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# HEPATOPROTECTIVE ACTIVITY OF *PTEROCARPUS MARSUPIUM* HEARTWOOD AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN FEMALE ALBINO WISTAR RATS

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**ABSTRACT**

In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus. The present study is designed to investigate the hepatoprotective activity of *Pterocarpus marsupium* heartwood against carbon tetrachloride ($CCl_4$) induced hepatotoxicity in female Albino Wistar rats. Forty-two Albino Wistar female rats were divided in to seven equal groups each containing six animals. The shade dried heartwood of *Pterocarpus marsupium* was subjected for extraction by continuous hot extraction with petroleum ether (40- 60ºC), chloroform, alcohol and water. All the extracts (200 mg/kg) were evaluated for their hepatoprotective activity in $CCl_4$ induced hepatotoxicated rats. Hepatotoxicity induced rats were orally treated with extracts for 5 days. Four groups received the different extracts of *Pterocarpus marsupium* heartwood and intraperitoneal (i.p.) administration of $CCl_4$ (2 ml/kg). One group was control, one treated with $CCl_4$ and one group received standard drug Liv-52. The hepatotoxicity was assessed by plasma concentration of serum bilirubin and enzymes activities. Biochemical analysis and histopathology studies were performed to support the hepatoprotective activity. Among all the extracts, the aqueous extract (200 mg/kg) of *Pterocarpus marsupium* heartwood significantly reduced carbon tetrachloride induces damages in the liver, which is measured by SGOT, SGPT, SALP and serum bilirubin levels in blood. Histopathological examination also supported the hepatoprotective effect of aqueous extract (200 mg/kg) of *Pterocarpus marsupium* heartwood.

**Keywords:** *Pterocarpus marsupium,* heartwood, carbon tetrachloride, hepatoprotective.

## INTRODUCTION

Liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturb body metabolism. In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus[1]. There are hardly any proven remedies for the prevalent liver disorders among people. No drug has been developed in the modem system of medicine which may stimulates the liver function, protect it from damage or help in the regeneration of hepatic cells. The only drugs, which are available for treatment of liver disorders, are corticosteroids and immuno-suppressive agents but their use is accompanied by serious side effects. There is an ever-increasing need for an agent, which could protect the liver against damages[2,3]. Due to lack of a consistent liver protective medicine in the modern system, a number of medicinal preparations in Ayurveda, the Indian system of medicine, are recommended for the cure of liver disorders. Natural remedies from medicinal plants are considered to be effective and safe option medicines for hepatotoxicity[4].

*Pterocarpus marsupium*is a medium sized to large tree grows to 10-15 meters in height, belonging to Family Papilionaceae. It is commonly known as India kino tree and Malabar kino tree.[5,6] It is found throughout India in deciduous and evergreen forests up to 4500 ft. also distributed in Sri Lanka[7]. The heartwood, leaves, flowers and gum of the tree mainly used for medicinal purpose. The heart wood is used to treat the conditions like inflammations, fractures, bruises, leprosy, leucoderma, ophthalmopathy and rheumatoid arthritis[8], rejuvenator, astringent, herpes.[5] The stem bark of *Pterocarpus marsupium* had been evaluated for the hepatoprotective activity.[9] The heartwood of the plant is rich source of flavonoids. Five new flavonoid were isolated from an aqueous extract of the heartwood of *Pterocarpus marsupium.*[10] The aqueous extract of heartwood of *P. marsupium* contains 5, 7, 2-4 tetrahydroxy isoflavone 6-6 glucoside which is potent antioxidant agent.[11] The plant also has exhibited remarkable antioxidant activity and/or free radical scavenging activity may be due to the presence of the different type of constituents specifically phenolic and flavonoid contents.[12] Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties[13] and was found to be useful in the treatment of liver damage.[10]

Taking into consideration the potential role of antioxidant agent in liver disorder, this study deals with Hepatoprotective activity of heartwood of *Pterocarpus marsupium* Linn.

## MATERIAL AND METHODS

### Plant Material

Procurement of drugs: The heartwood of *Pterocarpus marsupium* was collected from Pragati Pharma, Belagaum.

Authentication: *Pterocarpus marsupium* Linn. Papilionaceae were authenticated at SSVPS science college, Dhule (Maharashtra) by Dr. D. A. Patil. A voucher specimen was deposited at School of Pharmacy, KLERLSCI-1439

### Drying and Size Reduction

In the present study, heartwood of *Pterocarpus marsupium Linn*. were reduced to coarse powder (# 40 size mesh) using mechanical grinder.

**Extraction**

The shade dried heartwood of *Pterocarpus marsupium* was subjected for extraction by continuous hot extraction with petroleum ether (40- 60ºC), chloroform, alcohol and water. Each time before extracting with the next solvent the powder material dried in a hot air oven at 50°c for one hour. After the effective extraction, the solvents were distilled off, the extracts then concentrated on water bath and extract obtained with each solvent weighed. Its percentage is calculated in term of air-dried weight of plant material.

**Materials used**

Carbon tetrachloride $CCl_4$ was obtained from Poona Chemical Laboratory and Liv-52 syrup was obtained from Himalaya Drug Company, Bangalore. Olive oil was obtained from seven ships brand, Nirmal Chemicals, Bangalore.

**Estimation Kit (GOT and GPT)**

GOT kit –Dr. Reddy's lab, Hyderabad
GPT Kit- Span Diagnostic Ltd. Surat
All other chemicals used were of analytical grade.

**Animal Selection**

Female Albino Wistar strain rats weighing between 120-150 g were used for hepatoprotective model. The animals were breed in animal house, Sri. Venkateshwara Enterprises, Bangalore. (CPCSEA Reg. No. 276) Rats were kept in polypropylene cages and were allowed free access to food and water. The rats were housed in a group of six at 25 °C and were exposed to 12 hours of darkness and light each. The bedding materials of cages were changed every day.

**Determination of LD50 Study**

The acute oral toxicity study was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD), received draft guidelines 423, received from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of social Justice and Empowerment, Government of India.[14] It was carried out by administering large doses of extracts from 300 mg/Kg to 5000 mg/Kg. The substance is administered orally to a group of experimental animals (consisting of 3 rats) at one of the defined dose. At the dose of 2000 mg/Kg half the population of animals was found to be dead for every extract within first 24 hours. Thus, this dose was considered as LD50 and1/10$^{th}$ dose of the lethal dose is therapeutic dose (200 mg/kg) for pharmacological activity.

**Assessment of Hepatoprotective Activity**[15,16]

The SGOT, SGPT, SALP, and Serum Bilirubin estimation method has been used in this study. The different plant extracts were administered on group of 6 female albino wistar rats, weighing about 120-150 g, for recording enzymatic levels and histopathology during the evaluation. Animals were administered with carbon tetrachloride (2 ml/kg) i. p. to induce hepatotoxicity. Marked increased in the serum level or SGOT, SGPT, SALP and Serum Bilirubin was taken as indication of hepatotoxicity.

The procedure consists of:

Group A – Served as Control and received single daily dose of 1 ml/kg i.p. of sucrose solution for 4 days along with 1 ml/kg s. c. of olive oil on 2$^{nd}$ and 3$^{rd}$days.

Group B– Also received single daily dose of 1 ml/kg i.p. aqueous sucrose solution for 4 days with 2 ml/kg of Carbon tetrachloride by subcutaneous route dissolved in an equal volume of olive oil on 2$^{nd}$ and 3$^{rd}$ days.

Group C– Received standard drug Liv-52 as a single daily dose of 5 ml/kg of oral route for 4 days with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2$^{nd}$ and 3$^{rd}$days.

Group D, E, F and G received single daily dose of 200 mg/kg of extracts by oral route for 4 days respectively, with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2$^{nd}$ and 3$^{rd}$ days.

**Table 1: Schedule for Carbon Tetrachloride Model**

| S. No. | Group | Days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 1 | A-Control | SS | SS, OO | SS, OO | SS | Animals were sacrificed under light anesthetic ether |
| 2 | B-Carbon Tetrachloride | SS | SS, $CCl_4$ | SS, $CCl_4$ | SS | |
| 3 | C- Std drug Liv-52 | SD | SD, $CCl_4$ | SD, $CCl_4$ | SD | |
| 4 | D-Petroleum ether | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |
| 5 | E-Chloroform extract | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |
| 6 | F-Alcohol extract | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |
| 7 | G-Aqueous extract | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |

(SS — Sucrose solution, OO — Olive oil, $CCl_4$, — Carbon tetrachloride in olive oil in 1: 1 ratio, TS — Test Solution and SD — Standard Drug (Liv-52)

All the rats in all the groups were sacrificed on 5$^{th}$ day under light anesthetic ether. Blood from each rat was collected through cardiac puncture under ether anesthesia for biochemical investigation like SGOT, SGPT, SALP and serum bilirubin estimation. Blood was allowed to coagulate at 37$^0$C for 30 min and the serum was separated by centrifugation at 2500 rpm for 10 minutes. The liver of all the experimental animals were removed and processed immediately for histological investigation.

**Histological Investigation**

The liver from each animal was removed after dissection. The liver lobes were fixed for 48 h in 10% formalin and were embedded in paraffin. Subsequently, 5 sections of livers were stained with haematoxylin and eosin. These sections were observed under light microscope for histological changes and compound to normal liver physiology.

**Estimation of Serum Glutamate Pyruvate Transaminase (SGPT)**

SGPT or ALT is located in the cytosol of the liver cell. During liver cell inflammation, they are released into circulation due to increased permeability of cell membrane break down of liver cells; hence, determination of SGPT as index of the extent of liver damage. Diagnostic reagent kit was used for determination of

SGPT also called as "Alanine amino transaminase" (ALT) activity by method of Reitman and Frankel[17].

**Estimation of SGOT**

SGOT (AST) is located on the cytosol of liver cell. In addition, it is also found in the mitochondria. It is also found in many tissues such as heart, liver, skeletal muscle and kidney which are rich source of SGOT in that order, liver is being the second richest source of SGOT the importance of SGOT levels in hepatic damage of hepatic cells leads to increased levels of SGOT in blood serum.

SGOT Kit is based on Reitman and Frankel's method.[17] SGOT catalyzes the transfer of the amino group of L-aspartate (ASP) to α-ketoglutarate of the (α -KG) resulting in the formation of oxaloacetate (OAA) and L-glutamate (L-Glu). The oxaloacetate so formed, is allowed to react with 2,4-DNPH to form 2,4 dinitrophenyl hydrazone derivative which is brown colored in alkaline medium. The hydrazone derivative of oxaloacetate similar to pyruvate is considerably more chromogenic than that of α -KG. The final color developed does not obey Beer's law.

**Estimation of SALP/ALP**

SALP Kit is based on Kind and King Method.[18] Alkaline phosphatase (ALP) at an alkaline pH hydrolyses di Sodium Phenylphosphate to form phenol. The Phenol formed reacts with 4 – Aminoantipyrine in the presence of Potassium Ferricyanide, as an oxidizing agent, to form a red colored complex. The intensity of the color formed is directly proportional to the activity of ALP present in the sample.

**Serum Bilirubin Kit**

It is based on Jendrassik and Grof's method.[19] Bilirubin reacts with diazotized Sulfanilic acid to form a colored compound. The unconjugated bilirubin couples with the Sulfanilic acid in presence of caffeine — benzoate accelerator. The intensity of the color med is directly proportional to the amount of bilirubin present in the sample.

**Statistical Analysis**

Results of the biochemical estimation were expressed as mean ± S.D. for determination of significant intergroup difference each parameter was analyzed separately and one-way analysis of Variance (ANOVA) was carried out.[20] The calculated F ratio has been tabulated along with the critical value of F ratio. Dunnett's test was used for individual comparisons.[21,22]

**RESULT AND DISCUSSION**

The petroleum ether (40-60°C), chloroform, alcohol and aqueous extracts were tested for hepatoprotective activity. The color, consistency and percentage yield of different extracts of heartwood of *Pterocarpus marsupium* are tabulated in Table 2.

In acute toxicity studies, no mortality and no change in general behavior were observed in the animals treated with all the extracts up to a dose of 2000 mg/kg, p.o. At the dose of 2000 mg/Kg half the population of animals was found to be dead for every extract within first 24 hours. Thus, this dose was considered as LD50. $1/10^{th}$ dose of the lethal dose is therapeutic dose (200 mg/kg) for pharmacological activity.

To assess the hepatoprotective activity of heartwood of *Pterocarpus marsupium*, carbon tetrachloride induced hepatotoxicity was produced in female albino rats and parameters like enzyme study (SGOT and SGPT SALP and Serum bilirubin) and histopathological studies were carried out and the extent of regenerative changes were observed.

The degree of hepatotoxicity developed can be known by elevated levels of SGOT, SGPT, SALP and serum bilirubin activity which is attributed to generation of $CCl_4$ free radical during metabolism by hepatic microsomes which in turn causes peroxidation of lipids of cellular membrane.

Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells. Disarrangement of normal hepatic cells with necrosis is observed in $CCl_4$ intoxicated liver. The liver sections of the rat treated with aqueous extract (200 mg/kg p.o.) of *Pterocarpus marsupium* heartwood followed by $CCl_4$ intoxication showed absence of necrosis, the results observed were comparable with standard Liv-52.

In the Table 3 to 6 it is clear that when $CCl_4$, was used to induce liver toxicity there is a substantial increase in enzyme activity of SGOT, SGPT and SALP. Any decrease in the activity of above enzymes would indicate reversed of induced toxicity of liver.

Treatment with *Pterocarpus marsupium* heartwood extract has decreased the levels of lipid peroxidation and the elevated levels of above-mentioned biochemical markers to the near normal levels. The aqueous extract of *Pterocarpus marsupium* has reduced the increased SGOT levels from 138.7 IU/L to 70.33 IU/L and SGPT levels from 180.2 IU/L to 76.67 IU/L, SALP level from 89.17 IU/L to 45.17 IU/L and serum bilirubin level from 8.98 IU/L to 3.51 IU/L. The result of hepatoprotective activity of all the extracts of heartwood of *Pterocarpus marsupium* showed that aqueous extracts of the plant was having excellent hepatoprotective potential among all other extracts and comparable to standard as shown in Table 2 to 5, which is further confirmed by the histopathological study (Plate 1).

The various phytoconstituents present in the extracts are responsible for the pharmacological effect. Hence, the hepatoprotective activity of *Pterocarpus marsupium* heartwood might be due to the presence of phytoconstituents. Earlier works have reported the presence of flavonoids in the *Pterocarpus marsupium* heartwood and the antioxidant property of flavonoids and phenolics might contribute the hepatoprotective activity of plant.[11,14]

Therefore, *Pterocarpus marsupium* heartwood extract possess hepatoprotective activity which is comparable to standard Liv-52.

**CONCLUSION**

The result of this study clearly demonstrates that the histopathological alterations produced by $CCl_4$ in tissue were significantly reserved by the pretreatment of extracts of heartwood of *Pterocarpus marsupium* and standard Liv-52. In conclusion, an aqueous extract of the plant is having excellent hepatoprotective potential among all other extracts and comparable to standard. Thus, the present study demonstrates heartwood of *Pterocarpus marsupium* has promising hepatoprotective action; therefore, to isolate the phytoconstituent responsible for the hepataoprotective activity of heartwood of *Pterocarpus marsupium* is necessary to search for the novel herbal drug for the treatment of liver diseases with potent efficacy and safety.

**Table 2: % Yield of different extracts of *Pterocarpus marsupium***

| S. No. | Solvents | Color | Nature of Consistency | % yield |
|---|---|---|---|---|
| 1 | Pet-ether | Dark yellow | Semisolid | 1.49 |
| 2 | Chloroform | Dark brown | Semisolid | 2.7 |
| 3 | Alcohol | Dark brown | Semisolid | 15.5 |
| 4 | Aqueous | Dark brown | Semisolid | 15.38 |

**Table 3: SGPT level (IU/L) of different extracts of *Pterocarpus marsupium***

| S. No. | Control | $CCl_4$ | Standard | Pet-ether | Methanol | Chloroform | Aqueous |
|---|---|---|---|---|---|---|---|
| 1 | 38 | 159 | 40 | 155 | 143 | 166 | 69 |
| 2 | 55 | 188 | 69 | 178 | 113 | 156 | 79 |
| 3 | 51 | 205 | 58 | 199 | 146 | 169 | 71 |
| 4 | 48 | 209 | 55 | 203 | 198 | 175 | 78 |
| 5 | 60 | 143 | 72 | 123 | 184 | 187 | 82 |
| 6 | 59 | 147 | 70 | 140 | 123 | 200 | 81 |
| Mean | 51.83 | 180.2 | 69 | 166.3 | 151.2 | 175.7 | 76.67 |
| SD | 8.183 | 15.88 | 11.56 | 32.40 | 33.51 | 15.78 | 5.391 |
| SE | 3.341 | 10.57 | 4.719 | 13.23 | 13.68 | 6.443 | 2.201 |
| F ratio | 38.21 | | | | | | |
| P value | - | - | P < 0.001 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.001 |

P < 0.05 is significant

**Table 4: SGOT level (IU/L) of different extracts of *Pterocarpus marsupium***

| Sr. No | Control | $CCl_4$ | Standard | Pet-ether | Methanol | Chloroform | Aqueous |
|---|---|---|---|---|---|---|---|
| 1 | 48 | 132 | 49 | 131 | 132 | 137 | 63 |
| 2 | 55 | 135 | 58 | 138 | 144 | 126 | 71 |
| 3 | 58 | 130 | 62 | 121 | 123 | 129 | 68 |
| 4 | 60 | 145 | 68 | 133 | 122 | 140 | 73 |
| 5 | 45 | 149 | 55 | 140 | 132 | 138 | 77 |
| 6 | 59 | 141 | 69 | 120 | 129 | 127 | 70 |
| Mean | 54.17 | 138.7 | 60.17 | 130.5 | 130.3 | 132.8 | 70.33 |
| SD | 6.242 | 7.554 | 7.731 | 8.408 | 7.967 | 6.178 | 4.719 |
| SE | 2.548 | 3.084 | 3.156 | 3.433 | 3.252 | 2.522 | 1.926 |
| F ratio | 178.8 | | | | | | |
| P value | - | - | P < 0.001 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.001 |

P < 0.05 is significant

**Table 5: SALP level (IU/L) of different extracts of *Pterocarpus marsupium***

| Sr. No | Control | $CCl_4$ | Standard | Methanol | Pet-ether | Chloroform | Aqueous |
|---|---|---|---|---|---|---|---|
| 1 | 25 | 95 | 30 | 87 | 82 | 88 | 47 |
| 2 | 22 | 91 | 37 | 83 | 80 | 81 | 51 |
| 3 | 26 | 88 | 29 | 81 | 79 | 78 | 42 |
| 4 | 27 | 99 | 26 | 78 | 81 | 79 | 35 |
| 5 | 23 | 82 | 37 | 79 | 80 | 80 | 49 |
| 6 | 27 | 80 | 39 | 82 | 86 | 78 | 47 |
| Mean | 25 | 89.17 | 33 | 81.67 | 81.33 | 80.67 | 45.17 |
| SD | 0.2098 | 7.360 | 5.329 | 3.204 | 2.503 | 3.777 | 5.811 |
| SE | 0.8563 | 3.005 | 2.176 | 1.308 | 1.022 | 1.542 | 2.372 |
| F ratio | 200 | | | | | | |
| P value | - | - | P < 0.001 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.001 |

P < 0.05 is significant

**Table 6: Serum bilirubin level (IU/L) of different extracts of *Pterocarpus marsupium***

| Sr. No | Control | $CCl_4$ | Standard | Methanol | Pet-ether | Chloroform | Aqueous |
|---|---|---|---|---|---|---|---|
| 1 | 2.21 | 11.2 | 4.25 | 10.6 | 10.6 | 9.8 | 3.22 |
| 2 | 2.10 | 6.11 | 5.27 | 11.4 | 11.1 | 10.1 | 4.11 |
| 3 | 2.03 | 3.16 | 6.7 | 9.6 | 13.1 | 11.4 | 5.25 |
| 4 | 2.01 | 5.8 | 6.3 | 12.6 | 11.7 | 12.1 | 5.9 |
| 5 | 2.26 | 13.2 | 5.4 | 11.8 | 9.6 | 9.1 | 6.3 |
| 6 | 2.21 | 13.9 | 5.2 | 9.8 | 10.6 | 11.2 | 5.2 |
| Mean | 2.135 | 8.894 | 3.308 | 10.97 | 11.12 | 10.61 | 3.510 |
| SD | 0.1071 | 4.452 | 0.4674 | 1.176 | 1.192 | 1.130 | 0.4725 |
| SE | 0.04372 | 1.818 | 0.1908 | 0.4800 | 0.4868 | 0.4615 | 0.1929 |
| F ratio | 20.27 | | | | | | |
| P value | - | - | P < 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.05 |

P < 0.05 is significant

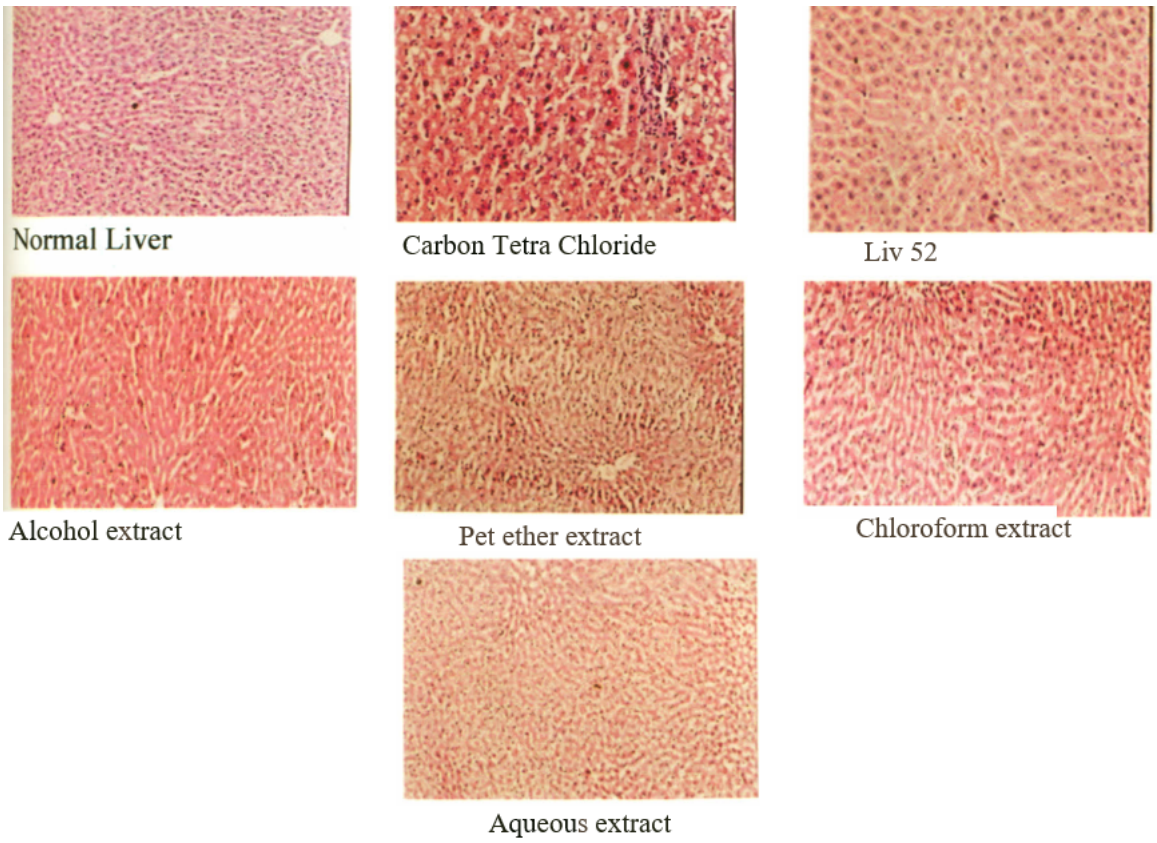


**Plate 1: Histopathology study of different extracts of *Pterocarpus marsupium***

Histology of hepatic tissue of different treatment groups against $CCl_4$ induced hepatic toxicity. (a) Control group with normal histological features, (b) toxicant $CCl_4$ group necrotic areas and vacuole formation, (c) showing almost normal histology after treatment with standard Liv-52 drug. (d) (e) (f) (g) The normal architecture of hepatic tissue in animals treated with alcohol, pet-ether, chloroform and aqueous extracts of heartwood of *Pterocarpus marsupium* respectively

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