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Phytochemical And Hepatoprotective Activity Of Plant Extract Of Dalbergia Sissoo Seeds

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ABSTRACT:
The current work aims to perform a photochemical analysis and a hepatoprotective activity study of various Dalbergia sissoo seed extracts. The Fabaceae family includes Dalbergia sissoo DC., which has ruminate medicinal properties. Aqueous, methanolic, and chloroform extractions were performed in succession to create the various plant extracts. After that, these extracts (AEDS, MEDS, and CEDS) were taken and subjected to a first phytochemical screening utilising accepted techniques. The hepatoprotective activity of the different extracts of Dalbergia sissoo was evaluated by using carbon tetrachloride induced liver damage in experimental rats. In hepatotoxicity induced animals, an oral dose of 200 mg/kg, of the aqueous, methanol and chloroform extracts of Dalbergia sissoo exhibited a compelling decrease in marker enzyme levels and increased levels of antioxidant enzymes. When tested animals were exposed to the above dosage of plant extracts, their levels of lipid peroxidase significantly decreased in response to liver damage generated by carbon tetrachloride. The greatest hepatoprotective efficacy was observed in the methanolic extract, which was also shown to be rich in phytochemical components. Our current study's results led us to the conclusion that Dalbergia sissoo DC's methanolic extract exhibited a strong antioxidant defence mechanism as well as hepatoprotective activities. Because of their antioxidant qualities, polyphenolic compounds may have hepatoprotective potential.

Key words: *Dalbergia sissoo, Fabaceae, Silymarin, Liver, Antioxidant, Hepatoprotective*

INTRODUCTION:

Because of its critical location in the body, the liver serves as the primary metabolic organ and is also involved in secretion and excretion. It is frequently exposed to a variety of xenobiotics, ambient toxins, and chemotherapy drugs. Liver disease is a worldwide concern.[1-4] Common medications used to treat liver problems can occasionally be ineffective and have dangerous side effects. Therefore, in order to replace the present medications used to treat liver disease with ones that are safer and more effective, alternative medications must be found. Despite the fact that little is known about how they work, medicines made from plant extracts are more often used to treat a variety of illnesses. [3] The pharmacological evaluation of several plants for potential therapeutic uses in the Indian traditional medical system is gaining popularity. A popular

model for rat liver damage is poisoning with carbon tetrachloride (CCl4). Hepatotoxicity is associated with a marked decline in cell defence mechanisms. The primary defining characteristic of the liver injury is the cytochrome P-450 dependent metabolism of CCl_4. Lipid peroxidation is initiated by free radicals, which results in the reduction of enzyme activity [5, 6].

The medium- to large-sized deciduous tree Dalbergia sissoo DC. (Family: Fabaceae) is locally called "shishu" in Bangladesh and is popularly known as sisu, sheesham, tahli, tali, and jag in different regions of the world. Typically, it is used to treat gonorrhoea, syphilis, diarrhoea, and sore throats [7, 8]. Blood purifier, leprosy, headaches, bronchitis, inflammations, infections, hernias, and skin problems are among the conditions for which it is utilised. Due to their numerous pharmacological qualities, such as their hepatoprotective and antioxidant effects, Dalbergia sissoo phytoconstituents, such as flavonoids, terpenoids, and steroids, have drawn a lot of interest lately [9, 10]. Research into the potential health advantages of some flavonoids, triterpenoids, and steroids has sparked an increasing amount of interest in their examination. Antioxidant activity is one of their primary features in this regard, which allow them to debilitate the development of tumor and inflammatory disease [11, 12]. Antioxidant plays a significant part in hindering and tramping radicals, thus act as a shield to humans against infections and generative illnesses [13-15]. According to the literature survey, this research was carried out to evaluate the hepatoprotective activity of Dalbergia sissoo seed extracts.

MATERIALS AND METHODS:

Collection, Procurement and Extraction: Dalbergia sissoo seeds were procured from Green Fields Herbal Grass and Forestry Seeds, Maheshpur, Jabalpur, Madhya Pradesh, India. The plant seeds were collected, cleaned, shade-dried and then powdered. Firstly, the dried coarse powder of seeds was subjected to successive extraction in a Soxhlet apparatus using Aqueous, methanol and chloroform. The solvents were distilled off after extraction and the extracts were concentrated under reduced pressure. The extracts were kept in refrigerator until tested. [16-17]

Preliminary phytochemical screening: The crude extracts of *D sissoo* was qualitatively tested for the detection of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, reducing sugars, proteins, glucosides, terpenoids, and steroids. Preliminary phytochemical screening was done for the extract as per standard methods. [18-19]

Animals: Albino Wistar rats (140–170 g) of both sexes were used in the studies. The animals were acquired from our institution's animal house. The animals were kept under regular laboratory settings, with a temperature of 25±2°C and a 14/10 h light and dark cycle, and were grouped and housed in appropriate 38 × 23 × 10 cm cages, with no more than six animals per cage. They had unrestricted access to water and the regular dry pellet feed (Hindustan Lever, Kolkata, India). Before the trial started, the rats were given ten days to become used to the lab environment.[20-21]

Experimental Design: For the pharmacological tests, the extracts were dissolved in 2% Tween-80 in normal saline solution to prepare 200 mg/kg concentrations. The animals were divided into 6 groups each containing 6 animals. [22]

- Group I: Normal control and received the vehicle alone (Sterile distilled water, 2 ml p.o.) for 5 days.
- Group II: toxic control – received carbon tetrachloride in liquid paraffin (2 ml/kg, 1:1 in olive oil, i.p).
- Group III received Aqueous extract 200 mg/kg p.o. with CCl_4 as maintained above.
- Group IV received methanolic extract 200 mg/kg p.o. with CCl_4 as maintained above.
- Group V received chloroform extract 200 mg/kg p.o. with CCl_4 as maintained above.
- Group VI: standard received the standard drug Silymarin (10 mg/kg p.o) once in a day and CCl_4 as maintained above.

Treatment duration was 10 days and the dose of CCl_4 was administrated after every 72 hour. Under the light anesthetic ether animals were sacrificed 24 hour after the last injection. Blood was collected and allowed to clot and serum separated. The liver was dissected out and used for biochemical studies. [23]

Histopathological studies: One animal from each group was utilized for histopathological study. The livers were fixed in 10% formalin for 24 h. [24]

Biochemical studies: Every animal's retro-orbital plexus was punctured to extract its blood. The blood samples were left to coagulate at room temperature for forty-five minutes. Using analytical kits from Span Diagnostics Ltd., India, serum was separated by centrifugation at 2500 rpm at 30°C for 15 minutes. The serum was then used to estimate many biochemical parameters, including SGPT, SGOT and serum bilirubin. [25-27] After collection of the blood samples the rats were slaughtered and their livers cut out, washed in ice cold normal saline followed by 0.15M Tris-HCl (pH 7.4) blotted dry and weighed. [28-30]

Statistical analysis: The findings are shown as mean ± SEM. Using SPSS 18.00 software, the statistical analysis was carried out utilising one-way analysis of variance (ANOVA) and, if necessary, Tukey's multiple comparison column test.

Differences across groups were considered significant at a level of $p < 0.001$. [31-32]

RESULTS AND DISCUSSION:

The initial phytochemical examination revealed that the aqueous extract had high levels of lipids, proteins, amino acids, phenolic compounds, steroids, and alkaloids. Alkaloids, sugars, glycosides (cardiac glycoside), tannins (phenolic chemicals), proteins (amino acids), flavones and flavonoids, and saponins are all present in methanol extract. Chloroform extract contained alkaloids, cardiac glycoside, tannins-phenolic chemicals, flavones & flavonoids, steroids & sterols. Hepatoprotective activity demonstrated that the all three extract (AEDS, MEDS, CEDS) of Dalbergia sissoo give protection against the harmful effect of CCl_4 on liver.

Table: 1. Effect of different extracts of *D. sissoo* on CCl_4 induced hepatotoxicity in rat

Groups	Treatment	Level of biochemical parameters				
		SGOT (U/L)	SGPT(U/L)	ALP(U/L)	Total Bilirubin (mg/dL)	Total Protein (mg/dL)
Group I	Normal Control	49.11±3.45	96.21±2.45	125.45±1.37	0.756±0.007	11.21±0.025
Group II	Toxic Control	299.23±2.65^{b***}	483.34±1.432^{b***}	249.11±2.432^{b***}	3.125±0.034^{b***}	5.183±0.047^{b***}
Group III	AEDS	286.11±2.58^{b***}	453.45±3.322^{b***}	242.67±3.032^{b***}	2.64±0.130^{b***}	6.543±0.033^{b**}
Group IV	MEDS	136.60±3.984^{ab***}	234.25±1.089^{ab***}	214.31±2.053^{ab***}	1.43±0.143^{b*}	8.54±0.222^{a**}
Group V	CEDS	278.01±3.804^{b***}	459.15±3.431***	244.64±3.970^{b***}	2.81±0.258^{b***}	8.11±1.269^{b**}
Group VI	Silymarin	85.43±2.052^{ab***}	129.18±1.439^{ab***}	140.42±1.070^{ab***}	1.12±0.251^{a***}	9.91±0.210***

Values are mean ± SEM (n=6) one-way ANOVA followed by Tukey's multiple comparison column test. Where, ***=P≤0.001, **= P≤0.01, *= P≤0.05 vs ^{a}Toxic Control ***= P≤0.001, **= P≤0.01, *= P≤0.05 vs ^{b}Control

Table 2: Effect of *D. sissoo* extracts on anti-oxidant parameters in CCl_4 induced hepatotoxicity in rats.

Groups	Treatment	Liver weight	Level of antioxidant parameters			
			SOD	LPO	GPx	Catalase
Group I	Normal Control	9.11 ± 0.43	35.62±0.792	11.05±0.43	49.11±2.098	63.21±0.084
Group II	Toxic Control	13.55 ±1.65^{b**}	20.86±0.705^{b***}	25.40±0.912^{b***}	23.78±0.53^{b***}	31.88±1.183^{b***}
Group III	AEDS	9.42 ±0.20^{a***}	21.53±0.708^{b***}	22.77±0.749^{b***}	24.44±0.51^{b***}	35.26±1.357^{b***}
Group IV	MEDS	8.19 ± 0.04^{b***}	24.97±.0425^{a,b**}	21.15±1.100^{a,b**}	30.50±0.45^{a,b**}	39.21±1.901^{a,b**}
Group V	CEDS	10.12 ± 0.01^{a***}	30.95±0.688^{a***}	14.48±0.875^{a***}	35.77±0.62^{a,b***}	48.91±1.195^{a,b***}
Group VI	Silymarin	8.11 ± 0.18^{a***}	31.29±0.819^{a***}	11.23±0.833^{a***}	39.68±0.51^{a,b***}	55.35±1.826^{a,b***}

Values are mean ± SEM (n=6) one-way ANOVA followed by Tukey's multiple comparison column test. Where, ***=P≤0.001, **= P≤0.01, *= P≤0.05 vs ^{a}Toxic Control ***= P≤0.001, **= P≤0.01, *= P≤0.05 vs ^{b}Control

The results observed in pre-treatment of *Dalbergia sissoo* extracts with respect to induction of hepatotoxicity using CCl_4 are given in (Table 1). A marked reduction in total protein levels was observed in the group treated with CCl_4 (5.183±0.047, $P < 0.001$) when compared to the normal control group. The group treated with CCl_4 significantly increased the level of SGOT, SGPT, ALP, total bilirubin. Rats treated with CCl_4 (toxic control) developed significant liver damage and it was well indicated by elevated levels of specific hepatic enzymes like SGOT (299.23±2.65), SGPT (483.34±1.432), ALP

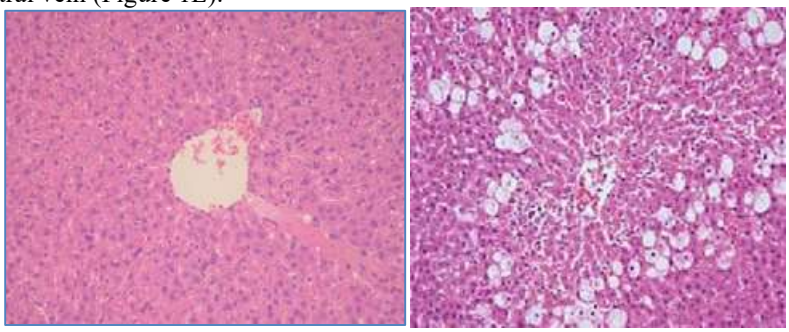
(249.11±2.432) and Total bilirubin (3.125±0.034) in serum.

The groups received the pre-treatment of Dalbergia sissoo extracts methanol extract (MEDS) and chloroform extract (CEDS) at dose levels of 200 mg/kg body weight significantly controlled the change in the biochemical parameters. The methanol extract (MEDS) and chloroform extract (CEDS) at dose levels of 200 mg/kg exhibited significant increase 8.54±1.269^{b**}mg/dL, 8.11±0.222^{a**} mg/dL, ($P < 0.01$, $P < 0.05$) respectively in the serum total protein level as compared to toxic control group and the effect was compared with the standard group 9.91±0.210*** mg/dL ($P < 0.001$) treated with silymarin (Sily-100).

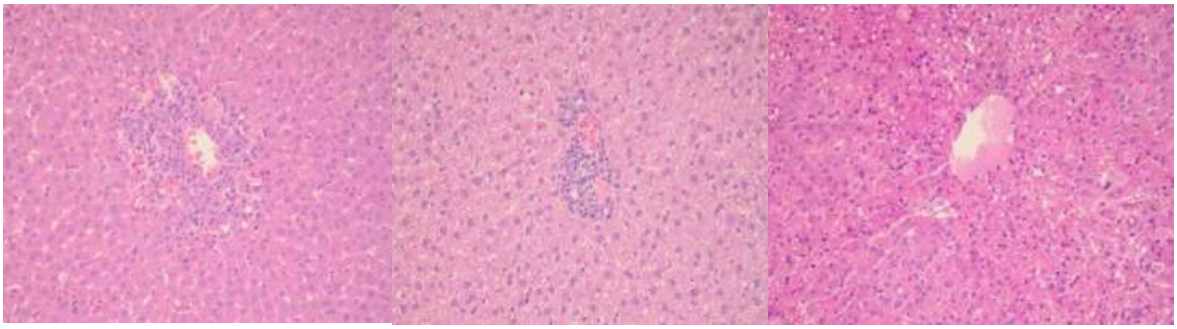
The SGOT, SGPT, ALP and Total bilirubin level decreased in drug treated groups to significant level. The SGOT 136.60±3.984 ab***, 85.43±2.052 ab*** ($P< 0.001$), SGPT 234.25±1.089 ab***, 129.18±1.439 ab*** ($P < 0.001$), ALP 214.31±2.053 ab***, 140.42±1.070 ab*** ($P < 0.001$) and Total bilirubin 1.43±0.143b*, 1.12±0.251a*** ($P < 0.001$) levels decreased significantly in methanol extract (ANME) and standard drug silymarin group as compared to toxic control group. The SGOT 278.01±3.804 b*** ($P< 0.01$), SGPT 459.15±3.431*** ($P < 0.05$), ALP 244.64±3.970 b*** ($P < 0.05$) and Total bilirubin 2.81±0.258 b*** ($P < 0.01$) levels significantly decreased in chloroform extract (ANCE) as compared to toxic control group. Administration of pet. ether extract (ANPE) and benzene extract (ANBE) did not display effect of increase in the serum enzyme levels as compared to toxic control group.

Table 2 displays the outcomes of antioxidant enzymes such as LPO, SOD, Catalse, and GPx in CCl4-induced hepatotoxicity. In comparison to the normal control group, the LPO, SOD, CAT, and GPx activities in the toxin control group dramatically decreased ($P < 0.001$), respectively. At both the methanol extract (MEDS), chloroform extract (CEDS), and standard medication silymarin, LPO, SOD, Catalase, and GPx activity increased.

Histopathological study of liver in CCl4 induced hepatotoxic rats: The normal liver lobular architecture and cell structure in the normal control animals were shown by the histological feature, as depicted in Figure 1A. Normal control animals showed no signs of pathogenic alterations. There was a vacuolar degeneration of hepatocytes around central vein with moderate to severe hepatocyte necrosis due to CCl4 toxicity in animals treated with CCl4. Microvesicular fatty change, extensive fatty change, and some centrilobular hepatocyte necrosis were observed on the midzonal or entire lobe (Figure 1B). The histological appearance of rat liver treated with CCl4 and Methanolic extract of Dalbergia sissoo (MEDS) revealed modest fibrosis and necrosis around the central veins, together with more apparent cellular degradation (Figure 1C). Rat liver treated with CCl4 and Dalbergia sissoo chloroform extract (CEDS) displayed mild to moderate portal inflammation histologically. It showed signs of development, with necrosis and fatty alterations diminishing (Figure 1D). Similar alterations were also detected in the silymarin treated animals. Rat liver tissue treated with CCl4 and silymarin sections demonstrated a satisfactory histological healing, free of fatty deposits and necrosis. There is not much portal inflammation in the central vein (Figure 1E).



A **B**



C D E

Fig. 1: A) Histological appearance of normal rat liver. B) Histological appearance of rat liver treated with CCl_4. C) Histological appearance of rat liver treated with methanolic extract of D. sissoo and CCl_4. D) Histological appearance of rat liver treated with Chloroform extract of D. sissoo and CCl_4. E) Histological appearance of rat liver treated with silymarin and CCl_4.

CONCLUSION:

The measurement of enzyme levels, such as SGPT and SGOT, is frequently employed in the evaluation of liver damage caused by CCl4 hepatotoxin. Since necrosis releases the enzyme into the bloodstream, serum can be used to measure it. Elevated SGPT levels are indicative of muscular injury, myocardial infarction, and liver damage. The similar mechanism is used by SGPT to release itself after catalysing the conversion of alanine to pyruvate and glutamate. As a result, SGPT is a better metric for identifying liver damage because it is more liver-specific. Our findings, which employed the rat model of CCl4-induced hepatotoxicity, showed that the test extract significantly reduced the levels of SGPT and SGOT. The function of the hepatic cell is correlated with serum bilirubin and cholesterol levels. Our test extracts (MEDS and CEDS) significantly reduced bilirubin levels, as shown by our results utilising the rat model of CCl4-induced hepatotoxicity. The study's findings, taken together, show that MEDS and CEDS have a strong hepatoprotective effect on rats' liver disease caused by carbon tetrachloride. Our results reveal that the hepatoprotective benefits of our test sample may be attributable to the antioxidant and free radical scavenging activities supplied by the presence of phytoconstituents like phenolic compounds and flavonoids.

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