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EVALUATION OF HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF PTEROLOBIUM HEXAPETALUM ON CARBONTETRACHLORIDE INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Background: Pterolobium hexapetalum is one of the important medicinal plants belonging to Caesalpiniaceae., with significant herbal uses like anti diabetic, antiulcer, antiurolithic, antibacterial, anti fungal effects Pterolobium hexapetalum also possess high quantities of significant phytoconstituents like alkaloids, flavonoids, phenols, glycosides, tannins, quinines and steroids, which lead to use in many tropical countries. Due to its remarkable medicinal, nutritional and socio-economic value. This study was designed to clarify the protective effect of ethanolic extract of *Pterolobium hexapetalum* against carbon tetrachloride ccl4 induced hepatotoxicity in rats. Materials and Methods: Thirty white Albino male rats were used in this study and after acclimatization rats were subjected to different treatments blood and tissue samples were collected after day 10 post administration, biochemical, and histopathological examinations were utilized to investigate hepatoprotective activity of ethanolic extract of Pterolobium hexapetalum. Result: Pterolobium hexapetalum showed significant protection with the depletion of alalinine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP) gamma glutanyl transpeptidase, in serum as it was raised due to ccl4 induction. Concentration of serum triglycerides, total cholesterol and low density lipo protein was were sceen to get reduced when treated with sylimarin as proven with administration of p.hexapetalum.there was amarkable increase in high density lipoprotein comparatively raise in total bilirubin and direct bilirubin was also reduced with administration of p.hexapetalum extract. Conclusion: From these results, it is suggested that Pterolobium hexapetalum possesses hepatoprotective properties.

Keywords :- *Pterolobium hexapetalum ,* hepatoprotective, CCl_4

INTRODUCTION

Liver disease is considered great public health trouble on a global scale. In spite of modern drugs have been utilizing to treat liver disturbances, these drugs have often side effects. Thence, advanced research studies have been performed to explore the safe and potent therapies without side effects to treat liver disorders. Natural therapies from medicinal plants are consider the most desirable and ravishing area as alternative treatment for hepato-toxicity, hepatoprotective effects of plants are related with phytocompounds rich in natural antioxidants as glycosides, saponin, flavonoids, tannin, alkaloids, vitamin A, C, E and other phenolic compounds.[1] *Pterolobium hexapetalum* (Family: Caesalpiniaceae.) is a valuable plant. It is widely distributed in a lot of countries of the world; *Pterolobium hexapetalum* is a perennial herb native to tropical region, central to *Pterolobium hexapetalum* is a good source of sugar, salts, minerals (calcium, potassium, phosphorus, and magnesium).

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Phytochemical compound (glycosides, flavonoids, saponin and tannin. In traditional medicine, *Pterolobium hexapetalum* has been used for management of various liver disorders. Hence, this study was designed to demonstrate the hepatoprotective effects of *Pterolobium hexapetalum* against CCl4 toxicity through determination of liver function, hepatoprotectivity and histopathological examination and these results will further support the protective effect of *Pterolobium hexapetalum* and will clarify the capacity of this plant in medication of liver diseases by a comparison with silymarin. It was given in a two doses (200 mg/kg b.wt and 400 mg/kg b.wt) orally for 30 day for hepatoproductive effect [2].

MATERIALS AND METHODS

Collection of plant material and authentication

The plant *Pterolobium hexapetalum* was collected from region, Perungalur, of Pudukkottai district and Maruthamalai hills of Coimbatore,

The collected plant material was identified botanically, confirmed and authenticated by Ministry of Environment, Forest & Climate Change, **Botanical Survey of India (BSI)**, TNAU campus, Coimbatore on 1st October 2020 by Scientist E & Head of Office, Dr.M.U.Shareeff. The herbarium specimen was preserved for further reference. No.BSI/SRC/5/23/2020 /Tech/09.

It was given in a two doses (200 mg/kg b.wt and 400 mg/kg b.wt) orally for 10 day for hepato productive effect.

Drugs

Silymarin (hepaticum®) Is a micronized silymarin, silybin in the form of dry standardized extract of milk thistle plant (Silybummarianum). Each 100 ml suspension contains 1gm silymarin standardized to a content of more than 45% silybin (50 mg /5ml)Silymarin suspension purchased from Micro labs, Bangalore. .It was used in a dose of 75mg/kg b.wt.

Carbontetrachloride: Is one of the most common hepatotoxin used for experimental induction of liver injury in animal studies. Carbon tetrachloride was purchased from Merk India Ltd, given in a dose 100 mg / kg b.wt intraperitoneally for three times in 10days

Preparation of ethanolic extract of *Pterolobium hexapetalum*

The plant material collected was dried under shade dried and coarsely powdered using a pulverizer and the extract was obtained using Soxhlet apparatus [3]. The extract was filtered and concentrated at temperature below 50º C to a syrup consistency (yield: 35.5 percentage w/w) and weighed.500gm of plant material was extracted. The dried and weighed marc was packed in the extractor and extracted with alcohol for 3hours to get the alcoholic extract of drug. The marc left after alcoholic extraction was dried and stored for further studies.

Preparation of CCl4

Carbon tetrachloride was administered along with the vehicle (30 percent in olive oil,3ml/kgbw).

Sylimarin (standard drug)

Sylimarin purchased asin suspension form and administered in rats as such as a standard drug.

Grouping and Acclimation of Animals

Procurement of adult albino rats wistar strain (Rattusnorvegicus) of either sex weighing between 200-250 gm from M/S Venkateshwara Enterprises, 4304, 13th main, 2nd cross, subramanyanagar Bangalore, Karnataka, India. . The procured animals were housed under standard environmental conditions (temperature of 25 ± 2°C with an alternating 12 hour light-dark cycle and relative humidity of 60 ± 5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethical Committee. They were feed with standard commercial rat feed supplied by SKM Feeds and Foods, Erode and water adlibitum. They were allowed to laboratory conditions for ten days after arrival before use.

Experimental Design

Table 1. Experimental Protocol

SL.NO	GROUPS		TREATMENT SCHEDULE
1	GROUP I	Control or untreated	Receive 5% CMC 10ml/kg body weight. The group served as a normal control.
2	GROUP II	Negative Control or inducing agent	Receive CCl_4 (0.7ml/kg) body weight intraperitonially on the days three and six
3	GROUP III	Standard drug	Receive Silymarin, the standard drug (25mg/kg) bodyweight
4	GROUP IV	Test I /curative Low dose	Receive Ethanolic extract of *Pterolobium hexapetalum* 200mg/Kg body weight
5	GROUP V	Test II /curative high dose	Receive ethanolic extract of *Pterolobium hexapetallum* 400mg/Kg bodyweight.

Sampling

Estimation of Biochemical Parameters

On 11th day blood was collected from animals under anaesthesia by cardiac puncture. Blood samples collected was centrifuged at 3500 rpm for 15 mins at room temperature for separation of serum. The clear, non-haemolysed sera was separated using clean dry disposable plastic syringe and stored at -20ºC for measurements of the following,

Serum parameters

- Alkaline phosphatase
- Serum glutamate oxaloacetate Transaminase
- Serum glutamate pyruvate Transaminase
- Bilirubin
- Total protein
- Albumin

Liver tissue parameters

- Alkaline phosphatase
- Glutamate oxaloacetate Transaminase
- Glutamate pyruvate Transaminase
- Protein
- Lipid peroxidation
- Histopathology of the liver

Serum biochemical analysis

Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, protein and albumin levels were determined in serum spectro-photometrically by specific kits [4]. Determination of AST, ALT according to Reitman and Frankel, Determination of ALP according to Haussament [5] and Determination of total protein and albumin level [6]

Histopathology study

Autopsy samples were taken from the liver of rats in several groups and fixed in 10% formalin saline for 7 days, after completion of fixation, the samples were dehydrated in a series of alcohols, cleared in toluene and then embedded in paraplast (Sherweed Medical Co, USA). Blocks were cut at 5-7 µm. Sections were de-waxed, re-hydrated in a series of alcohols, stained in Harris hematoxylin (Cole Parmer, USA) and counterstained in 1% aqueous eosin (Sigma, USA). Sections were then mounted in DePeX (GURR, BDH, UK) and examined under a light microscope. Representative slides were photographed under an Olympus Vanox photomicroscope (Olympus, USA) [7].

Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS 16 Inc. Released, 2009) to determine if variables differed among groups , Comparison among means was conducted by one-way ANOVA and subsequent Ducann's multiple range test, probability values of less than 5% ($p \leq 0.05$) were considered significant.

RESULTS

Ethanolic extract of *Pterolobium hexapetallum* was colorless semisolid texture. The extraction process gave a yield of 35.5% from 2000 gm of the plant. Carbontetrachloride induced a significant ($P<0.05$) increase in the level of SGOT,SGPT and other enzyme parameters like serum AST, ALT, ALP, etc compared to the control group and sylimarin (SL) treated group as standard ,administration of ethanolic extract of *Pterolobium hexapetalum* at both doses with carbontetrachloride restore the toxic effect of carbontetrachloride.

PHARMACOLOGICAL EVALUATION:

Assessment of liver tissue enzyme parameters:

Ethanol extract of *Pterolobium hexapetalum* possessed a good hepatoprotective activity on rats as shown in the table. At varying dose levels, (200 mg/kg and 400 mg/kg), this extract attenuated altered biochemical parameters produced by CCl_4 was dose dependent. This extract possessed significant hepatoprotective activity at both 200 and 400 mg/kg dose level [8].

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Group II compared with group I and Groups III, IV & V were compared with group II.Values are presented as means ± SE. Means within a column with different letter superscripts are significantly different. P.hexapetalum, ccl4-carbontetrachloride group; SL: silymarin treated group. AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase) The concentrations of total protein and albumin level was significantly [9].

Assessment of serum biochemical parameters:

Direct bilirubin and total bilirubin:

Elevation of direct and total bilirubin levels after administration of CCl_4 indicate its hepatotoxicity. Pretreatment with Silymarin, and ethanolic extract of the plant significantly reduced levels of direct and total bilirubin levels when compared to toxic control group indicating Hepatoprotective effect of ethanolic extract of *Pterolobium hexapetalum* which is shown in the table [10].

Values are mean ± SEM (n=6) one way ANOVA.Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, and *** represents very significant at $p<0.001$. All values are compared with toxicant.

Assessment of Lipid profiles:

The serum lipid profile such as total cholesterol, triglycerides, LDL & VLDL were elevated, were as HDL level was decreased and this indicated deterioration in hepatic function due to the damage caused by CCl_4 administration [11].

Whereas treatment of *Pterolobium hexapetalum* extract significantly declined the effect of CCl_4 induced damage and it was evidenced by the decreased level of total cholesterol, triglycerides, LDL & VLDL and increased level of HDL in extract group.

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Group II compared with group I and Groups III, IV & V were compared with group II [12].

HISTOPATHOLOGICAL EVALUATION

Physical parameters:

Wet liver weight and Wet liver volume:

Carbon tetrachloride treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and ethanolic extract of *Pterolobium hexapetalum* showed significant restoration of wet liver weight and wet liver volume nearer to normal. The EEPH at 200mg/kg b.wt and 400mg/kg body weight showed reduction of wet liver weight and wet liver volume significantly at $p<0.05$.The results are shown in table no.

Values are mean ± SEM (n=6) one way ANOVA. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, and *** represents very significant at $p<0.001$. All p values are compared with toxicant [13,14].

Description of Histopathological evaluation

The histopathological evaluation of CCl_4 toxicity in all the groups was examined and shown in below figure , The description is as follows,

- Group I: Section of rat liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance.
- Group II: Section of liver in toxicant control group shows partially effaced architecture. Some of the hepatocytes show apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity.
- Group III: Section of liver in silymarin treated group shows liver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids.
- Group IV: Section of liver in EEPH drug treated groups (200mg/kg& 400mg/kg) shows intact architecture, few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group.
- Group V: Liver tissue section shows that hepatocytes were regenerative and showed no visible changes and prominent nuclei, reduced score of necrosis and no fatty changes. Thus, confirming the safety of the extract.

Table 2: Effect of ethanolic extract of *Pterolobium hexapetalum* on liver tissue enzyme parameters in CCl4 induced hepatotoxic rats

LIVER TISSUE ENZYME PARAMETERS							
GROUP	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**ACP (U/L)**	**LDH (U/L)**
Control 0.5% CMC	98.01±1.41	83.75±1.48	36.06±0.12	57.88±1.789	153.64±0.95	44.88±0.75	205±0.5
Negative Control CCl_4-0.7 ml/kg	298.50±1.50***	364.00±1.99***	53.47±0.11***	114.28±1.02**	220.2±0.92***	65.99±0.76***	261±1.25**
Standard-Silymarin-75mg/Kg	117.56±1.80***	107.73±2.98***	37.84±1.13**	62.47±0.84**	168.12±0.57**	49.26±1.07**	200±1.85**
EEPH 200 mg/Kg	204.40±1.37**	198.00±1.42***	45.0±2.72**	67.28±1.01***	191.65±0.87**	55.43±0.86**	220±0.5***
EEPH 400 mg/Kg	128.20±1.03***	119.06±0.98**	39.98±1.11***	64.95±0.95**	182.95±1.62***	51.77±0.71**	246±0.4**

Chart 1. Liver tissue Enzyme parameters

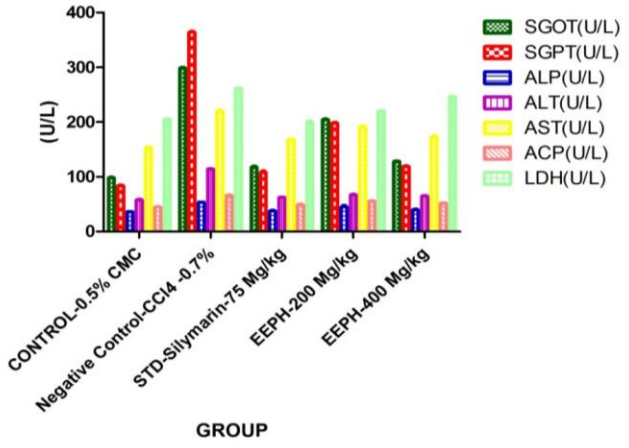


Chart 2. Serum biochemical parameters (bilirubin – mg/dl)

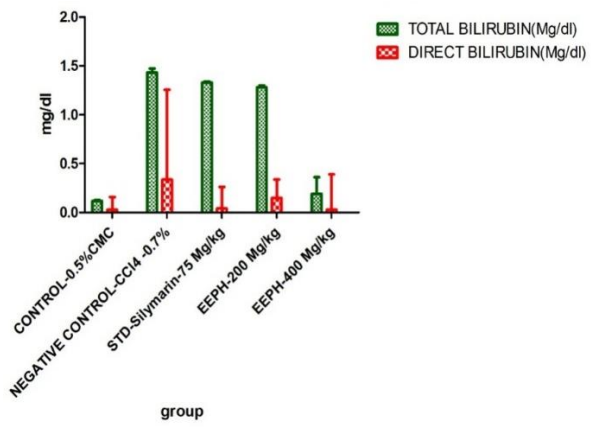


Chart 3. Serum biochemical parameters (lipid profiles)

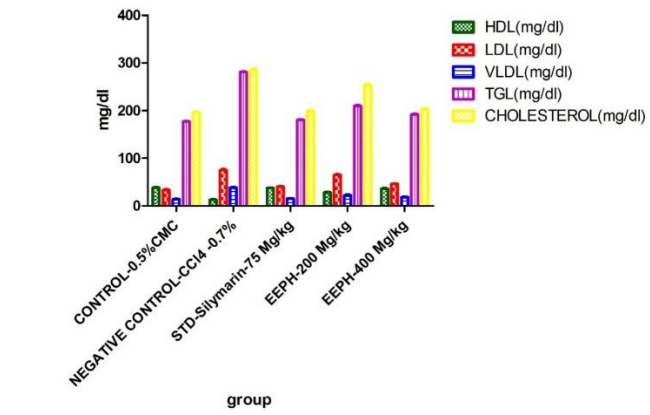


Chart 4. Effect of ethanolic extract of *Pterolobium hexapetalum* on wet liver weight in CCl4 induced hepatotoxic rats

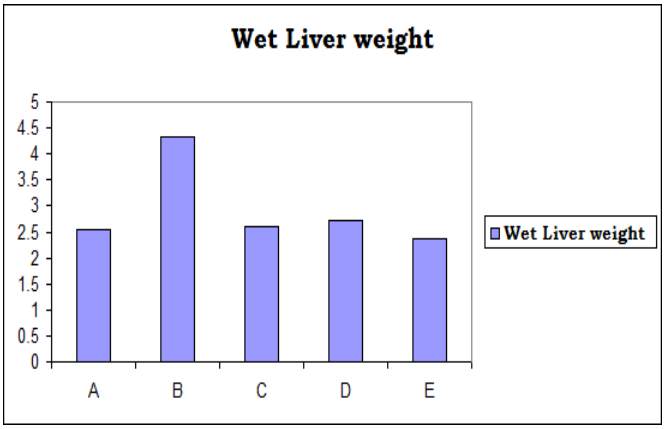


Chart 5. Effect of ethanolic extract of *Pterolobium hexapetalum* on wet liver volume levels in CCl_4 induced hepatotoxic rats

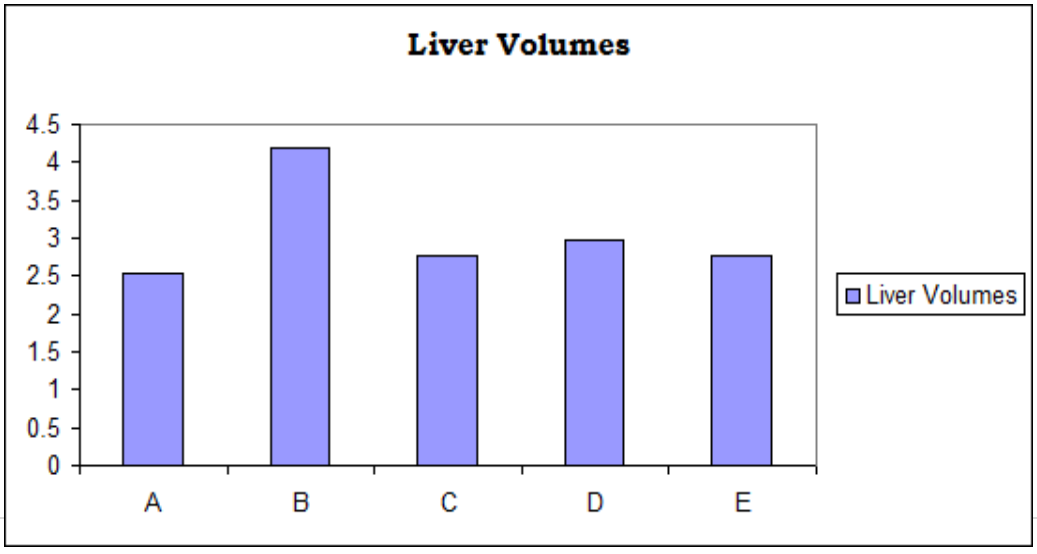


Table 3: Effect of ethanolic extract of *Pterolobium hexapetalum* on serum biochemical parameters (bilirubin) (mg-dl) in CCl4 induced hepatotoxic rats

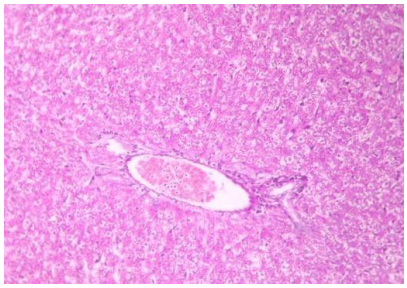
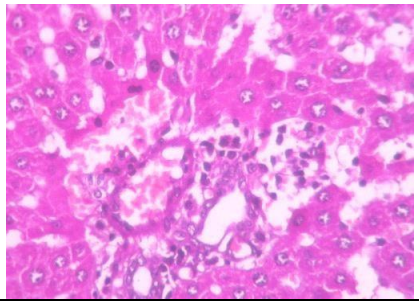
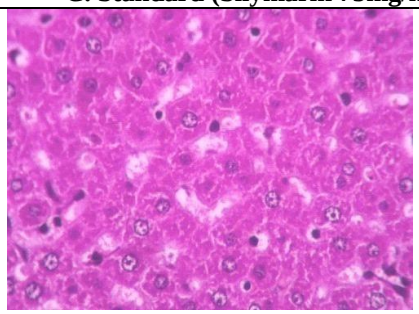
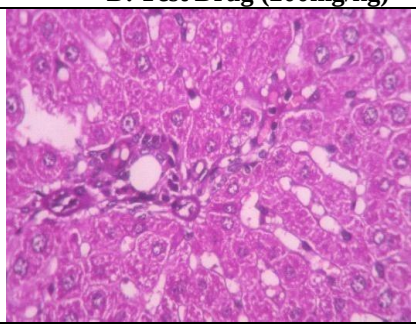
Serum Biochemical Parameters (Bilirubin) (mg/dl)		
Group	**Total bilirubin**	**direct bilirubin**
CONTROL-0.5% CMC	0.117±0.01	0.0274±0.13
NEGATIVE CONTROLL CCL_4-0.7ml/kg	1.4334±0.04***	0.3356±0.92**
STANDARD SILYMARIN 75mg/Kg	0.1330±0.01***	0.0415±0.22**
EEPH200mg/Kg	1.2805±0.03**	0.1481±0.19***
EEPH400mg/Kg	0.1908±0.1***	0.0290±0.36***

Table 4:Effect of ethanolic extract of *Pterolobium hexapetalum* on lipid profile levels in CCl4 induced hepatotoxic rats.

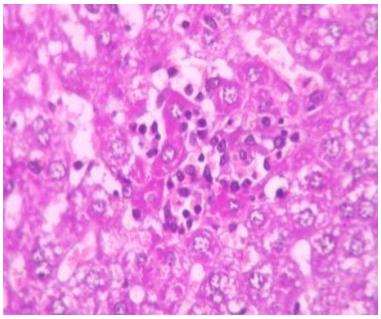
Serum Biochemical Parameters (mg/dl)					
GROUP	**HDL**	**LDL**	**VLDL**	**TGL**	**CHOLESTEROL**
Control 0.5%	38.40±1.41	33.40±1.51	14.00±0.90	177.07±0.77	196.28±1.11
Negative control CCL_4- 0.7 ml/kg	13.33±1.03***	75.20±1.98**	38.56±1.36***	280.82±1.30**	284.74±2.96***
Standard silimarin 75mg/Kg	37.73±0.75***	40.00±1.42***	15.42±1.04***	180.83±0.54**	198.79±1.45**
EEPH 200mg/Kg	28.10±1.75**	64.78±1.65**	22.47±1.52**	210.20±1.016***	253.47±1.79**
EEPH400mg/Kg	36.58±1.16**	46.23±0.75***	18.43±1.55***	192.25±1.230**	203.66±0.50***

Table 5.Effect of ethanolic extract of *Pterolobium hexapetalum* on wet liver weight and wet liver volume in Ethanol induced hepatotoxic rats.

Group	Treatment	Dose	Wet Liver weight (gm/100gm)	Wet liver volumes (ml/100gm)
1	Normal control	5% cmc	2.53 ± 0.535	2.535±0.53
2	ToxicantControl	CCl_4 .7 ml/kg	4.34 ± 0.095	4.19±0.04
3	Standard	Silymarin 75mg/kg	2.61 ± 0.110*	2.78±0.23*
4	EEPH	EEPH 200mg/kg	2.73 ± 0.120*	2.973±0.07*
5	EEPH	EEPH 400mg/kg	2.36 ± 0.27*	2.77±0.11*

A. Normal Control	**B. Toxicant (Carbon tetrachloride)**
	
C. Standard (Silymarin 75mg/kg)	**D. Test Drug (200mg/kg)**
	

E. Test Drug (400mg/kg)



Histopathology of Liver of Rat

Photomicrograph of liver shows a normal hepatic cellular arrangements in Group 1 (A) whereas, in group II (B) showing loss of hepatic architecture with intense peripheral central vein necrosis, fat*9ty changes, crowding of central vein. In rats treated with silymarin (C), a normal hepatic architecture with moderate mild degree of necrosis. Group IV and group V (D & E) reduces the hepatic injury score of fatty degeneration and necrosis, clearly indicating the protection offered by EEPH [15,16].

Summary and Conclusion

The present study was undertaken to determine the hepatoprotective activity of ethanolic extract from *Pterolobium hexapetalum [17]*.

The Pharmacognostical studies made on the powdered plant of *Pterolobium hexapetalum* like ash values, extractive value, loss on drying gave valuable information. This helped for correct identification of the plant [18,19].

The preliminary phytochemical investigation showed the presence of carbohydrates, Flavonoids, Terpenoids, phenolic compounds, fixed oils and fats, steroidsin ethanol extract. Histopathological studies on isolated liver revealed that ethanolic extract of *Pterolobium hexapetalum* reversed the liver damage caused by CCl_4. The normal pattern of histology of liver was observed.

Based on the results obtained from the present study, it can be concluded ethanolic extract of *Pterolobium hexapetalum* (EEPH) is found to be more potent hepatoprotective [20,21].

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