Pharmacological Evaluation of Leaves Extract of *Shorea Robusta* on Carbon- Tetra Chloride Induced Hepatotoxicity in Wistar Rats

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Abstract:

Background: The liver is a vital organ for metabolic homeostasis and xenobiotic detoxification, making it highly susceptible to chemical-induced assaults. Carbon tetrachloride (CCl_4) is widely used to induce experimental hepatotoxicity via free-radical-mediated lipid peroxidation and inflammatory necrosis. This study evaluated the ethnopharmacological potential of *Shorea robusta* leaf extract against CCl_4-induced liver injury in Wistar rats.

Methods: Wistar rats were divided into five groups. Hepatotoxicity was induced using CCl_4 (1 ml/kg, i.p.) once a week on days 7 and 14. Treatment groups received a 70% methanolic leaf extract of *Shorea robusta* at doses of 200 mg/kg or 400 mg/kg orally for 14 days. Silymarin (100 mg/kg) served as the standard reference drug. On day 15, serum biochemical markers (SGPT, SGOT, ALP, Total Bilirubin, Total Protein, and Albumin) were measured, and liver tissues were subjected to histopathological evaluation.

Results: CCl_4 administration caused severe hepatotoxicity, marked by significantly elevated levels of SGPT, SGOT, ALP, and Total Bilirubin, along with diminished Total Protein and Albumin levels. While the 200 mg/kg dose provided partial protection, the 400 mg/kg dose achieved robust, highly significant hepatoprotection ($p < 0.001$). It suppressed SGPT (47.17 ± 1.472 IU/L) and SGOT (32.17 ± 1.602 IU/L) and restored Total Bilirubin (3.238±0.07055 mg/dl) to a level statistically equivalent to Silymarin (3.203±0.06346 mg/dl ($p = 0.995$). Histopathological analysis confirmed that the 400 mg/kg dose completely prevented CCl_4-induced ballooning degeneration, interface hepatitis, and drop-out necrosis.

Conclusion: The methanolic leaf extract of *Shorea robusta* possesses potent, dose-dependent hepatoprotective and anti-inflammatory properties. This efficacy is likely due to the antioxidant and membrane-stabilizing synergy of its secondary metabolites, confirming its potential as a natural phytopharmaceutical candidate.

Keywords: *Shorea robusta*, Carbon tetrachloride, Hepatotoxicity, Transaminases, Histopathology.

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Introduction:

The liver is the largest internal organ and a vital metabolic engine responsible for maintaining systemic physiological homeostasis [1]. It coordinates central processes including carbohydrate, lipid, and protein metabolism, alongside key functional capacities like nutrient storage, plasma protein synthesis, and bile secretion [1, 2]. Crucially, the liver is the primary site for the biotransformation and detoxification of endogenous waste and foreign xenobiotics [1, 3]. Because of its unique dual blood supply and pivotal role in drug clearance, it is continuously exposed to highly reactive intermediates, environmental pollutants, and diverse toxic chemical assaults [4, 5].

Hepatotoxicity arises when chemical compounds overwhelm the structural and functional integrity of hepatocytes, causing an elevation of vital clinical diagnostic markers like serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) [6, 7]. Toxic liver injury poses a major global public health challenge, contributing extensively to human morbidity and mortality via complex clinical presentations such as steatosis, chronic cirrhosis, liver failure, and hepatocellular carcinoma [5, 6]. In experimental pharmacology, carbon tetrachloride (CCl_4) remains a robust, highly reliable, and clinically relevant model for inducing liver damage in laboratory animals [7]. Hepatic biotransformation of CCl_4 via the cytochrome P450 enzyme framework (specifically

CYP2E1) triggers free radical generation by releasing highly reactive trichloromethyl (CCl_3) and trichloromethyl peroxy (.$OOCCl_3$) intermediates [7, 8]. These active free radicals execute widespread lipid peroxidation across organelle membranes, precipitating profound mitochondrial dysfunction, intracellular calcium disruption, and extensive centrilobular necrosis [7, 9]. The secondary consequence is a massive pro-oxidant state that triggers acute inflammatory cell infiltration through the Kupffer-cell-mediated expression of pro-inflammatory cytokines like TNF-alpha, IL-1, and IL-6. [8, 10].

To mitigate these severe pathways, scientific inquiry has focused closely on natural products and medicinal plants as sources of safe and effective hepatoprotective agents due to their benign safety profiles [11, 12]. *Shorea robusta* Gaertn. f. (Family: Dipterocarpaceae), traditionally known as the Sal tree, is a large deciduous tree widely distributed across India, Nepal, and Bhutan, with an extensive history of ethnopharmacological use for treating wounds, ulcers, skin diseases, and inflammatory conditions [13]. Phytochemical profile studies reveal that *Shorea robusta* leaves contain a rich spectrum of bioactive secondary metabolites, including phenolic acids, flavonoids (such as quercetin and kaempferol), condensed tannins, triterpenoids (like ursolic and oleanolic acids), sterols, and glycosides [13, 14]. These diverse compounds exhibit powerful antioxidant, free radical scavenging, anti-inflammatory, and membrane-stabilizing attributes [14].

Consequently, evaluating *Shorea robusta* leaf extract against CCl_4-induced hepatotoxicity in Wistar rats provides a vital experimental avenue to investigate its capacity to reduce lipid peroxidation, scavenge destructive free radicals, suppress downstream inflammatory markers, and enhance the baseline expression of natural endogenous antioxidant networks including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) [14, 15].

Materials and Methods

The research was conducted at Pharmacology Research laboratory in Siddhartha Institute of Pharmacy, Dehradun.

Experimental Animals:

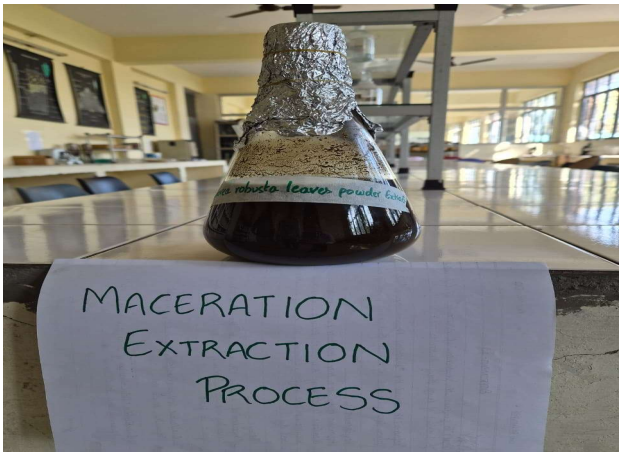
Wistar Rats weighing 150-200 g were used for the experiment which have been obtained from Shri Guru Ram Rai University. They were housed in laboratory environment with regular housing conditions (temperature- 25C under 12 hrs. light and 12 hrs. dark cycle) with a standard pellet feed. All animal procedures were performed according to the regulations specified by theInstitutional Animal Ethics Committee.

Collection of plant materials:

The plant material *Shorea Robusta* investigated in the present study was collected from Munakot, Pithoragarh, Uttarakhand in the months of January, 2026. The plant was identified and authenticated by Botanical survey of India, Dehradun, Uttarakhand, India. Plant Authentification identification reference number- BSI/ NRC. Tech./Herb (Ident.)/2026-27/194.

Preparation of the plant extract:

The collected plant leaves of Shorea robusta were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Shorea robusta* was macerated with 70% methanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. [16].



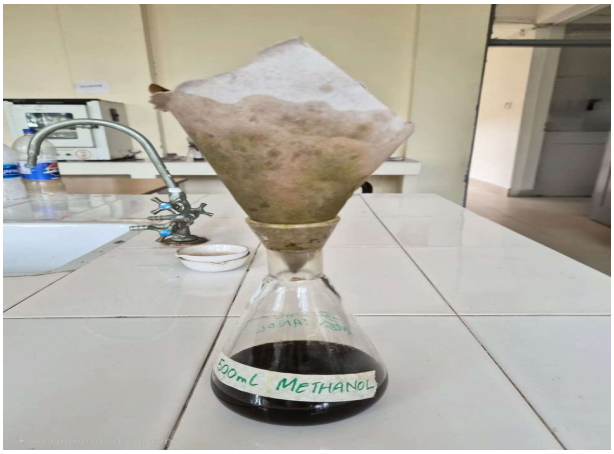


Figure 1. Methanolic Extraction of *Shorea robusta* leaves using a Maceration Process.

Induction of Hepatotoxicity:
Hepatotoxicity will be induced by Carbon Tetrachloride (CCl_4) 1mg/kg intraperitoneally (i.p.) on the 7th and 14th days. [17]

Experimental design:

Rats will be divided into 5 groups (6 rats / group) and classified as the following:

Group 1: (Normal control group): Rats administrated with normal saline water and food.

Group 2 (Disease control group): Rats will be given with 1 ml /kg CCl_4 (i.p) once a week (7th & 14th day).

Group 3 (Test Group): Rats will be treated with leaves extract of *Shorea Robusta* (200 mg/kg) OD, orally for 14 days. **[18]**

Group 4 (Test Group with higher dose): Rats will be treated with leaves extract of *Shorea Robusta* (400 mg/kg) OD, orally for 14 days. **[18]**

Group 5 (Standard group): Rats will be treated with Silymarin (100mg/kg, orally) OD, for 14 days.

After 14 days of *Shorea Robusta* administration, various biochemical studies will be performed which are as follows:

Biochemical and Oxidative Stress Markers

On Day 15, animals were anaesthetized and sacrificed. Blood and liver samples were collected for estimation of SGOT, SGPT, ALP, total bilirubin levels.

Histopathology

Liver tissues were fixed in 10% formalin for histopathological evaluation.

RESULT

Statistical analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey test. P value of $p < 0.05$ was considered statistically significant ($p < 0.01$, $p < 0.001$).

Biochemical and Antioxidant Effects

Liver enzymes—SGOT, SGPT, ALP, and total bilirubin—were estimated as per the lab's standard validated protocols and internal quality controls.

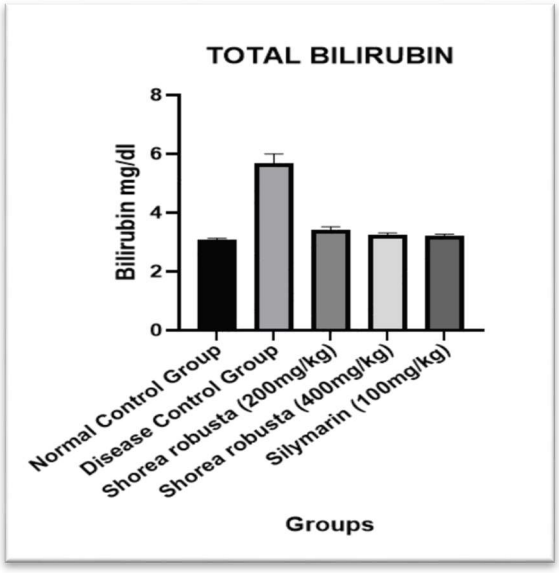
Histological Observations

The CCl_4 control group exhibited widespread hepatocellular degeneration, necrosis, and inflammatory infiltration. Extract-treated groups, particularly at 400 mg/kg, showed improved preservation of hepatic cords, reduced necrotic zones, and restoration of sinusoidal structure. The silymarin group revealed near-normal hepatic architecture with signs of regeneration.

S. No	Group Name	Total Bilirubin Level	S. no	Tukey's multiple comparison test s	Mean Difference	95.00 % CI of diff.	Significant?	Adjusted P Value
1	Normal Control	3.080 ± 0.04427	1	Normal vs. Disease	-2.597	-2.862 to -2.331	Yes	<.001

2	Disease Control (CCl_4)	5.677 ± 0.3181	**2**	Disease vs. Treatment (*Shorea Robusta*) 200 mg/kg	2.260	1.994 to 2.526	Yes	<.001
3	Treatment group (*Shorea Robusta*) 200mg/kg+ CCl_4	3.417 ± 0.1023	**3**	Disease vs. Treatment (*Shorea Robusta*) 400 mg/kg	2.438	2.173 to 2.704	Yes	<.001
4	Treatment group (*Shorea Robusta*) 400mg/kg+ CCl_4	3.238 ± 0.07055	**4**	Disease vs. Standard (Silymarin 100 mg/kg)	2.473	2.208 to 2.739	Yes	<.001
5	Standard Group (Silymarin) 100mg/kg+ CCl_4	3.203 ± 0.06346	**5**	Treatment 200 mg/kg vs Treatment 400 mg/kg	0.1783	-0.08720 to 0.4439	No	.308
			6	Treatment 400 mg/kg vs Silymarin 100 mg/kg	0.03500	-0.2305 to 0.3005	No	.995

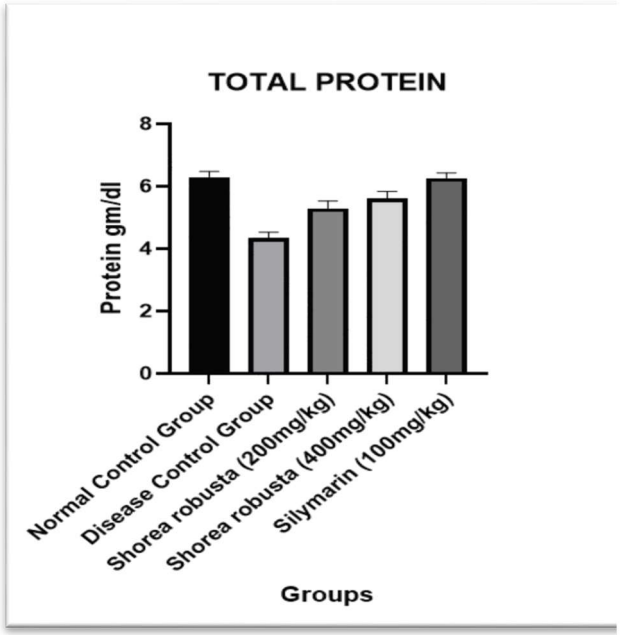
Table 1. Shows Effect of various pharmacological interventions on level of Total Bilirubin (mg/dl)



Graph 1. Effect of *Shorea robusta* with CCl_4 on Total Bilirubin level

Table 2. Effect of various pharmacological interventions on level of Total Protein. (gm/dl)

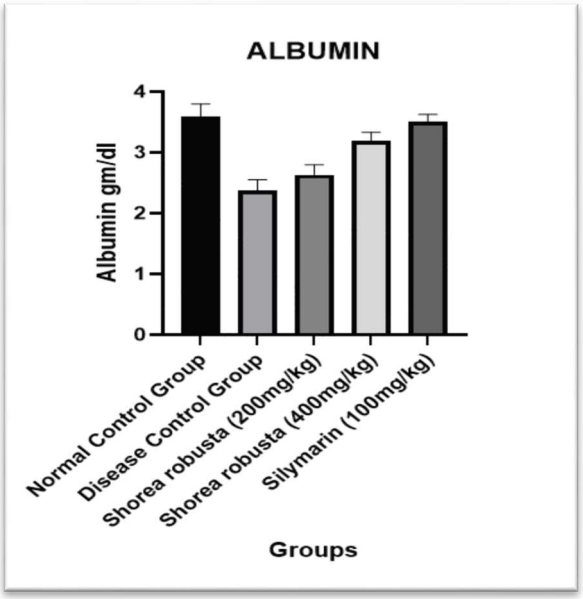
S.No	Group Name	Total Albumin Level	S.no	Tukey's multiple comparison tests	Mean Difference	95.00% CI of diff.	Significant?	Adjusted P Value
1	Normal Control	3.583 ± 0.2137	**1**	Normal vs. Disease	1.217	0.9282 to 1.505	Yes	<.001
2	Disease Control (CCl_4)	2.367 ± 0.1862	**2**	Disease vs. Treatment (*Shorea Robusta*) 200 mg/kg	-0.2667	-0.5551 to 0.02175	No	.080
3	Treatment group (*Shorea Robusta*) 200mg/kg+	2.633 ± 0.1633	**3**	Disease vs. Treatment (*Shorea Robusta*) 400 mg/kg	-0.8167	-1.105 to -0.5282	Yes	<.001



Graph 2. Effect of *Shorea robusta* with CCl_4 on Total Protein level

	CCl_4							
4	Treatment group (*Shorea Robusta*) 400mg/kg+ CCl_4	3.183 ± 0.1472	**4**	Disease vs. Standard (Silymarin 100 mg/kg)	-1.133	-1.422 to -0.8449	Yes	<.001
5	Standard Group (Silymarin) 100mg/kg+ CCl_4	3.500 ± 0.1265	**5**	Treatment 200 mg/kg vs Treatment 400 mg/kg	-0.5500	-0.8384 to -0.2616	Yes	<.001
			6	Treatment 400 mg/kg vs Silymarin 100 mg/kg	-0.3167	-0.6051 to -0.02825	Yes	.026

Table 3. Effect of various pharmacological interventions on level of Albumin (gm/dl)

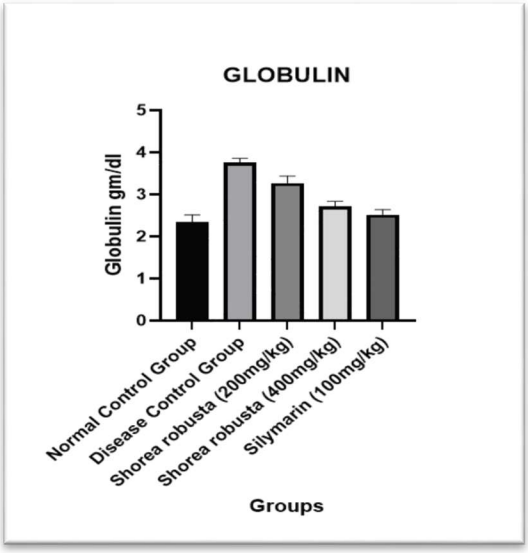


Graph 3. Effect of *Shorea robusta* with CCl_4 on Albumin level.

S. No	**Group Name**	**Total Globulin Level**	**S. no**	**Tukey's multiple comparison tests**	**Mean Difference**	**95.00 % CI of diff.**	**Significant?**	**Adjusted P Value**
1	Normal Control	2.333 ± 0.1751	**1**	Normal vs. Disease	-1.417	-1.651 to -1.182	Yes	<.001
2	Disease		**2**	Disease				

	e Control (CCl_4)	3.750 ± 0.1049		e vs. Treatment (*Shorea Robusta*) 200 mg/kg	0.4833	0.2488 to 0.7179	Yes	<.001
3	Treatment group (*Shorea Robusta*) 200mg/kg+ CCl_4	3.267 ± 0.1633	**3**	Disease vs. Treatment (*Shorea Robusta*) 400 mg/kg	1.033	0.7988 to 1.268	Yes	<.001
4	Treatment group (*Shorea Robusta 4*00mg/kg+ CCl_4	2.717 ± 0.1169	**4**	Disease vs. Standard (Silymarin 100 mg/kg)	1.233	0.9988 to 1.468	Yes	<.001
5	Standard Group (Silymarin) 100mg/kg+ CCl_4	2.517 ± 0.1169	**5**	Treatment 200 mg/kg vs Treatment 400 mg/kg	0.5500	0.3155 to 0.7845		<.001
			6	Treatment 400 mg/kg vs Silymarin 100 mg/kg	0.2000	-0.03454 to 0.4345	No	.122

Table 4. Effect of various pharmacological interventions on level of Globulin (gm/dl)

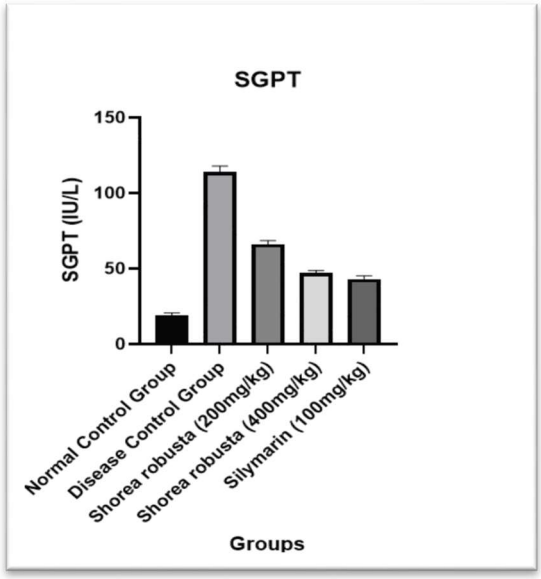


Graph 4. Effect of *Shorea robusta* with CCl_4 on Globulin level.

S.	Group	SGP	S.	Tukey's	Mean Dif	95.00 %	Signifi	Adjuste

No	Name	TLevel	no	multiple comparison tests	ference	CI of diff.	cant?	dP Value
1	Normal Control	19.17 ± 1.472	**1**	Normal vs. Disease	-94.67	-98.88 to -90.45	Yes	<.001
2	Disease Control (CCl_4)	113.8 ± 3.971	**2**	Disease vs. Treatment (*Shorea Robusta*) 200 mg/kg	47.83	43.62 to 52.05	Yes	<.001
3	Treatment group (*Shorea Robusta*) 200 mg/kg + CCl_4	66.00 ± 2.449	**3**	Disease vs. Treatment (*Shorea Robusta*) 400 mg/kg	66.67	62.45 to 70.88	Yes	<.001
4	Treatment group (*Shorea Robusta*) 400 mg/kg + CCl_4	47.17 ± 1.472	**4**	Disease vs. Standard (Silymarin 100 mg/kg)	70.83	66.62 to 75.05	Yes	<.001
5	Standard Group (Silymarin) 100 mg/kg + CCl_4	43.00 ± 2.191	**5**	Treatment 200 mg/kg vs Treatment 400 mg/kg	18.83	14.62 to 23.05	Yes	<.001
			6	Treatment 400 mg/kg vs Silymarin 100 mg/kg	4.167	-0.04854 to 8.382	No	.054

Table 5. Effect of various pharmacological interventions on level of SGPT (IU/L)

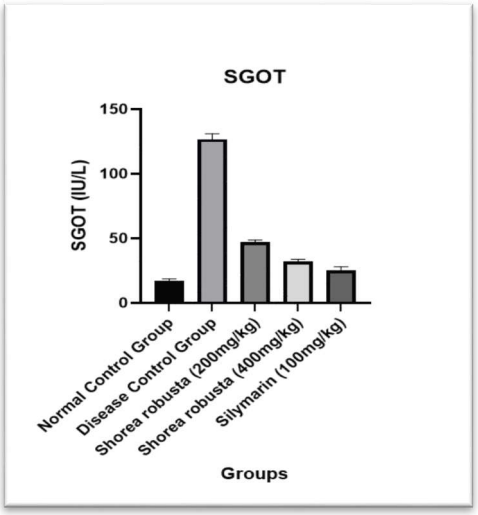


Graph 5. Effect of *Shorea robusta* with CCl_4 on SGPT

S. No	Group Name	SGOT Level	S. no	Tukey's multiple comparison tests	Mean Difference	95.00% CI of diff.	Significant?	Adjusted P Value
1	Normal Control	17.17 ± 1.472	**1**	Normal vs. Disease	-109.5	-114.0 to -105.0	Yes	<.001
2	Disease Control	126.7 ± 4.412	**2**	Disease vs. Treatment (*Shorea Robusta*) 200 mg/kg	79.50	75.03 to 83.97	Yes	<.001
3	Treatment group (*Shorea Robusta*) 200 mg/kg+ CCl_4	47.17 ± 1.472	**3**	Disease vs. Treatment (*Shorea Robusta*) 400 mg/kg	94.50	90.03 to 98.97	Yes	<.001
4	Treatment group (*Shorea Robusta*) 400 mg/kg+ CCl_4	32.17 ± 1.602	**4**	Disease vs. Standard (Silymarin 100 mg/kg)	101.7	97.20 to 106.1	Yes	<.001
5	Standard Group (Silymarin) 100 mg/kg+	25.00 ± 2.898	**5**	Treatment 200 mg/kg vs Treatment	15.00	10.53 to 19.47	Yes	<.001

	CCl_4			400 mg/kg				
			6	Treatment 400 mg/kg vs Silymarin 100 mg/kg	7.167	2.696 to 11.64	Yes	<.001

Table 6. Effect of various pharmacological interventions on level of SGOT (IU/L)

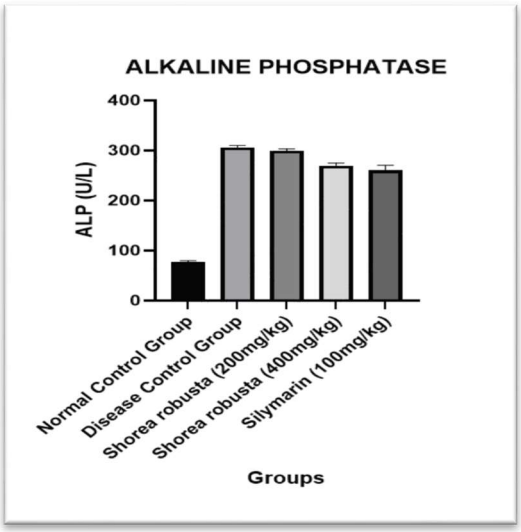


Graph 6. Effect of *Shorea robusta* with CCl_4 on SGOT

S.No	**Group Name**	**Total ALP Level**	**S.no**	**Tukey's multiple comparison tests**	**Mean Difference**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
1	Normal Control	77.50±1.871	**1**	Normal vs. Disease	-228.8	-238.6 to -219.0	Yes	<.001
2	Disease Control (CCl_4)	306.3±3.670	**2**	Disease vs. Treatment (*Shorea Robusta*) 200 mg/kg	6.500	-3.296 to 16.30	No	.319
3	Treatment group (*Shorea Robusta*) 200mg/kg+ CCl_4	299.8±3.312	**3**	Disease vs. Treatment (*Shorea Robusta*) 400 mg/kg	36.50	26.70 to 46.30	Yes	<.001
4	Treatment group (*Shorea Robusta*)400mg/kg+ CCl_4	269.8±5.	**4**	Disease vs. Standard (Silymari	46.33	36.54 to 56.13	Yes	<.001

		076		n 100 mg/kg)				
5	Standard Group (Silymarin) 100mg/kg+ CCl_4	260.0 ± 10.64	**5**	Treatment 200 mg/kg vs Treatment 400 mg/kg	30.00	20.20 to 39.80	Yes	<.001
			6	Treatment 400 mg/kg vs Silymarin 100 mg/kg	9.833	0.03691 to 19.63	Yes	.049

Table 7. Effect of various pharmacological interventions on level of ALP (U/L)



Graph 7. Effect of *Shorea robusta* with CCl_4 on ALP

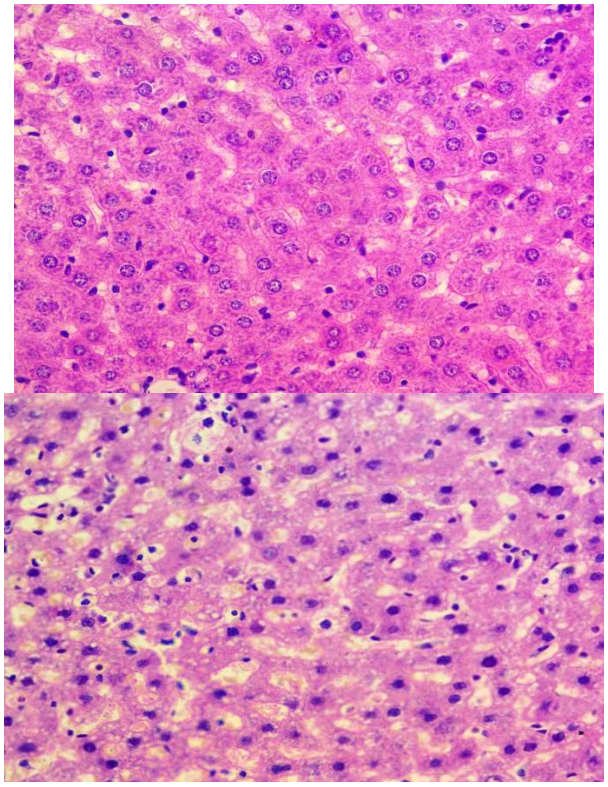


Fig- (a) Normal Control Group

Fig- (b) Disease Control Group

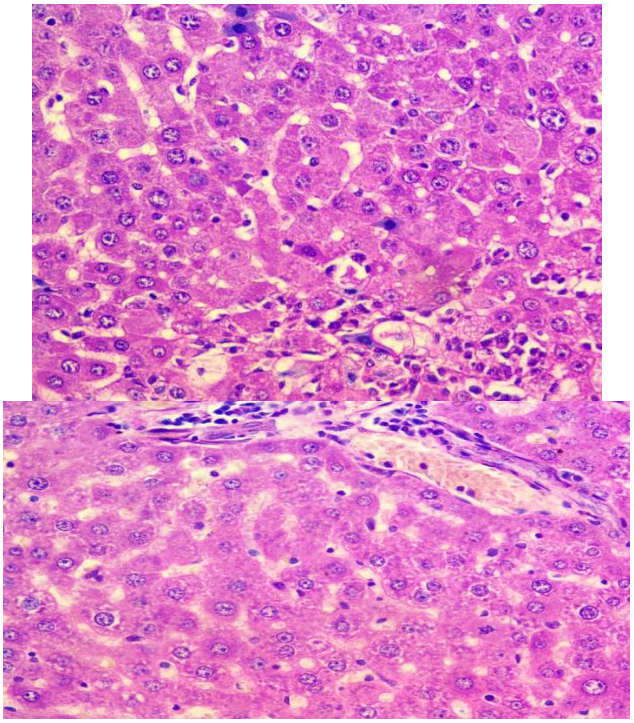


Fig- (c)- *Shorea Robusta* 200mg/kg

Fig- (d)- *Shorea Robusta* 400mg/kg

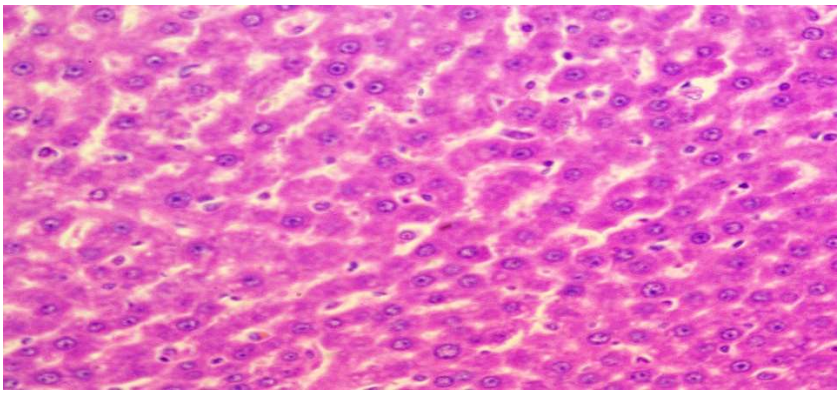


Fig- (e)- Silymarin 100mg/kg

(Fig. a) Liver section showing normal appearing portal area. Bile ducts and portal area. Bile ducts and portal vessels are within normal limits. Hepatocytic lobular architecture is maintained. No evidence of any obstruction or inflammatory changes noted. Sinusoidal integrity is maintained. Kupffer cells are within normal limits. No inflammatory cells, congestion, hemorrhage, collagen deposition and granuloma seen. **(Fig. b)** Liver sections showing expended portal triad, interface hepatitis noted at places. Portal vessels are within normal limits. Hepatocytic lobular architecture is somewhat maintained but cholestasis noted along with ballooning degeneration. Lobular inflammation noted and drop out necrosis noted. Sinusoids show cholestasis and mildly dilated, showing Kupffer cell hyperplasia. Mild congestion noted. No hemorrhage, collagen deposition and granuloma seen. **(Fig. c)** Liver sections showing expended portal triad, exhibiting mild bile duct proliferation. Lymphoplasmacytic infiltration along with eosinophilic infiltration noted focally in portal triad, interface hepatitis noted at places. Portal vessels are within normal limits. Hepatocytic lobular architecture is somewhat maintained but cholestasis noted along with ballooning degeneration at places. Lobular inflammation noted and drop out necrosis noted. Sinusoids show cholestasis and mildly dilated, showing Kupffer cells hyperplasia. Mild congestion noted. No hemorrhage, collagen deposition and granuloma seen. **(Fig. d)** Liver sections showing expended portal triad, exhibiting mild bile duct proliferation. Lymphoplasmacytic infiltration along with eosinophilic infiltration noted occasionally in portal triad, interface hepatitis not noted. Portal vessels are within normal limits. Hepatocytic lobular architecture is somewhat maintained but cholestasis noted focally, no ballooning degeneration noted. Lobular inflammation noted occasionally without drop out necrosis, Sinusoids show minimal cholestasis and mildly dilated, showing moderate Kupffer cell hyperplasia. Mild congestion noted. No hemorrhage, collagen deposition and granuloma seen. **(Fig. e)** Liver sections showing expended portal triad, exhibiting minimal bile duct proliferation. Mild Lymphocytic infiltration along with eosinophilic infiltration noted occasionally in portal triad, interface hepatitis not noted. Portal vessels are within normal limits. Lobular inflammation noted occasionally without drop out necrosis. Sinusoids showing minimal cholestasis occasionally and mildly dilated, showing mild Kupffer cell hyperplasia. Mild congestion noted. No hemorrhage, collagen deposition and granuloma seen.

Discussion-

The principal objective of the present study was to investigate the hepatoprotective and anti-inflammatory efficacy of a 70% methanolic leaf extract of *Shorea robusta* against carbon tetrachloride (CCl_4)-induced liver injury in Wistar rats. The liver stands as the vital metabolic hub responsible for xenobiotic biotransformation and systemic homeostasis; however, this critical functional role renders it uniquely vulnerable to chemical-induced assaults. In experimental pharmacology, the CCl_4-induced hepatotoxicity model is a widely validated standard for evaluating potential therapeutic agents. The toxicity of CCl4 is driven by its metabolic activation via the hepatic cytochrome P450 system (specifically CYP2E1) into highly reactive trichloromethyl (CCl_3) and trichloromethyl peroxy ($.OOCCl_3$) free radicals. These aggressive radicals attack adjacent hepatocyte membranes, initiating an autocatalytic chain of lipid peroxidation that causes profound mitochondrial dysfunction, intracellular

calcium accumulation, cellular ballooning, and extensive centrilobular necrosis.

The immediate consequence of this structural membrane breakdown is the leakage of cellular enzymes into the systemic circulation. In this study, CCl_4 intoxication (Group 2) produced severe liver damage, marked by a massive surge in serum transaminases: SGPT rose to 113.8 ± 3.971 IU/L (from a baseline of 19.17 ± 1.472 IU/L) and SGOT rose to 126.7 ± 4.412 IU/L (from a baseline of 17.17 ± 1.472 IU/L). Alkaline phosphatase (ALP) levels also accelerated dramatically to 306.3 ± 3.670 U/L, highlighting severe biliary canalicular distress and cholestasis. Furthermore, a significant near-doubling of Total Bilirubin (5.677 ± 0.3181 mg/dl) underscored a compromised metabolic clearance capacity, while a sharp decline in Total Protein (4.350 ± 0.1761 gm/dl) and Albumin (2.367 ± 0.1862 gm/dl) exposed a severely impaired hepatic biosynthetic machinery.

Oral administration of *Shorea robusta* leaf extract over 14 days produced a powerful, dose-dependent therapeutic reversal of these pathological parameters. At the lower dose of 200 mg/kg (Group 3), the extract initiated an intermediate recovery, significantly reducing SGPT to 66.00 ± 2.449 IU/L and SGOT to 47.17 ± 1.472 IU/L ($p < 0.001$). However, its modulation of ALP was statistically insignificant at this dose (299.8 ± 3.312 U/L, $p = 0.319$), and it failed to significantly restore Albumin (2.633 ± 0.1633 gm/dl, $p = 0.080$), signifying an incomplete therapeutic correction. In contrast, the high-dose regimen of 400 mg/kg (Group 4) established a highly robust hepatoprotective profile. It effectively suppressed SGPT to 47.17 ± 1.472 IU/L, SGOT to 32.17 ± 1.602 IU/L, and ALP to 269.8 ± 5.076 U/L ($p < 0.001$). Notably, Tukey's multiple comparison test revealed no statistically significant difference in Total Bilirubin levels between the 400 mg/kg group (3.238 ± 0.07055 mg/dl) and the standard Silymarin group (3.203 ± 0.06346 mg/dl. $p = 0.995$), demonstrating that the high-dose extract achieves a functional clearance capacity virtually identical to the reference drug. It also successfully revived hepatic synthetic functions, restoring Total Protein and Albumin close to baseline levels (5.617 ± 0.2229 gm/dl and 3.183 ± 0.1472 gm/dl).

These biochemical findings are completely supported by microscopic histopathological evaluations of the liver matrices. The Normal Control group (Group 1, represented by image displayed a flawless lobular architecture with intact sinusoids and healthy, uninjured portal areas. Conversely, the CCl_4 disease control group (Group 2, represented by image exhibited prominent tissue damage, including expanded portal triads, interface hepatitis, profound cholestasis, Kupffer cell hyperplasia, widespread ballooning degeneration, and drop-out necrosis. The lower extract dose (Group 3, represented by image, showed partial protection, characterized by focal lymphoplasmacytic and eosinophilic infiltration alongside persistent localized zones of necrosis and ballooning. The high dose of 400 mg/kg (Group 4, represented by image however, achieved a striking architectural recovery: interface hepatitis and drop-out necrosis were entirely absent, ballooning degeneration was completely prevented, and cholestasis was minimal. The presence of moderate Kupffer cell hyperplasia without tissue degradation indicated a healthily active cellular regenerative response, closely mimicking the clean structural restoration observed in the standard Silymarin cohort (Group 5, represented by image.

This multi-level protection is driven by the rich phytochemical profile of *Shorea robusta* leaves, which are packed with bioactive secondary metabolites including phenolic acids, condensed tannins, triterpenoids, and flavonoids like quercetin and kaempferol. These compounds act as potent free-radical scavengers that directly intercept CCl_3 intermediates, thereby terminating the lipid peroxidation cascade and preventing the downstream release of pro-inflammatory cytokines. Concurrently, specialized triterpenoids like ursolic and oleanolic acids provide crucial membrane-stabilizing effects, maintaining hepatocyte integrity and preventing the structural leakage of transaminases into the blood. Taken together, these findings demonstrate that the methanolic leaf extract of *Shorea robusta* possesses highly effective, dose-dependent hepatoprotective properties. This study successfully validates its traditional ethnopharmacological use and establishes the extract as a highly promising natural candidate for managing toxic liver disorders.

Conclusion-

In conclusion, the present investigation successfully validates the traditional ethnopharmacological use of *Shorea robusta* leaves as a highly effective, natural therapeutic intervention against chemical-induced hepatotoxicity. The experimental findings clearly demonstrate that the 70% methanolic leaf extract of *Shorea robusta* exerts a potent, dose-dependent hepatoprotective and anti-inflammatory action against CCl_4-induced acute liver injury in Wistar rats. While the lower dose (200 mg/kg) initiated an intermediate level of cellular repair, the higher dose of the extract (400 mg/kg) achieved a highly robust therapeutic response. This high-dose regimen successfully

reversed severe hepatocellular damage by significantly reducing elevated serum biomarkers (SGPT, SGOT, ALP, and Total Bilirubin) and restoring the metabolic clearance and biosynthetic machinery of the liver, as evidenced by the revival of Total Protein and Albumin levels. These biochemical outcomes were unequivocally confirmed by microscopic histopathological examinations using selected representative slides. The high-dose extract achieved an excellent structural restoration of the liver matrix—entirely preventing ballooning degeneration, interface hepatitis, and drop-out necrosis—thereby closely mirroring the standard commercial drug, Silymarin. This comprehensive dual-action protection is primarily driven by the rich synergy of its secondary metabolites, particularly its free-radical-scavenging flavonoids (quercetin and kaempferol) and membrane-stabilizing triterpenoids (ursolic and oleanolic acids). These compounds successfully intercept toxic free radicals, halt destructive lipid peroxidation, and preserve structural hepatocyte integrity. Consequently, this study establishes the leaf extract of *Shorea robusta* as a highly promising, safe, and scientifically validated phytopharmaceutical candidate for the management of toxic liver disorders and oxidative stress-associated hepatic pathologies.

Conflict of Interest: The author has no conflict of interest.

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Author Contribution

KS- Writing original

Draft RSD- Original concept

SS- Supervision

Ethical Approval:

The research study was conducted at Siddhartha institute of pharmacy, Near IT park, Dehradun 248001. The animal house is CPCSEA approval. And the registration no. of the animal house – 1435/PO/RE/S/11/CPCSEA.

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