# Evaluation Of Hepatoprotective Activity Of Grevillea Robusta Leaf Extract In Experimental Animal Models

**Santosh Yadav[1], Aaditya Singh[1], Rahul Kumar Sharma[1]**

1. Aryakul College College Of Pharmacy Gauri, Natkur, Po: Chandrawal, Bijnor Rd, near CRPF Base Camp, Sarojini Nagar, Lucknow, Uttar Pradesh 226002.
Email - santoshyadav2735@gmail.com, Email - rahul.pcol@gmail.com

***Corresponding Authour**
**Prof. Aaditya Singh**
Department of Pharmacy,
Aryakul College College Of Pharmacy
Gauri, Natkur, Po: Chandrawal, Bijnor Rd,
near CRPF Base Camp, Sarojini Nagar, Lucknow,
Uttar Pradesh 226002.

Email: aaditysingh85120@gmail.com

**Abstract**

This study explores the hepatoprotective potential of the ethanolic leaf extract of Grevillea robusta in mitigating chemically induced liver damage in experimental animals. Liver disorders, often triggered by oxidative stress and hepatotoxic agents, remain a significant global health concern. Using models of carbon tetrachloride ($CCl_4$)- and paracetamol-induced hepatotoxicity, the extract was evaluated for its effects on biochemical markers, antioxidant enzyme activity, and liver histology.

The findings revealed significant reductions in serum levels of ALT, AST, ALP, and bilirubin in extract-treated groups compared to disease controls, alongside enhanced antioxidant activity (SOD and CAT) and reduced lipid peroxidation (MDA). Histological assessments further confirmed the extract's efficacy, showing improved liver architecture with reduced necrosis and inflammation. These effects are attributed to the presence of bioactive compounds such as polyphenols and flavonoids identified in the extract.

The study concludes that Grevillea robusta holds promise as a natural therapeutic agent for managing liver disorders, providing a foundation for future research into its active constituents and clinical applications.

**Keywords:** Grevillea robusta, hepatoprotective activity, antioxidants, liver injury, medicinal plants, oxidative stress.

## 1. Introduction

The liver plays a central role in the body's metabolism, detoxification, and immune defense, making it one of the most vital organs in maintaining overall health. It is highly susceptible to damage caused by toxins, drugs, infections, and oxidative stress. Liver disorders, including hepatitis, cirrhosis, and fibrosis, represent significant global health challenges. These conditions are frequently induced by reactive oxygen species (ROS), inflammatory processes, and hepatotoxic agents, ultimately leading to liver failure if left untreated (1)**.**

Hepatotoxicity models induced by chemicals such as carbon tetrachloride ($CCl_4$) and paracetamol are widely utilized in research to evaluate the efficacy of potential hepatoprotective agents. These agents typically act by mitigating oxidative stress, enhancing the liver's antioxidant defenses, and reducing inflammation (2)(3)**.**

In recent years, there has been a growing interest in plant-based remedies for liver diseases due to their efficacy, safety, and affordability. Medicinal plants are rich in bioactive compounds such as polyphenols, flavonoids, and terpenoids, which possess antioxidant and anti-inflammatory properties. These phytochemicals have shown promise in protecting liver cells from oxidative damage and facilitating tissue regeneration (4,5)**.**

***Grevillea robusta***, commonly known as the Silky Oak, is a member of the Proteaceae family and native to the eastern regions of Australia. Traditionally known for its ornamental and timber uses, its leaves have recently attracted attention for their potential medicinal properties. Preliminary studies indicate that Grevillea robusta leaves contain phenolic compounds, flavonoids, and tannins, all of which are known for their antioxidant capabilities. Despite its widespread availability, the hepatoprotective properties of Grevillea robusta remain largely unexplored (6,7)**.**

This study aims to investigate the hepatoprotective activity of ethanolic leaf extracts of Grevillea robusta in experimental animal models. By evaluating its effects on biochemical markers of liver function, antioxidant enzyme activity, and histopathological changes, this research seeks to establish the potential of ***Grevillea*** robusta as a natural therapeutic agent for liver disorders (8)**.**

## Materials and Methods

### 1. Plant Material and Extraction

Fresh leaves of *Grevillea robusta* were collected from healthy plants and authenticated by a botanist. The leaves were washed, air-dried under shade, and powdered using a mechSanical grinder. The powdered material was subjected to ethanol extraction using a Soxhlet apparatus. The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C for further use (9).

### 2. Phytochemical Screening

The ethanolic extract of *Grevillea robusta* leaves was analyzed for the presence of phytochemicals, including flavonoids, phenolic compounds, tannins, and alkaloids, using standard qualitative methods (10,11).

### Quantification of Total Phenolic Content (TPC)

The total phenolic content in the hydrochloric fraction of *Grevillea robusta* leaves (GREE) was quantified using the method described by Ahmed et al. (2014). A concentrated GREE solution (1 mg/mL) was prepared and mixed with 3.16 mL of distilled water and 40 µL of Folin–Ciocalteu reagent (FCR). The mixture was incubated in the dark for 8 minutes, after which 600 µL of a 7% sodium carbonate solution was added and mixed thoroughly. The reaction mixture was incubated for 30 minutes at 40°C. The absorbance was measured at 765 nm against a blank. A calibration curve for gallic acid was plotted, and the TPC was expressed as micrograms of gallic acid equivalent (GAE) per milliliter, as described by (12,13).

### Quantification of Total Flavonoid Content (TFC)

The total flavonoid content in GREE was determined using the methodology outlined by Ahmed et al. (2014). Briefly, 301 µL of the GREE solution was mixed with 3.5 mL of methanol (30% concentration), 150 µL of 0.5 M sodium nitrite, and 150 µL of 0.3 M aluminum chloride. After 5 minutes of incubation, 1 mL of 1.0 M sodium hydroxide solution was added, and the mixture was blended thoroughly as per Dirar et al. (2019). Absorbance was measured at 506 nm against a blank. The results were expressed as micrograms of rutin equivalent (RE) per milliliter (14,15).

### 3. GC-MS Analysis

GC-MS analysis of GREE was performed using an Agilent Technologies 7890A GC System coupled with a 5975C inert MSD Detector. An HP-5MS column (31 m × 0.65 mm × 0.45 µm) was employed. The NIST 5 software library was used to analyze the chromatograms and mass spectra. The GC temperature program began at 60°C, increased by 10°C per minute until 310°C, and was held constant for 5 minutes. Helium served as the carrier gas at a flow rate of 1 mL/min, with the injector port set at 200°C. The ionization voltage was 70 eV. The injection was conducted in a splitless mode, and the mass spectra were scanned over a range of 45–500 m/z. The total GC-MS run time was 30 minutes. The identified compounds were compared with known patterns in the NIST library (16,17).

### 4. Antioxidant Activity Assessment: DPPH Radical Scavenging Assay

The antioxidant activity of GREE was evaluated using the DPPH radical scavenging assay, following the protocol of Trinh et al. (2020). A solution was prepared by mixing 3 mL of DPPH solution (with an absorbance of 0.98 at 517 nm) with 100 µL of either GREE or a standard antioxidant (BHT). The mixture was incubated at 37°C for 30 minutes, and absorbance was recorded at 517 nm. The antioxidant activity was calculated using the following formula:

$$\%Activity = \frac{\|A_c - A_s\|}{Ac}[100] \quad (1)$$

Where Ac represents the absorbance of the control (without sample), and As denotes the absorbance of the sample.

The EC50 {50} value was determined using the regression equation Y=0.8357x−2.2379Y = 0.8357x - 2.2379 (18,19).

**5. Experimental Animals**

Wistar albino rats (150–200 g) of either sex were procured and housed under standard laboratory conditions (temperature: 22 ± 2°C; humidity: 55 ± 5%; 12-hour light/dark cycle). The animals were fed a standard pellet diet and provided water ad libitum. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) (20)**.**

**Experimental Design**

Liver damage was induced in experimental animals using two models:

**$CCl_4$ - Induced Hepatotoxicity:** Rats were injected intraperitoneally with $CCl_4$ (1:1 v/v in olive oil) at a dose of 1 mL/kg body weight.

**Paracetamol-Induced Hepatotoxicity:** A single oral dose of paracetamol (2 g/kg body weight) was administered (20,21).

The animals were divided into the following groups (n = 6 per group):
Group I: Normal control
Group II: Disease control ($CCl_4$ or paracetamol).
Group III: Standard treatment (silymarin, 100 mg/kg).
Group IV-VI: Treated with Grevillea robusta extract at doses of 100, 200, and 400 mg/kg body weight, respectively.

| Groups (n=6) | Treatment and route of administration | Dose and duration |
|---|---|---|
| Normal/ control | CCl4 | 10 ml/kg |
| Positive control/ standard | Silymarine | 10 mg/kg for 7 days twice a day |
| Treatment low dose | Aqueous extract of Gravillea robusta | 100 mg/kg for 14 days |
| Moderate dose | Aqueous extract of Gravillea robusta | 200 mg/kg for 14 days |
| Treatment group high dose | Aqueous extract of Gravillea robusta | 400 mg/kg for 14 days |

Total Number of Animals used = 30

**6. Biochemical Analysis**

At the end of the treatment period, blood samples were collected via retro-orbital puncture and serum was separated. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin were measured using standard diagnostic kits (22).

**7. Measurement of Blood Cell Indices**

The blood, which had been treated with an anticoagulant, was examined for blood cell characteristics using a modern cell analyzer from Roche in Switzerland, following the guidelines provided by the manufacturer (23).

**8. Histopathological Examination**

Liver tissues were excised, fixed in 10% formalin, and processed for paraffin embedding. Thin sections were stained with hematoxylin and eosin (H&E) for histological evaluation under a light microscope (24).

**9. Statistical Analysis**

Data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

**Results**

**Phytochemical Screening**

The analysis of GREE revealed the existence of secondary compounds produced by plants, such as phenolic compounds, flavonoids, esters, and organic acids. Table 5.1 displayed a high quantity of 250 ± 30.40 µg of GAE per mg of dried mass for GREE, along with 163 ± 34.67 µg of RE per mg of processed mass. Despite that, the GCMS analysis confirmed the presence of polymers and organic carboxylylated acids, as illustrated in Figure 1 and outlined in Table 1.

**Table 1:** The overall levels of phenolic and flavonoid compounds in GREE

| **The number of phenolic compounds is expressed in micrograms of gallic acid equivalent per milligram of the dried material.** | **The number of flavonoids in micrograms of rutin equivalent per milligram of dried weight.** |
|---|---|
| 240± 25.50 | 141± 63.77 |

The values are expressed as mean ±SD (*n*= 3)

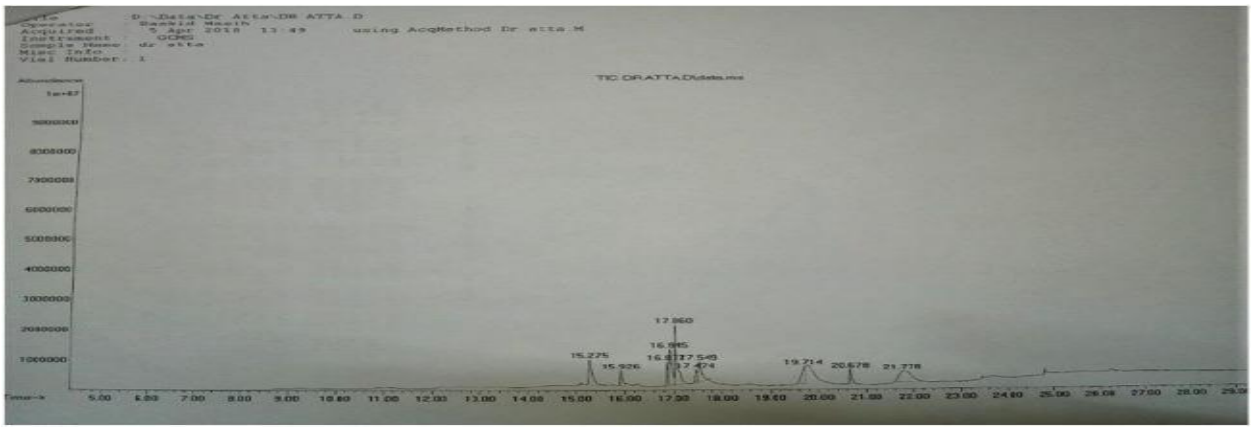

**Fig 1: Chromatogram of GREE obtained through GCMS analysis.**

**Table 2: GC-MS chromatograph of GREE**

| **Peak No.** | **Name** | **Mol. Wt.** | **Retention time** | **Corrected area** | **% of total** |
|---|---|---|---|---|---|
| 1 | 9,12-Octadecadienoic acid, methyl ester, (E, E)-C19H34O2 | 271 | 25.275 | 3536124 | 15.480 |
| 2 | Phytol C20H40O | 285 | 25.926 | 1526122 | 5.743 |
| 3 | Hexa-decanoic acid methyl ester, C17H34O2 | 296 | 26.877 | 1336998 | 6.066 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | (Z, Z, Z)-9,12,15-Octadecatrienoic acid, methyl ester, $C_{19}H_{32}O_2$ | 293 | 26.945 | 424034 | 22.174 |
| 5 | Hexa-decanoic acid ethyl ester, $C_{18}H_{36}O_2$ | 292 | 27.060 | 8182629 | 26.573 |
| 6 | (Z, Z, Z)- 9,15-Octadecatrienoic acid, $C_{19}H_{32}O_2$ | 304 | 27.474 | 938604 | 1.883 |
| 7 | Linoleic acid ethyl ester, $C_{20}H_{36}O_2$ | 302 | 27.549 | 2101807 | 7.172 |
| 8 | 1,2-Benzenedicarboxylic acid, di-is octyl ester, $C_{24}H_{38}O_4$ | 263 | 29.714 | 2613407 | 6.497 |
| 9 | 4,7,10-trimethyl-2,11-tetraoxatetradecan-13ol$C_{13}H_{28}O_5$ | 256 | 10.678 | 1977021 | 5.599 |
| 10 | 2-(3Acetooxy-4, trimethylandrost-8-17-yl)- propionic acid, $C_{27}H_{42}O_4$ | 435 | 31.778 | 6350349 | 17.813 |

The solution was mixed with ethanol and then passed through a filter. The analysis of chromatograms and fragments was carried out by consulting the NIST-5 GCMS library

**NMR of GREE**

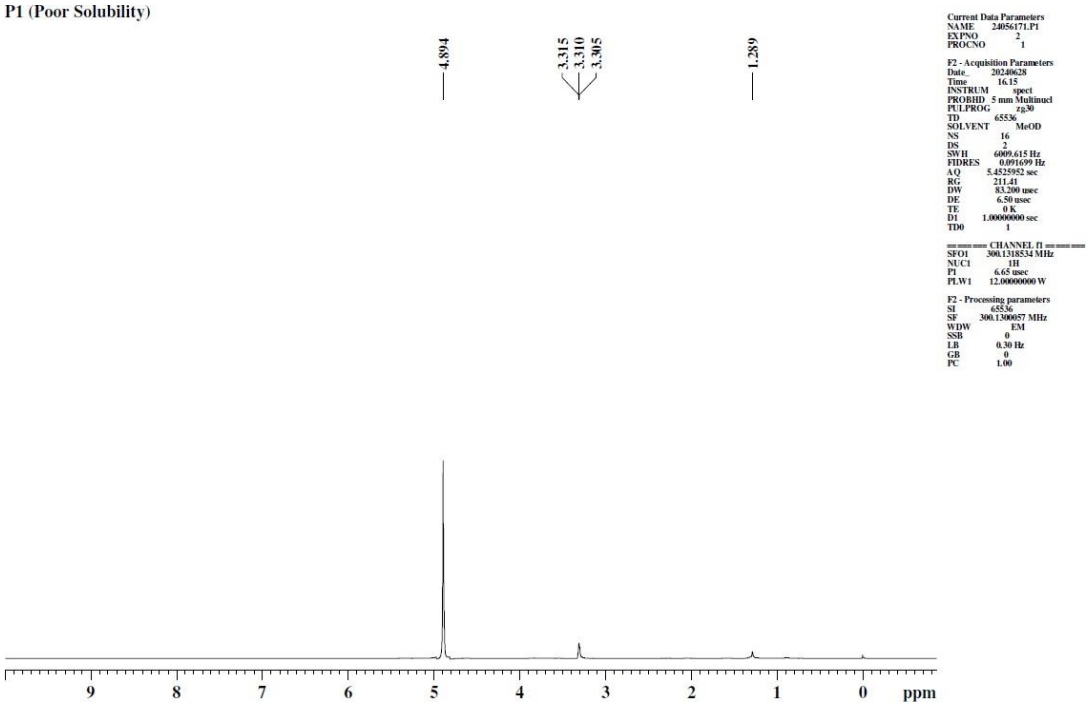


**Fig 2. NMR of GREE**

***2. Invitro* Antioxidant Activity**

Table 5.3 shows that GREE exhibited notable free radical scavenging activity compared to the standard antioxidant butylated hydroxytoluene (BHT). BHT maximally scavenged 89.35% of DPPH at a concentration of 100 µg/ml, while GREE achieved a maximum scavenging of 68% of DPPH at the same concentration. The effective concentration required to scavenge 50% of the DPPH (EC50) for GREE was 74.26 ± 0.46 µg/ml, whereas the EC50 for BHT was 41.23 ± 0.27 µg/ml (Table 5.3).

**Table 3: DPPH scavenging activity of GREE and BH**

| Concentration of BHT or GREE (ug/ml) | % scavenging activity | |
|---|---|---|
| | BHT | GREE |
| 0 | 0.00 | 0.00 |
| 16 | 35.05±0.94 | 7.04±0.35 |
| 32 | 46.94±0.68 | 16.48±1.21 |
| 46 | 64.66±3.34 | 28.94±3.22 |
| 65 | 74.64±0.73 | 41.94±6.75 |
| 78 | 78.88±0.42 | 52.36±2.15 |
| 95 | 84.97±0.31 | 62.94±2.08 |
| 100 | 86.36±0.43 | 69.11±1.38 |
| $EC_{50}$ | 43.24±0.37 | 75.25±1.49 |

The correlation between the dose and the proportion of DPPH elimination by GREE (µg approximate of phenolic content) is demonstrated, and it is contrasted with BHT as the benchmark. The EC50 represents the dose at which it eliminates half of the DPPH radicals. The shaded gray values are those that closely resemble the EC50 values. The results are shown as the mean value with the standard deviation added and subtracted, based on three repetitions of the experiment.

***In Vivo* Test Results**

**Effect of GREE and $CCl_4$ on Animal Weights**

In Table 4, the fluctuations in the body weight of rats are displayed in reaction to various treatments throughout the duration of the study. By the conclusion of the sixth week, the animals in the control group experienced a 22.5% growth in their average weight. The rats remained healthy.

After receiving a dosage of 500 mg per kilogram of body weight; their average body weight in this group increased by 5.8%. Within the group treated with $CCl_4$, there was an 8.9% reduction in body weight in comparison to the weight on day 1. Administering GREE to the group exposed to $CCl_4$ at doses of 100, 300, and500mg/kg resulted a mean rise in body weight of 5.6%, 10.2%, and 8.8%, respectively.

**Table 4: Effect of GREE on the body weight of animals**

| WEEK | CONTROL | GREE 500 | CCL4 | CCL4+GREE 100 | CCL4+GREE 300 | CCL4+GREE 500 |
|---|---|---|---|---|---|---|
| Week 1 | 195± 38.7 | 195.6 ±15. 5(0) | 187.2 ± 19.3 (0) | 197.1±14.3 (0) | 202.2±13.1 (0) | 175.2±4.15 (0) |
| Week 2 | 209±46.8 (+9.3) | 196±6.6 (−1.8) | 179± 16.6 (−4.3 ) | 206.5 ±1.5 (+1.2) | 204.2±9.1 (+3.4) | 184.1±9.5 (+3.9) |
| Week 3 | 223.2 ± 42.1(+35.6 ) | 201 ± 26.7 (0.67) | 186± 14.3 (−2.2 ) | 204.3 ±9.2 (+2.0) | 216.2±15.2 (+6.4) | 187.2±8.19 (+6.8) |
| Week 4 | 215±52.6 (+13.5) | 196.3 ± 13.7 (−1.6) | 173.6 ± 12.0 (−4.5 ) | 206 ± 24.9 (+3.9) | 207 ±13.9 (+3.8) | 194 ±16.3 (+12.4 |
| Week 5 | 225.6 ± 55.4 (+12.5) | 207.7 ± 15.6(+3.4 ) | 174.6 ± 12.4 (−6.4 ) | 215.5±13.2 (+5.7) | 219.7±9.5 (+16.2) | 195.7±16.6 (+9.8) |
| Week 6 | 236.2 ± 65.7 (+32.8) | 223.5 ± 18.4 (+4.4) | 164.7 ± 16.8 (−6.4 ) | 225.7±15.9 (+) | 214.7±27.8 (+13.2) | 198.7±15.5 (+6.9) |

Each value is represented by the mean with a margin of error defined by the standard deviation. The comparison reveals a remarkable difference: $p < 0.001$ concerning collective A and ### $p < 0.001$ regarding group B.

**Influence of GREE and CCl4 on the Levels of Liver Damage Indicators in the Blood**

During the 6th week of the research, Figure 5.2 illustrates the serum bioassay levels associated with the liver damage (ALT, and GGT) in rats after being given GREE and CCl4. Among all the groups that were treated, the lowest levels of ALT, AST, and GGT. The use of GREE at a concentration of 500 mg/kg did not lead to a rise in the levels in comparison to the control groups. On the other hand, the addition of CCl4 resulted in a significant surge ($p < 0.001$) in the concentrations of ALT, AST, and GGT in the blood, six-fold, escalating by fourfold and 2.5-fold, respectively, in comparison to the control group. Furthermore, in rats belonging to the experimental groups, the administration of 301 and 501 (mg/kg body weight) resulted in a significant reduction in AST levels (by 50% and 75%, respectively), ALT levels (by 50% and 60%, respectively), and GGT levels (by 47% and 58%, respectively) when compared to the CCl4 treatment group.

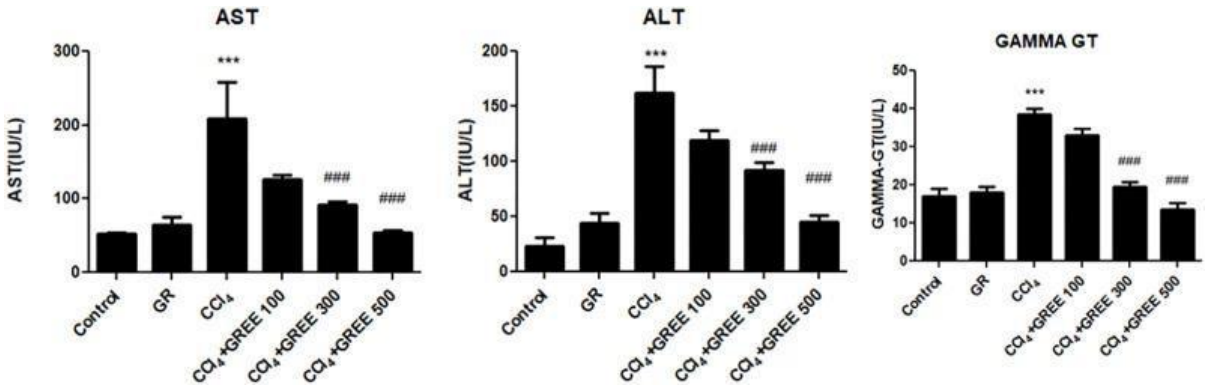


**Fig 3: Measurement of liver injury indicators in different sets of treatments. The levels of AST, ALT, and gamma-GT in the blood of animals from different groups were measured using diagnostic kits that were commercially available. The values are shown as the average plus or minus the standard deviation, with a sample size of 4. Importance: ***p < 0.001 in relation to group A and ### p < 0.001 when contrasted with group B. Influence of GREE and CCl4 on blood parameters.**

**Table 5: Hematological analysis of the treated groups**

| **Hematological parameter (unit)** | **Control** | **GRE E 500** | **CCl4** | **CCl4 +GRE E 100** | **CCl4+GRE E 300** | **CCl4+GRE E 500** |
|---|---|---|---|---|---|---|
| RBC(×10⁶/µl) | 15.4 ± 5.6 | 14.8± 2.5 | 39.2± 5.4* | 25.6±2.6 | 25.6±11.8 | 21.3±5.3 |
| WBC (×10³/µl) | 9.5 ± 0.6 | 7.0 ± 1.6 | 6.9 ± 0.6*** | 7.4±0.56 | 9.3±1.4### | 9.7±1.41 |
| HCT (%) | 15.2 ± 0.8 | 16.1± 1.4 | 17.5± 0.4* | 11.37±0.28 | 13.5±0.9## | 13.6±0.41 |
| Hb(g/dl) | 36.7 ± 6.1 | 48.9± 1.4 | 46.6± 6.0 | 43.8±2.4 | 44.8±3.1 | 42.6±2.7 |
| PLT(×10³/µl) | 893 ± 147.1 | 1166 ± 56.9 | 741.5 ± 56.1 | 814.1±25.1 | 897.5 ± 221.3 | 917.4±37.5 |

Every value is expressed as the average plus or minus the standard deviation. The results are statistically significant with a p-value below 0.001 when compared to group A and highly significant with a p-value below 0.001 when compared to group B.

In Table 5, the impact of different treatments on blood cell indices levels in reaction to GREE and CCl4 is displayed. According to the data, the CCl4 test caused a doubling in the number of white blood cells (WBC), along with a notable drop in red sanguine cell numbers and hemoglobin levels. The presence of CCl4 did not impact the hematocrit (HCT) or the number of platelets. After the CCl4 challenge, the average WBC count in the control group rose significantly from $14.3 \times 10^3/\mu l$ to $31.2 \times 10^3/\mu l$($p<0.05$). Within the group that was not treated, the number of redbloodcellswas7.5 $\times 10^8/\mu l$, while in the group treated with CCl4,the count of red blood cells dropped to $5.7 \times 10^6/\mu l$ ($p < 0.001$). Moreover, the administration of GREE at different levels to rats intoxicated with CCl4 brought back the number of red blood cellsto$8.3 \times 10^6/\mu l$($p <0.001$),with only as light decrease in the count of white blood cells.

**Liver-to-Body Weight Ratio**

Table 6 showcases the proportion of liver weight in relation to bodyweight for every treatment group. In the group that was not exposed to any treatment, the ratio of liver weight to body weight was 3.22, while it significantly increased to 4.81 in the group that was challenged with CCl4 ($p < 0.001$). Conversely, the most potent dosage of GREE treatment resulted in a ratio of 3.41 ($p < 0.001$).

**Table 6: Impact of GREE on the proportion of liver weight in relation to body weight**

| **Liver wt/bw (%)** | **Control** | **GREE 500** | **CCl4** | **CCl4+GREE 100** | **CCl4+GREE 300** | **CCl4+GREE 500** |
|---|---|---|---|---|---|---|
| | 3.22 ± 0.4 | 3.41 ± 0.07## | 4.81 ± 0.6*** | 4.01±0.7 | 3.51±0.45 | 3.1±0.1 |

The values are presented as the mean accompanied by the standard deviation range, based on a sample of

4. **Influence of GREE on the mRNA Levels of Genes Associated with Hepatic Fibrosis**

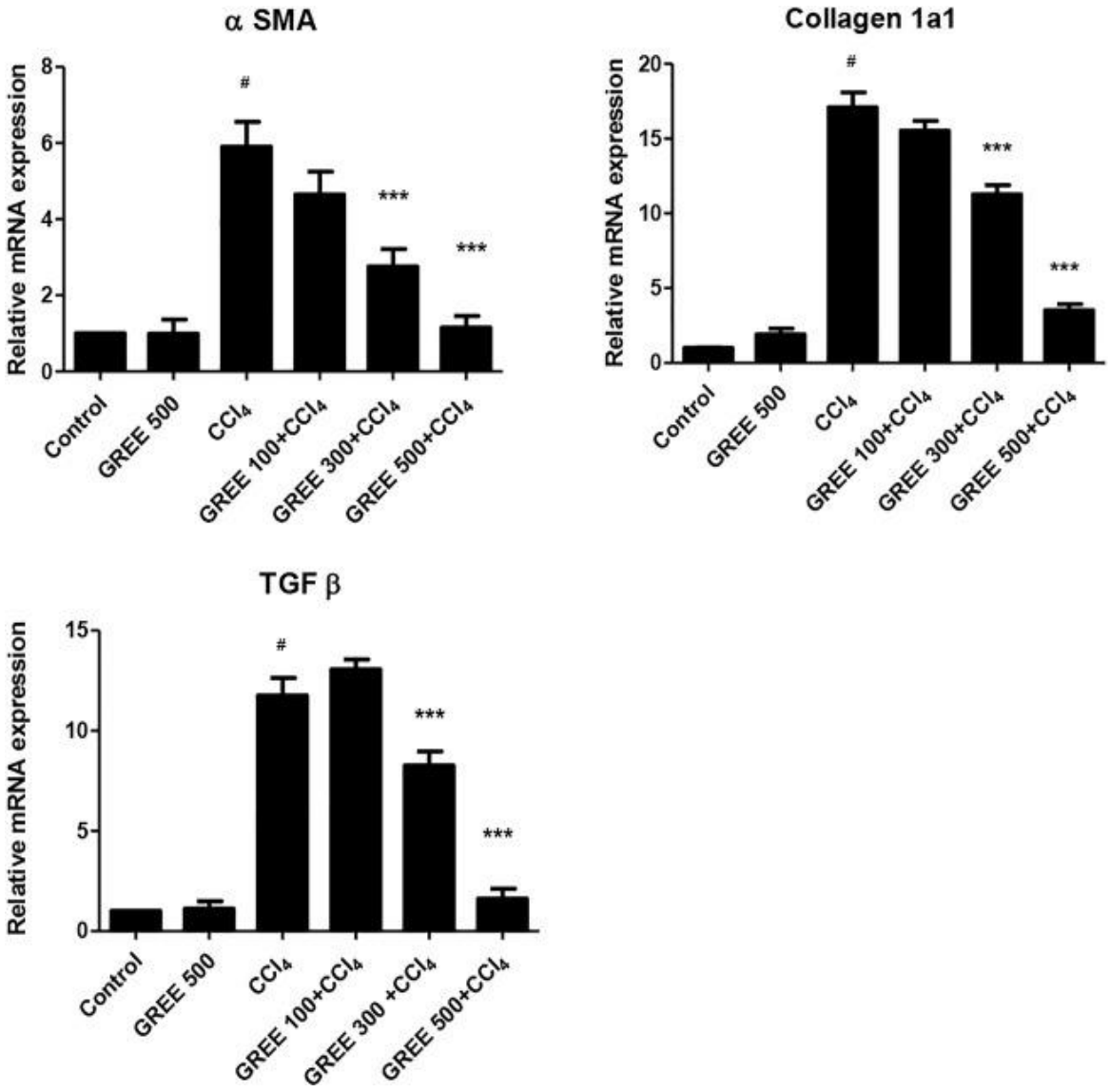


**Fig 4: Expression of mRNA in different groups of treatments. Them RNA levels of smooth musculus actin, collagenoids, and TGF-beta in liver samples from various animal groups were examined using real-time PCR. The values are presented as the mean with a margin of error represented by the standard deviation, based on a sample of 4. Significance: # p < 0.001 when compared to group A and ***p < 0.001 when contrasted with group B.**

Figure 4 illustrates the varying levels of α-SMA mRNA expression among all the groups. The levels of α-SMA mRNA expression in both and the group treated only with GREE stayed at a comparable baseline level. None the less, with in the CCl4 group, there was a notable sixfold rise in α-SMA mRNA expression, whereas in the experimental group, the decreased significantly in a manner that depended on the dosage. Coll's reactions to the treatments with CCl4 and GREE exhibited a comparable pattern.

WithinthegroupexposedtoCCl4, the transcriptase levels of Coll surged significantly, increasing by a remarkable 17-fold compared to the restricted group. Similarly, a notable reduction in mRNA levels ($p < 0.001$) was observed in the treated groups, displaying a dosage-dependent trend.

The patterns of TGF-β expression following exposure to CCl4 and GREE treatments exhibited a comparable tendency. Within the group exposed to CCl4, them RNA levels of Coll surged

significantly, multiplying by a factor of 12 when compared to the restricted group. Despite this, there was a significant reduction in mRNA doses ($p < 0.001$) only in the groups that were administered the higher dose; the lower dose (100 mg/kg bw) did not demonstrate any impact.

**Histology of Liver Tissue**

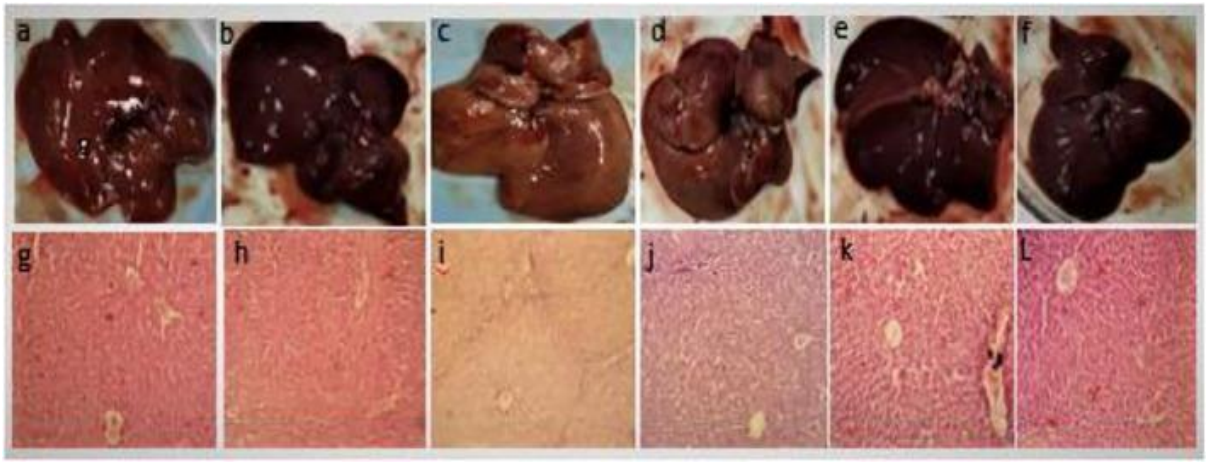


**Fig 5: Physical characteristics of liver. Group A and G served as the control group, while group B and H were treated with GREE. Group C and I were exposedtoCCl4, group Dand J received CCl4 along with 100mg/kg bw of GREE, group E and K were treated with CCl4 and 300mg/kg bw of GREE, and group F andLwereadministeredCCl4alongwith500mg/kg of GREE. The livers were taken out, cleansed with saline solution, and pictures were taken.**

In Figure 5.4, the overall appearance of liver samples stained with hematoxylin is displayed to analyze tiny alterations caused byCCl4 and GREE treatments. Ingroup B, significant transformations in the liver are evident when compared to group A. The livers of animals treated withCCl4 displayed rough and uneven surfaces with a nodular texture, as well as a shift in color from a reddish-brown hue to a lighter brown shade, as depicted in Figure 5c. Upon touching, the liver felt firm and it seemed to have decreased in size. These characteristics undeniably validate the beginning of cirrhosis within this particular group. The impact of the treatment on a broadscale is illustrated in groups D-F(Figure5 D-F). The liver displayed a smoother and more uniform surface texture, with the regularity of its lobes similar to those in group A.

**Microscopic Features**

In the lower part of Figure 5, you can see liver slices that have been stained with H&E. Images 5G-H showcase the characteristic histological arrangement of the liver, highlighting a central vein, strong hepatocytes, and the lack of immune cell penetration. In Figure 5H, we can see cells that have perished, showing a concentrated central vein, lack of nuclei, and a portal triad surrounded by a considerable accumulation of collagen between the portal regions, creating a nodular design and fibrous links. The features of

Figure5 J-L closely mirror those of the supervising group, showing no signs of extracellular matrix accumulation

## Discussion

The findings of this study reveal the hepatoprotective potential of Grevillea robusta leaf extract in mitigating chemically induced liver damage. Significant reductions in ALT, AST, ALP, and bilirubin levels in the extract-treated groups compared to the disease control groups highlight the efficacy of the extract in preserving liver function. These biochemical findings are supported by histopathological evidence, where treated groups demonstrated improved liver architecture with reduced necrosis and inflammation (3,9,25).

The antioxidant assays further underscore the role of Grevillea robusta in combating oxidative stress, a critical factor in liver injury. Enhanced activities of SOD and CAT and decreased MDA levels indicate the extract's ability to neutralize free radicals and prevent lipid peroxidation. This aligns with the phytochemical analysis, which identified the presence of polyphenols and flavonoids, compounds known for their potent antioxidant properties (14–17).

Comparatively, the hepatoprotective effects observed in this study are similar to those reported for other medicinal plants, reinforcing the potential of Grevillea robusta as a natural therapeutic agent. However, the exact mechanisms underlying these effects, including the specific bioactive components involved, warrant further investigation (26,27).

## Conclusion

The ethanolic extract of Grevillea robusta leaves exhibits significant hepatoprotective activity in experimental models of liver injury. By reducing biochemical markers of liver damage, enhancing antioxidant defenses, and improving histological outcomes, the extract demonstrates its therapeutic potential. These findings provide a foundation for further research to isolate active constituents, explore underlying mechanisms, and evaluate clinical applicability. Grevillea robusta holds promise as a cost-effective, natural remedy for managing liver disorders.

## Conflict of interest

All authors declare no possible conflict of interest.

## Author Contribution

SY- carried out the experiments and wrote the manuscript; RKS- performed data analysis and interpretation; AS- perceived the idea, supervised the work, and proofread the final version of the manuscript.

**Reference**


1. Banerjee P, Gaddam N, Chandler V, Chakraborty S. Oxidative Stress–Induced Liver Damage and Remodeling of the Liver Vasculature. Am J Pathol [Internet]. 2023;193(10):1400–14. Available from: https://www.sciencedirect.com/science/article/pii/S0002944023002122
2. Saha S, Alshammari A, Albekairi NA, Zulfiquar TN, Shakil MS, Mondal KR, et al. Exploring the antioxidant and protective effects of Marsdenia thyrsiflora Hook.f. leaf extract against carbon tetrachloride-induced hepatic damage in rat models. Front Pharmacol. 2024;15:1463922.
3. Pandey B, Baral R, Kaundinnyayana A, Panta S. Promising hepatoprotective agents from the natural sources: a study of scientific evidence. Egypt Liver J [Internet]. 2023;13(1):14. Available from: https://doi.org/10.1186/s43066-023-00248-w
4. Tran N, Pham B, Le L. Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology (Basel). 2020 Aug;9(9).
5. Gonfa YH, Tessema FB, Bachheti A, Rai N, Tadesse MG, Nasser Singab A, et al. Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. Curr Res Biotechnol [Internet]. 2023;6:100152. Available from: https://www.sciencedirect.com/science/article/pii/S2590262823000345
6. Kalinganire A, Harwood CE, Slee MU, Simons AJ. Floral Structure, Stigma Receptivity and Pollen Viability in Relation to Protandry and Self-incompatibility in Silky Oak ( Grevillea robusta A. Cunn.). Ann Bot [Internet]. 2000;86(1):133–48. Available from: https://doi.org/10.1006/anbo.2000.1170
7. Rojas-Sandoval J. Grevillea robusta (silky oak). CABI Compend [Internet]. 2022; Available from: https://doi.org/10.1079/cabicompendium.25866
8. Laosuwan T, Uttaruk Y, Sangpradid S, Butthep C, Leammanee S. The Carbon Sequestration Potential of Silky Oak (Grevillea robusta A.Cunn. ex R.Br.), a High-Value Economic Wood in Thailand. Forests [Internet]. 2023;14(9). Available from: https://www.mdpi.com/1999-4907/14/9/1824
9. Hameed S, Ur Rehman A, Massey S, Syed NIH, Anwar F, Ahmed D, et al. Grevillea robusta Delayed the Progression of Experimentally Induced Hepatic Fibrosis and Cirrhosis in Wistar Rats by Attenuating the Expression of Smooth Muscle Actin, Collagen, and TGF-β. Front Pharmacol. 2022;13:904584.
10. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. J Exp Pharmacol. 2023;15:51–62.
11. Youl O, Moné-Bassavé BRH, Yougbaré S, Yaro B, Traoré TK, Boly R, et al. Phytochemical Screening, Polyphenol and Flavonoid Contents, and Antioxidant and Antimicrobial Activities of Opilia amentacea Roxb. (Opiliaceae) Extracts. Appl Biosci [Internet]. 2023;2(3):493–512. Available from: https://www.mdpi.com/2813-0464/2/3/31
12. Siddiqui N, Rauf A, Latif A, Mahmood Z. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sci. 2017 Aug;12(4):360–3.
13. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants [Internet]. 2019;8(4). Available from: https://www.mdpi.com/2223-7747/8/4/96
14. Sagar NA, Pareek S, Gonzalez-Aguilar GA. Quantification of flavonoids, total phenols and antioxidant properties of onion skin: a comparative study of fifteen Indian cultivars. J Food Sci Technol. 2020 Jul;57(7):2423–32.
15. Ahmed D, Fatima K, Saeed R. Analysis of Phenolic and Flavonoid Contents, and the Anti-Oxidative Potential and Lipid Peroxidation Inhibitory Activity of Methanolic Extract of Carissa opaca Roots and Its Fractions in Different Solvents. Antioxidants (Basel, Switzerland) [Internet]. 2014 Oct;3(4):671—683. Available from: https://europepmc.org/articles/PMC4665497
16. Vilas-Boas ACM, Tarelho LAC, Moura JMO, Gomes HGMF, Marques CC, Pio DT, et al. Methodologies for bio-oil characterization from biomass pyrolysis: A review focused on GC-MS. J Anal Appl Pyrolysis [Internet]. 2025;185:106850. Available from:

https://www.sciencedirect.com/science/article/pii/S0165237024005059
17. Ano Y, Kutsukake T, Hoshi A, Yoshida A, Nakayama H. Identification of a Novel Dehydroergosterol Enhancing Microglial Anti-Inflammatory Activity in a Dairy Product Fermented with Penicillium candidum. PLoS One [Internet]. 2015;10(3):e0116598. Available from: https://app.dimensions.ai/details/publication/pub.1000095448
18. Gulcin İ, Alwasel SH. DPPH Radical Scavenging Assay. Processes [Internet]. 2023;11(8). Available from: https://www.mdpi.com/2227-9717/11/8/2248
19. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022 Feb;27(4).
20. Reppucci CJ, Brown LA, Chambers AQ, Veenema AH. Wistar rats and C57BL/6 mice differ in their motivation to seek social interaction versus food in the Social versus Food Preference Test. Physiol Behav. 2020 Dec;227:113162.
21. Frank D, Savir S, Gruenbaum BF, Melamed I, Grinshpun J, Kuts R, et al. Inducing Acute Liver Injury in Rats via Carbon Tetrachloride (CCl4) Exposure Through an Orogastric Tube. J Vis Exp. 2020 Apr;(158).
22. Sarhan MAA, Shati AA, Elsaid FG. Biochemical and molecular studies on the possible influence of the Brassica oleracea and Beta vulgaris extracts to mitigate the effect of food preservatives and food chemical colorants on albino rats. Saudi J Biol Sci [Internet]. 2014;21(4):342–54. Available from: https://www.sciencedirect.com/science/article/pii/S1319562X13001010
23. Cavagnetto C, Alejo Blanco R, McKenna H, Willmott L, Aydogdu E, Akinyemi N, et al. Residual red cells in blood components: A multisite study of fully automated enumeration using a hematology analyzer. Transfusion. 2021 Feb;61(2):568–78.
24. Thurberg BL, Wasserstein MP, Schiano T, O'Brien F, Richards S, Cox GF, et al. Liver and skin histopathology in adults with acid sphingomyelinase deficiency (Niemann-Pick disease type B). Am J Surg Pathol. 2012 Aug;36(8):1234–46.
25. Flores-Estrada J, Cano-Martínez A, Vargas-González Á, Castrejón-Téllez V, Cornejo-Garrido J, Martínez-Rosas M, et al. Hepatoprotective Mechanisms Induced by Spinach Methanolic Extract in Rats with Hyperglycemia—An Immunohistochemical Analysis. Antioxidants [Internet]. 2023;12(11). Available from: https://www.mdpi.com/2076-3921/12/11/2013
26. Kim SY, Oh KJ, Seo YR, Kim YW, Song PH, Song CH. Comparative Study on Hepatoprotective Effects of Traditional Herbs, Roots of Angelica gigas Nakai, Glycyrrhiza uralensis Fischer, Zizyphus jujuba Mill., and Fruits of Paeonia lactiflora Pall., on Ethanol-Induced Liver Injury in Mice. Antioxidants [Internet]. 2024;13(9). Available from: https://www.mdpi.com/2076-3921/13/9/1137
27. Ali M, Khan T, Fatima K, Ali QUA, Ovais M, Khalil AT, et al. Selected hepatoprotective herbal medicines: Evidence from ethnomedicinal applications, animal models, and possible mechanism of actions. Phytother Res. 2018 Feb;32(2):199–215.