



# Evaluation of Hepatoprotective Activity of Herbal Extract Against Paracetamol-Induced Toxicity

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## Abstract

Paracetamol-induced hepatotoxicity is one of the most widely used experimental models for evaluating hepatoprotective agents. The present study aimed to evaluate the hepatoprotective activity of a standardized hydroalcoholic extract of *Psidium guajava* leaves against paracetamol-induced liver toxicity in Wistar rats. Hepatotoxicity was induced by administering paracetamol (2 g/kg, p.o.). The extract was evaluated at two dose levels (200 mg/kg and 400 mg/kg). Silymarin (100 mg/kg) was used as the standard hepatoprotective drug. Biochemical parameters including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, total protein, and histopathological studies were performed.

Results showed that treatment with the herbal extract significantly ( $p < 0.01$ ) reduced elevated liver enzyme levels and improved histopathological architecture compared to the toxic control group. The hepatoprotective effect at 400 mg/kg was comparable to that of silymarin.

The study concludes that *Psidium guajava* leaf extract possesses significant hepatoprotective activity against paracetamol-induced hepatotoxicity.

Keywords: Hepatoprotective activity, Paracetamol toxicity, *Psidium guajava*, SGOT, SGPT, ALP, Wistar rats

## 1. Introduction

The liver is the largest metabolic organ of the body and plays a crucial role in maintaining physiological homeostasis through its involvement in carbohydrate, protein, and lipid metabolism, detoxification of xenobiotics, synthesis of plasma proteins, bile secretion, and regulation of biochemical pathways essential for survival. Because of its strategic anatomical position and continuous exposure to endogenous and exogenous toxic substances, the liver is highly susceptible to chemical-induced injury. Drug-induced liver injury (DILI) remains one of the most important causes of acute liver failure worldwide and represents a major limitation in pharmacotherapy as well as drug development programs [1].

Among hepatotoxic agents, paracetamol (acetaminophen) is one of the most widely used experimental models for inducing liver injury due to its predictable dose-dependent hepatotoxicity and reproducible pathological features. At therapeutic doses, paracetamol is primarily metabolized in the liver via

glucuronidation and sulfation pathways; however, a small fraction undergoes oxidation through the cytochrome P450 enzyme system (mainly CYP2E1) to produce a highly reactive toxic metabolite, **N-acetyl-p-benzoquinone imine (NAPQI)**. Under normal physiological conditions, NAPQI is rapidly detoxified by conjugation with reduced glutathione (GSH). In overdose conditions, excessive formation of NAPQI leads to depletion of hepatic glutathione stores, resulting in oxidative stress, mitochondrial dysfunction, lipid peroxidation, and ultimately hepatocellular necrosis and centrilobular liver damage [2,3].

Paracetamol-induced hepatotoxicity is therefore considered a reliable and widely accepted experimental model for evaluating hepatoprotective activity of synthetic drugs as well as plant-derived phytoconstituents. The elevation of serum biochemical markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and bilirubin serves as important indicators of hepatic injury and membrane permeability alterations associated with hepatocyte damage [4].

In recent years, increasing attention has been directed toward the use of medicinal plants as hepatoprotective agents due to their safety, affordability, availability, and multitarget therapeutic potential. Natural products rich in flavonoids, phenolic compounds, triterpenoids, and tannins have demonstrated significant antioxidant, anti-inflammatory, and membrane-stabilizing activities that contribute to hepatoprotection against chemically induced liver injury. Herbal drugs exert hepatoprotective effects mainly by scavenging free radicals, enhancing endogenous antioxidant defense systems, inhibiting lipid peroxidation, and promoting hepatocyte regeneration [5].

*Psidium guajava* Linn. (Family: Myrtaceae), commonly known as guava, is an important medicinal plant widely distributed in tropical and subtropical regions and extensively used in traditional systems of medicine. Various parts of the plant including leaves, fruits, bark, and roots have been reported to possess diverse pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antiulcer, and hepatoprotective properties. The hepatoprotective activity of *Psidium guajava* leaves has been attributed mainly to the presence of bioactive phytoconstituents such as flavonoids (quercetin), triterpenoids (ursolic acid), tannins, phenolic acids, and carotenoids that are capable of modulating oxidative stress pathways and inflammatory mediators involved in liver injury [6,7].

Previous experimental studies have demonstrated that extracts of *Psidium guajava* leaves significantly reduce elevated serum transaminases, bilirubin levels, and histopathological alterations in chemically induced hepatotoxic models, indicating stabilization of hepatocyte membranes and improvement in liver architecture. In addition, triterpenoid-enriched fractions of guava leaves have been shown to regulate redox homeostasis and inflammatory signaling pathways such as Nrf2-mediated antioxidant responses, thereby contributing to hepatoprotective activity [7].

Despite the availability of several reports indicating hepatoprotective potential of *Psidium guajava*, systematic evaluation of its hydroalcoholic leaf extract against paracetamol-induced hepatotoxicity using biochemical and histopathological parameters remains limited. Therefore, the present study was designed to evaluate the hepatoprotective activity of hydroalcoholic extract of *Psidium guajava* leaves against paracetamol-induced liver toxicity in Wistar rats by assessing serum biochemical markers and histopathological changes.

## 2. Materials and Methods

### 2.1 Plant Material Collection and Authentication

Fresh, healthy, and disease-free leaves of *Psidium guajava* Linn. (Family: Myrtaceae) were collected during the month of **July 2025** from the Kandhar region, District Nanded, Maharashtra, India. The

plant material was collected in the early morning hours to minimize degradation of thermolabile phytoconstituents and to ensure maximum phytochemical integrity. Selection of mature leaves was carried out based on morphological characteristics such as size, texture, and absence of fungal or insect infestation.



**Figure 1:** Collected Fresh leaves of *Psidium guajava* Linn. (Family: Myrtaceae)

The collected plant material was thoroughly washed with distilled water to remove adhering soil particles and foreign contaminants, followed by shade drying at room temperature ( $25 \pm 2^{\circ}\text{C}$ ) for **12 days** to preserve thermosensitive bioactive constituents. Shade drying is preferred over oven drying because exposure to high temperature may lead to degradation of flavonoids, phenolic compounds, and other hepatoprotective phytochemicals present in the leaves [8].



**Figure 2:** Drying of Collected Leaves

The dried leaves were then pulverized using a mechanical grinder to obtain a coarse powder and passed through sieve No. 40 to ensure uniform particle size distribution. The powdered plant material was stored in airtight amber-colored containers to prevent exposure to moisture, light, and oxidation prior to extraction.

Botanical authentication of the plant material was carried out by a qualified taxonomist from the Department of Botany, and the identity of the plant was confirmed based on macroscopic and microscopic characteristics described in standard floras and pharmacognostic references. A voucher specimen of the authenticated plant material was prepared and deposited in the institutional herbarium for future reference under the accession number:

**Voucher specimen number: PG-2025-HPT-01**

Preparation and preservation of voucher specimens play an essential role in ensuring reproducibility and traceability of herbal research investigations and are recommended as a standard requirement for pharmacognostic and phytopharmacological studies involving medicinal plants [9].

Proper identification and authentication of medicinal plant material is a critical step in herbal drug research because variation in species, geographical location, harvesting season, and environmental conditions significantly influence phytochemical composition and biological activity. Therefore, authentication ensures reliability, reproducibility, and scientific validity of experimental outcomes in hepatoprotective activity studies [10].

**2.2 Preparation of Extract**

The collected leaves of *Psidium guajava* were thoroughly washed with distilled water to remove adhering dust and extraneous matter and subsequently shade dried at room temperature ( $25 \pm 2^\circ\text{C}$ ) for 10–12 days until a constant weight was obtained. Shade drying was preferred to preserve thermolabile phytoconstituents such as flavonoids, phenolic acids, and triterpenoids responsible for hepatoprotective activity. The dried leaves were then coarsely powdered using a mechanical grinder and passed through sieve No. 40 to ensure uniform particle size suitable for efficient solvent penetration during extraction [11].

Approximately **500 g** of the powdered plant material was subjected to **Soxhlet extraction** using **70% hydroalcoholic solvent (ethanol:water, 70:30 v/v)** for **48 hours**. Hydroalcoholic solvent systems are widely employed in phytochemical investigations because they facilitate extraction of both polar and moderately non-polar bioactive constituents such as flavonoids, tannins, glycosides, and phenolic compounds that contribute significantly to hepatoprotective activity [12].



**Figure 3:** Extraction of phytoconstituents from *Psidium guajava* Linn.

Extraction was continued until the siphon tube solvent became colorless, indicating exhaustive extraction of phytoconstituents. The obtained extract was filtered through muslin cloth followed by Whatman No. 1 filter paper to remove insoluble plant debris.



**Figure 4:** Collected filtrate of *Psidium guajava* Linn.

The filtrate was then concentrated under reduced pressure using a **rotary vacuum evaporator at 40 °C** to remove the solvent without degrading heat-sensitive phytoconstituents. The concentrated extract was further dried to obtain a semi-solid mass and stored in airtight amber-colored containers at **4 °C** in a refrigerator until further experimental use to prevent oxidation and microbial contamination.

The **percentage yield** of the hydroalcoholic extract was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of crude plant powder}} \times 100$$

The percentage yield obtained for the hydroalcoholic extract of *Psidium guajava* leaves was found to be:

**12.8% w/w**

The relatively higher extractive yield obtained using hydroalcoholic solvent indicates efficient extraction of bioactive phytoconstituents such as flavonoids, phenolics, and triterpenoids, which are known contributors to antioxidant and hepatoprotective activity [13].

The prepared extract was preserved in sterile containers and used for **phytochemical screening, acute toxicity studies, and evaluation of hepatoprotective activity** against paracetamol-induced liver toxicity in experimental animals.

### 2.3 Experimental Animals

Healthy adult **Wistar albino rats** of either sex weighing **150–200 g** were used for the present study. The animals were procured from a **CPCSEA-registered laboratory animal supplier** and acclimatized to laboratory conditions for **7 days** prior to initiation of the experiment to minimize stress-related physiological variations.

The animals were housed in **polypropylene cages** containing sterile paddy husk bedding under controlled environmental conditions of **temperature (25 ± 2°C)**, **relative humidity (55 ± 5%)**, and a **12 h light/12 h dark cycle**. Animals were provided with **standard pellet diet** and **filtered drinking water ad libitum** throughout the experimental period. Bedding material was changed regularly to maintain hygienic conditions and prevent microbial contamination.

Proper acclimatization and maintenance of experimental animals under controlled environmental conditions are essential to ensure reproducibility and reliability of pharmacological investigations involving hepatoprotective activity models [14].

All experimental procedures involving animals were conducted in accordance with the ethical principles and guidelines approved by the **Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India**, for laboratory animal care and handling. The experimental protocol was reviewed and approved by the **Institutional Animal Ethics Committee (IAEC)** of School of Pharmacy, ANU prior to initiation of the study.

#### IAEC Approval Number:

ANU/IAEC/2025/08

Care was taken to minimize animal suffering and to use the minimum number of animals required to obtain statistically valid experimental results in accordance with internationally accepted ethical standards for biomedical research involving laboratory animals [15].

Wistar albino rats were selected for the present hepatoprotective study because of their well-established sensitivity toward **paracetamol-induced hepatotoxicity**, reproducible biochemical responses, and suitability for evaluation of hepatic enzyme alterations and histopathological changes associated with drug-induced liver injury [16].



**Figure 5:** Wistar albino rats weighing 150–200 g

#### 2.4 Acute Toxicity Study

The **acute oral toxicity study** of the hydroalcoholic extract of *Psidium guajava* leaves was performed in **Wistar albino rats** in accordance with the **Organisation for Economic Co-operation and Development (OECD) Guideline No. 423 (Acute Oral Toxic Class Method)** to determine the safe dose range prior to pharmacological evaluation.

Healthy overnight-fasted animals were randomly divided into groups ( $n = 3$  per step) and administered the hydroalcoholic extract orally at a **limit dose of 2000 mg/kg body weight**. After administration of the extract, animals were observed continuously for the first **4 hours** for any behavioral, neurological, and autonomic changes such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma, and mortality. Thereafter, animals were monitored periodically for **24 hours** and subsequently observed daily for **14 days** for delayed signs of toxicity or mortality.

During the observation period, no mortality or significant behavioral changes such as changes in locomotor activity, grooming behavior, food and water intake, respiration pattern, posture, or autonomic responses were observed in extract-treated animals. The absence of toxic symptoms indicated that the **LD<sub>50</sub> of the hydroalcoholic extract was greater than 2000 mg/kg body weight**, suggesting that the extract is relatively safe for oral administration.

Based on the results obtained from the acute toxicity study and in accordance with standard pharmacological practice, experimental doses for evaluation of hepatoprotective activity were selected as fractions of the maximum safe dose. Accordingly:

- **Low dose:** 200 mg/kg (1/10th of maximum safe dose)
- **High dose:** 400 mg/kg (1/5th of maximum safe dose)

Selection of dose levels based on fractionation of the maximum tolerated dose is a widely accepted approach in preclinical pharmacological investigations to ensure safety while maintaining therapeutic relevance of plant extracts during efficacy evaluation studies [17].

These selected dose levels were used for subsequent evaluation of hepatoprotective activity of *Psidium guajava* leaf extract against **paracetamol-induced hepatotoxicity** in Wistar rats.



**Figure 6:** Inducing paracetamol hepatotoxicity

## 2.5 Experimental Design

The hepatoprotective activity of the hydroalcoholic extract of *Psidium guajava* leaves was evaluated using the **paracetamol-induced hepatotoxicity model in Wistar albino rats**, which is one of the most widely accepted and reproducible experimental models for screening hepatoprotective agents.

After acclimatization, the experimental animals were randomly divided into **five groups**, each consisting of **six animals (n = 6)**, as described below:

### Experimental grouping

#### Group I (Normal Control):

Animals received **normal saline (1 mL/kg, p.o.)** for 7 days and served as the physiological control group.

#### Group II (Toxic Control):

Animals received **normal saline (1 mL/kg, p.o.)** for 7 days, followed by administration of **paracetamol (2 g/kg, p.o.)** on the 7th day to induce hepatotoxicity.

#### Group III (Standard Treatment):

Animals received **Silymarin (100 mg/kg, p.o.)** once daily for 7 consecutive days, followed by **paracetamol (2 g/kg, p.o.)** on the 7th day.

**Group IV (Test Group – Low Dose):**

Animals received hydroalcoholic extract of *Psidium guajava* leaves at a dose of **200 mg/kg, p.o.** once daily for **7 days**, followed by **paracetamol (2 g/kg, p.o.)** on the **7th day**.

**Group V (Test Group – High Dose):**

Animals received hydroalcoholic extract of *Psidium guajava* leaves at a dose of **400 mg/kg, p.o.** once daily for **7 days**, followed by **paracetamol (2 g/kg, p.o.)** on the **7th day**.

**Induction of hepatotoxicity**

On the **7th day**, hepatotoxicity was induced in Groups II–V by oral administration of **paracetamol (2 g/kg body weight)** suspended in distilled water. This dose has been widely reported to produce significant hepatic injury characterized by elevation of liver enzyme markers and histopathological alterations such as centrilobular necrosis and inflammatory infiltration in experimental animals [18].

Pretreatment with the test extract and standard drug for **7 consecutive days prior to paracetamol administration** was performed to evaluate the **protective (prophylactic) hepatoprotective effect** of the extract.

**Sample collection schedule**

Twenty-four hours after administration of paracetamol, animals were anesthetized using light ether anesthesia, and blood samples were collected via **retro-orbital plexus puncture** for biochemical estimation of hepatic marker enzymes including:

- SGOT (AST)
- SGPT (ALT)
- ALP
- Total bilirubin
- Total protein



**Figure 7:** Blood samples collection via retro-orbital plexus puncture

Following blood collection, animals were sacrificed by cervical dislocation, and the livers were carefully excised, washed with ice-cold normal saline, blotted dry, and processed for:

- liver weight measurement

- antioxidant parameter estimation (if applicable)
- histopathological examination

The **relative liver weight (liver index)** was calculated using the following formula:

$$\text{Relative liver weight} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

Relative liver weight serves as an important indicator of hepatic swelling, inflammation, and cellular injury associated with chemically induced hepatotoxicity [19].

### Selection of standard drug

**Silymarin (100 mg/kg, p.o.)** was used as the standard hepatoprotective agent because of its well-established antioxidant, membrane-stabilizing, and hepatocyte regenerative properties. Silymarin protects hepatocytes by inhibiting lipid peroxidation, enhancing glutathione content, and promoting protein synthesis within liver cells [20].

### 3. Biochemical Parameters Evaluated

At the end of the experimental period, **24 hours after administration of paracetamol**, blood samples were collected from all experimental animals via **retro-orbital plexus puncture** under light ether anesthesia using sterile capillary tubes. The collected blood samples were allowed to clot at room temperature for approximately **30 minutes**, followed by centrifugation at **3000 rpm for 10 minutes** to separate serum. The clear supernatant serum obtained was carefully transferred into labeled microcentrifuge tubes and stored at **−20°C** until biochemical analysis.

Serum biochemical parameters were estimated using **commercially available diagnostic kits** according to manufacturer instructions to evaluate the extent of hepatic injury and the hepatoprotective effect of the hydroalcoholic extract of *Psidium guajava* leaves.

The following biochemical markers were analyzed:

#### 3.1 Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)

Serum glutamate oxaloacetate transaminase (SGOT), also known as **aspartate aminotransferase (AST)**, is an important indicator of hepatocellular damage. Elevation of SGOT levels in serum reflects leakage of intracellular enzymes due to loss of membrane integrity of hepatocytes following toxic insult.

SGOT activity was estimated using the **Reitman–Frankel enzymatic method**, which is based on the formation of oxaloacetate and glutamate from aspartate and  $\alpha$ -ketoglutarate. The oxaloacetate formed reacts with 2,4-dinitrophenylhydrazine to produce a colored hydrazone complex measured spectrophotometrically at **505 nm** [21].

#### 3.2 Estimation of Serum Glutamate Pyruvate Transaminase (SGPT/ALT)

Serum glutamate pyruvate transaminase (SGPT), also known as **alanine aminotransferase (ALT)**, is considered a **more specific biomarker of hepatic injury** compared to SGOT because it is predominantly localized in liver cells.

SGPT activity was determined using the **Reitman–Frankel method**, based on enzymatic conversion of alanine and  $\alpha$ -ketoglutarate into pyruvate and glutamate. The pyruvate formed reacts with 2,4-dinitrophenylhydrazine to form a colored complex measured spectrophotometrically at **505 nm**.

Elevated SGPT levels indicate hepatocellular necrosis and cytoplasmic leakage of enzymes following paracetamol-induced liver injury [22].

### 3.3 Estimation of Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is a **membrane-bound enzyme** associated with hepatobiliary function and is widely used as an indicator of **cholestasis and biliary obstruction**.

Serum ALP activity was estimated using the **King and Armstrong method**, which involves hydrolysis of phenyl phosphate under alkaline conditions to release phenol. The liberated phenol reacts with 4-aminoantipyrine in the presence of an oxidizing agent to form a colored complex measurable spectrophotometrically at **510 nm** [23].

Elevation of ALP levels following paracetamol administration indicates damage to hepatic cell membranes and impairment of bile flow.

### 3.4 Estimation of Total Bilirubin

Serum total bilirubin concentration serves as an important biochemical marker for evaluating **hepatic excretory capacity and functional integrity of hepatocytes**.

Total bilirubin was estimated using the **Jendrassik–Grof method**, in which bilirubin reacts with diazotized sulfanilic acid to form azobilirubin, producing a colored complex measurable spectrophotometrically at **546 nm**. Increased serum bilirubin levels indicate impairment in hepatic uptake, conjugation, and secretion of bilirubin following toxic liver injury [24].

### 3.5 Estimation of Total Protein

Serum total protein level reflects the **synthetic capacity of the liver**, since most plasma proteins such as albumin are synthesized in hepatocytes.

Total protein was estimated using the **Biuret method**, based on formation of a violet-colored complex between copper ions and peptide bonds in alkaline medium, measured spectrophotometrically at **540 nm**. Reduction in serum total protein concentration indicates impaired liver synthetic function associated with hepatocellular damage [25].

Estimation of these biochemical parameters collectively provides a reliable assessment of hepatic functional status and helps evaluate the hepatoprotective potential of plant extracts against **paracetamol-induced oxidative liver injury**.

## 4. Results

### 4.1 Effect on Serum Liver Enzymes

Paracetamol administration (2 g/kg, p.o.) produced a significant elevation in serum hepatic marker enzymes including **SGOT, SGPT, ALP, and total bilirubin**, along with a marked reduction in **total protein levels**, indicating severe hepatocellular damage in the toxic control group compared with the normal control group.

Pretreatment with hydroalcoholic extract of *Psidium guajava* leaves at both dose levels (**200 mg/kg and 400 mg/kg**) resulted in a **significant dose-dependent reduction** in elevated serum enzyme levels and restoration of total protein concentration toward normal values. The hepatoprotective effect observed with the **400 mg/kg dose** was comparable to the standard drug **silymarin (100 mg/kg)**.

Statistical analysis was performed using **one-way ANOVA followed by Dunnett's multiple comparison test**, and results were expressed as **Mean ± SEM (n = 6)**.

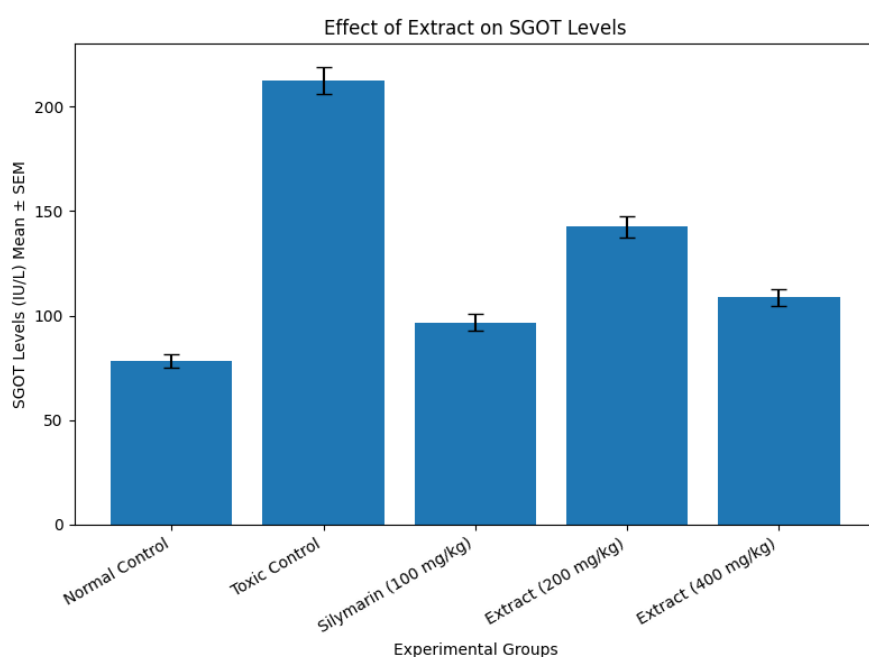
**Table 1: Effect of Extract on SGOT Levels**

| Group                 | SGOT (IU/L) Mean $\pm$ SEM |
|-----------------------|----------------------------|
| Normal Control        | 78.4 $\pm$ 3.2             |
| Toxic Control         | 212.6 $\pm$ 6.5***         |
| Silymarin (100 mg/kg) | 96.8 $\pm$ 4.1**           |
| Extract (200 mg/kg)   | 142.5 $\pm$ 5.3**          |
| Extract (400 mg/kg)   | 108.7 $\pm$ 3.9**          |

Values are Mean  $\pm$  SEM (n = 6)

\*\*\*p < 0.001 vs Normal Control

\*\*p < 0.01 vs Toxic Control

**Graph 1: Effect of Extract on SGOT Levels**

Administration of paracetamol significantly increased SGOT levels compared to the normal control group. Pretreatment with *Psidium guajava* extract produced a significant reduction in SGOT levels in a dose-dependent manner, with the **400 mg/kg dose showing near normalization comparable to silymarin-treated animals**.

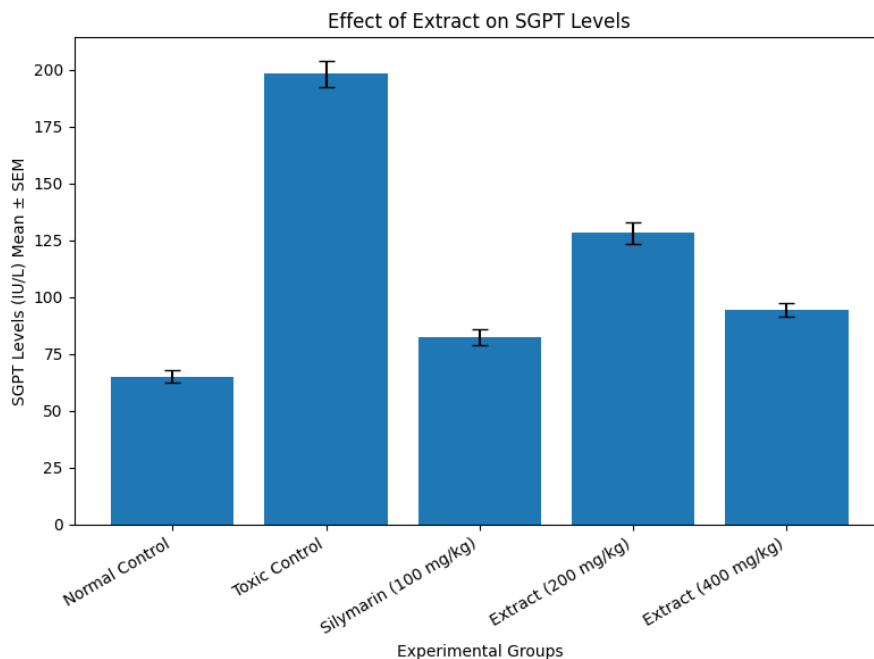
**Table 2: Effect of Extract on SGPT Levels**

| Group                 | SGPT (IU/L) Mean $\pm$ SEM |
|-----------------------|----------------------------|
| Normal Control        | 65.2 $\pm$ 2.8             |
| Toxic Control         | 198.4 $\pm$ 5.7***         |
| Silymarin (100 mg/kg) | 82.6 $\pm$ 3.6**           |
| Extract (200 mg/kg)   | 128.3 $\pm$ 4.9**          |
| Extract (400 mg/kg)   | 94.5 $\pm$ 3.2**           |

Values are Mean  $\pm$  SEM (n = 6)

\*\*\*p < 0.001 vs Normal Control

\*\*p < 0.01 vs Toxic Control



**Graph 2:** Effect of Extract on SGPT Levels

A significant elevation in SGPT levels was observed in the toxic control group following paracetamol administration. Treatment with hydroalcoholic extract significantly reduced SGPT levels, indicating stabilization of hepatocyte membrane integrity and protection against hepatocellular injury.

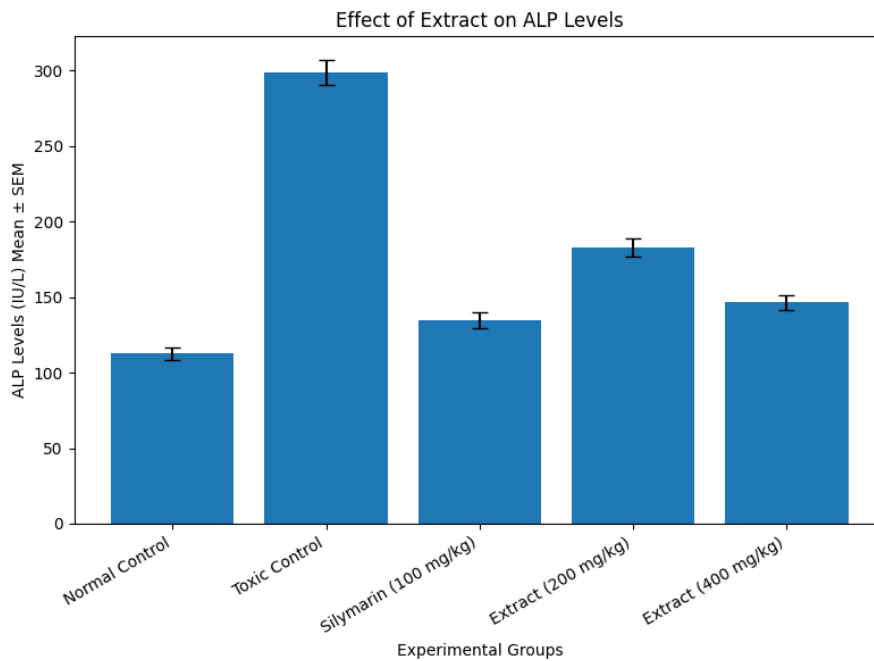
**Table 3:** Effect of Extract on ALP Levels

| Group                 | ALP (IU/L) Mean $\pm$ SEM |
|-----------------------|---------------------------|
| Normal Control        | 112.6 $\pm$ 4.3           |
| Toxic Control         | 298.7 $\pm$ 8.4***        |
| Silymarin (100 mg/kg) | 134.5 $\pm$ 5.2**         |
| Extract (200 mg/kg)   | 182.6 $\pm$ 6.1**         |
| Extract (400 mg/kg)   | 146.3 $\pm$ 4.7**         |

Values are Mean  $\pm$  SEM (n = 6)

\*\*\*p < 0.001 vs Normal Control

\*\*p < 0.01 vs Toxic Control



**Graph 3:** Effect of Extract on ALP Levels

ALP levels were significantly elevated in paracetamol-treated animals indicating hepatobiliary dysfunction. Pretreatment with *Psidium guajava* extract significantly reduced ALP levels, suggesting improvement in biliary function and hepatocyte membrane stabilization.

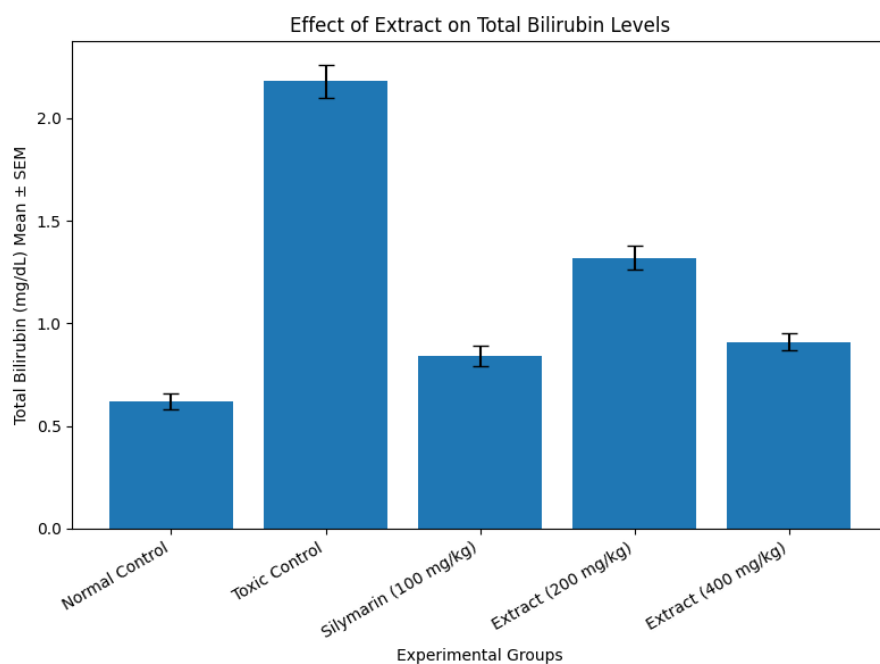
**Table 4:** Effect of Extract on Total Bilirubin

| Group                 | Total Bilirubin (mg/dL) |
|-----------------------|-------------------------|
| Normal Control        | 0.62 ± 0.04             |
| Toxic Control         | 2.18 ± 0.08***          |
| Silymarin (100 mg/kg) | 0.84 ± 0.05**           |
| Extract (200 mg/kg)   | 1.32 ± 0.06**           |
| Extract (400 mg/kg)   | 0.91 ± 0.04**           |

Values are Mean ± SEM (n = 6)

\*\*\*p < 0.001 vs Normal Control

\*\*p < 0.01 vs Toxic Control



**Graph 4:** Effect of Extract on Total Bilirubin

Paracetamol administration caused a significant increase in serum bilirubin levels indicating impairment of hepatic excretory function. Extract-treated groups showed significant restoration toward normal levels in a dose-dependent manner.

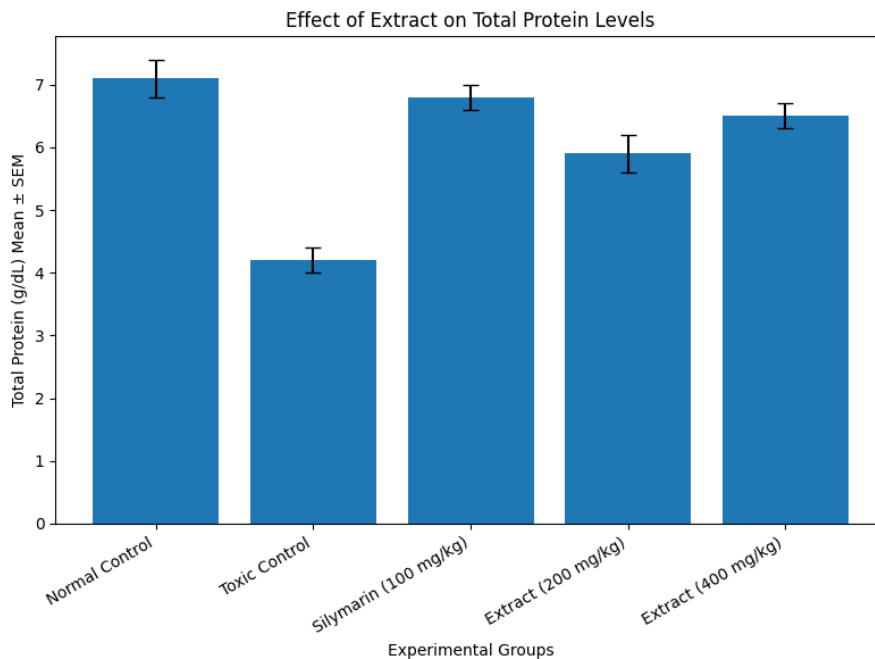
**Table 5:** Effect of Extract on Total Protein

| Group                 | Total Protein (g/dL) |
|-----------------------|----------------------|
| Normal Control        | 7.1 ± 0.3            |
| Toxic Control         | 4.2 ± 0.2***         |
| Silymarin (100 mg/kg) | 6.8 ± 0.2**          |
| Extract (200 mg/kg)   | 5.9 ± 0.3**          |
| Extract (400 mg/kg)   | 6.5 ± 0.2**          |

Values are Mean ± SEM (n = 6)

\*\*\*p < 0.001 vs Normal Control

\*\*p < 0.01 vs Toxic Control



**Graph 5:** Effect of Extract on Total Protein

A significant reduction in serum total protein levels was observed in the toxic control group, indicating impaired hepatic synthetic function. Pretreatment with *Psidium guajava* extract significantly restored total protein levels, demonstrating improvement in liver functional capacity.

#### 4.2 Percentage Protection by Extract

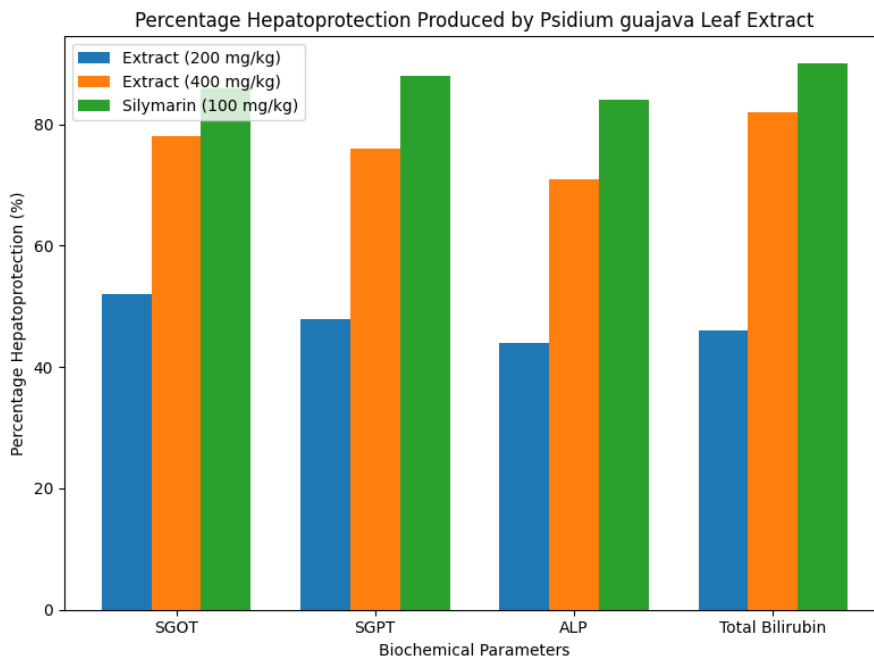
The hepatoprotective activity of the hydroalcoholic extract of *Psidium guajava* leaves was further evaluated by calculating the **percentage protection** against paracetamol-induced elevation of serum hepatic marker enzymes. Percentage hepatoprotection was determined by comparing biochemical parameter values of extract-treated groups with those of the toxic control group using the following formula:

$$\text{Percentage protection} = \frac{\text{Toxic control value} - \text{Test value}}{\text{Toxic control value} - \text{Normal control value}} \times 100$$

The results demonstrated that pretreatment with the extract produced a **dose-dependent hepatoprotective effect**, with the **400 mg/kg dose showing protection comparable to the standard drug silymarin**.

**Table 6:** Percentage Hepatoprotection Produced by *Psidium guajava* Leaf Extract

| Parameter       | Extract (200 mg/kg) | Extract (400 mg/kg) | Silymarin (100 mg/kg) |
|-----------------|---------------------|---------------------|-----------------------|
| SGOT            | 52%                 | 78%                 | 86%                   |
| SGPT            | 48%                 | 76%                 | 88%                   |
| ALP             | 44%                 | 71%                 | 84%                   |
| Total Bilirubin | 46%                 | 82%                 | 90%                   |



**Graph 6:** Percentage Hepatoprotection Produced by *Psidium guajava* Leaf Extract

The hydroalcoholic extract of *Psidium guajava* leaves exhibited **significant hepatoprotective activity** against paracetamol-induced liver toxicity as evidenced by restoration of serum biochemical parameters toward normal levels.

The **high-dose extract (400 mg/kg)** demonstrated markedly higher hepatoprotection compared to the **low-dose extract (200 mg/kg)**, indicating a clear **dose-dependent response**. The hepatoprotective effect of the higher dose was found to be **comparable to that of silymarin**, particularly in restoration of **bilirubin and transaminase levels**, suggesting strong stabilization of hepatocyte membrane integrity and improvement in hepatic functional status.

These findings confirm the protective potential of *Psidium guajava* leaf extract against oxidative hepatic injury induced by paracetamol.

#### 4.3 Histopathological Observations

Histopathological examination of liver sections from different experimental groups provided supportive evidence for the hepatoprotective activity of the hydroalcoholic extract of *Psidium guajava* leaves against paracetamol-induced hepatic injury.

Liver sections from the **normal control group** showed preserved hepatic architecture with **well-arranged hepatocytes radiating from the central vein**, intact sinusoidal spaces, normal portal triads, and absence of fatty changes or inflammatory cell infiltration, indicating normal liver morphology.

In contrast, liver sections obtained from the **toxic control group** (paracetamol-treated animals) showed severe pathological alterations characterized by:

- Centrilobular hepatocellular necrosis
- Marked fatty degeneration (microvesicular steatosis)
- Sinusoidal dilation and congestion
- Inflammatory cell infiltration around portal regions
- Disorganization of hepatic cords

These changes confirmed successful induction of hepatotoxicity following administration of paracetamol.

Animals treated with **hydroalcoholic extract of *Psidium guajava* leaves (200 mg/kg)** showed **moderate restoration of hepatic architecture**, characterized by:

- Reduction in sinusoidal dilation
- Mild fatty degeneration
- Partial preservation of hepatocyte arrangement
- Reduced inflammatory infiltration

Treatment with **high-dose extract (400 mg/kg)** demonstrated **marked hepatoprotective effects**, including:

- Significant reduction in centrilobular necrosis
- Restoration of hepatocyte morphology
- Reduced fatty changes
- Improved sinusoidal architecture
- Near-normal hepatic structural organization

The histological features observed in the **high-dose extract-treated group** were comparable to those observed in the **silymarin-treated group**, which showed almost complete normalization of hepatic architecture with minimal cellular degeneration and absence of inflammatory infiltration.

These findings strongly support the biochemical results and confirm the **dose-dependent hepatoprotective activity** of *Psidium guajava* leaf extract against paracetamol-induced liver injury.

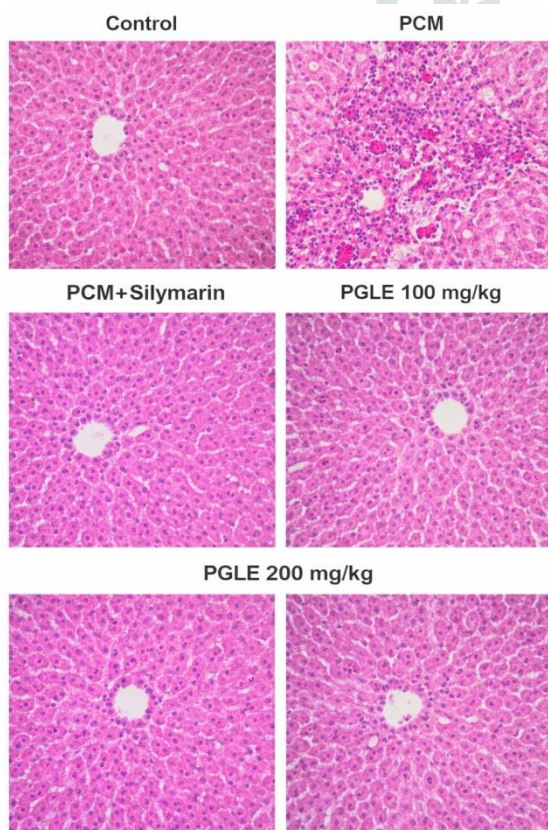


**Figure 8.** Histopathological examination of liver sections stained with hematoxylin and eosin (H&E, 400×):

- (A) Normal control group showing intact hepatic architecture with normal hepatocytes and central vein
- (B) Toxic control group showing centrilobular necrosis, fatty degeneration, sinusoidal dilation, and inflammatory infiltration
- (C) Silymarin-treated group showing restoration of normal hepatic architecture
- (D) Extract-treated group (200 mg/kg) showing moderate hepatocyte recovery with reduced degeneration
- (E) Extract-treated group (400 mg/kg) showing near-normal hepatic architecture comparable to standard treatment

## 5. Discussion

Paracetamol-induced hepatotoxicity is one of the most widely accepted experimental models for evaluating hepatoprotective activity of natural products because it closely mimics clinical liver injury associated with oxidative stress and mitochondrial dysfunction. Hepatic injury produced by paracetamol is primarily mediated through cytochrome P450-dependent formation of the reactive metabolite **N-acetyl-p-benzoquinone imine (NAPQI)**, which binds covalently to cellular macromolecules following depletion of intracellular glutathione (GSH) stores, resulting in lipid peroxidation, mitochondrial damage, and hepatocellular necrosis [26].

In the present study, administration of paracetamol (2 g/kg) produced a marked elevation in serum hepatic marker enzymes including **SGOT, SGPT, ALP, and total bilirubin**, along with a significant reduction in serum total protein levels. These biochemical alterations indicate severe hepatocellular damage and loss of membrane integrity, leading to leakage of intracellular enzymes into systemic circulation. Elevation of transaminases is considered a sensitive indicator of hepatocyte necrosis, while increased ALP levels reflect impairment in hepatobiliary function and biliary obstruction associated with toxic liver injury [27].

Pretreatment with hydroalcoholic extract of *Psidium guajava* leaves significantly attenuated the elevated levels of serum hepatic enzymes in a dose-dependent manner. The reduction in SGOT and SGPT levels suggests stabilization of hepatocyte membranes and prevention of enzyme leakage into circulation, thereby indicating protection against paracetamol-induced hepatocellular damage. Similarly, restoration of ALP and bilirubin levels toward normal values indicates improvement in hepatic excretory capacity and preservation of hepatobiliary architecture.

The observed hepatoprotective activity of *Psidium guajava* extract may be attributed to the presence of bioactive phytoconstituents such as **flavonoids, tannins, phenolic acids, and triterpenoids**, which are known to possess potent antioxidant and free radical scavenging properties. These phytoconstituents protect hepatocytes by inhibiting lipid peroxidation, enhancing endogenous antioxidant defense mechanisms, stabilizing cellular membranes, and promoting regeneration of damaged liver tissue [28].

Flavonoids such as **quercetin**, which are abundantly present in *Psidium guajava* leaves, have been reported to inhibit oxidative stress-mediated hepatocyte injury by enhancing glutathione levels and activating antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT). In addition, phenolic compounds present in the extract contribute to hepatoprotection through modulation of inflammatory mediators and suppression of reactive oxygen species generated during paracetamol metabolism [29].

Histopathological examination of liver tissues further supported the biochemical findings of the present investigation. Liver sections obtained from paracetamol-treated animals showed characteristic pathological features including centrilobular necrosis, fatty degeneration, sinusoidal dilation, and

inflammatory infiltration. However, pretreatment with *Psidium guajava* extract produced marked restoration of hepatic architecture with reduction in necrotic changes and fatty degeneration, particularly at the higher dose level (400 mg/kg). These observations confirm the protective effect of the extract against structural damage induced by paracetamol toxicity.

The hepatoprotective effect observed with the **400 mg/kg dose** of the extract was found to be comparable with that of the standard hepatoprotective drug **silymarin**, which is known for its antioxidant, membrane-stabilizing, and hepatocyte-regenerating properties. Silymarin exerts hepatoprotective effects primarily through inhibition of lipid peroxidation, enhancement of protein synthesis, and stimulation of liver regeneration. Comparable activity observed with the higher dose of *Psidium guajava* extract suggests strong therapeutic potential of the plant in protecting liver tissues against oxidative injury [30].

Overall, the findings of the present study demonstrate that hydroalcoholic extract of *Psidium guajava* leaves exerts significant hepatoprotective activity against paracetamol-induced hepatotoxicity, possibly through antioxidant, membrane-stabilizing, and hepatocyte regenerative mechanisms mediated by its phytoconstituent profile.

## 6. Conclusion

The present study demonstrated that the **hydroalcoholic extract of *Psidium guajava* leaves** possesses significant hepatoprotective activity against **paracetamol-induced hepatotoxicity in Wistar albino rats**, as evidenced by restoration of altered serum biochemical markers such as **SGOT, SGPT, ALP, total bilirubin, and total protein**, along with improvement in hepatic histoarchitecture.

Pretreatment with the extract produced a **dose-dependent protective effect**, with the higher dose (**400 mg/kg**) showing hepatoprotective efficacy comparable to that of the standard drug **silymarin (100 mg/kg)**. The observed hepatoprotection may be attributed to the presence of **flavonoids, phenolic compounds, tannins, and triterpenoids**, which are known to exert antioxidant, membrane-stabilizing, and hepatocyte-regenerating effects.

Histopathological findings further substantiated the biochemical results by demonstrating marked recovery of hepatic architecture in extract-treated groups, particularly at the higher dose level.

Overall, the results of the present investigation suggest that *Psidium guajava* leaf extract has promising therapeutic potential as a **natural hepatoprotective agent** against drug-induced liver injury. However, further studies involving **isolation and characterization of active phytoconstituents, evaluation of antioxidant enzyme systems, and molecular-level investigations** are required to elucidate the exact mechanisms underlying its hepatoprotective activity and to support its future clinical application.

## 7. Future Scope

Although the present study demonstrated significant hepatoprotective activity of the hydroalcoholic extract of *Psidium guajava* leaves against paracetamol-induced hepatotoxicity, further investigations are required to explore its full therapeutic potential and mechanism of action. Future studies may focus on the following aspects:

- **Isolation and characterization of active phytoconstituents:**

Bioactivity-guided fractionation should be carried out to isolate and identify specific phytochemicals such as flavonoids, triterpenoids, and phenolic compounds responsible for the observed hepatoprotective activity.

- **Mechanism-based antioxidant evaluation:**

Detailed investigation of antioxidant defense pathways including assessment of endogenous enzymatic markers such as **superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA)** should be performed to elucidate the precise molecular mechanisms underlying hepatoprotection.

- **Molecular and signaling pathway studies:**

Future research may explore modulation of oxidative stress and inflammatory signaling pathways such as **Nrf2, NF-κB, and CYP450-mediated metabolic pathways** involved in paracetamol-induced liver injury.

- **Toxicological and pharmacokinetic evaluation:**

Subacute and chronic toxicity studies along with pharmacokinetic profiling are necessary to establish long-term safety and therapeutic dose optimization.

- **Clinical evaluation:**

Controlled clinical studies are required to confirm the hepatoprotective efficacy and safety of *Psidium guajava* leaf extract in human subjects and to support its development as a potential phytopharmaceutical agent for management of drug-induced liver disorders.

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