HEPATOPROTECTIVE ACTIVITY OF NATURAL PLANTS IN ALBINO WISTAR RATS

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ABSTRACT

The aim of this study was Evaluation of hepatoprotective activity of natural plants in albino Wistar rats. In this study three plant was selected *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* and its ethanolic and hydro-alcoholic extract was prepared. Albino Wistar rats selected as animal for this study. Extracted sample was used for the hepatoprotective activity in CCI4 induced hepatotoxicity in rats. Silymarin was taken as standard drug. Various biochemical parameters as SGPT, SGOT, alkaline phosphatase, total bilirubin, total protein, Cholesterol were estimated by using commercially available diagnostic kit and histopathological studies were performed in albino Wistar rat's liver. The hepatoprotective study was performed for separately prepared extract of all three plant and combined extract of all plant. The result of the present study show that in all three plant ethanolic extract of *Dalbergia Sissoo* was more effective than other Hibiscus rosa and Quisqualis Indica and The combined extract of all test sample was found to be more significant than separately prepared extract in CCl4 induced hepatotoxicity in albino Wistar rats.

Keywords: Hepatoprotective Activity, CCl4 induced liver toxicity, *Dalbergia Sissoo*, *Hibiscus Rosa, Quisqualis Indica*, Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), alanine phosphatase

Introduction

The Liver is a reddish-brown organ, cone or wedge shape. It is situated above the spleen and stomach. The liver weight is 3 to 3.5 pounds [1,2

]. 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] 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 etc.[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, Medications, Surgery [20] Get Regular Exercise, Drink Coffee, Try Milk Thistle. [21, 22]

Materials and Methods

Animals albino Wistar rats weighing 150-250 gm obtained from Authentic venders were used. The animals received standard pellet diet and allowed free access to food and water ad libitum and acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25 ± 2°C relative humidity 44 –56% and light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiments. The experiments were approved by CPCSEA and 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 used. Animals were examined for the physical fitness before conducting the experiments.

Collection and authentication of plant materials:

Collection of plant based on botanical survey, traditional use and literature survey. The leaves of *Dalbergia Sissoo* and mature flowers and leaves of *Quisqualis indica Linn* and leaves and flowers of *Hibiscus rosa* were collected from the nursery and garden from Noida, U.P. in the month of November 2020. The plant was 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 then 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 (500 g) were defatted with petroleum ether and then extracted with ethanol (90%) in a Soxhlet extractor. The ethanolic extract was filtered (Whatman paper) and concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rota vapour.[23] The obtained dried extract was further triturated in ethanol at room temperature, and the alcohol-soluble part was concentrated and dried and its weight was taken 34.83 g to obtain yield value was 6.96. ***Quisqualis indica: About 180 gm of dry powder was taken in a closed bottle and it was defatted with Petroleum ether. Defatting was carried out for 9-10 days with occasional shaking, using petroleum ether as the solvent. The petroleum ether extract was filtered, and the residual marc was dried under shade. The dried marc was then subjected to cold maceration extraction with methanol and water (hydroalcoholic) for 9-10 days with occasional shaking. The hydroalcoholic extract was filtered, concentrated under reduced pressure to a semisolid mass, and then completely freed from solvent. The final extract was weighed and it was 8.33 g, yield calculated as 4.62, and stored in a cool place.[24] ***Hibiscus rosa:*** 500 g of fine leaves and flower powder was suspended for 24 hour at room temperature in 1500 ml of ethanol. The mixture was first filtered through a fine muslin cloth and then passed through Whatman No. 1 filter paper for further purification. The filtrate was placed in a water bath to dry at 40°C and the final ethanol-free clear residue weight was taken 51.12 g and its percentage yield was 10.22. All extract w used for the phytochemical study.[25]

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 Sisoo*** **(A)**	***Quisqualis Indica*** **(B)**	***Hibiscus Rosa*** **(C)**
Alkaloids	+	+	+
Glycosides	+	+	-

Flavonoids	+	+	+
Steroids	+	_	+
Tannins	+	+	+
Carbohydrates	+	-	-
Saponins	+	+	+
Terpenoids	+	-	+
Protein	+	+	-
Reducing sugar	+	+	-
Amino acids	+	+	-

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. The control group received saline, while the other groups were administered the test extract orally at doses ranging from 50 to 2000 mg/kg. Observations were made and recorded continuously for the first 4 h for any behavioural changes. The animals were then kept under observation for up to 14 days after drug administration to monitor for any mortality. 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. 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 is a known hepatoprotective agent and it was used as reference standard.[26,27,28] Animals were grouped as follows:[29]

Experimental protocol

CCL4 induced hepatotoxicity

Table 2: **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 CCL4.

Group III	Treated with silymarin (25 mg p.o.) daily for 7 days followed by CCl4.
Group IV	Treated with ethanolic extract of *Dalbergia Sissoo* bark (100 mg/kg p.o.) daily for 7 days followed by CCl4.
Group V	Treated with hydroalcoholic extract of *Quisqualis Indica* (400 mg/kg p.o.) daily for 7 days followed by CCl4.
Group VI	Treated with ethanolic extract of *Hibiscus Rosa* (200 mg/kg p.o.) daily for 7 days followed by CCl4.
Group VII	Treated with combined extracts of *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* (200 mg/kg p.o.) daily for 7 days followed by CCl4 [30]

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. SGPT. SGOT, alkaline phosphatase, 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.[31]

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:

Blood collected by cardiac puncture, [32 by using Syringe of 20gauge needle, approximately 10 mL blood taken out 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.[33]

Histopathological 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 CCl4 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 CCl4 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, Preparation of microscopic slides of liver tissue. Liver tissues was section by microtome and 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 all plant extract. 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.)

Table 3: Effect of plant extracts of *Dalbergia Sissoo, Hibiscus Rosa, Quisqualis Indica* in CCl_4 induced hepatotoxicity

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	35.33± 1.20	73.01± 1.20	92.3±0.77	3.23±0.14	7.98 ±0.04	0.45± 0.067	66.17± 0.84
Toxicant	98.83± 0.68$^{\#}$	210.17± 4.02$^{\#}$	278.32± 2.93$^{\#}$	1.01±0.05$^{\#}$	5.03±0.35$^{\#}$	1.91± 0.162$^{\#}$	92.17± 0.73$^{\#}$
Silymarin	39.92± 0.67**	81.08± 0.87*	104.16± 1.08**	3.36±0.21^{ns}	6.16±0.24**	0.92± 0.179*	71.33± 1.25**
100 mg/kg (DS)	42.17± 0.85**	97.03± 1.40**	132.32± 1.57**	2.08±0.15**	5.40±0.18**	1.19± 0.068**	79.83± 0.80**
200 mg/kg (HR)	74.83± 1.03**	123.05± 1.47**	187.70± 2.16**	1.98±0.19**	5.21±0.10**	1.25± 0.089**	86.03± 0.56**
400 mg/kg (QI)	49.00± 1.02**	133.18± 1.65**	212.50± 2.81**	1.91±0.21**	5.25±0.13**	1.31± 0.121**	85.12± 0.97**
Combined (DS+HR+QI)	40.09± 0.74**	86.22± 0.66**	113.63± 2.30**	2.45±0.18*	6.72±0.26**	1.10± 0.064**	72.00± 1.13**

Values are expressed as Mean ±SEM, where n= 6 and the significance was performed by One way ANOVA followed by Dunnett's comparison test. $^{ns}P > 0.05$, $*P < 0.05$, $**P < 0.01$, as compared to normal control group. $^{\#}P < 0.01$ is significant increase in levels of enzymes in comparison to normal control group.

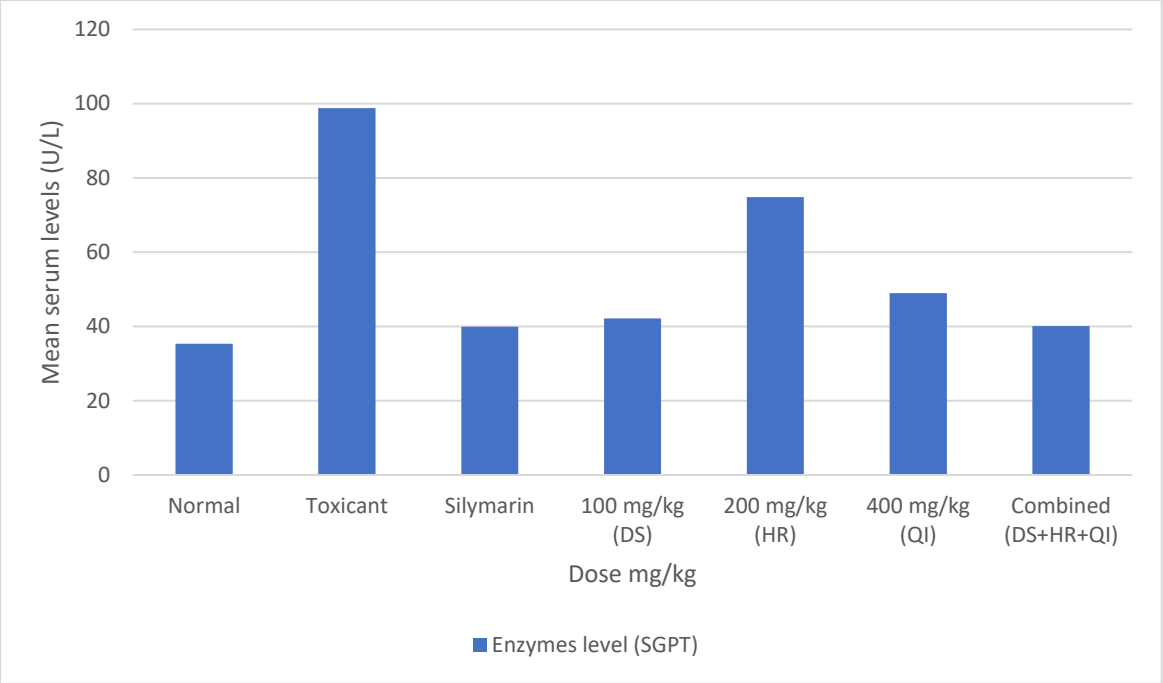


Figure 28: **Comparative effects on serum SGPT levels in CCl4 intoxicated rats.**

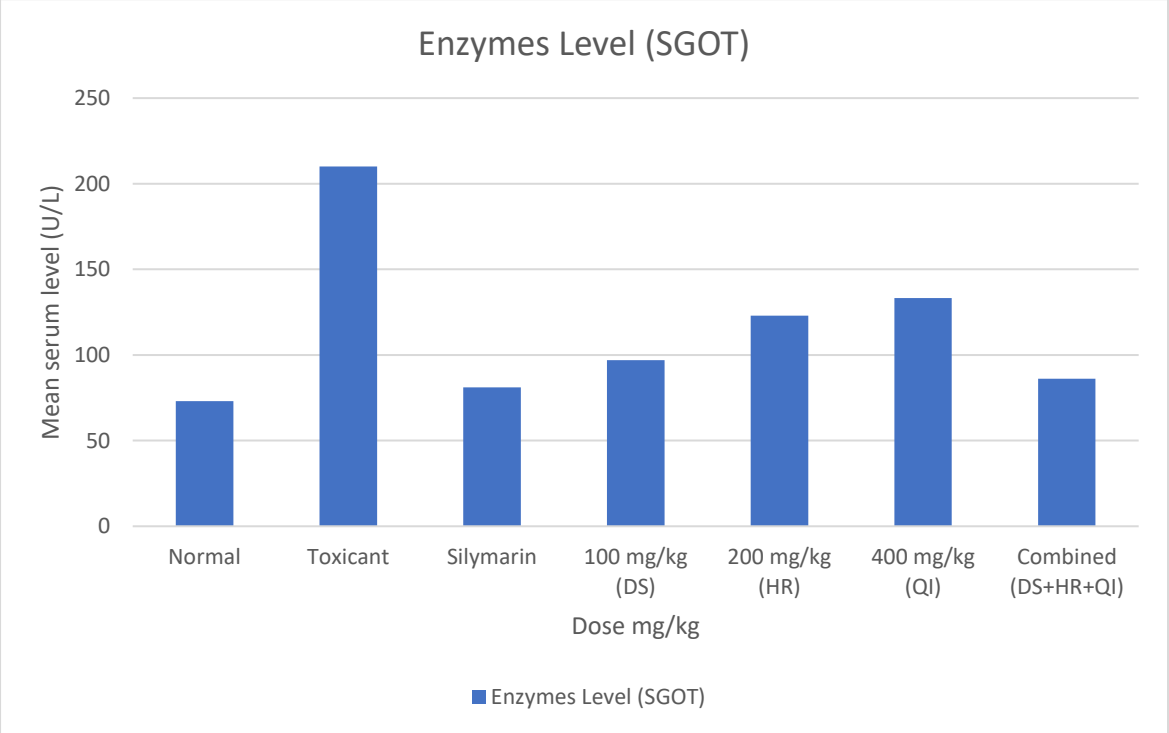


Figure: 29: **Comparative effects on serum SGOT levels in CCl4 intoxicated rats.**

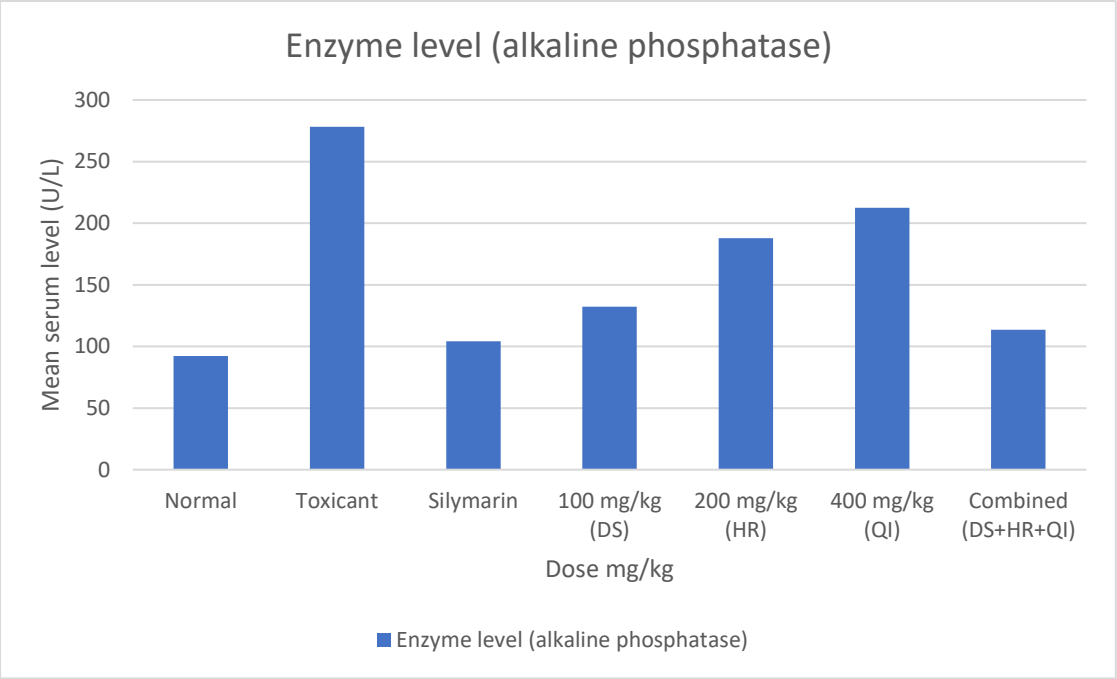


Figure 30: **Comparative effects on serum alkaline phosphatase levels in CCL4 intoxicated rats.**

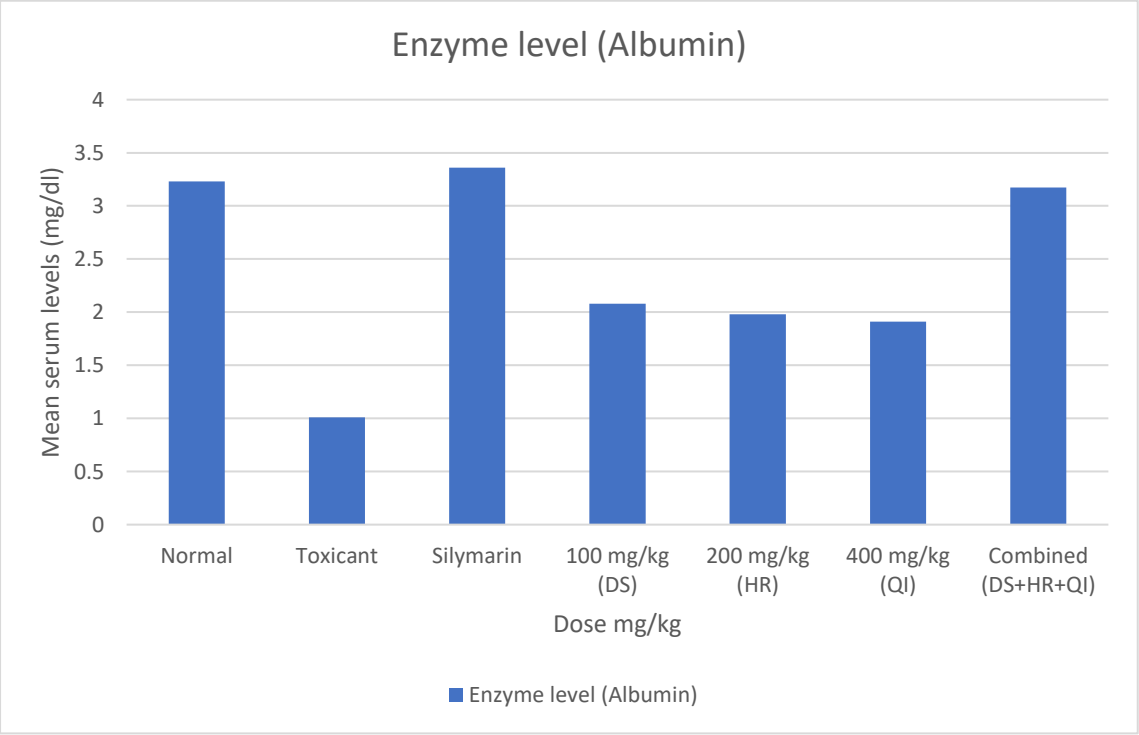
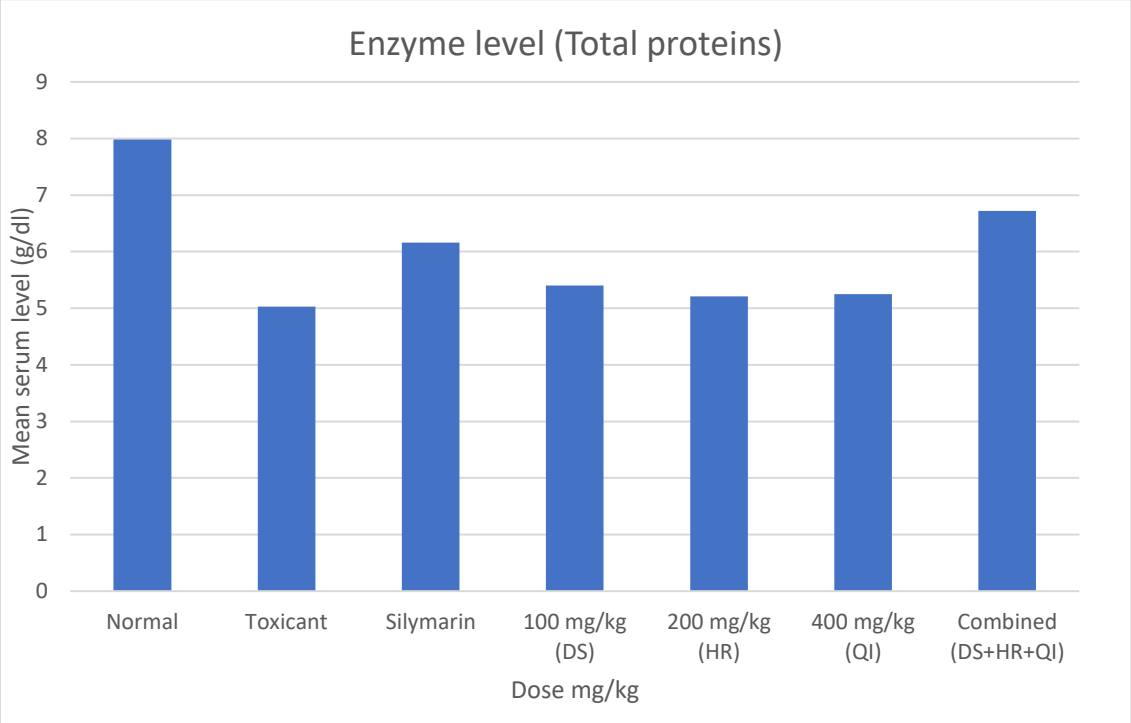


Figure 31: Comparative effects on serum albumin levels in CCl4 intoxicated rats.



32: Comparative effects on serum total proteins levels in CCl4 intoxicated rats.

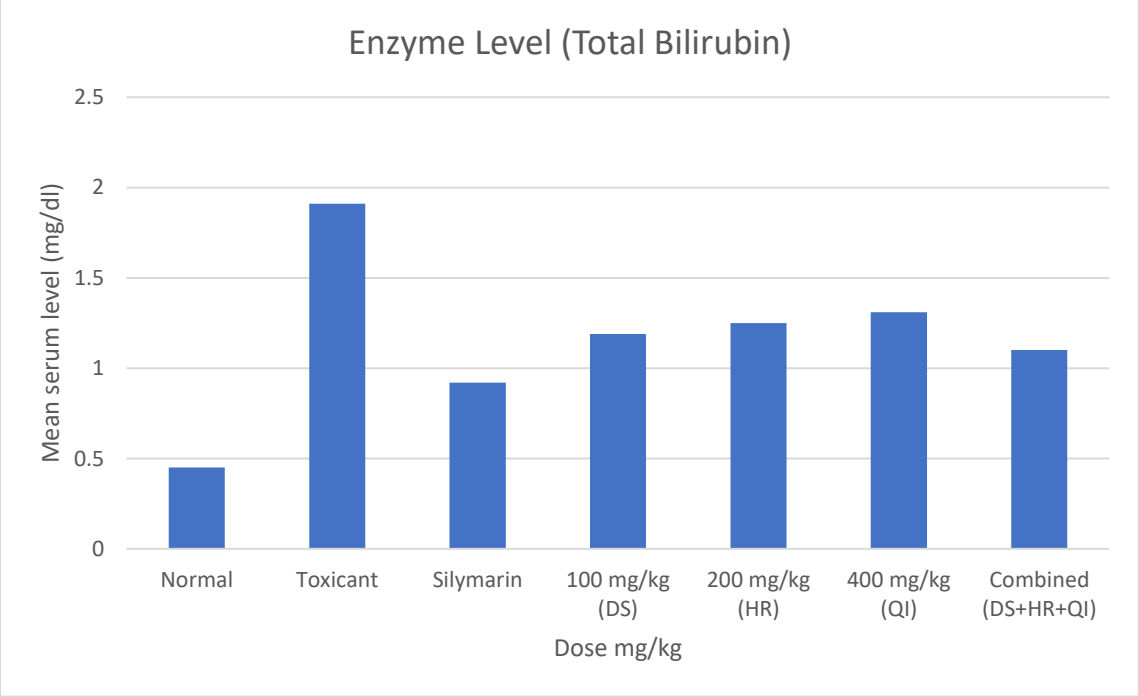


Figure 33: Comparative effects on serum total bilirubin levels in CCl4 intoxicated rats.

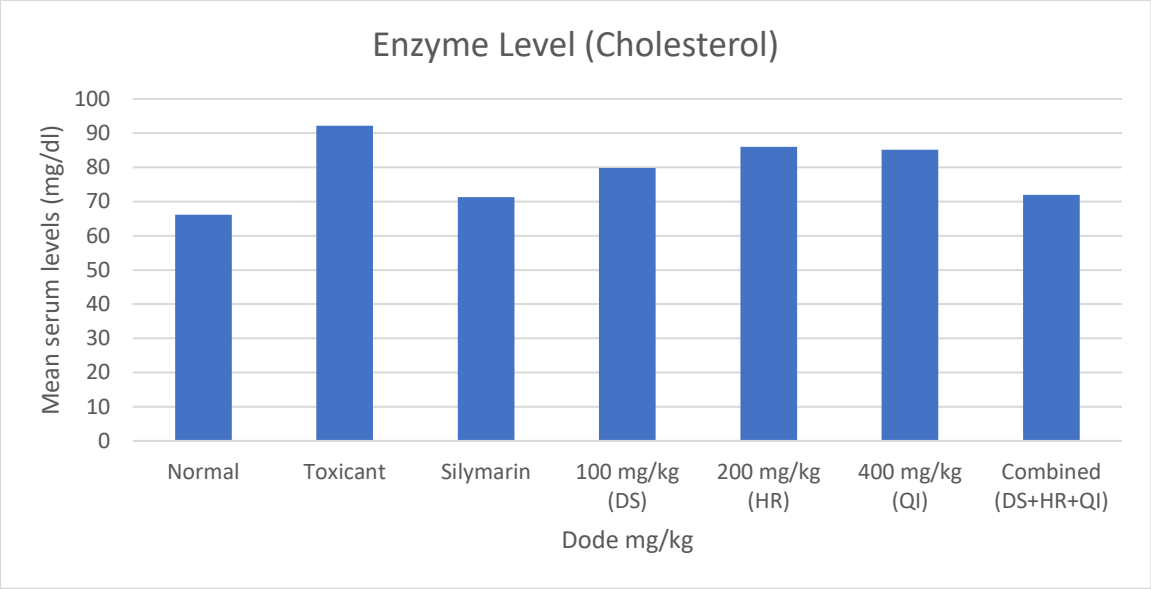


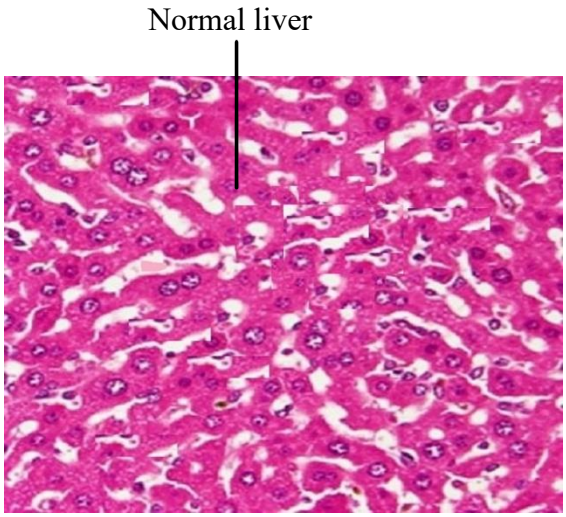
Figure 34: **Comparative effects on serum cholesterol levels in CCl4 intoxicated rats.**

Analysis of variance:

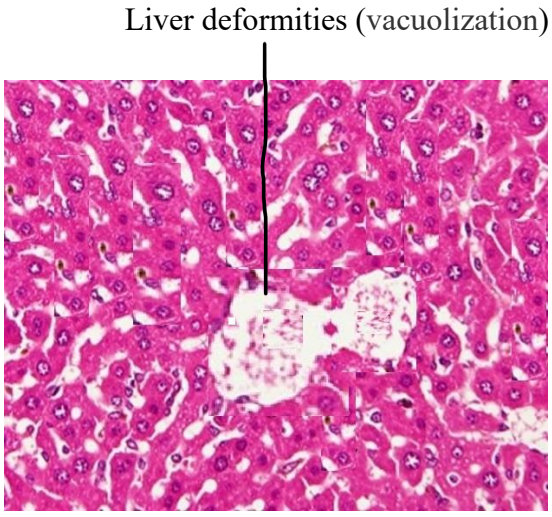
Statistical study on the mean differences of estimated serum levels of enzymes for the various groups were analysed by analysis of variance (ANOVA) test followed by Dunnett test by using the software GraphPad InStat. The P value estimated to determine the significant level.

Histopathological examination of liver tissue:

Following observations were found in the histopathological examination of liver tissue of CCl4 treated rats.

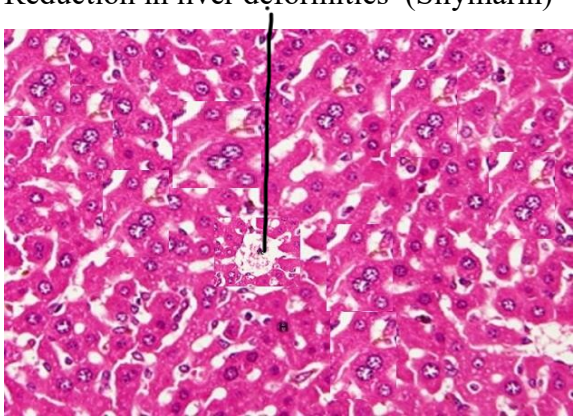


Gr. I: Section of liver with normal cell Structure



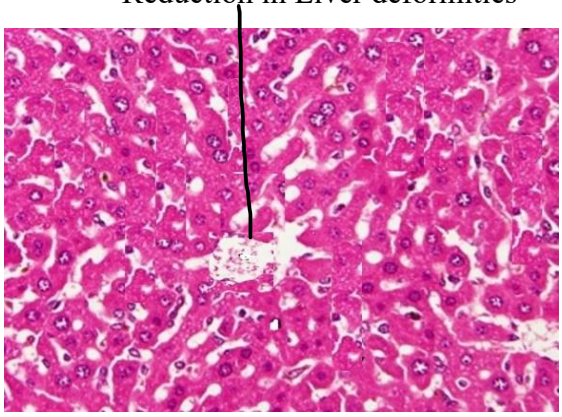
Gr. II: Section of liver of CCl4 intoxicated rats.

Reduction in liver deformities (Silymarin)



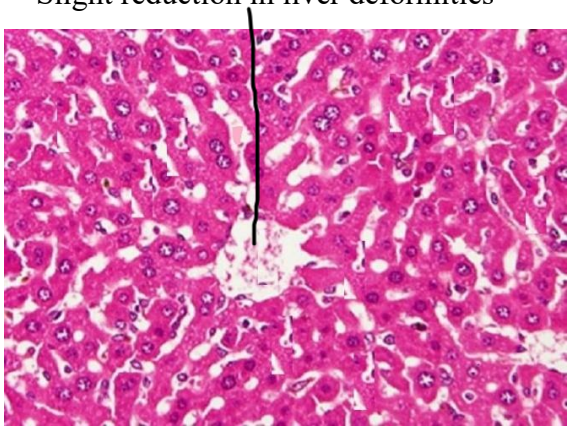
Gr. III: Section of liver of standard treatment+ CCl4 intoxicated rats.

Reduction in Liver deformities



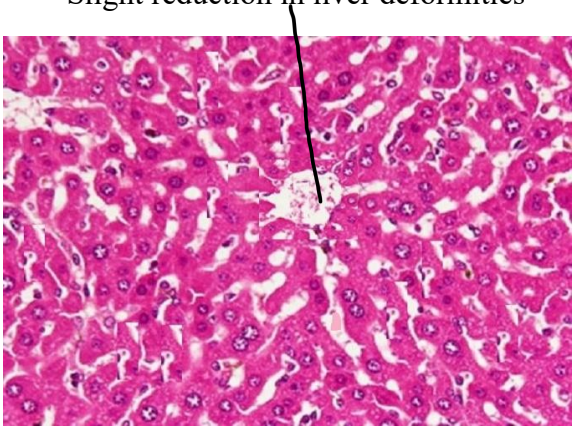
Gr. IV: Section of liver of 100 mg/kg of *Dalbergia Sissoo*+CCl4.

Slight reduction in liver deformities



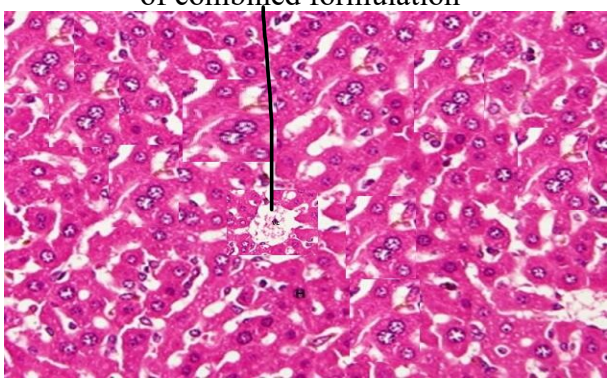
Gr.V: Section of liver of 200 mg/kg treatment with Hibiscus Rosa+CCl4

Slight reduction in liver deformities



Gr. VI: Section of liver of 400 mg/kg treatment with Quisqualis Indica +CCl4

Significant hepatoprotective effect of combined formulation



Gr. VII: Section of liver of 200 mg/kg treatment with combined test sample of (Dalbergia sissoo+ Hibiscus Rosa+ Quisqualis Indica) +CCl4 intoxicated rats

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 showed fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities are prominent. Gr. III 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 showed comparatively reduced in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities but less than group IV. Gr. VI howing less reduced in fatty liver change centriolobular necrosis hepatocytes degeneration and sinusoidal deformities. Gr. VII have significantly more reduction in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities, showing satisfactory results as compared to other groups.

Discussion

The present study indicated that all the combined formulation of extracts of *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* provide significant protection against CCl_4 induced hepatotoxicity in rats rather than separately prepared extract formulation. **CCl_4 is used for the hepatotoxicity in rats.** Phytoconstituents like flavonoids, triterpenoids,[34] saponins [35] and alkaloids[36] are known to possess hepatoprotective activity. The reduced in the concentrations of SGPT, SGOT and ALP, albumin, total protein. Total bilirubin and cholesterol due to the presence of flavonoids in selected plants.[37] All liver enzymes were estimated and its assay was performed by using commercially available kits. Histological analysis was performed. Using a microscope and inspected the microscopic slides for hepato-protective activity. The results were expressed as mean ± SEM. Statistical significance was determined by one way analysis of variance (ANOVA) tests followed by Dunnett tests.

Conclusion

On the basis of results obtained, it can be concluded that the extract of Dalbergia sisso bark seems to possess hepatoprotective in rats. No toxic symptom or mortality was observed in 14 days of study in rats. Histopathological examination of the liver and kidney section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated

with extract of all plants with toxicant showed sign of protection against these toxicants to considerable extent as evident from absence of necrosis and vacuoles.

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