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EVALUATION OF HEPATOPROTECTIVE ACTIVITY IN NATURAL PLANTS

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Abstract

This study aimed at Evaluation of hepatoprotective activity in natural plants. In this study Ethanolic extract of powder of plant *Dalbergia Sissoo*, *Hibiscus Rosa* and hydroalcoholic extract of *Quisqualis Indica* was prepared. Albino Wistar rats selected as animal for this study. Extracted sample was used for the hepatoprotective activity in CCl_4 induced hepatotoxicity in rats. Various biochemical parameters as SGPT, SGOT, Total Bilirubin, Total Protein, Cholesterol were estimated and histopathological studies were also performed in albino Wistar rat's liver. The combined extract of all test sample was found to be more significant than separately in CCl_4 induced hepatotoxicity in albino Wistar rats. Silymarin used as a standard drug for the hepatoprotective agent in CCl_4 induced hepatotoxicity in rats. The results of the present study show that ethanolic extract of *Dalbergia Sissoo* was more effective than *Hibiscus rosa* and *Quisqualis Indica* and combined test sample extract of *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* has significant hepatoprotective activity than separate extract.

Keywords: Hepatoprotective Activity, CCl_4 induced liver toxicity, *Dalbergia Sissoo*, *Hibiscus Rosa*, *Quisqualis Indica*, Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), alanine phosphatase (ALP)

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Introduction:

The Liver is a reddish-brown organ, characterised by its distinctive cone or wedge shape. It is situated above the spleen and stomach, its larger end extends over the small intestine, it resides in the right upper abdomen below the lungs. The liver weight is 3 to 3.5 pounds [1]. In macroscopically the outer surface is enveloped by the fibrous Glisson's capsule [3,4]. The main function of liver is digestion, metabolism, detoxification, storage, production, immunity.[5] It accomplishes this crucial task by processing venous blood delivered via the portal vein. [2]

Liver diseases represent significant health challenges globally. Annually, they contribute to two million deaths, comprising 4% of all global mortality, equating to one out of every 25 deaths worldwide. [6, 7, 8]. Various types of liver disease such as Chronic liver disease which changes in liver structure and function can lead to significant impairment of liver function and ultimately contribute to the development of cirrhosis. Hepatitis. [9] Alcoholic liver disease [10] Fascioliasis **[11]**. Fatty liver disease. The Etiology of liver disease include drug-induced liver disease, Exposure to Toxins, Harmful Supplements.[12] Genetics , Lifestyle Risk Factors, Drug use, exposure to Toxins, Viral infections.[13] Autoimmune Causes.[14] Herbal supplements **[15]**The major symptoms of liver problems are Jaundice, Weakness, Weight loss, Vomiting, Fatigue, Swelling of the limbs, Itches and rashes, Pain in the abdominal region, Swelling in the abdominal region, decrease in appetite, bruising more easily than usual.[16, 17] Diagnosis of liver disease is performed by certain blood tests. Laboratory examination, Radiological studies[4] Liver.[18] Liver function tests help to check liver's health and detect liver damage. Liver Enzyme Tests **(**ALT, AST, ALP, GGT). [19] Liver disease is generally treated and managed with Lifestyle modifications, Dietary changes or Eat a Healthy Diet, Medications, Surgery [20], Maintain a Healthy Weight, Get Regular Exercise, Drink Coffee, Try Milk Thistle. [21, 22]

Materials and method

Animals Wistar rats weighing 150-200 gm obtained from Authentic venders were used. The animals received standard pellet diet and allowed free access to food and water ad libitum and were maintained under standard environmental conditions were approved by the Institutional Animal Ethics committee of **INSTITUTE OF BIOMEDICAL AND INDUSTRIAL RESEARCH** from 6th February 2023. Project proposal no: (**1737/PO/Rc/S/14/CPCSEA**) Chemicals and standard drug Silymarin, ethanol, normal saline solution was procured and used.

Collection and authentication of plant materials

Material and Methods Selection and Collection of plants based on botanical survey, traditional use and literature survey. The leaves of *Dalbergia sissoo* (Family: *Fabaceae*), Leaves and flower of plant *Quisqualis Indica* **(Family:** *Combretaceae***),** Leaves and flowers of plant *Hibiscus Rosa* (*Malvaceae*) were collected from the nursery and garden from Noida, U.P. in the month of November 2020. The plant was collected and authenticated from Botanical Garden of Indian Republic (BGIR), Botanical Survey of India, Noida, U.P. for all the plants. Delbergia Sisoo (**Authentication No.:** BGIR 344), Quisqualis Indica (**Authentication No:** BGIR 343), Hibiscus rosa (**Authentication No.:** BGIR 342)

Preparation of extract:

After the collection, identification and authentication of plant part. It was washed properly with distilled water and dried in shade. After the shade drying of leaves of *Dalbergia Sissoo*, flower parts and leaves of *Quisqualis Indica* and *Hibiscus Rosa* were powered separately with a mechanical grinder and passed through a 40-mesh sieve. For the extraction below method used**:**

***Dalbergia Sissoo*:** The shade dried and pulverized leaves (1000 g) were defatted with petroleum ether and then extracted with ethanol (90%) in a Soxhlet extractor. The ethanolic extract was filtered using Whatman paper and concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rota vapour.[23] The obtained dried Dalbergia Sissoo leaves extract was further triturated in ethanol at room temperature, and the alcohol-

soluble part was concentrated and dried to obtain yield value. The physical and chemical properties of the extract should be identified. [24] The dark brown extract was then subjected to various qualitative phytochemical investigations for the identification of the different phytochemical component.[25]

Quisqualis indica:180 gm of dry powder was defatted with Petroleum ether in a closed bottle with occasional shaking and this process was continued for 9-10 days. Filtered the extract of petroleum ether. The marc obtained after defatting was dried in shade to get a dry mass and after that it was extracted with methanol and water (hydroalcoholic) by using cold maceration extraction and this process was continued for 9-10 days with occasional shaking. Filtered the extract and concentrated under reduced pressure to obtain a semisolid mass and made it free from solvent. Taken out the weight of final extract and percentage yield was calculated and stored in a cool place.[26]

Hibiscus rosa: 500 g of fine flower powder was suspended in 1500 ml of ethanol for 24 h at room temperature. Filtered the mixture with a fine muslin cloth and then Whatmann No: 1) filter paper. The filtrate was placed in a water bath to dry at 40°C and the final ethanol-free clear residue was used for the study.[27]

Phytochemical screening of plant extracts:

Table 1: Phytochemical study of the extracts of leaves of *Dalbergia sissoo, Quisqualis indica, Hibiscus rosa*

+=Presence; - = Absence, Dalbergia sissoo= A, Quisqualis Indica= B, Hibiscus Rosa = C

Phytoconstituents/ Extracts	**Dalbergia Sissoo (A)**	**Quisqualis Indica (B)**	**Hibiscus Rosa (C)**
Alkaloids	+	+	+
Glycosides	+	+	-
Flavonoids	+	+	+
Steroids	+	_	+
Tannins	+	+	+
Carbohydrates	+	-	-
Saponins	+	+	+
Terpenoids	+	-	+
Protein	+	+	-
Reducing sugar	+	+	-
Amino acids	+	+	-

Pew's test:

Few mL aqueous extract solution + 0.1gm metallic zinc + 8mL conc. H2SO4 = A red colour {flavonols}

Analysis of powder of plant extracts *Dalbergia sissoo, Hibiscus rosa, Quisqualis indica*

Few common test conducted for the standardization of powder of plant extracts (*Dalbergia sissoo, Hibiscus rosa, Quisqualis indica*) among the various pharmacopeial analysis of herbal origin. Following are the results obtained in the physical standardization of powder.

Table 2: Analysis of powder of plant

S. No.	**Parameters**	**Value obtained (%W/W)**		
		A	B	C
1	Ash content	7.0	6.3	7.1
2	Acid-insoluble ash	1.5	1.8	1.2
3	Water-soluble ash	0.8	1.1	0.6
4	Ethanol soluble extractive	47	32	38
5	Water soluble extractive	51	47	42

A= *Dalbergia sissoo*, B= *Hibiscus rosa*, C= *Quisqualis indica*

PHARMACOLOGICAL EVALUATION OF HEPATOPROTECTIVE ACTIVITY

Acute oral toxicity study

The acute toxicity studies were conducted over albino Wistar rats as per OECD guidelines 423, where rats were divided into 7 groups of six animals each. Saline received by control group and other group given 100 to 2000 mg/kg p.o. of test extract respectively. Observations were made and recorded continuously for the first 4 h for any behavioural changes. After the drug administration animals were kept under observation up to 14 days to find out the mortality if any. One-tenth of the maximum tolerated dose of, *Dalbergia sissoo* (100-200 mg/kg), *Quisqualis Indica* (400 mg /kg), *Hibiscus Rosa* (200 mg/kg, body weight, p.o.) was selected and used for animals. For hepatoprotective activity Hepatic injury was induced in rats by intraperitoneal administration of a single dose of CCl_4 (1.0 ml/kg), castor oil (1.0 ml/kg). Silymarin was used as a reference standard hepatoprotective agent was used as reference standard. Animals were grouped as follows:

EXPERIMENTAL PROTOCOL

CCl_4 induced hepatotoxicity

Table No. 3: Animals were grouped as follows for hepatoprotective activity:

GROUPS	**TREATMENT**
Group I	Control group, treated with vehicle (2.0 ml, p.o.) daily for 7 days.
Group II	Treated with vehicle (2.0 ml, p.o) daily for 7 days followed by CCL_4.
Group III	Treated with silymarin (25 mg p.o.) daily for 7 days followed by CCL_4
Group IV	Treated with ethanolic extract of *Dalbergia Sissoo* bark (100 mg/kg p.o.) daily for 7 days followed by CCL_4
Group V	Treated with hydroalcoholic extract of *Quisqualis Indica* (400 mg/kg p.o.) daily for 7 days followed by CCL_4
Group VI	Treated with ethanolic extract of *Hibiscus Rosa* (200 mg/kg p.o.) daily for 7 days followed by CCL_4
Group VII	Treated with combined extracts of *Dalbergia Sissoo, Hibiscus Rosa* and *Hibiscus Rosa* (200 mg/kg p.o.) daily for 7 days followed by CCL_4

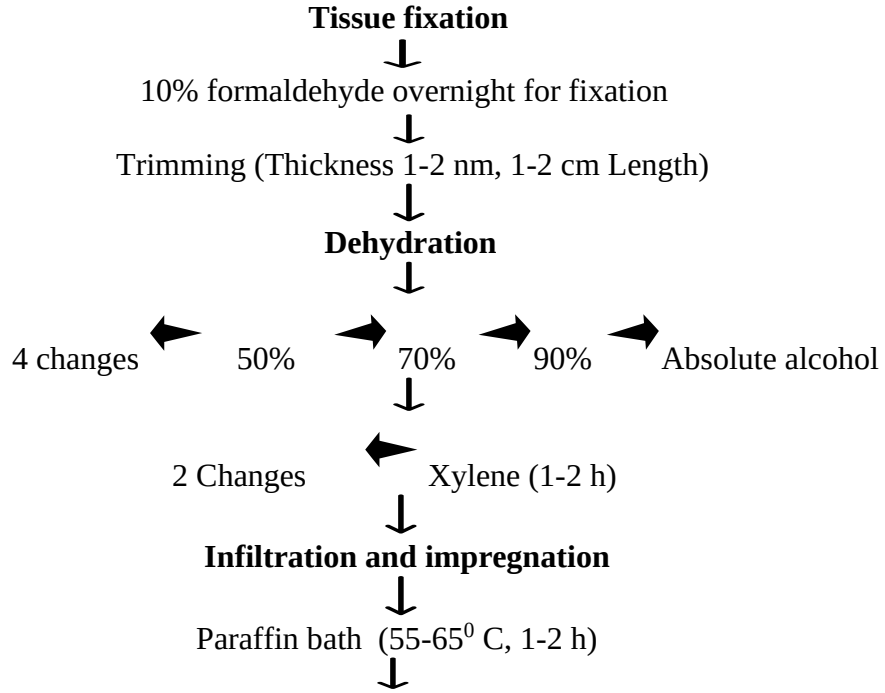
In Vitro hepatoprotective activity

For in-vitro hepatoprotective activity cardiac puncture done by a centrifuge tubes and separated serum was used for the assay of hepatic water enzymes. ASAT, ALAT, ALP along with albumin, total protein, total bilirubin, and cholesterol were estimated using diagnostic kits in clinical autoanalyzer. All animals of the experimental groups were sacrificed under anaesthesia. Liver of all animals were incised out and preserved in 10% formalin solution for histopathological examination.

Calculation and measurement of dose:
Dose of extract, silymarin, carbon tetrachloride (30 ml of Carbon tetrachloride was taken by a pipette and mixed with a 70 ml of castor oil) and vehicle were calculated according to the body weight of each group of experimental rats.

Withdrawal and collection of blood:
Cardiac puncture is a suitable technique to obtain a single, large, good quality sample from a anaesthetized rats (Anaesthetic diethyl ether was used for the induction of anaesthesia in rats). Approximately 10 mL of blood was collected by cardiac puncture. Syringe of 20gauge needle was used for the withdrawal and collection of blood under deep surgical anaesthesia. Blood samples were taken from the heart. Preferably, the left side of the chest. Blood was withdrawal slowly so that heart can be preventing from the collapse.

Histo-pathological examination of liver tissue
Histopathological examination of liver was conducted on all the groups of experimental rats for the evaluation of hepatoprotective activity of all ethanolic extracts and hydroalcoholic extracts. Protective extracts of all ethanolic and hydroalcoholic extracts and silymarin (standard) were evaluated and compared with CCl_4 intoxicated rats. In this study the gross microscopic examination of liver histology were conducted in all groups of rats. Thus, the pattern of liver damage caused by CCl_4 and its protection by plant extracts and silymarin were evaluated. Thes process followed for the histopathological examination Fixation, Processing, dehydration, clearing and infiltration, Embedding, Sectioning, Staining, Collection and preservation of liver tissue, 2 Preparation of microscopic slides of liver tissue,



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Cutting section

Staining of tissue section
Sections of tissue were cleaned with xylene till paraffin removed. Then dehydrated the tissue section were with absolute alcohol 90%, 70%, 50% alcohol for 1-2 minutes in each of the above concentrations. Therefore, sections were washed with water and finally rinsed with distilled water.

Microscopic examination of stained tissue
The prepared section of the tissue was examined to evaluate the protective effects of ethanolic extract of *Dalbergia sissoo, Hibiscus rosa* and hydroalcoholic extract of *Quisqualis indica*. The liver tissue section was prepared from all the groups experimented rats. So that comparative evaluation could be achieved against normal and toxicated rats. (Light binocular microscope was used for the histopathological examination of the liver tissues.)

STATISTICAL ANALLYSIS
The results were expressed as mean ± SEM. One way analysis of variance (ANOVA) is used for the determination of statistical significance. Significant difference between the mean was accepted when $P < 0.05$. Statistical analysis performed with software (SPSS 17). The data were evaluated by one-way ANOVA. Statistically significant represented by P values ± 0.01.

Table 3: Effect of plant extracts of *Dalbergia Sissoo, Hibiscus Rosa, Quisqualis Indica* on CCl_4 induced hepatotoxicity [Values are mean ±SEM from 6 animals in each group]

Group	**SGPT**	**SGOT**	**alkaline phosphatase**	**Albumin**	**total proteins**	**total bilirubin**	**cholesterol**
	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM
Normal	25.33 ± 0.882	73.00 ± 2.266	7.83 ± 0.601	3.23 ± 0.187	7.68 ± 0.291	0.45 ± 0.042	66.17 ± 1.424
Toxicant	78.83 ± 1.537	210.17 ± 4.086	38.00 ± 0.816	1.01 ± 0.162	5.90 ± 0.073	1.61 ± 0.094	92.17 ± 1.195
Silymarin	25.17 ±1.329	78.17 ±1.909	8.67 ±0.882	3.36 ±0.212	6.367 ±0.996	0.53 ±0.084	68.17 ±1.167
100 mg/kg (DS)	27.17 ±1.250	76.15 ±4.868.	9.16 ±0.574	2.90 ±1.650	6.93 ±0.464	0.69 ±0.136	71.33 ±1.679
200 mg/kg (HR)	74.83 ±1.092	91.05 ±2.043	9.81 ±0.442	3.147 ±0.285	7.10 ±0.330	1.00 ±0.089	73.00 ±2.053
400 mg/kg (QI)	49.00 ±1.483	98.50 ±4.202	30.67 ±0.955	2.35 ±0.17	6.58 ±0.130	0.767 ±0.088	77.83 ±1.19
Combined (DS+HR+QI)	26.09 ±1.815	25.12±1.379	8.93±0.464	3.173±0.319	6.913±0.601	0.59±0.121	69.21±1.611

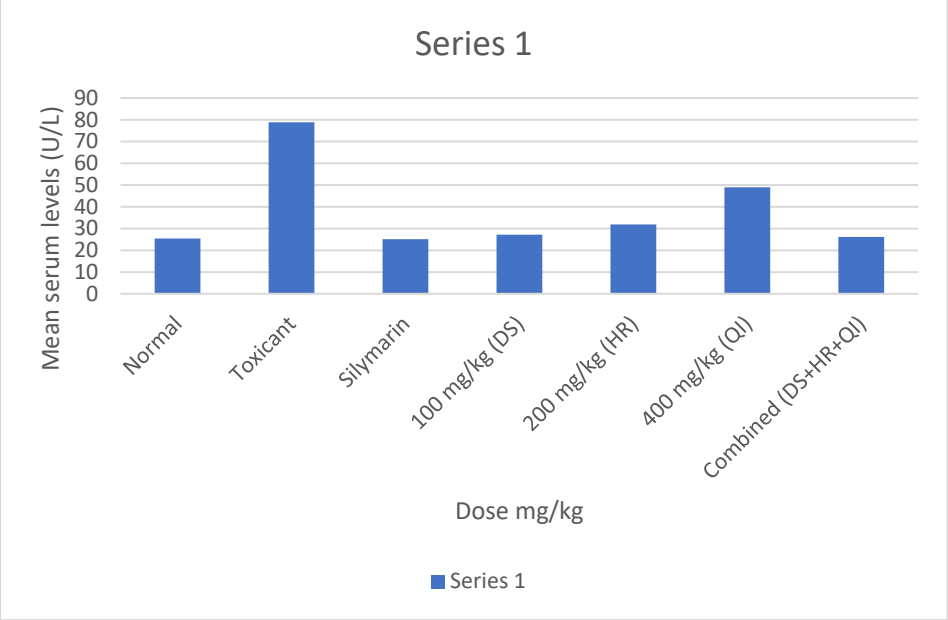

Figure 1: Comparative effects on serum SGPT levels in CCl_4 intoxicated rats.

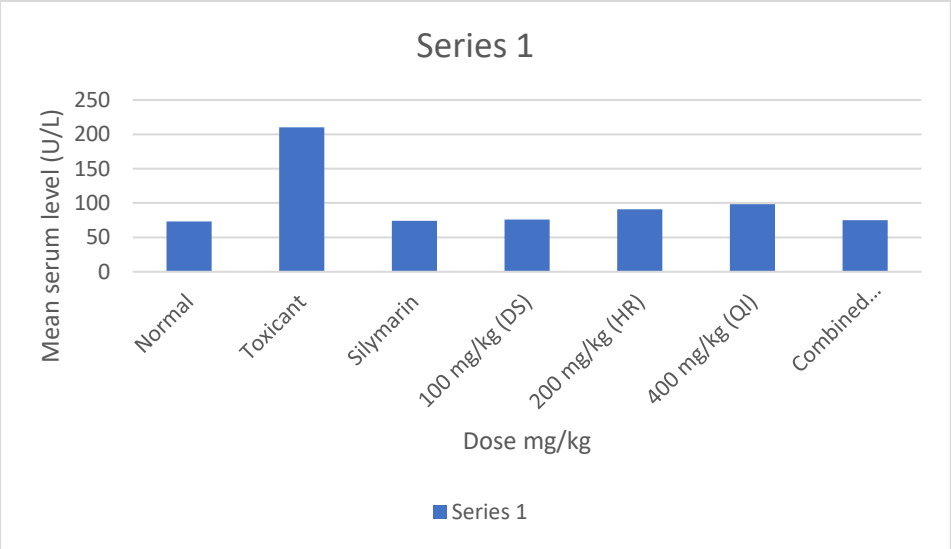

Figure: 2: Comparative effects on serum SGOT levels in CCl_4 intoxicated rats.

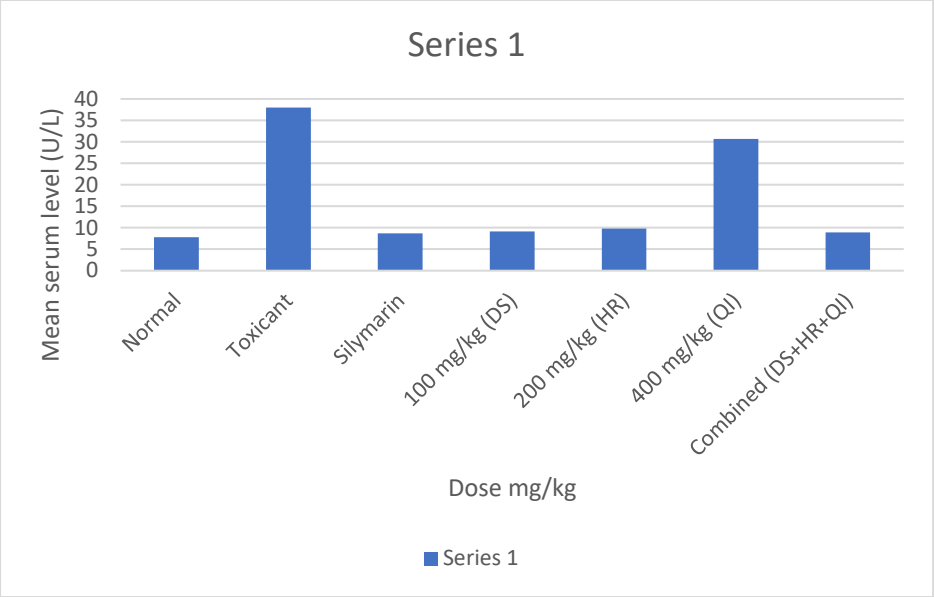

Figure 3: Comparative effects on serum alkaline phosphatase levels in CCl_4 intoxicated rats.

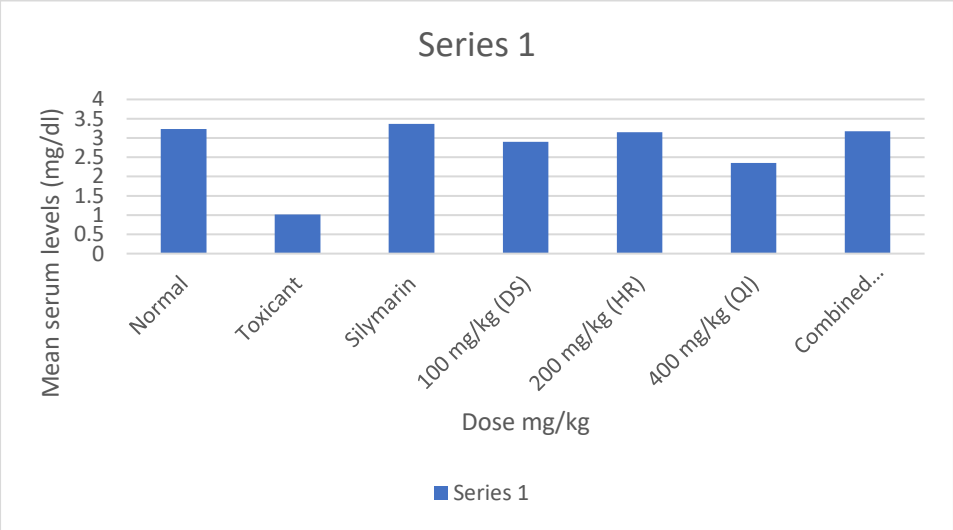

Figure 4: Comparative effects on serum albumin levels in CCl_4 intoxicated rats.

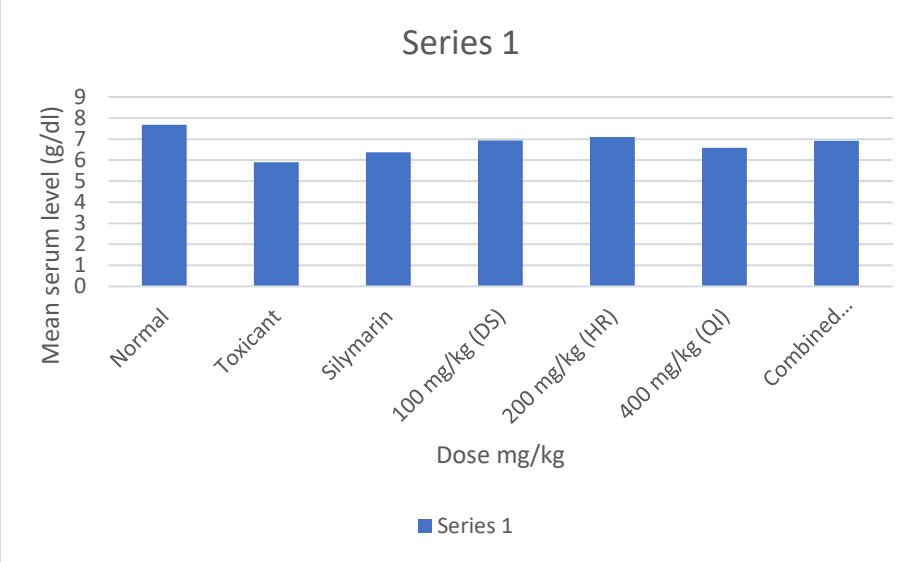

Figure 5: Comparative effects on serum total proteins levels in CCl_4 intoxicated rats.

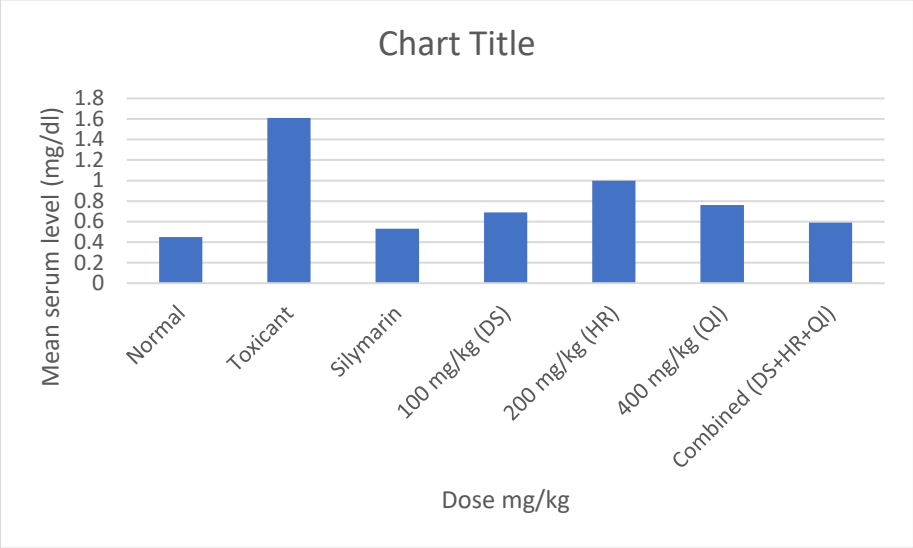

Figure 6: Comparative effects on serum total bilirubin levels in CCl_4 intoxicated rats.

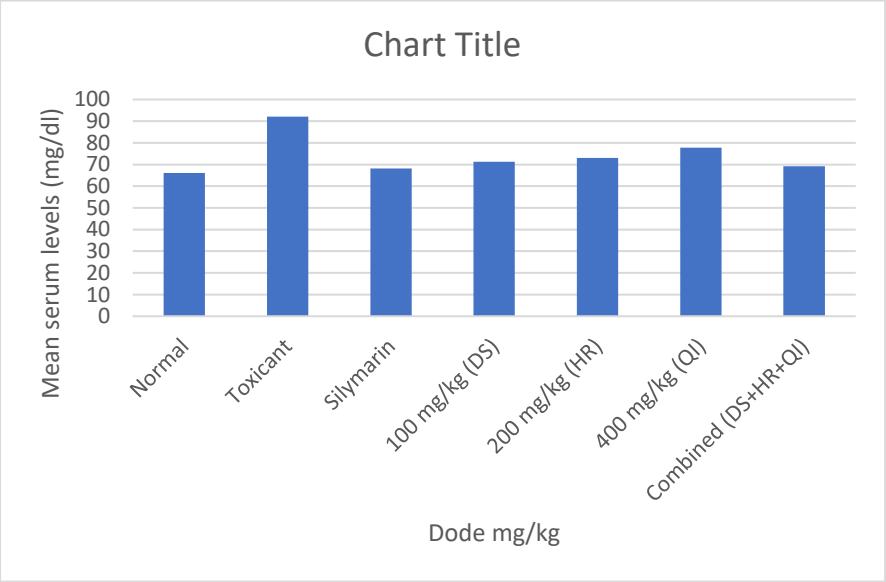

Figure 7: Comparative effects on serum cholesterol levels in CCl_4 intoxicated rats.

Statistical study on the mean differences of estimated serum levels of enzymes for the various groups were analysed by analysis of variance (ANOVA) test.

Histopathological examination of liver tissue

Following observations were found in the histopathological examination of liver tissue of CCl_4 treated rats.

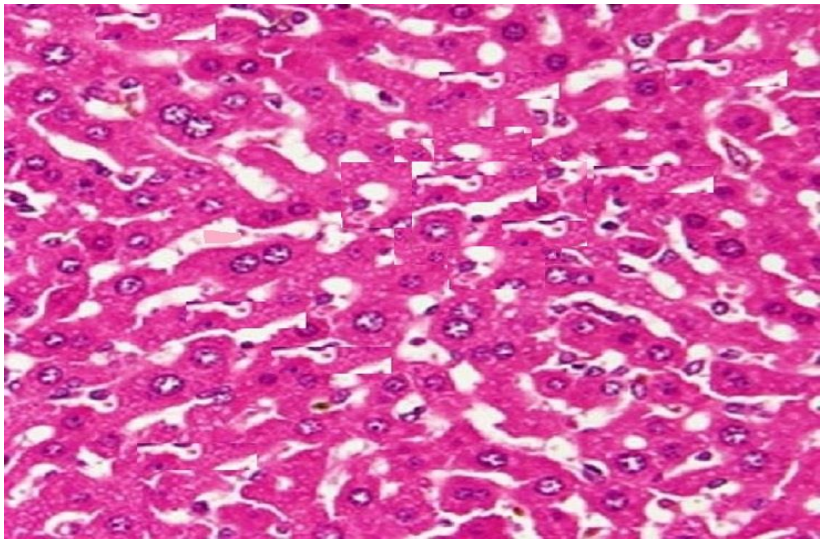

Figure 8: Group 1: Section of liver with normal cell structure

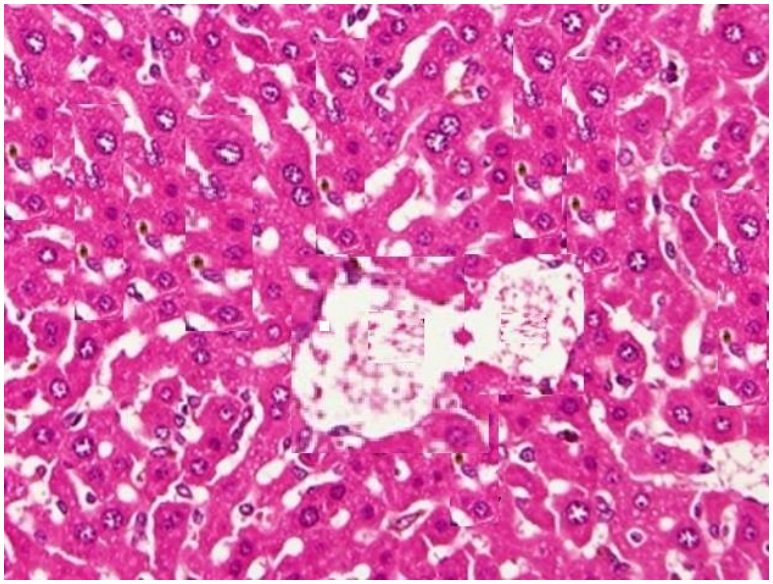

Figure 9: Group II: Section of liver of CCl_4 intoxicated rats.

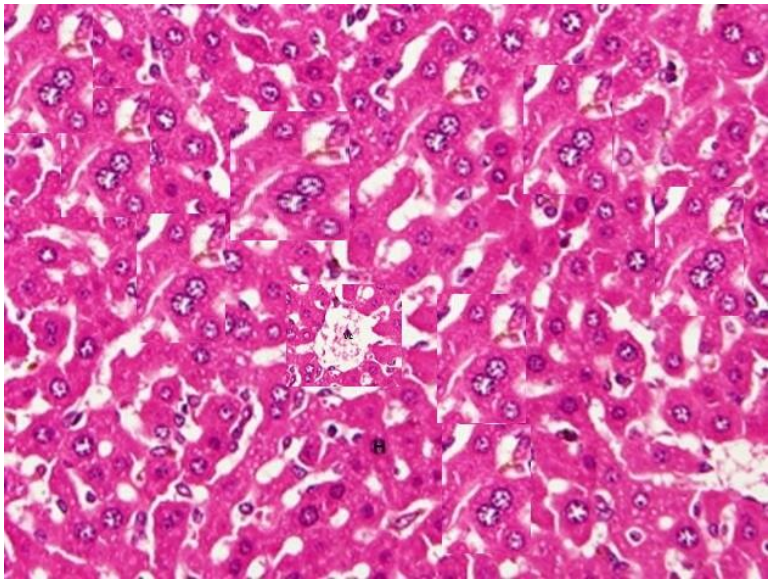

Figure 10: Group III: Section of liver of standard treatment+ CCl_4 intoxicated rats, 40 x

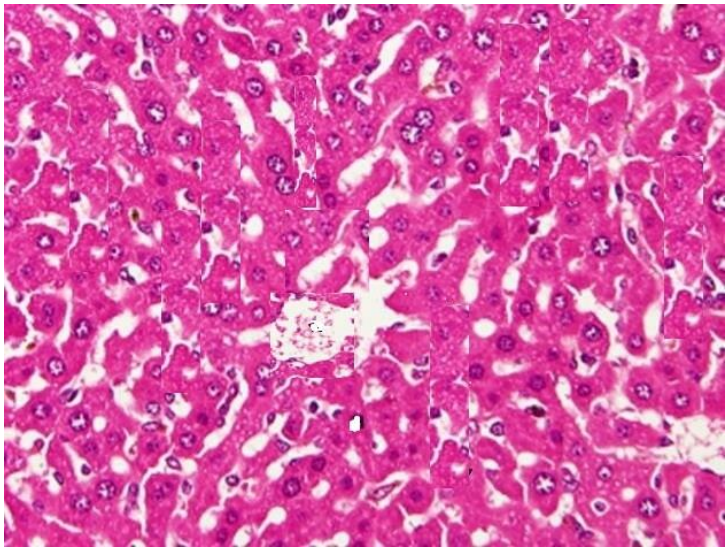

Figure 11: Group IV: Section of liver of 100 mg/kg treatment test sample of *Dalbergia Sissoo* + CCl_4 intoxicated rats, 40 X

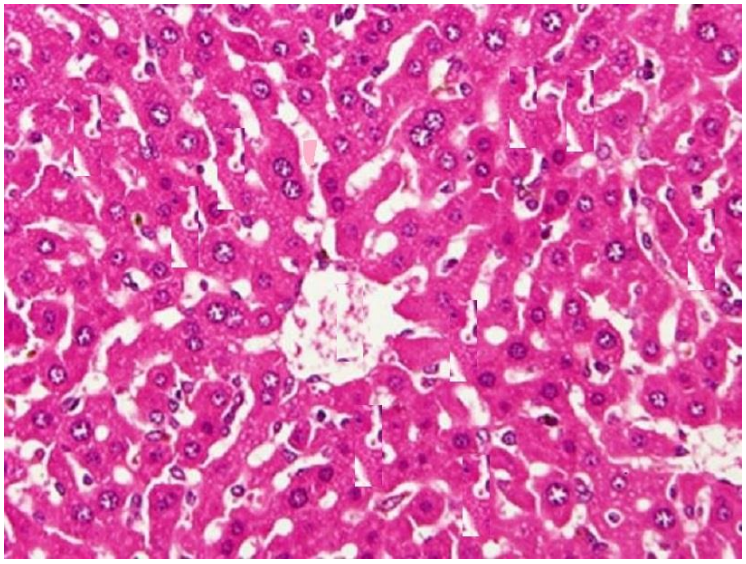

Figure 12: Group V: Section of liver of 200 mg/kg treatment with test sample *Hibiscus Rosa* + CCl_4 intoxicated rats, 40 X

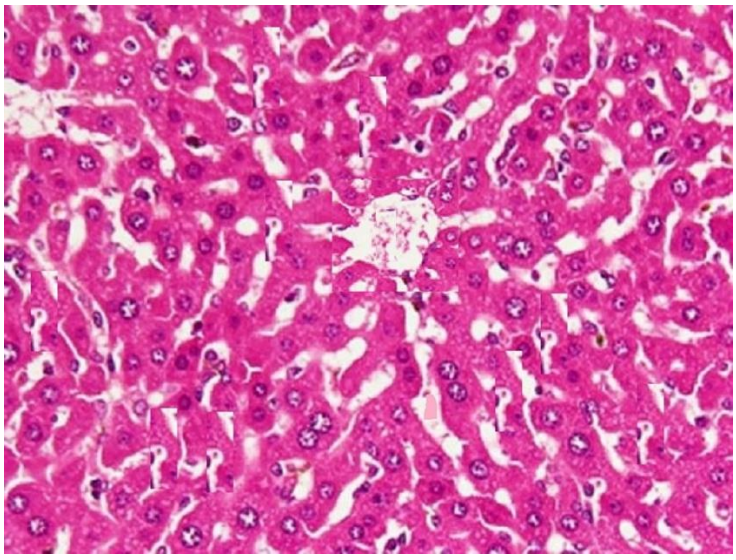

Figure 13: Group IV: Section of liver of 400 mg/kg treatment with test sample *Quisqualis Indica* + CCl_4 intoxicated rats, 40 X

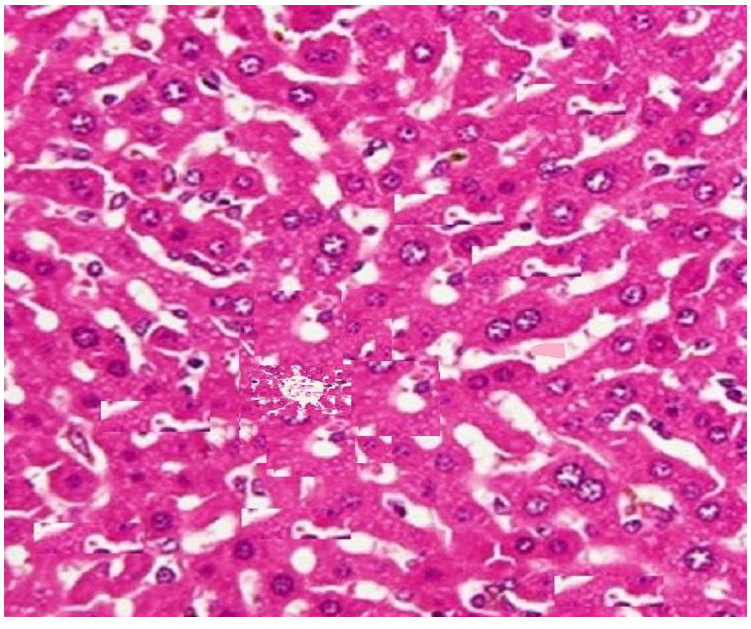

Figure 14: Group VII: Section of liver of 200 mg/kg treatment with combined test sample of (*Dalbergia sissoo+ Hibiscus Rosa+ Quisqualis Indica*) + CCl_4 intoxicated rats, 40 X

Microscopic examination of the liver histology

Gr. I animal indicates that the pattern of liver histology among the group is following animal architecture of liver tissue.
Gr. II animal indicates complete fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities are prominent.
Gr. III animals showing reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities.
Gr. IV animals have significantly reduction in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities.
Gr. V animals showing comparatively reduced in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities but less than group IV.
Gr. VI animals showing less reduced in fatty liver change centriolobular necrosis hepatocytes degeneration and sinusoidal deformities.
Gr. VII animals have significantly reduction in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities, showing satisfactory results as compared to other groups.

Discussion

The present investigation indicated that all the extracts of Dalbergia Sissoo, Hibiscus Rosa and Quisqualis Indica provide significant protection against CCl_4 induced hepatotoxicity in rats. CCl_4 is widely used as hepatotoxin in the experimental studies.[28] CCl_4 metabolically activated to form trichloromethyl free radical (CCl_3) which bind with cellular lipids and proteins which cause the structural change of endoplasmic reticulum and other membrane. [29] Several plants viz., *Cassia aungustifolia*,[30] *Wrightia tinctoria*, [31] *Foeniculum vulgare*[32]. and *Panax notoginseng*[33] have been tested for their efficacy in controlling the CCl_4 induced liver damage. Several phytoconstituents induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of lipid peroxidation induced by CCl_4.[34]. Phytoconstituents like flavonoids, triterpenoids,[35]. saponins[36] and alkaloids[37] are known to possess hepatoprotective activity. Phytochemical investigations of all extracts of *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* revealed the presence of alkaloids, phenols, saponins, glycosides, flavonoids, triterpenoids, sterols and tannins. The present study revealed that all the extract of plants found to possess significant protective effect against hepatotoxicity induced by CCl_4 which may be attributed to the individual or combined action of phytoconstituents present in it. Conventional drugs used in treatment of liver diseases are sometimes inadequate and offer serious adverse effects. Therefore, herbal medicines are in great demand in developed as well as developing countries for primary healthcare because of their wide biological/medicinal activities with higher safe. The elevations of these enzymes are due to the extensive liver damage induced by toxin. The reduced concentrations of SGPT,

SGOT and ALP because of plant extract administration observed during the present study might be due to the presence of flavonoids. [38].

Conclusion

Present study demonstrated that carbon tetrachloride induction causes prominent increase of the liver enzymes such as SGPT, SGOT, Bilirubin, total protein, cholesterol which may be due to the decrease of antioxidant system in serum in CCl_4 induced rat and oxidative stress is an important mechanism of organ damage. We chose the natural source such as *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* depending upon its potential activities through literature review. A positive effect has been observed for all plant extract against CCl_4 induced hepatotoxicity. In conclusion, we can say that combined extract of all test sample is a prominent source to treat CCl_4 induced liver damage. By comparing with standard (Silymarin) is has been revealed that herbal products specially plant source is a great source for treating hepatic diseases. The combined extract are more significant than separately used for hepatoprotective activity. [28]

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