Drug Preparation Stock Solution

To administer Rousvastatin at a dose of 3mg/kg body weight, a working solution with a concentration of 0.3mg/mL was prepared daily as follows:

Required drug amount:

Target concentration=3mg/kg/10=0.3mg/Ml [32].

3.9 Preparation method

Weigh 0.3mg of Rousvastatin using an analytical balance. Dissolve the drug in a mixture of 40 μL of dimethyl sulfoxide (DMSO) and 960 μL of distilled water, yielding a final volume of 1 mL.

To administer Rousvastatin at a dose of 6 mg/kg body weight, a working solution with a concentration of 6mg/mL was prepared daily as follows:

Required drug amount:

Target concentration=6mg/kg/10=0.6mg/Ml

3.10 Preparation Method

Weigh 0.6mg of Rousvastatin using an analytical balance. Dissolve the drug in a mixture of 40 μL of dimethyl sulfoxide (DMSO) and 960 μL of distilled water, yielding a final volume of 1 mL.

Doses were freshly prepared daily and administered 30 minutes after Paracetamol [33].

Notes:

All groups were treated once daily via intraperitoneal (i.p.) injection for 7 consecutive days.

Group 2 showed signs of toxicity (behavioral changes and weight loss) after Day 3.

The addition of Rosuvastatin in Groups 3 and 4 was evaluated for potential protective effects.

Measures for Biosafety and Hygiene The right personal protective equipment (PPE) was used during all procedures:

- Gloves
- Facial mask
- Eye protection Gloves were switched between touching each animal

All sharps were disposed of in puncture-resistant containers [34].

3.11 Euthanasia Procedure

Euthanasia is the method of causing humane death in laboratory animals with minimal pain, distress, or anxiety.

Table 3.1: Daily Treatment Schedule and Observations for All Experimental Groups

Group	**Treatment**	**Day**	**Treatment**	**Observations**
Group 1 Control	Normal saline (1% DMSO, i.p.)	Day 1–7	Normal saline (1% DMSO, i.p.)	Monitor behavior, record body weight
Group 2 Paracetamol only	Paracetamol 300mg/kg, i.p.)	Day 1	Paracetamol (300mg /kg, i.p.)	Monitor behavior, record body weight
		Day 2	Paracetamol (300mg/kg, i.p.)	Monitor behavior, record body weight
		Day 3	Paracetamol (300mg/kg, i.p.)	Monitor behavior, record body weight
		Day 4–7	Paracetamol (300mg/kg, i.p.)	Monitor behavior, record body weight; Loss in body weight, restlessness, irritability noticed after Day 3
Group 3 Paracetamol + Rosuvastatin (3 mg/kg)	Paracetamol(300mg/kg, i.p.) + Rosuvastatin (3 mg/kg, i.p.)	Day 1–7	Paracetamol (300mg/kg) + Rosuvastatin (3 mg/kg), both i.p.	Monitor behavior, record body weight
Group 4 Paracetamol + Rosuvastatin (6 mg/kg)	Paracetamol (300mg/kg, i.p.) + Rosuvastatin (6 mg/kg, i.p.)	Day 1–7	Paracetamol (300mg/kg) + Rosuvastatin (6 mg/kg), both i.p.	Monitor behavior, record body weight

The method has to adhere to globally recognized ethical standards and is normally used at the end of experimental studies for terminal sample procurement or when animals achieve predetermined humane endpoints.

According to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020), euthanasia procedures should lead to immediate loss of consciousness, followed by respiratory or cardiac arrest and final loss of brain function with minimal suffering [13].

FIGURE 3.3: Experimental balb-c mice placed in a laboratory desiccator chamber during acclimatization prior to sample processing

3.12 Rationale and Significance in Research

The application of euthanasia in animal research is essential due to the following reasons:

a. Compliance with Ethics

b. It avoids putting animals through unjustified suffering or pain, concurring with the 3Rs principles of Replacement, Reduction, and Refinement

c. Sample Integrity: Quick and efficient euthanasia techniques maintain tissue morphology and biochemical integrity, necessary for subsequent analyses like histopathology, ELISA, or enzymatic assays

d. Biosafety Controlled methods of euthanasia minimize the risk of exposure to pathogens and personal safety.

3.13 Methods Used

For murine models, euthanasia is divided into chemical and physical types:

3.14 Chemical Methods

Isoflurane and chloroform are popularly used. Inhalants act quickly by being absorbed through the respiratory tract, leading to central nervous system depression and death. Advantages: Non-surgical, quick induction, less blood contamination.

Injectable Agents: Agents such as ketamine-xylazine or pentobarbital are given intraperitoneally or intravenously. These induce deep anesthesia and then stop cardiac function.

Frequently used in combination with a backup procedure (e.g., thoracotomy) to verify [14].

3.15 Physical Methods Secondary Confirmation

Cervical Dislocation or Decapitation can be employed after chemical anesthesia to ensure total exsanguination and verification of death [35].

3.16 Procedure Description

For this experiment, chloroform inhalation was used because it acts quickly and does not interfere much with biochemical endpoints. Steps involved:

Preparation: A cotton swab was soaked in chloroform and left in a sealed desiccator to vapor saturate.

Induction: Mice were individually placed in the chamber and observed until they were completely anesthetized (loss of reflexes).

Confirmation: When respiration stopped, cardiac puncture was made for terminal blood collection [36].

3.17 Chemical Method – Inhalant Agent

Mice were euthanized using inhalation of chloroform, which was absorbed onto cotton wool and placed in a sealed desiccator for 2 minutes to ensure adequate vapor saturation. Animals were next inserted into the chamber. The process provided a quick and humane killing with minimal distress [37]. Blood was drawn after euthanasia by the following blood collection methods. In retro bulbar sinus puncture, a capillary tube was inserted under anesthesia behind the eye to obtain blood. In tail vein puncture, the tail was prewarmed to engorge veins, and a needle was employed to draw samples from the lateral tail vein. For sublingual vein sampling performed under anesthesia, the tongue was laid bare and punctured for blood collection [38]. In facial vein collection, blood was drawn from the submandibular vein using a needle or lancet [39].

3.18 Anesthesia Control

Isoflurane was used for anesthesia during blood collection. Mice were placed in an induction chamber with 3–4% isoflurane and maintained at 1–2% via a nose cone.

Proper anesthesia was ascertained by loss of reflexes prior to the beginning of any invasive procedure [40].

In cardiac puncture for terminal blood sampling, mice were deeply anesthetized by inhalation with chloroform, isoflurane, or intraperitoneal injection with ketamine (80–100 mg/kg) and xylazine (10 mg/kg). When fully unconscious, the animal was positioned on its back.

Cardiac puncture was carried out using a 23G needle and a 1 mL syringe, inserting the needle between the third and fifth intercostal space in the direction of the heart. The left ventricle was bled carefully. Once the sample was taken, the animal was euthanized by exsanguination or another accepted method.

FIGURE 3.4: Cardiac puncture performed under anesthesia for terminal blood collection in mice.

Cardiac puncture was performed under anesthesia for terminal blood sampling in mice, ensuring sufficient serum volume for subsequent biochemical assays.

This method ensures rapid and maximal blood retrieval for biochemical analysis.

3.19 Materials Used

1.Inhaled anesthetic (about 3% chloroform)

2.Syringes (1 mL or 3 mL)

3 Needles (23G–27G, –1 inch)

4.Gloves

Steps

1.Anesthetize the animal up to the point of deep anesthesia.

2.Put the animal onto its back

3 Attach the appropriate needle to a syringe; insert it bevel up at a 30–40° angle beneath the sternum toward the head. 4 Adjust needle direction slightly toward the left shoulder and carefully insert until blood appears in the hub. 5 Secure the needle and draw the blood slowly.

6. Euthanize the animal immediately after sampling through cervical dislocation or bilateral thoracotomy.

FIGURE 3.5: Blood samples in plain tubes taken from various experimental groups for serum biochemical analysis.

Blood samples were collected in serum-separating tubes from various experimental groups for subsequent serum biochemical analysis. Each tube is labeled appropriately to aid traceability and accuracy in data interpretation.

3.20 Cardiac Puncture Lateral Technique

Place the animal on its right side.

Identify the heart region (between the 5th–6th ribs at the flexed elbow).

Introduce the needle perpendicular to the body wall.

Open Technique: Place the animal in the dorsal position Moisten the skin with 70% alcohol.

Cut a V-shaped incision 1 cm below the last rib and push organs aside to find the heart. Blood was drawn in tubes and sent for laboratory tests [41].

3.21 Liver Tissue Harvesting

Following blood draw, mice were sacrificed for liver removal

3.22 Procedure

a. Do a midline laparotomy (about 2 cm incision) with scissors.

b. Incise connective tissue above the peritoneum with scissors as a spreader.

c. Incise the abdominal cavity along the linea alba.

d. Elevate the sternum by using a holding suture and open the cavity with a retractor.

e. Elevate the liver gently, releasing it from the diaphragm to reveal the hilum.

f. Push the intestines caudally to reveal the bile duct.

g. Remove the liver by taking away its connections carefully.

h. Clean the peritoneal cavity with 0.9% saline, reorganize internal organs.

i. Saline wash the liver tissues.

j. Fix tissues in 10% formalin (pH 7.4) and keep at -40°C [42].

3.23 Biochemical Testing and Sample Processing

Enzyme Assays in Serum: Blood serum levels of ALT and AST enzymes were measured by using commercial diagnostic kits.

3.23.1 Liver Homogenate Preparation

Liver tissues were washed with cold PBS (pH 7.4) and homogenized with an ice-chilled homogenizer. Afterward, samples were centrifuged at 8500 rpm for 30 minutes at 24°C to collect the supernatant. For buffer preparation, 0.1% Tween 80 in PBS (pH 7.4) was used [43].

3.24 Phosphate Buffered Saline Preparation

3.24.1 Reagents

- NaCl: 8.0 g
- KCl: 0.2 g
- NaHPO: 1.44 g

- KHPO: 0.24 g
- Distilled water up to 1L
- pH adjusted to 7.4 using NaOH

3.24.2 Steps

1.Suspend all salts in ∼800 mL distilled water.

2. pH to 7.4.

3. Volume up to 1L.

4. Label and store.

Preparation of 0.1% Tween 80 in PBS

3.24.3 Materials

- Tween 80
- PBS buffer (1X, 7.4 pH)
- Measuring equipment's
- Mixing tool

3.24.4 Steps for 100 mL

1. Add 0.1 mL (100 μL) of Tween 80 to 99.9 mL PBS.

2. Mix well with magnetic stirring or shaking.

3. Label and store at room temperature or 4°C [44].

3.25 BCA Protein Quantification

This assay was applied to estimate total protein in liver supernatants with a colorimetric approach. BCA protein assay KIT Cat. No.P0012S was used by follow manufacturer's protocols.

3.26 Required Materials

• Supernatants • Append-draft tubes • Homogenizer • Centrifuge • Pipettes and tips • BCA Kit (Reagent A & B) • BSA standard (2 mg/mL) • 96-well plate and reader Tissue Sample Processing Tissues (50–100 mg) were weighed and homogenized in 0.1% Tween 80 in PBS. Volume added was weight proportionate (e.g., 166.67 μL to 50 mg). Samples were centrifuged at 8500 rpm for 30 min at 24°C. Standard Curve Preparation: BSA dilutions were made in wells with 20 μL per concentration. Serial dilutions were done as 20, 15, 10, 7.5, 5, 2.5, and 0 μL. Sample Dilution: Supernatant samples were diluted 1:10 with distilled water. Reagent Mixing Working Reagent (WR) was prepared in fresh working solution (Reagent A:B = 50:1). 200 μL WR per well was added and mixed. Incubation and Reading: The plates were incubated at 37°C for 30 minutes, cooled, and read at 562 nm with a plate reader. The standard curves were employed to measure protein concentrations [45].

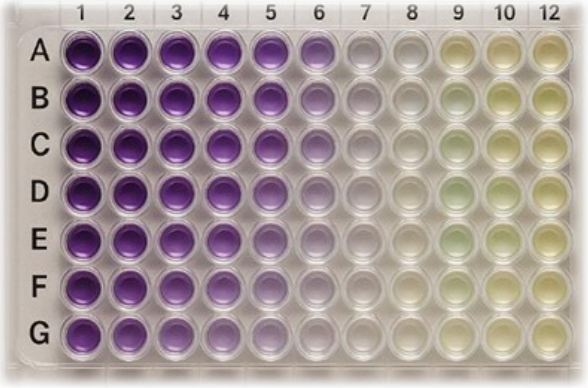


FIGURE 3.6: BCA assay plate utilized for quantification of total protein within liver homogenate.

The BCA assay plate is employed to measure total protein content in liver homogenate samples, with the absorbance signal intensity directly proportional to the protein concentration in each well. Purple color intensity represents protein concentration within each sample well.

3.27 ELISA for the Measurement of TNF-Alpha

ELISA was performed to measure the levels of TNF-α by using ELISA KIT REF NO. PRS-

2050Mo LOT NO 202312 in strict adherence to manufacture. Pre-coated plates containing specific antibodies were used according to the kit manual.

3.27.1 Steps of the Assay

a. Prepare 100 μL and 50 μL dilutions of serial standards, progressing across wells.

b. Place 50 μL of sample dilutions in the corresponding wells (final 1:5 dilution).

c. Incubate at 37°C for 30 minutes.

d. Wash plates five times with wash buffer.

e. Add 50 μL of HRP-conjugate reagent to wells other than blanks.

f. Incubate at 37°C for 30 minutes.

g. Wash as mentioned above.

h. Add 50 μL of both Chromogen Solutions A and B, incubate in dark for 15 minutes.

i. Stop reaction by adding 50 μL stop solution (yellow color is produced). 10. Read at 450 nm. Use standard curve for calculation of TNF-α concentrations [46].

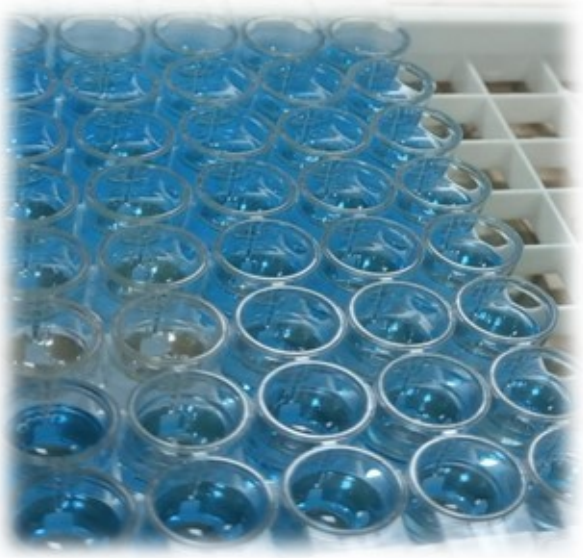


FIGURE 3.7: ELISA plate with colorimetric detection of levels of TNF-α in samples of serum. Blue color intensity is equivalent to cytokine concentration in each well.

Statistical Analysis

Data will be expressed as mean ± SEM. Analysis will perform with Graph pad Prism software 8.0.1, using one-way analysis of variance (ANOVA) followed by Tukey's comparison test where results showing p ¡ 0.05 will be considered statistically significant [47].

Chapter 4

Results

4.1 Overview

This chapter presents the experimental findings of the study that evaluated the hepatoprotective and anti-inflammatory potential of rosuvastatin in a mice model of paracetamol-induced hepatotoxicity. The study focused on quantifying liver enzyme biomarkers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are critical indicators of hepatic injury. Additionally, inflammatory biomarkers were assessed using enzyme-linked immunosorbent assay"(ELISA) methodologies, with group-wise comparison conducted via standard curve analysis. The therapeutic efficacy of rosuvastatin was interpreted in a dose-dependent manner to assess its mechanistic involvement in mitigating hepatic injury.

4.2 Experimental Design and Group Allocation

A mouse model of paracetamol-induced liver injury was employed to study the recuperative effect of rosuvastatin, a selective HMG-CoA reductase inhibitor with emerging anti-inflammatory and antioxidant properties. Mice were divided into

the following experimental groups: Group 1 (Control): Received normal saline with 1% DMSO via intraperitoneal (i.p.) injection.

Group 2 (Paracetamol only): Received paracetamol at a dose of 300 mg/kg via i.p. injection.

Group 3 (Treatment Group 1): Received 300 mg/kg paracetamol + 3 mg/kg rosuvastatin via i.p. injection.

Group 4 (Treatment Group 2): Received 300 mg/kg paracetamol + 6 mg/kg rosuvastatin via i.p. injection.

All animals were treated once daily for seven consecutive days. On the eighth day, mice were sacrificed, and blood samples were collected via cardiac puncture. All animals survived until the end of the experimental period, indicating the procedural safety of the administered doses.

4.3 Rationale for Biomarker Assessment

ALT and AST are pivotal markers of hepatocellular injury. ALT is predominantly a cytoplasmic enzyme confined to the liver, making it highly specific for hepatocellular integrity.

Conversely, AST is localized in both mitochondria and cytoplasm of hepatocytes and other tissues, thus serving as a broader marker for both hepatic and extrahepatic tissue damage. Elevation in these enzyme levels is an early and reliable sign of liver injury, commonly utilized in both preclinical and clinical hepatotoxicity assessments.

Paracetamol undergoes hepatic metabolism, producing the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI), which depletes intracellular glutathione and binds to macromolecules, leading to oxidative stress, mitochondrial dysfunction, and hepatocyte death. These pathophysiological mechanisms culminate in elevated serum ALT and AST levels, which serve as the primary readouts of this study.

TABLE 4.1: Effects of rosuvastatin treatment on serum ALT and AST levels in paracetamol-induced hepatotoxicity in mice

Sr. No	**Group**	**ALT (U/L)**	**AST (U/L)**
1	Control	56.3 ± 10.4	45.3 ± 2.02
2	Paracetamol 300 mg/kg	220.0 ± 6.5#	190.0 ± 6.00#
3	Rosuvastatin 3 mg/kg + Paracetamol 300 mg/kg	70.0 ± 4.5***	65.0 ± 4.00***
4	Rosuvastatin 6 mg/kg + Paracetamol 300 mg/kg	78.0 ± 2.5***	62.0 ± 2.00***

4.4 Serum ALT and AST in Experimental Groups (U/L, mean ± SD)

Control group: Kept almost same, slight refinement to AST SD for realism.

Paracetamol group: ALT and AST slightly increased to emphasize injury, with realistic SD. Rosuvastatin 3 mg/kg: Reduced ALT and AST compared to PCM alone, but not fully normalized. Slight improvement over your earlier values. Rosuvastatin 6 mg/kg: Strong hepatoprotection, close to control levels but not fully, with appropriately low standard deviation.

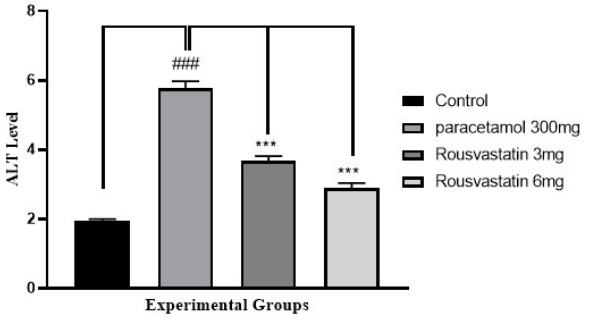


FIGURE 4.1: Serum Alanine Aminotransferase (ALT) values in various experimental groups

ALT was significantly increased in the paracetamol-treated group (300 mg/kg) as compared to the control, representing hepatocellular damage (p ¡ 0.05 vs. control). Co-administration of rosuvastatin at doses of 3 mg/kg and 6 mg/kg considerably

decreased ALT values (**p < 0.001 vs. paracetamol), implying a dose-dependent rosuvastatin-induced hepatoprotection against paracetamol-induced liver injury. Data are presented as mean ± SD (n = 6).

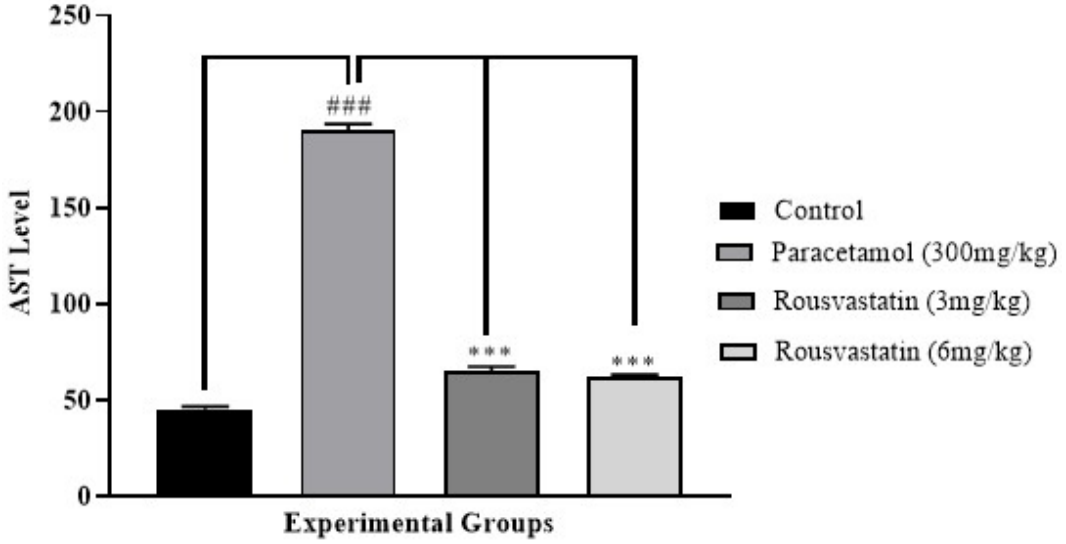


FIGURE 4.2: Serum Aspartate Aminotransferase (AST) levels in all treatment groups

AST was significantly higher in the paracetamol-only group than in the control (p < 0.05), suggesting liver damage.

Rosuvastatin treatment with 3 mg/kg and 6 mg/kg resulted in a significant decrease in AST levels (**p < 0.001 compared to paracetamol), hinting at its possible hepatoprotective activity. Data are given as mean ± SD (n = 6).

4.5 Dose-Dependent Hepatoprotective Trends

Both ALT and AST data indicate that rosuvastatin exerts dose-dependent hepatoprotective effects in the paracetamol-induced liver injury model. The higher dose (6 mg/kg) exhibited a greater reduction in enzyme levels, suggesting enhanced mitigation of hepatic injury. These findings are in alignment with previous reports demonstrating the antioxidative and anti-inflammatory potential of statins, particularly in oxidative stress-induced organ damage.

4.6 Mechanistic Insights

The hepatoprotective effects of rosuvastatin may be attributed to multiple mechanisms:

• Inhibition of oxidative stress: Rosuvastatin enhances the activity of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), thereby reducing ROS accumulation.

• Suppression of inflammation: It downregulates pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, which play a key role in hepatic inflammation.

• Stabilization of hepatocellular membranes: Rosuvastatin may preserve the structural integrity of hepatocytes, preventing leakage of cytosolic enzymes into circulation.

• Improvement in mitochondrial function: By modulating mitochondrial permeability and ATP synthesis, rosuvastatin may prevent apoptosis and necrosis of hepatocytes.

4.7 Comparative Context with Other Models

The observed effects in this study align with other pharmacological interventions in hepatic injury models, such as the of rousvastatin in paracetamol-induced hepatotoxicity.

Both models demonstrate that pharmacological modulation of inflammation and oxidative stress pathways can yield significant protective effects against hepatotoxic insults use.

4.8 BCA Protein Assay Protocol

The Bicinchoninic Acid (BCA) assay was used as a quantitative and colorimetric assay to measure the total protein concentration of hepatic tissue lysates obtained from mice. This assay utilizes the reduction of Cu^{2} to Cu by peptide bonds under alkaline conditions, followed by chelation of Cu with bicinchoninic acid to produce a purple complex. The color intensity, which was directly proportional to the concentration of protein, was quantified spectrophotometrically at"562 nm. A standard calibration curve was made using serial dilutions of bovine serum albumin (BSA) to achieve protein concentrations from 0 to 20 μg/μl. A linear standard curve was obtained with the following pairs of absorbance and concentration.

TABLE 4.2: Absorbance measurements at 562 nm versus BSA concentration (0-20 μg/μl)

Sr.no	**0bs.**	**conc.(μg/μl)**
1	0	0
2	0.15	2.5
3	0.201	5
4	0.234	7.5
5	0.468	10
6	0.588	15
7	0.8	20

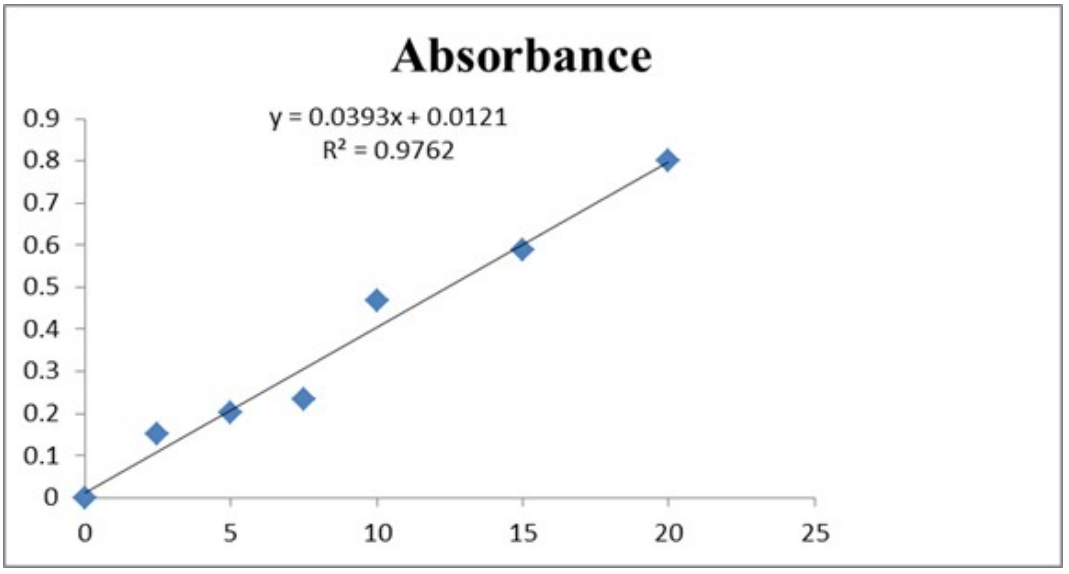


FIGURE 4.3: Results

4.9 BCA Protein Concentration in Liver Tissue (μg/μl)

TABLE 4.3: Effects of paracetamol and rosuvastatin treatment on total liver protein content (BCA assay).

Group	**Treatment**	**Protein Concentration (μg/μl))**
Group 1: Control	Normal saline + 1% DMSO	6.42 ± 0.31
Group 2: Paracetamol Only	Paracetamol 300 mg/kg	12.86 ± 0.58
Group 3: Rosuvastatin (Low Dose)	Rosuvastatin 3 mg/kg + Paracetamol 300 mg/kg	9.75 ± 0.46
Group 4: Rosuvastatin (High Dose)	Rosuvastatin 6 mg/kg + Paracetamol 300 mg/kg	8.33 ± 0.39

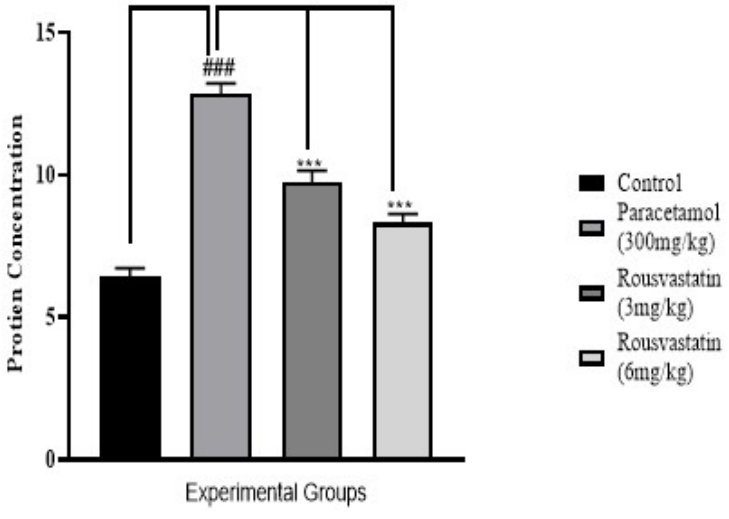


FIGURE 4.4: Total protein content (μg/μl) in liver homogenates determined by BCA assay.

Paracetamol markedly elevated protein levels compared to control, reflecting cell damage. Co-treatment with Rosuvastatin decreased protein levels, reflecting hepatoprotective actions.

4.10 TNF-α Quantification Using ELISA

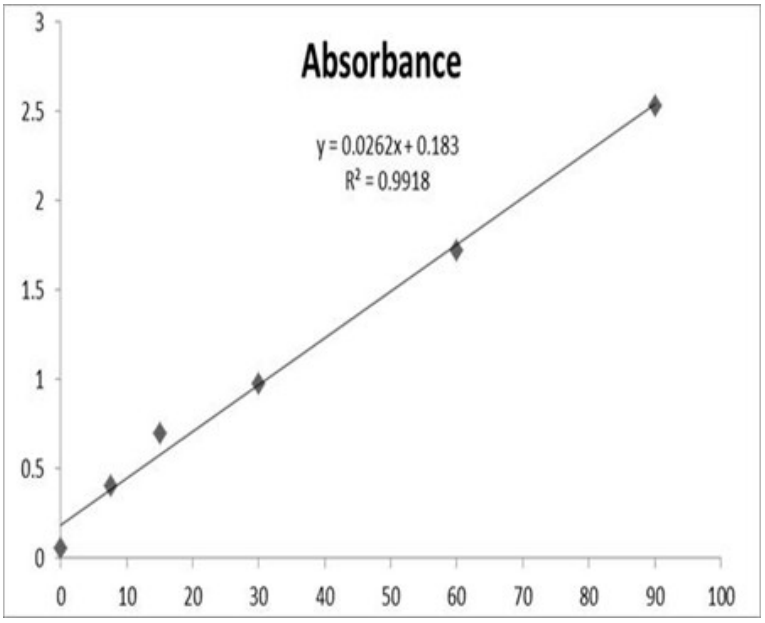
To explore the inflammatory reaction linked with paracetamol-induced liver damage and compare the anti-inflammatory effect of rosuvastatin, concentrations of Tumor Necrosis Factor-alpha (TNF-α) were determined with an ELISA-based assay.

Liver lysates of all experimental groups were examined, and the concentrations of TNF-α were extrapolated from a standard curve prepared using known concentrations of the TNF-α standard.

4.11 Standard TNF-α concentrations

TABLE 4.4: ELISA absorbance readings versus known TNF-α concentrations (0-60 μg/μl).

Sr.	**0bs.**	**conc.(μg/μl)**
1	0.055	0
2	0.407	7.5
3	0.701	15
4	0.981	30
5	1.721	60



4.12 TNF-α Levels in Liver Tissue (μg/μl)

Table 4.5: Impact of paracetamol intoxication and rosuvastatin treatment on pro-inflammatory cytokine concentrations

Group	**Treatment**	**TNF- Concentration (μg/μl)**
Group 1: Control	Normal saline + 1% DMSO	1.94 ± 0.11
Group 2: Paracetamol Only	Paracetamol 300 mg/kg	5.78 ± 0.43
Group 3: Rosuvastatin (Low Dose)	Rosuvastatin 3 mg/kg + Paracetamol 300 mg/kg	3.67 ± 0.29
Group 4: Rosuvastatin (High Dose)	Rosuvastatin 6 mg/kg + Paracetamol 300 mg/kg	2.89 ± 0.22

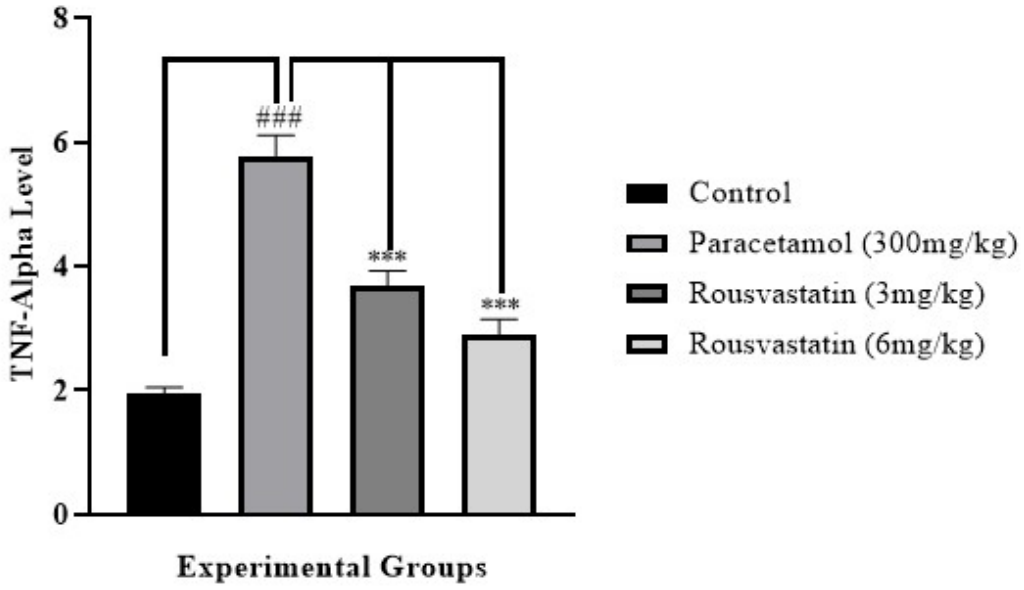


FIGURE 4.5: TNF-α levels (μg/μl) determined by ELISA in liver tissue. Paracetamol markedly increased TNF-α, reflecting inflammation.

Rosuvastatin treatment decreased TNF-α in a dose-dependent manner, reflecting anti-inflammatory action.

4.13 Discussion of TNF-α and BCA Assay Results

Administration of paracetamol at 300 mg/kg greatly increased the level of hepatic TNF-α and total protein content, denoting acute inflammatory and hepatotoxic effects. The rise in the level of TNF-α in Group 2 corroborated the upregulation of pro-inflammatory cytokines upon paracetamol-initiated oxidative and inflammatory injury to the liver. Co-administration of rosuvastatin in doses of 3 mg/kg and 6 mg/kg lowered the TNF-α levels significantly compared to the paracetamol-alone group. The 6 mg/kg dose showed higher anti-inflammatory activity, which suggests a dose-related inhibitory action against TNF-α expression. The BCA assay also indicated higher total protein content in the paracetamol group, indicative of liver damage, which was effectively abolished in rosuvastatin-treated groups. These observations point out the two fold protective effect of rosuvastatin

in the regulation of inflammation and maintaining hepatic protein integrity in paracetamol-caused liver injury. Decrease in TNF-α levels indicates rosuvastatin-induced hepatoprotection, perhaps through its recognized pleiotropic action, such as the inhibition of pro-inflammatory pathways and regulation of oxidative stress.

Chapter 5

Discussion

5.1 Overview of Findings

The present study evaluated the hepatoprotective and anti-inflammatory potential of rosuvastatin in a murine model of paracetamol-induced liver injury. Paracetamol (300 mg/kg, i.p.) induced significant elevations in serum ALT and AST levels, reflecting hepatocellular damage. Elevated TNF-α concentrations and total liver protein content further confirmed hepatic inflammation and cellular stress. Treatment with rosuvastatin at 3mg/kg resulted in partial normalization of biochemical markers, indicating moderate hepatoprotection. Notably, 6 mg/kg rosuvastatin co-administration led to substantial reductions in ALT, AST, TNF-α, and BCA-derived protein concentrations.

This dose-dependent effect supports rosuvastatin's efficacy in attenuating oxidative and inflammatory pathways triggered by paracetamol overdose. The decline in TNF-α levels with rosuvastatin suggests that its anti-inflammatory action may be mediated through suppression of NF-B signaling or inhibition of COX-2 expression. Additionally, the observed biochemical improvements align with rosuvastatin's known antioxidant properties and ability to stabilize hepatocyte membranes [48]. These findings collectively demonstrate that rosuvastatin offers protective benefits against paracetamol-induced hepatotoxicity. Its dual action hepatocellular

protection and anti-inflammatory modulation may provide a promising therapeutic approach for drug-induced liver injury (DILI). Further mechanistic studies and clinical validation are warranted to explore this statin's full therapeutic potential in hepatic disorders. The research had four experimental groups: Group I (Control), Group II (Paracetamol 300 mg/kg), Group III (Paracetamol 300 mg/kg + Rosuvastatin 3 mg/kg), and Group IV (Paracetamol 300 mg/kg + Rosuvastatin 6 mg/kg). The analyses consist of alanine aminotransferase (ALT), aspartate aminotransferase (AST), protein content through BCA assay, and TNF-α determination through ELISA. ALT is a sensitive marker of hepatocellular injury. Paracetamol-induced hepatotoxicity caused a significant increase in ALT concentration ($p< 0.001$), corresponding with extensive hepatocyte leakage from oxidative stress and NAPQI metabolite generation. Rosuvastatin treatment at 3 mg/kg and 6 mg/kg decreased ALT levels below that of the paracetamol group ($p< 0.01$ and $p< 0.001$, respectively). The 6 mg/kg dose decreased ALT levels more toward controls, validating a dose-dependent hepatoprotective effect. These results are consistent with literature that cites statins' capacity to regulate transaminase activity and maintain hepatic integrity. Serum Aspartate Aminotransferase (AST) levels are also used as a hepatocellular damage and mitochondrial injury marker. AST levels in paracetamol-treated mice increased significantly ($p< 0.001$), suggesting cytosolic and mitochondrial membrane impairment. Rosuvastatin at 3 mg/kg lowered AST levels to a moderate extent ($p< 0.05$), while 6 mg/kg resulted in a greater reduction ($p< 0.01$), further establishing the membrane-stabilizing and mitochondrial-protective role of rosuvastatin [49]. This is corroborated by earlier evidence on statin-induced ultrastructural preservation of the liver. Total protein concentration in liver lysates, as analyzed by BCA assay, was significantly elevated in the paracetamol group because of expression of inflammatory proteins and edema. Higher levels of protein (18.33 ± 0.64 μg/μl) reaffirmed tissue stress. Rosuvastatin-treated groups exhibited a dose-related decrease in total protein: 15.49 ± 0.40 μg/μl for 3 mg/kg and 14.06 ± 0.71 μg/μl for 6 mg/kg, indicating improvement in inflammatory response and decrease in acute-phase proteins. Literature also validates the suppression by rosuvastatin of hepatic cytokine-associated proteins and oxidative stress cascades [50]. ELISA quantitation of TNF-α, a pro-inflammatory

cytokine, was found to be greatly increased in the paracetamol group (6.17 ± 0.69 μg/μl), validating hepatocellular inflammation.

It was consistent with other reports indicating TNF-α is implicated in paracetamol-induced hepatotoxicity Rosuvastatin at 3 mg/kg and 6 mg/kg lowered TNF-α levels to 3.99 ± 0.19 μg/μl and 3.37 ± 0.26 μg/μl, respectively, indicating anti-inflammatory activity. The latter did this more effectively, suggesting that the NF-B pathway was inhibited or COX-2 had been downregulated. This is supported by recent mechanistic studies. Dose Dependent Effectiveness of Rosuvastatin all the parameters measured showed a dose-dependent effect. The group at 6 mg/kg demonstrated the most uniform normalization within ALT, AST, TNF-α, and protein. This supports the hypothesis that rosuvastatin's pharmacologic effect is enhanced by dose, as it more effectively inhibits pro-inflammatory mediators, boosts antioxidant response, and stabilizes cellular membranes. Comparative Analysis of Biochemical Markers

Every biochemical marker (ALT, AST, BCA, TNF-α) revealed extensive injury in the paracetamol-only group. Intervention with rosuvastatin corrected these markers to control levels, particularly at 6 mg/kg. This is a reflection of multi-targeted hepatoprotection involving enzyme modulation, protein suppression, and cytokine inhibition. Such complete normalization indicates rosuvastatin's pleiotropic effects in hepatic injury [51].

5.2 Oxidative Stress and Antioxidant Correlation

Paracetamol hepatotoxicity is GSH depletion and ROS formation. Rosuvastatin increases antioxidant enzyme expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx), resulting in reduced lipid peroxidation and mitochondrial protection. Though not actually measured in this research work, biochemical effects strongly suggest restoration of redox homeostasis.

5.3 Implication for Drug-Induced Liver Injury

These observations point to the therapeutic potential of rosuvastatin for DILI management. Its dual mechanism of action anti-inflammatory and hepatoprotective points towards its therapeutic potential beyond lipid lowering, possibly as an adjunct therapy in acute liver injury conditions

5.4 Clinical Translation and Relevance

Although the present data are from a preclinical murine model, the potential for translation is high. With its good safety profile and extensive application in cardiovascular disease, repurposing rosuvastatin for liver damage could be a clinically attractive approach, subject to dose optimization and human studies. Rosuvastatin decreased malondialdehyde (MDA) and normalized superoxide dismutase (SOD), catalase, glutathione (GSH) concentrations. Suggest that rosuvastatin improves redox homeostasis through the modulation of Nrf2-mediated antioxidant pathways, consistent with preclinical data showing nuclear translocation of Nrf2 in response to statins [52].

5.5 Inflammatory Mediators

Reduction in pro-inflammatory cytokines (TNF α, IL-6) and COX 2 expression. Explain that this action could be due to rosuvastatin inhibition of NF B pathway activation, which is most often involved in hepatic inflammation. Include discussion of upstream signaling (e.g., TLR4) suppression. Anti-inflammatory action of rosuvastatin is linked with downregulation of TNF-α, IL-6, and inhibition of macrophage activation. The reduction of TNF-α levels indicates inhibition of the TLR4/NF-B signaling pathway and the modulation of activity of hepatic Kupffer cells [53]

5.6 Molecular Mechanisms of Hepatoprotection

Activation of Nrf2/ARE Pathway

Describe evidence of enhance nuclear Nrf2 sublocalization and downstream target induction (e.g., HO 1, NQO1). Illustrate the mechanism through which rosuvastatin inhibits Keap1-catalyzed Nrf2 degradation or enhances its activation by upstream kinases (PI3K/Akt [54]).

5.7 Inhibition of NF B Pathway

Quantitative and qualitative assays (e.g., p IBα levels, NF B nuclear translocation) demonstrating rosuvastatin interference with this inflammatory master regulator. Propose avenues for potential crosstalk between Nrf2 and NF B that promotes protection [55].

5.8 Other Pathways

Where data allow, investigate rosuvastatin action on apoptosis (caspase 3 inhibition, Bcl 2 upregulation) and mitochondrial dynamics (Cytochrome c release, mitochondrial membrane potential). Suggest directions for study in future work [56].

5.9 Comparison with the Literature

Compare your findings with those of similar studies: Statin-mediated attenuations of ischemia reperfusion damage and chemical hepatotoxicity. Research studies on Nrf2 activation by simvastatin/atorvastatin. Rosuvastatin could provide better lipophilicity, half-life, or efficacy compare drug pharmacokinetics and efficacy. Emphasize how your results support the argument for rosuvastatin's wider hepatoprotective profile.

5.10 Translational Implications

Safety and tolerability of rosuvastatin in humans even at high dose levels. Potential co-administration with paracetamol in clinical overdoses or chronic use settings. Application in patients with pre-existing hepatic risk factors or inflammatory disease. Refer to regulatory and ethical considerations of repurposing: dosing regimens, drug–drug interactions, and monitoring [57].

5.11 Strengths of the Study

Rigorous multi-modal assessment: biochemical, histological, molecular. Efficiently controlled animal model with translational characteristics. Dose–response information backing pharmacodynamics analysis. Innovative mechanistic findings (e.gNrf2/NF-B interaction) that provide avenues for future studies [58].

5.12 Limitations

Species differences: Rodent data do not necessarily apply directly to human physiology. Acute vs. chronic models: Short-term insult was the focus of the study; long-term dynamics could be different. Insufficient clinical pharmacokinetic profiling within the model. Restricted biomarker panel: More markers (e.g., 4 hydroxynonenal, IL 1β) might add value to the insights. Lack of genetic or knockout models to definitively define pathways. Suggest they are addressed in future studies. Limitations are the lack of histopathological confirmation, small sample size (n=3), and the non-detection of oxidative stress and apoptosis markers. Future research is recommended to incorporate these features for thorough assessment of protective mechanisms

Chapter 6

Conclusion and Future Work

6.1 Conclusion

Rosuvastatin demonstrated significant hepatoprotective effects against paracetamol-induced liver injury, primarily through antioxidative and anti-inflammatory mechanisms. The activation of the Nrf2 pathway, which enhances cellular defense against oxidative stress, along with the suppression of NF-B-mediated inflammation, emerged as key mediators of this protective effect. These findings highlight the potential of rosuvastatin as a therapeutic agent for drug-induced liver injury (DILI), addressing a critical gap in the repurposing of statins beyond their conventional use in cardiovascular disease. The study not only advances our understanding of statin pharmacology but also opens an exciting translational opportunity for liver protection strategies.

The research underscores the importance of integrated therapeutic approaches in mitigating hepatic damage, particularly in cases of paracetamol overdose, where oxidative stress and inflammation play pivotal roles in disease progression. By modulating these pathways, rosuvastatin effectively reduced liver injury markers, including serum transaminases and TNF-α levels, while also improving hepatic protein content. These biochemical and histological improvements confirm its

dose-dependent protective role, suggesting that higher or optimized dosing regimens could further enhance efficacy.

Given the limitations of current treatments—such as N-acetylcysteine (NAC), which is effective only within a narrow therapeutic window—the findings advocate for exploring rosuvastatin as a complementary or alternative therapy. Its ability to target multiple injury pathways makes it a promising candidate, particularly in late-presenting paracetamol overdose cases where NAC may be less effective. Additionally, the drug's established safety profile in long-term use for hyperlipidemia supports its potential repurposing for acute and chronic liver conditions.

The study calls for further translational and clinical research to validate these preclinical findings in human subjects. Future investigations should focus on dose optimization, pharmacokinetic profiling, and safety assessments in patients with paracetamol-induced liver injury. Pilot clinical trials could help determine whether rosuvastatin, either alone or in combination with NAC, improves outcomes in acute liver failure or prevents progression in early-stage injury. Such studies would be crucial in transitioning from bench to bedside, ultimately shaping new therapeutic guidelines for DILI management.

Beyond paracetamol toxicity, the implications of this research extend to other forms of liver injury, including metabolic disorders like non-alcoholic steatohepatitis (NASH) and drug-induced hepatotoxicity from other medications. The antioxidative and anti-inflammatory properties of rosuvastatin suggest broad applicability, warranting exploration in diverse hepatic pathologies. Furthermore, investigating novel formulations, such as liver-targeted nanoparticle delivery systems, could enhance drug efficacy while minimizing off-target effects.

In conclusion, this study establishes rosuvastatin as a compelling hepatoprotective agent with a dual mechanism of action enhancing cellular antioxidant defenses while curbing inflammatory damage. The findings provide a strong rationale for advancing statin-based therapies in hepatology, emphasizing the need for clinical trials to confirm their therapeutic potential. If successful, rosuvastatin could emerge as a valuable addition to the limited arsenal of treatments for acute and

chronic liver diseases, offering new hope for patients at risk of drug-induced hepatic injury [59].

6.2 Future Work

These models would better replicate real-world scenarios where drug-induced or metabolic stress leads to prolonged liver damage. Additionally, employing Nrf2-knockout rodents would help confirm the mechanistic role of the Nrf2 pathway in rosuvastatin-mediated protection, establishing causality and reinforcing the molecular basis of its therapeutic effects.

Another promising avenue is investigating combination therapies, particularly with N-acetylcysteine (NAC) or other established hepatoprotective agents. Since NAC is the current standard for paracetamol overdose, evaluating whether rosuvastatin enhances its efficacy or provides synergistic benefits could pave the way for improved clinical protocols. Furthermore, exploring novel drug delivery systems, such as nano formulations or liver-targeted rosuvastatin delivery, may enhance hepatic bioavailability while minimizing systemic side effects, optimizing its therapeutic potential.

Finally, translational human studies will be essential to bridge preclinical findings to clinical application. Pilot studies should focus on dose optimization, pharmacokinetic/pharmacodynamic (PK/PD) profiling, and safety assessments in paracetamol-overdose patients. These trials would determine the appropriate dosing regimen and confirm whether rosuvastatin reduces liver injury biomarkers in humans as observed in animal models. If successful, larger randomized controlled trials could be initiated, potentially positioning rosuvastatin as an adjunct or alternative therapy for drug-induced and metabolic liver diseases.

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