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Evaluation of hepatoprotective effect of leaf extracts of grewia hirsute on paracetamol induced hepatotoxicity in male albino rats.

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ABSTRACT

Background: plant parts of *Grewia hirsute* is one of the important medicinal plants belonging to the family plantae., with significant herbal uses like the bitter leaves lessen inflammation; useful in nose and eye disease; anthelmintic. The root of this variety is astringent to bowels; useful in cholera, hydrophobia, kidney pain, piles. The tasteless leaves used as purgative, expectorant, carminative, bortiofacient, emmenogaugue, vulnerary galactogoggue; useful in splenic enlargement, eye troubles, piles, rheumatism, pain in the joints and in the breasts. The plant *Grewia hirsute* also possess high quantities of significant phytoconstituents like alkaloids, flavonoids, phenols, glycosides, and coumarins 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 petroleum ether,ethyl acetate and ethanolic extract of *Grewia hirsute* against paracetamol induced hepatotoxicity in rats. Materials and Methods: Thirty six 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 all the three extract of *Grewia hirsute*. Result: *Grewia hirsute* 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 paracetamol induction. Concentration of serum triglycerides, total cholesterol and low density lipo protein were sceen to get reduced when treated with sylimarin as proven with administration of *Grewia hirsute*. There was amarkable increase in high density lipoprotein comparatively raise in total bilirubin and direct bilirubin was also reduced with administration of *Grewia hirsute* extract. Conclusion: From these results, it is suggested that *Grewia hirsute* possesses hepatoprotective properties.

Keywords: *Grewia hirsute*, hepatoprotective, paracetamol

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. *Grewia hirsute* (Family: planteae.) is a valuable plant. It is widely distributed in a lot of countries of the world; *Grewia hirsute* is a perennial herb native to the region of foot hills of himalayas, Especially found in Bihar and Orissa. *Grewia hirsute* is a good source of sugar, salts, minerals (calcium, potassium, phosphorus, and magnesium).

Phytochemical compound (glycosides, flavonoids, saponin and tannin. In traditional medicine, *Grewia hirsute* has been used for management of various liver disorders. Hence, this study was designed to demonstrate the hepatoprotective effects of *Grewia hirsute* against paracetamol induced toxicity through determination of liver function, hepatoprotectivity and histopathological examination and these results will further support the protective effect of *Grewia hirsute* and will clarify the capacity of this plant in medication of liver diseases by a comparison with silymarin. The three different extracts were given in a two doses (200 mg/kg b.wt and 400 mg/kg b.wt) orally for 10 day for hepatoproductive effect [2].

MATERIALS AND METHODS

Collection of plant material and authentication

The plant *Grewia hirsute* was collected from krishnankoil during the month of April 2018. The collected plant material was identified botanically, confirmed and authenticated by Dr. D. Stephen Asst. Professor, Department of Botany, The American College, Madurai. The herbarium specimen was preserved for further reference. The three different extracts were 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**:** Petroleum ether was purchased from RFCL LTD., MAHARASHTRA Ethyl Acetate was purchased from RFCL LTD., MAHARASHTRA Ethanol from HIMEDIA LABORATORIES PVT, LTD.,MUMBAI and Silymarin (hepaticum®) Is a micronized silymarin, silybin in the form of dry standardized extract of milk thistle plant (Silybummarianum). SILYBON 140MG TAB, MICROLAB PVT LTD. Paracetamol was purchased from XYKAA 650, TROKIAA PHARMACEUTICALS LTD., AHEMADABAD, paracetamol is one of the most common hepatotoxin used for experimental studies. All the substance was prepared immediately before use and the reagents were used as analytical grade.

Grouping and acclimation of animals

Adult male albino rats (180 – 220 gm) were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan limited, Bangalore, India) and water ad libitum. The study was approved by the Institutional Ethical lever Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA).

Preparation of the extracts

Extraction involves the separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure.

The coarse powder of *Grewia hirsuta* leaves was extracted by soxhlet apparatus with Petroleum ether, ethyl acetate and Ethanol for 48 hours. The extracts were collected by filtration, the marc was separated and the extraction was repeated with fresh solvents for two times. The extracts were combined and concentrated at 55˚C on water bath, till it acquires a maximum concentration.

Then the small fractions of all the extracts were subjected to various chemical tests for the identification of various plant constituents as in the procedure given below and the findings are reported in the table.

Petroleum ether extract of leaves of *Grewia hirsuta* (PEGH)

The dried coarse powder of leaves of *Grewia hirsuta* was extracted with one litre of petroleum ether (60 - 80˚C) by continuous percolation method using soxhlet apparatus, after 74 hours the extraction was completed then petroleum ether was taken and the solvent was redistilled. A dark Greenish colour was obtained.

Ethyl acetate extract of leaves of *Grewia hirsuta* (EAGH)

The march left after extraction, was dried and subsequently extracted with 1 litre of ethyl acetate by continuous percolation method. After 74 hours, the

extraction was completed, it was filtered and the solvent was removed by distillation under reduced pressure. A dark green coloured residue was stored in a desiccator and the marc was dried for further extraction.

Ethanol extract of leaves of *Grewia hirsuta* (EGH)

The marc left after extraction, was dried and subsequently extracted with 1 litre of ethanol by continuous percolation method. After 74 hours the extraction was completed, it was filtered and the solvent was removed by distillation under reduced pressure. The green coloured residue was stored in a desiccator and the marc was dried for further extraction.

CHROMATOGRAPHIC TECHNIQUE - THIN LAYER CHROMATOGRAPHY

Separation and isolation of plant constituents by chromatographic methods [56]

The various methods of separating and isolating of plant constituents, is done according to the chromatographic procedure originated by Tswettis. All finely divided solids have the powder to absorb other substance are capable of being adsorbed, some much more readily than others, this phenomenon of selective adsorption is the fundamental principle of chromatography. In the present study, thin layer chromatography methods were used.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography is so widely used that it has become an essential technique for analyst and research workers. TLC is the almost universal analytical technique in chemical analysis for organic and inorganic matter. TLC is a simple and rapid method carried out using thin layer of adsorbents on plates. TLC not only combines the advantage of paper and column chromatography but in certain aspects it is found to be superior method.

TLC is an important tool in the separation, identification and estimation of different classes of natural products. When a mixture containing different components is made to ascend in a TLC plate with the help of a solvent which acts as mobile phase, there will be a preferential adsorption of different components at different places on the plate. The result is the separation of components.

Preparation of TLC Plate

80gm of silica gel G was weighed and shaken to a homogenous suspension with 85 ml of distilled water for 90seconds.This suspension was poured in TLC applicator which was adjusted to 0.25 mm thickness. The plates were dried in hot air oven at 110^0 C for 30 minutes (activation). The plates were then stored in a dry atmosphere and used whenever required.

Application of extracts for separation

The various diluted extracts spotted on a TLC plate 2 cm above its bottom using capillary tube. Mostly solution for application was between 0.1-1% strength. The starting point was equally sized as far possible and spots had diameter ranging from 2-5 mm.

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The various extracts of leaves powder of *Grewia hirsuta* were subjected to thin layer chromatography using different mobiles phases that are suitable for detecting various phytoconstituents like alkaloids, glycosides, flavonoids, tannins and phenols of the three extracts, chromatogram for the petroleum ether, ethyl acetate, ethanol was carried out using the procedure recommended by Indian pharmacopoeia.

Solvent system used for running TLC

1. Chloroform and methanol (9:1)

Characterization of phytoconstituents using FT-IR spectroscopy

FT-IR stands for Fourier Transform Infra-Red, the preferred method of infrared spectroscopy. In Infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample.

All the separated compounds from Petroleum ether, Ethyl acetate and Ethanol extract of Grewia hirsuta leaf extract was characterized by FTIR spectroscopy.

Pharmacological evaluation

Hepatoprotective activity [58-61]

Male albino rats weighing between 180 – 220 gm used in the experiment were kept in the animal house under standard environmental conditions and had free access to feed and water *adlibitum*. Totally the animals were fasted for 16 hours before experiment but allowed free access to water. The rats were divided into nine groups each containing four rats.

Group I : Control group of animals received Tween 80
Group II : treated with silymarin (100 mg/kg)
Group III : Paracetamol control which received paracetamol (3 g/kg)
Group IV : treated with Petroleum ether extract of leaves of *Grewia hirsuta* (200 mg/kg)
Group V : treated with Petroleum ether extract of leaves of *Grewia hirsuta* (400 mg/kg)
Group VI : treated with Ethyl acetate extract of leaves of *Grewia hirsuta* (200 mg/kg)
Group VII : treated with Ethyl acetate extract of leaves of *Grewia hirsuta* (400 mg/kg)
Group VIII : treated with Ethanol extract of leaves of *Grewia hirsuta* (200 mg/kg)
Group IX : treated with Ethanol extract of leaves of *Grewia hirsuta* (400 mg/kg)

The animals of the test group were given the pre-treatment of petroleum ether, Ethyl acetate and Ethanol extracts of *Grewia hirsuta* (200–400 mg/kg p.o.) in 50% Tween 80 solution once daily for 10 days in succession followed by single administration of paracetamol 3 g/kg p.o., 1h after Petroleum ether, Ethyl acetate and Ethanol extract administration. The animals of paracetamol control groups received vehicle (50% Tween 80 solution) for 10 days in succession followed by single oral administration of paracetamol. The control group received vehicle alone. Twenty-four hours after the paracetamol administration, the rats of each group were anaesthetized and blood was collected directly from the heart. The blood samples were allowed to clot for 20–30min. Serum was separated by centrifugation at 37◦C and used for estimation of various biochemical parameters such as SGOT, SGPT, ALP and total bilirubin.

Parameters to assess hepatotoxicity

Assessment of liver function test

Serum parameters

- Alkaline phosphatase
- Serum glutamate oxaloacetate Transaminase
- Serum glutamate pyruvate Transaminase
- Bilirubin

Histopathological studies

Histopathology study of liver

A portion of liver tissue in each group was fixed in 10% formalin and proceeded for histopathological studies for evaluation of hepatocytes cells, sinusoidal congestion and mononuclear inflammatory cells of different groups.

RESULTS

Alkaloids, flavonoids, tanins, coumarins, phenolic compounds are found to be present in *Grewia hirsute.*
Thin layer chromatography.

TLC petroleum ether extract of *Grewia hirsuta* leaves

Solvent system: Chloroform and Methanol (9:1)

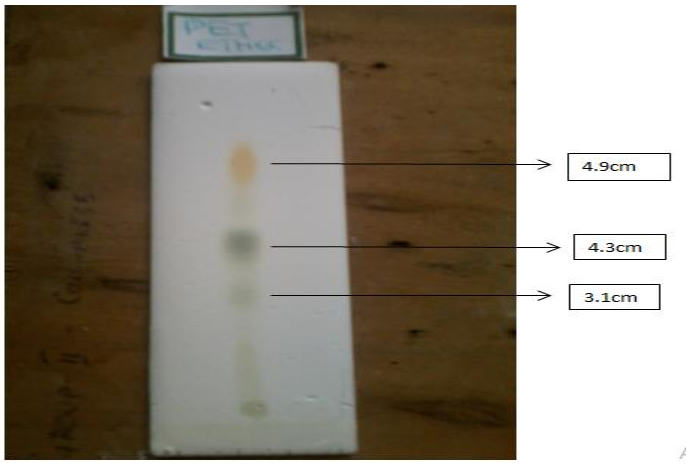


Fig 1. TLC plate of pet ether extract of *Grewia hirsuta*

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Distance travelled by the solute = 5.2
Distance travelled by the solvent
Spot 1 Rf value = 3.1 / 5.2 = 0.59
Spot 2 Rf value = 4.3 / 5.2 = 0.82
Spot 3 Rf value = 4.9 / 5.2 = 0.94

TLC of Ethyl acetate extract of *Grewia hirsuta* leaves

Solvent system: Chloroform and Methanol (9:1)

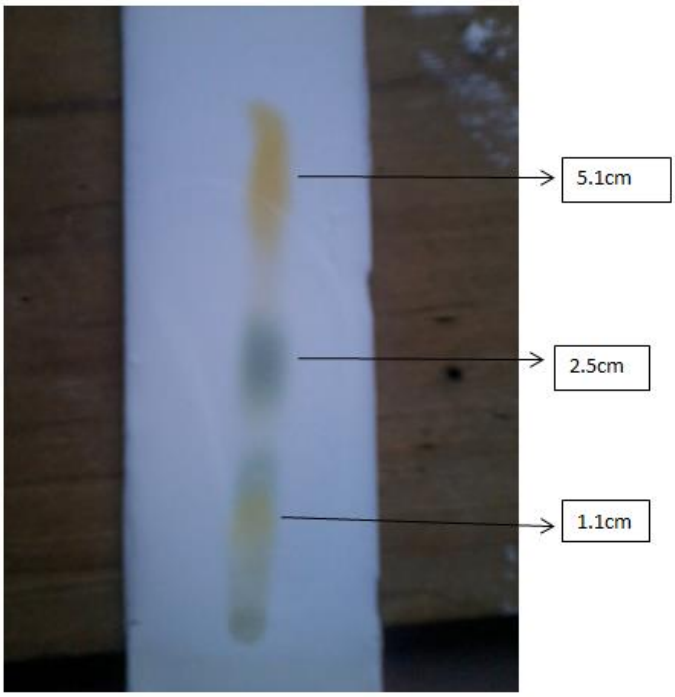


Fig.2. TLC plate of Ethyl acetate extract of *Grewia hirsute*

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Distance travelled by the solute = 5.6
Distance travelled by the solvent
Spot 1 Rf value = 1.1/ 5.6 = 0.19
Spot 2 Rf value = 2.5 / 5.6 = 0.44
Spot 3 Rf value = 5.1 / 5.6 = 0.9

TLC of Ethanol extract of Grewia hirsuta leaves

Solvent system: Chloroform and Methanol (9:1)

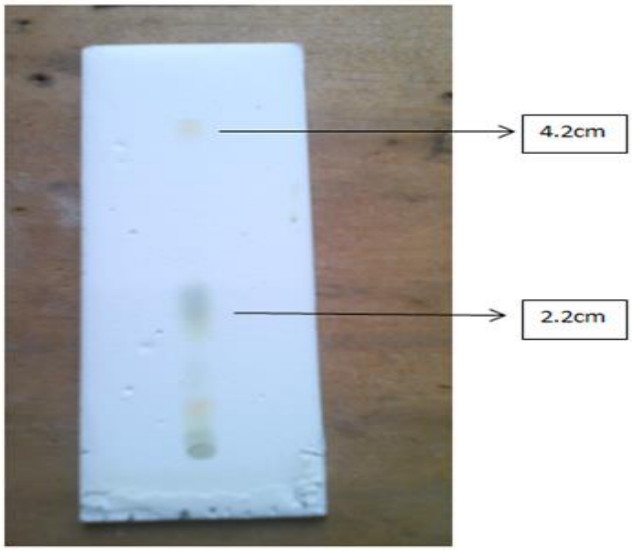


Fig.3: TLC plate of chloroform extract of *Grewia hirsute*

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Distance travelled by the solute = 5.1
Distance travelled by the solvent
Spot 1 Rf value = 2.2 / 5.1 = 0.43
Spot 2 Rf value = 4.2 / 5.1 = 0.82

Table 1: Rf Values of Petroleum ether and Ethyl acetate and Ethanolic extract of *Grewia hirsuta* leaves

EXTRACTS	SOLVENT SYSTEM	Rf VALUE
Petroleum ether extract	Chloroform and methanol (9:1)	Spot 1 = 0.59 Spot 2 = 0.82 Spot 3 = 0.94
Ethyl acetate extract	Chloroform and methanol (9:1)	Spot 1 = 0.19 Spot 2 = 0.49 Spot 3 = 0.9
Ethanol extract	Chloroform and methanol (9:1)	Spot 1 = 0.43 Spot 2 = 0.82

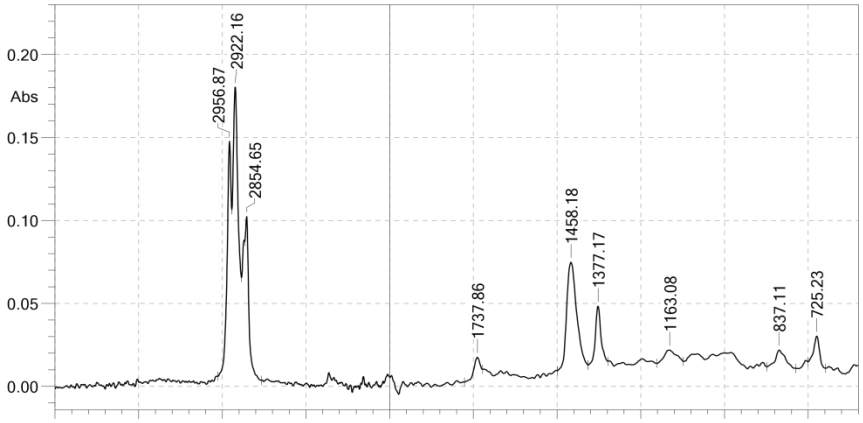


Fig.4: FT-IR studies on *Grewia hirsuta* pet ether extract

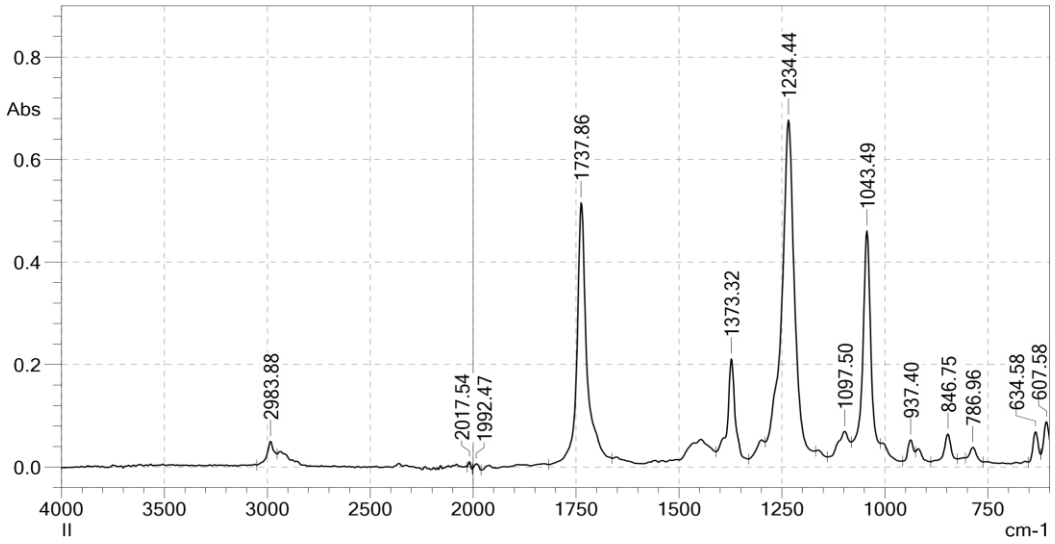


Fig.5: FT-IR Studies on *Grewia hirsuta* Ethyl acetate extract

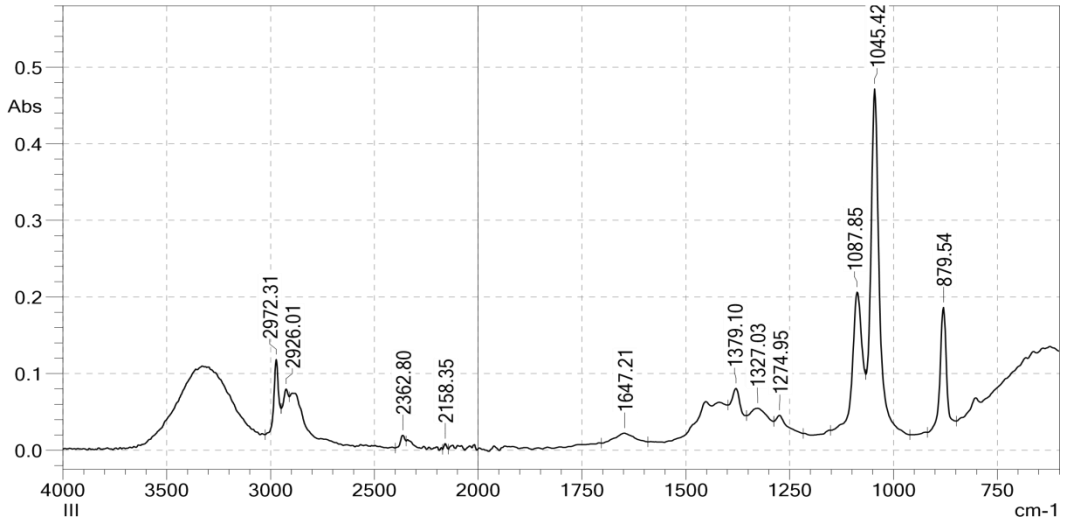


Fig.6: FT-IR Studies on *Grewia hirsuta* Ethanolic extract

Interpretation of FT-IR studies

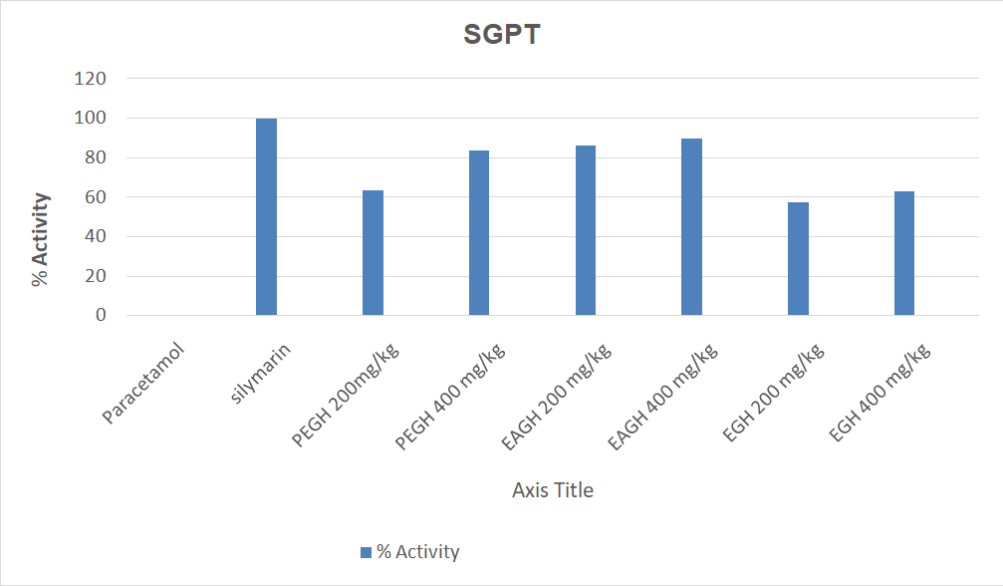
Table 2: Interpretation of FT-IR studies on Petroleum ether, Ethyl acetate and Ethanol extract of *Grewia hirsuta*

EXTRACT OF *Grewia hirsuta*	PEAK	CORRELATION FUNCTION GROUP	RANGE
Petroleum ether extract of *Grewia hirsuta*	725.23	CH2 bending	
	1377.17	Methyl (m)-bend	1450&1375
	1737.86	CO in aldehyde	1730
	2854.65	CH stretch	Near 3000
	2922.16	CH stretch	Near 3000
	2956.87	CH stretch	Near 3000
Ethyl acetate extract of *Grewia hirsuta*	1234.44	CO ester SP2	
	1373.44	Methyl (m)-bend	1450&1375
	1737.86	Aldehyde (s)	1740-1720
	2983.88	CH Stretching	
Ethanol extract of *Grewia Hirsute*	1045.42	CO bending (may be alcohol)	
	1087.85	CO bending (may be alcohol)	
	1379.10	Methyl (m)-bend	1450&1375
	2926.01	CH stretching	<3000
	2972.31	CH stretching	<3000

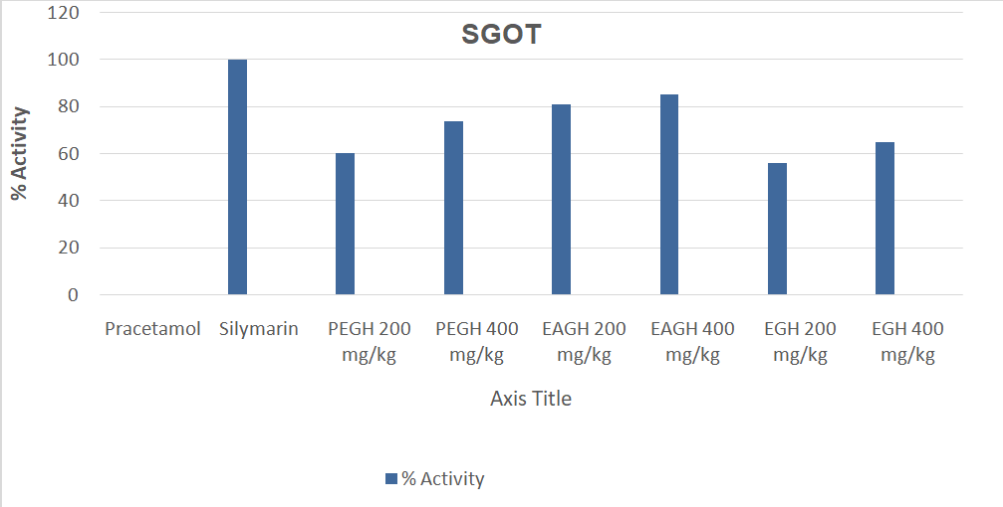
Table 3: Effect of extracts of *Grewia hirsuta* leaves on serum enzyme SGPT and SGOT on Paracetamol – intoxicated rats

Treatment	Dose (mg/kg)	SGPT level Mean ± SEM	% Activity	SGOT Level Mean ± SEM	% Activity
Control	Tween 80	270.98 ± 0.742	-	306.82 ± 0.55	-
Paracetamol	3000	793.91 ± 0.95	-	824.96 ± 1.05	-
Standard (Silymarin)	200	322.85 ± 1.4***	100%	328.26 ± 1.1***	100%
PEGH	200	506.02 ± 0.92**	63.8%	543.69 ± 0.92	60.37%
PEGH	400	384.46 ± 1.58**	83.97%	444.28 ± 0.71**	73.88%
EAGH	200	372.77 ± 1.43**	86.6%	404.69 ± 0.83**	81.1%
EAGH	400	358.34 ± 1.42**	90.09%	385.43 ± 0.71**	85.16%
EGH	200	562.12 ± 1.45**	57.43%	584.52 ±0.86**	56.15%
EGH	400	511.97 ± 1.07**	63.06%	504.79 ±1.4**	65.02%

Results are expressed as means ± SEM, n=4 **p<0.01, ***p<0.001 when compared to normal control group



Graph.1: Percentage reduced in the elevated level of Serum SGPT

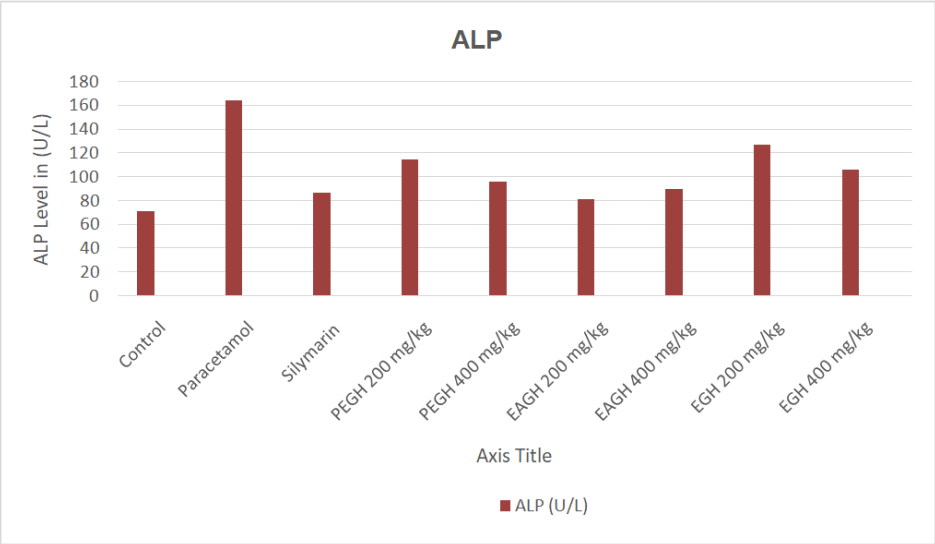


Graph.2: Percentage reduced in the elevated level of Serum SGOT

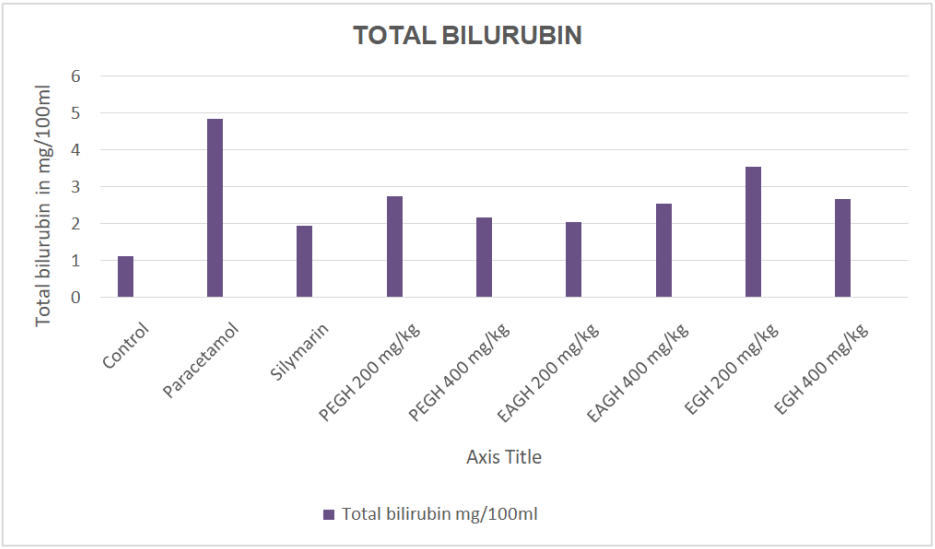
Table 4: Effect of pet ether, ethyl acetate and ethanol extract of *Grewia hirsute* on ALP and Total bilirubin level in Paracetamol intoxicated rat

Treatment	ALP (U/L)	Total Bilirubin (mg/100ml)
Normal Control	71.73 ± 1.82	1.12 ± 0.11
Paracetamol	164.61 ± 2.58	4.86 ± 0.34
Silymarin	86.62 ± 1.84**	1.95 ± 0.18***
PEGH 200	114.75 ± 2.29*	2.76 ± 0.25*
PEGH 400	96.51 ± 1.88**	2.18 ± 0.13**
EAGH 200	81.52 ± 2.24*	2.04 ± 0.12**
EAGH 400	90.24 ± 1.78	2.55 ± 0. 22*
EGH 200	127.38 ± 2.32	3.56 ± 0.26
EGH 400	106.5 ± 2.10**	2.68 ± 0.27**

Results was expressed in Mean ± SEM *p<0.05, **p<0.01, ***p<0.001 as comparison with the control group



Graph.3: Effect of extracts of *Grewia hirsuta* on ALP enzyme on Paracetamol intoxicated rats

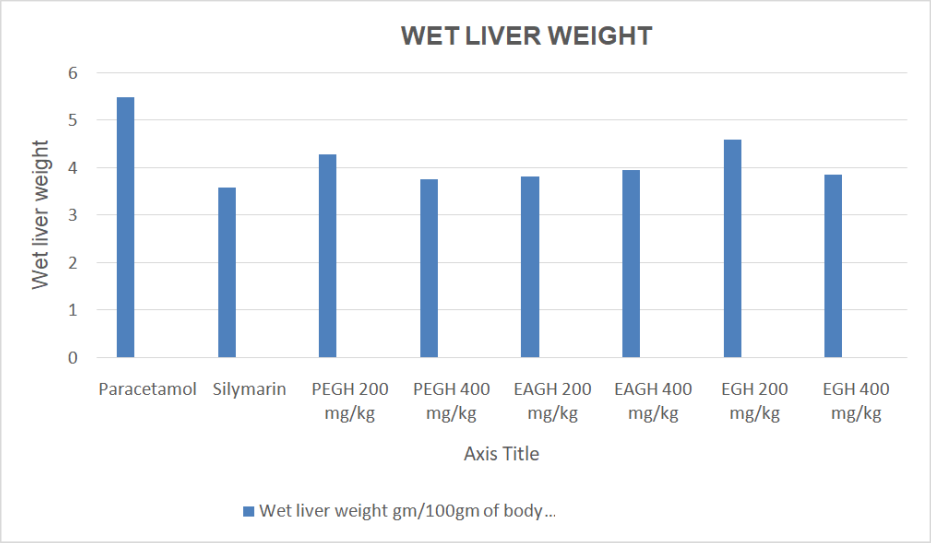


Graph.4: Effect of extracts of *Grewia hirsuta* on Total Bilirubin on Paracetamol intoxicated rats

Table 5: Effect of Pet ether, Ethyl acetate and Ethanol extract of *Grewia hirsuta* leaves on paracetamol induced hepatotoxicity (Wet liver weight)

Treatment	Dose (mg/kg)	Wet liver weight (gm/100 gm of Animal) Mean ± SEM
Paracetamol	3000	5.5 ± 0.169
Standard (Silymarin)	200	3.6 ± 0.136***
PEGH	200	4.3 ± 0.110*
PEGH	400	3.77 ± 0.165**
EAGH	200	3.82 ± 0.172**
EAGH	400	3.97 ± 0.179*
EGH	200	4.6 ± 0.108
EGH	400	3.87 ± 0.103**

Results are expressed as means ± SEM, n=4 *P<0.01 and **P<0.01 when compared with the paracetamol intoxicated group.



Graph.5: Effect of extract of *Grewia hirsuta* on wet liver on paracetamol intoxicated rats

Histopathological studies of the liver in paracetamol induced hepatotoxicity

The histopathological evaluation of paracetamol toxicity in all the groups was examined and shown in figure. The description is as follows, the section of rat liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Section of paracetamol induced toxic control group show tridities with scattered inflammatory infiltration within the parenchyma which is due to the toxicity. Section of silymarin treated group shows liver parenchyma with intact architecture. Section of liver in the test drugs pet ether, ethyl acetate and ethanol extract treated groups shows intact architecture, few regenerative hepatocytes and sinusoidal congestion which is similar to silymarin treated group.

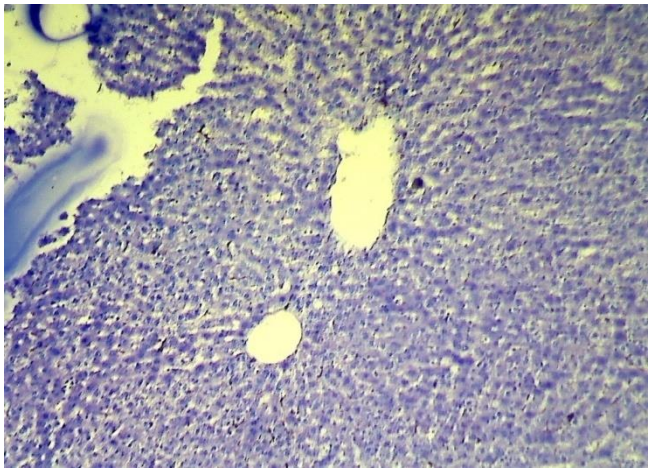


Fig.7: Normal control group, showing normal hepatocytes

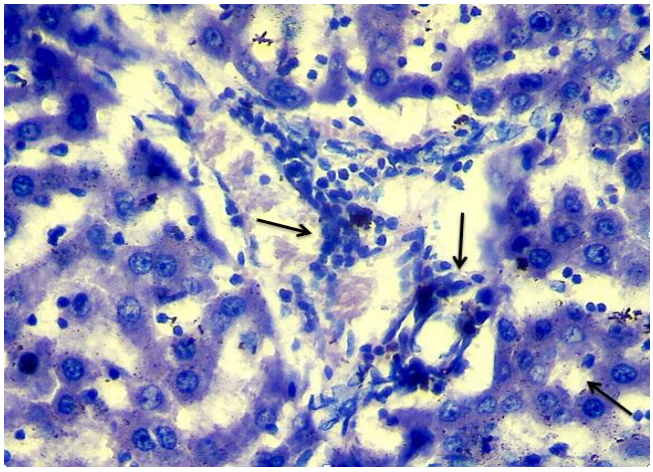


Fig.8: Paracetamol treated animal group shows hepatic damage, sinusoidal congestion of liver and kupffer cell hyperplasia.

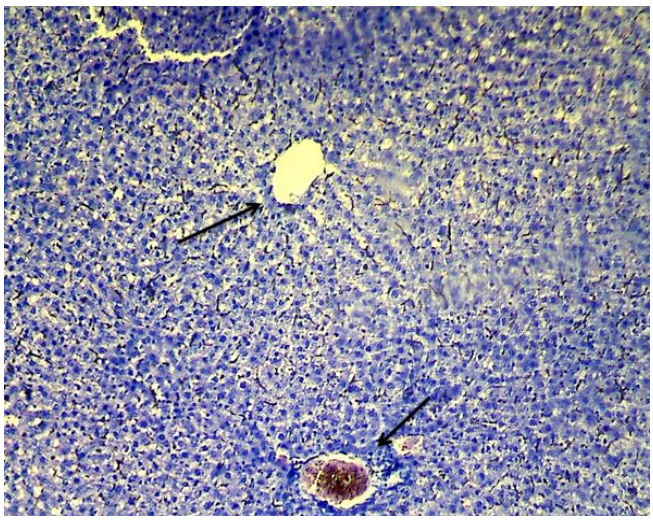


Fig.9: Hepatocytes of Standard (Silymarin 100 mg/kg) treated group

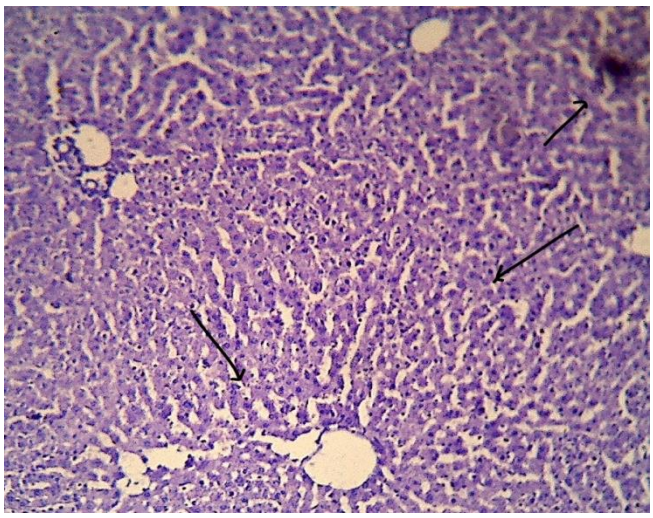


Fig.10: Pet ether 100 mg/kg extract treated group shows show regenerative hepatocytes and Triaditis

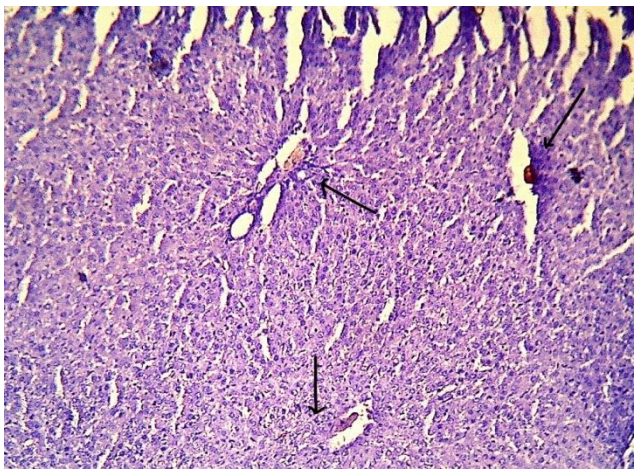


Fig.11: Pet ether 400mg/kg treated group shows few regenerative hepatocytes and sinusoidal congestion

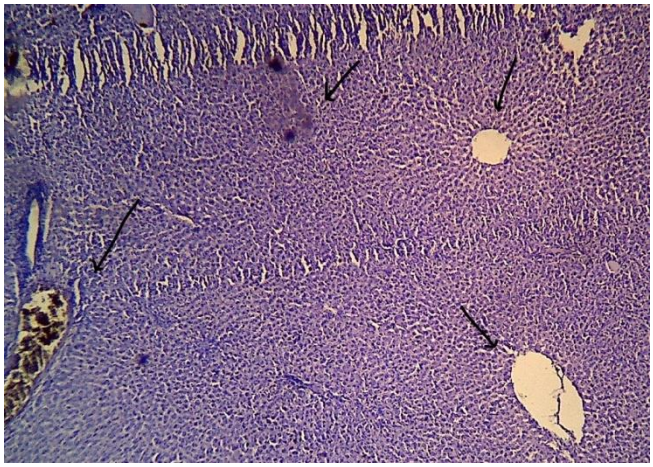


Fig.12: Ethyl acetate extract 100 mg/kg treated group shows that regenerative hepatocytes, Triaditis and sinusoidal collections of lymphocytes.

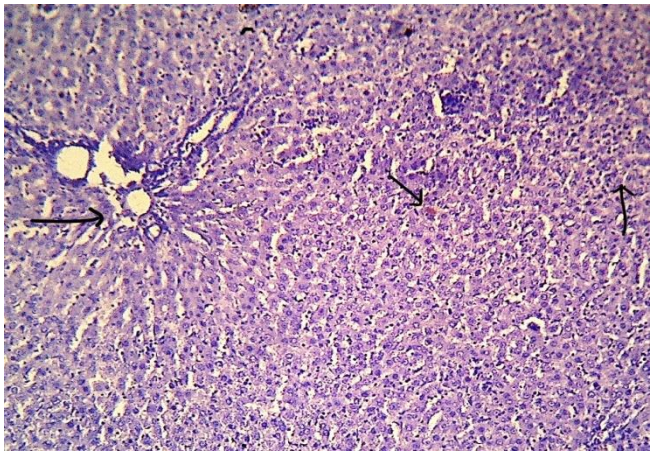


Fig.13: Ethyl acetate extract 400 mg/kg treated group shows that regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group.

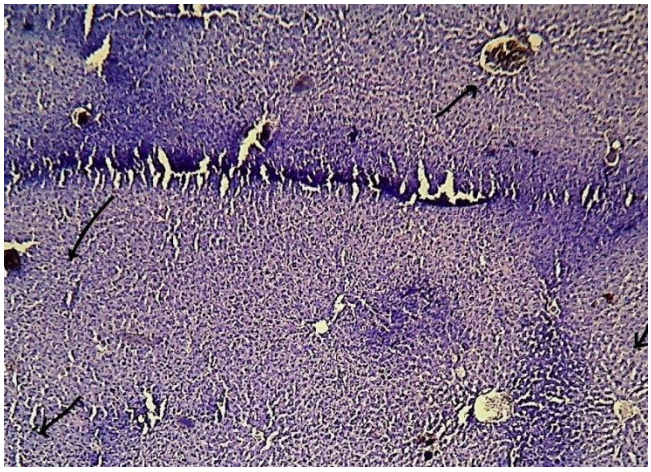


Fig.14: Ethanol 100 mg/kg treated group shows few regenerative hepatocytes

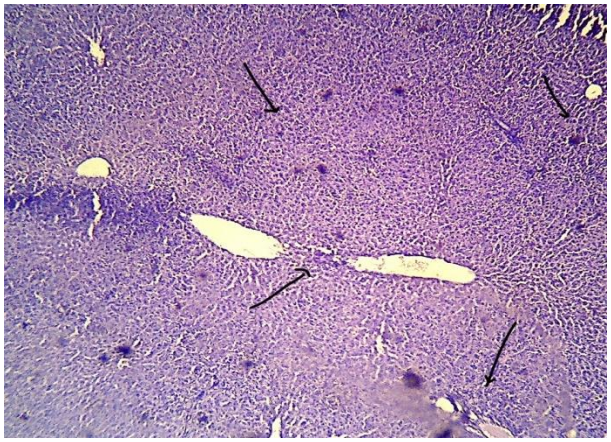


Fig.15: Ethanol 400 mg/kg treated group shows regenerative hepatocytes, Triaditis and sinusoidal collections of lymphocytes.

DISCUSSION

Hepatoprotective activity

Paracetamol is one of commonly used antipyretic agent; which is safe up to level of preferred dose. When this medication taken in higher dose (toxic dose) which cause severe damage to liver. Because major metabolism of paracetamol take place in liver, with help hepatic enzymes mainly by Cytochrome P-450. Mostly in safe dose liver detoxify the drug by converting them to sulfate and glucuronide conjugation which are in active. Then a small amount of drug which is oxidized by cytochrome P-450 hepatic enzyme which converts into a highly reactive intermediate product known as N-acetyl-P-benzoquinone imine (NAPQI) which is deactivated by glutathione. During the toxic dose level, where there high concentration of drug which cause inactivate the sulfate and glucuronide pathways which lead to shut down the action of Cytochrome P-450 activity. The excess amount of glutathione is excreted from the liver which lead to glutathione depletion which cause liver damage. Because glutathione is important for the viability of liver and promoting the regeneration of liver cells. [69] Evidence for the parcetamol inducing toxicity was reported earlier.

When liver damaged by any cause, it can be identified by the analysing the increased level of serum biochemical enzymes such as SGOT, SGPT, ALP and Total bilirubin. In this study the liver damage in induced by intoxication of animals with paracetamol (3000 mg/kg). Likewise, by analysing the blood sample for estimating the level of serum biochemical after the 48 hours of paracetamol intoxication shows marked increase in the serum enzymes level in case of paracetamol toxic group and this elevated level of serum enzymes was significantly recovered in the standard group and in the petroleum ether, Ethyl acetate and Ethanolic extract treated group shows somewhat near towards the normal level was observed. All extracts show better hepatoprotective activity at 400 mg/kg and at lower dose shows marked activity.

In the Wet liver weight assesment paracetamol control group has increased liver weight. Then the P 0.05> significant reduction in the weight of liver is observed in all extracts at 400 mg/kg.

Furthur Histopathological liver section of paracetamol treated animal show sinusoidal collection and kupffer cell hyperplasia. In case of petroleum ether, Ethyl acetate and ethanol extract of *Grewia hirsuta* shows decrease in severity of liver damage and shows regenerative cells which further indicates that the supplement of Plant extract of *Grewia hirsuta* shows hepatoprotective action.

CONCLUSION

In conclusion, the hepatoprotective effect of petroleum ether, Ethyl acetate and Ethanol extract of leaves of *Grewia hirsuta* was confirmed by the following measures; such as level of SGPT, SGOT, ALP and total bilirubin observed in paracetamol induced hepatotoxicity. Finally, the Petroleum ether, Ethyl acetate and ethanol extract of *Grewia hirsuta* shown significant hepatoprotectivity. Thus, they effectively overcome the toxic mechanism of paracetamol. This proves the hepatoprotective potential of leaves extract of *Grewia hirsuta.*

In support to this study, histopathological results also show significant activity of the plant extract. In toxicant treated animals there will be severe disturbances in the cytoarchitecture of the liver. But in the petroleum ether, Ethyl acetate and Ethanol extract of *Grewia hirsuta* shows regeneration of hepatocytes was observed, which confirms the hepatoprotective activity and supports the traditional application of the same under light of modern science.

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