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Hapatoprotective Effect Of Grewia Tiliaefolia Vahl Extracts On Wistar Albino Rats

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Abstract

Grewia tiliaefolia Vahl has been evaluated for its anti free radical activity and hepatoprotective activity in wistar albino rats. The aqueous and methanolic extracts were subjected to antioxidant study using 2, 2- diphenyl-1-picrylhydrazyl, Hydrogen peroxide and Nitrous oxide methods and hepatoprotective activity of aqueous and methanolic extract activity was carried out on paracetamol induced heptotoxic rats . In conclusion methanolic extract exhibited high antioxidant activity and hepatoprotective activity.

Keywords: Hepatoprotective activity, Antioxidant, Grewia tiliaefolia, Paracetamol

Introduction

Paracetamol is one of the universally recommended as antipyretic agent. Due to over dosage of drug taken accidental or deliberate leads to liver damage as liver is the largest organ it mainly filters blood coming from the digestive tract, before passing into the rest of the body, it also detoxifies chemicals and has been attributed to the formation of toxic highly reactive metabolite n-acetyl parabenzoquineimine (NAPQI) . NAPQI causes an imbalance between the production and removal of free radicals and leading to oxidative strees (tezcan etal 2018,jaeschke,2015) proinflammatory cytokinins released from liver kuffer cells are responsible for chronic liver disease and chronic inflammation (lopez-reyes 2008). In hepatotoxicity the activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes increased 24 h after drug administration (Aycan 2014).Synthetic or conventional drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects .Traditional medicinal plants are in use for the treatment of liver ailments (Mitra 2009) as they have very less to no side affects. The literature survey revealed that there were no detailed studies carried out on the free radical scavenging activity and hepatoprotective activity of Grewia tiliaefolia Vahl. Thus we took an opportunity to study the antioxidant and hepatoprotective activities of different extracts of Grewia tiliaefolia .

Materials and Methods

2, 2- diphenyl-1-picrylhydrazyl, Hydrogen peroxide, Sodium dihydrogen phosphate Disodium hydrogen phosphate, Potassium ferricyanide, Ferric chloride, Hydrochloric acid, Sodium hydroxide and all other chemicals and reagents used were of analytical grade obtained from Sigma Chemical Company, St.Louis, USA and Fine Chemicals Ltd., Mumbai, India. Paracetamol was procured from Science Lab.Com, Bombay, India, Silymarin from Sigma Aldrich,

Mumbai, India, sodium CMC purchased from Merk, India, Animal feed was supplied by Krish scientists Shoppe, Bangalore, India. Kits for the estimation of selected biochemical parameters were purchaesd fron SINDU LAB-CHEM, Vasakhapatnam, India manufactured by EXCEL DIAGNOSTICS PVT.LTD, Hyderabad, India, Oral gavage, Tuberculin syringe, crude methanol extract and crude aqueous extract of Grewia tiliaefolia, and Semi autoanalyzer (Excel Crex EA-112) for measuring the biomarker enzymes of serum, Centrifuge (Remi) .

Determination of 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca et al., 2001. An aliquot of 3 ml of 0.004 % DPPH solution in methanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady-state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of the respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated as $[(A_0-A_1)/A_0] \times 100$.

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Determination of Hydrogen Peroxide assay

The hydrogen peroxide scavenging assay was carried out by following the procedure of Ruch et al., (Ruch et al., 1989). A solution of hydrogen peroxide (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The extracts (100 µg/mL) were added to 0.6 ml of hydrogen peroxide solution (0.6 ml, 43 mM). The absorbance values of the reaction mixtures were recorded at 230 nm after 10 mins against a blank solution in phosphate buffer without sample and hydrogen peroxide.

The percentage of scavenging of hydrogen peroxide of plant extracts and the standard compound was calculated using the following equation:

Percentage Inhibition= $(A_0 – A_1) /A_0 \times 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Reducing Power Assay

Reducing power was determined by the method (Oyaizu et al.¸1986). Various samples in methanol at various concentrations each 2.5 was mixed with a phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%), and the mixture was incubated at 50°C for 20 min. Next, 2.5ml of trichloroacetic acid (10%) was added to the reaction mixture, which was then centrifuged at 650 RPM for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5ml) and ferric chloride (1 ml, 0.1 %), and the absorbance was measured at 700 nm. A stronger absorbance will indicate higher reducing power.

Selection of animals

Albino Wistar rats of either sex (170-200 g) were obtained from the Mahaveer Enterprises, Hyderabad, India. They were kept under the temperature of (23 ± 2) °C, the humidity of 50 % and light and dark cycles of 12 h: 12 h. They were fed with commercial pellet diet purchased from Krish scientists Shoppe, Bangalore, India and water was provided ad libitum. The protocol was approved by the Institutional Animal Ethics Committee (Regd. No. 516/PO/c/01/IAEC/07**)** and the lab of Au college of Pharmaceutical Sciences, Andhra University was approved by CPCSEA (Regd. No. 516/01/A/CPCSEA), Government of India**.**

Hepatoprotective Activity

In this method, Wistar albino rats of either sex weighing between 170-200 g were used for the study. The rats were housed under standard conditions of constant temperature and lighting (12 hours light/dark cycle). They had access to a standard pellet diet and water adlibitum. The rats were selected and divided into 18 groups each containing six rats. Paracetamol powder suspended in saline solution and different extracts, nanoparticles, and silymarin were dissolved in 1 % sodium carboxymethyl cellulose suspension. The treatment protocol was planned to study the effect of herbal medicine in the preventive aspect of paracetamol-induced hepatotoxicity (Shenoy et al., 2002). The doses of the plant extracts were selected as 100 mg/kg (LD), 200 mg/kg (MD) and 400 mg/kg (HD) of body weight. The dose of silymarin used was 50 mg/kg (Hermenean et al., 2017). All doses were given 1 hour prior to Paracetamol 2 g/kg b.w orally for 7days. The percentage of protection is calculated as given below.

$$\%\,\mathrm{Pr}\,otection = \frac{\left\{\begin{matrix} AST\,/\,ALT\,/\,ALP\,/\,T.BIL \\ in\,Toxicant\,group \end{matrix}\right\} - \left\{\begin{matrix} AST\,/\,ALT\,/\,ALP\,/\,T.BIL \\ in\,Drug + Toxicant\,group \end{matrix}\right\}}{\left\{\begin{matrix} AST\,/\,ALT\,/\,ALP\,/\,T.BIL \\ in\,Toxicant\,group \end{matrix}\right\} - \left\{\begin{matrix} AST\,/\,ALT\,/\,ALP\,/\,T.BIL \\ in\,before\,treatment \end{matrix}\right\}} x100$$

Table 01.The treatment protocol is summarized as given below

Groups	Treatment
Group-I	Normal control, 1 % w/v Sodium CMC suspension orally, 1ml/kg
Group-II	Paracetamol as toxicant 2 g/kg b.w orally once daily for 7 days
Group-III	Silymarin 50 mg/kg b.w orally
Group-IV	Methanolic extract of G. tiliaefolia 100 mg/kg orally,
Group-V	Methanolic extract of G. tiliaefolia 200 mg/kg orally
Group-VI	Methanolic extract of G. tiliaefolia 400 mg/kg orally
Group-VII	Aqueous extract of G. tiliaefolia 100 mg/kg orally
Group-VIII	Aqueous extract of G. tiliaefolia 200 mg/kg orally
Group-IX	Aqueous extract of G. tiliaefolia 400 mg/kg orally

Statistical analysis

All the results were expressed as Mean ± SEM. Statistical analysis was done using Two-way ANOVA followed by Bonferroni's Post test using Graph Pad Prism version 5 software (Graph Pad Inc, USA). The statistical significance was determined at $P<0.05$.

Results and discussion

In the present study, the crude methanol, and crude aqueous extracts were found to possess concentration-dependent scavenging activity on DPPH radicals. The percentage inhibition of crude methanolic of Grewia tiliaefolia Vahl, and ascorbic acid on dpph free radicals at 400 µg were 83.24 %, and 99.44 % respectively and the results were given Table-6.2 and fig.6.1. The mean IC50 values on DPPH radical of Grewia tiliaefolia were found to be 106.22 µg/mL .The mean IC50 value of ascorbic

acid was found to be 41.89 µg/mL. The results were given in Table-6.8 and Fig. 6.7.

The crude methanolic extract on Hydrogen peroxide radicals at 400 µg were 73.74 %, and 90.61 % respectively and the results were given in Table-6.3and Fig.6.2. The mean IC_{50} values on DPPH radical of Grewia tiliaefolia were found to be 184.35 µg/mL and ascorbic acid was found to be 78.47 µg/mL.

The crude methanol extract possess concentration-dependent scavenging activity on free radicals of reducing power assay. The percentage inhibition of crude methanolic extract and successively extracted hexane, ethyl acetate and methanolic leaf extracts of Grewia tiliaefolia Vahl and ascorbic acid on reducing power assay radicals at 400 µg were 85.96 % and 97.19 % respectively and the results were given in Table-6.4 and Fig.6.3. The mean IC_{50} values of crude methanolic of Grewia tiliaefolia Vahl was found to be 122.5 µg/mL and ascorbic acid was found to be 44.79 µg/mL. The results were given in Table-6.8 and Fig. 6.7

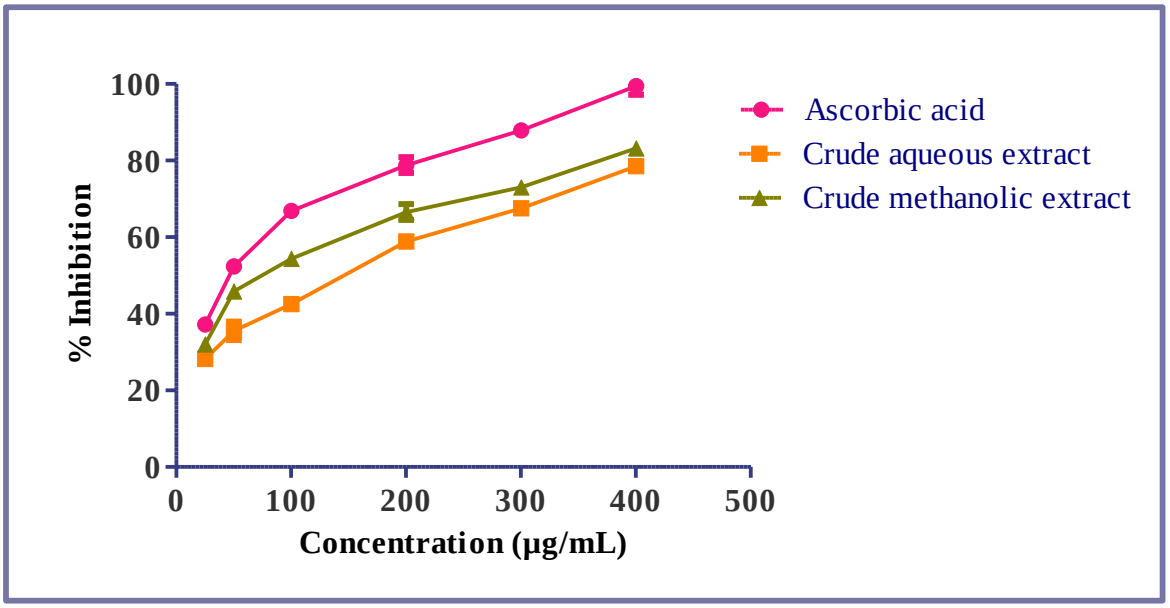

Fig. 1. Concentration-dependent percentage inhibition of different extracts of Grewia tiliaefolia on DPPH radicals

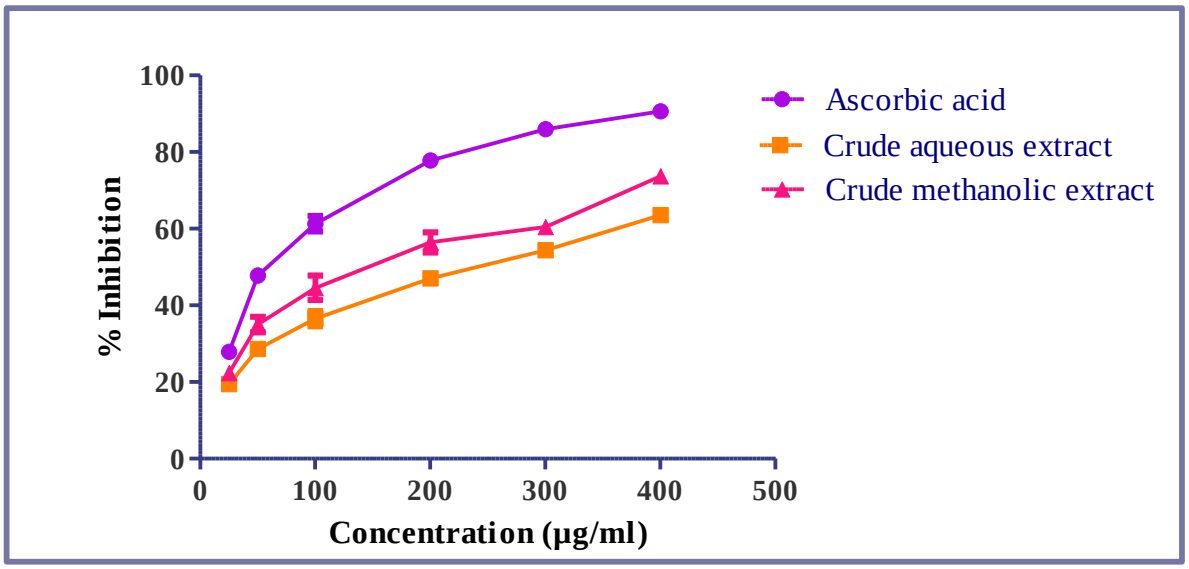

Fig. 2. Concentration-dependent percentage inhibition of different extracts of Grewia tiliaefolia on hydrogen peroxide radical

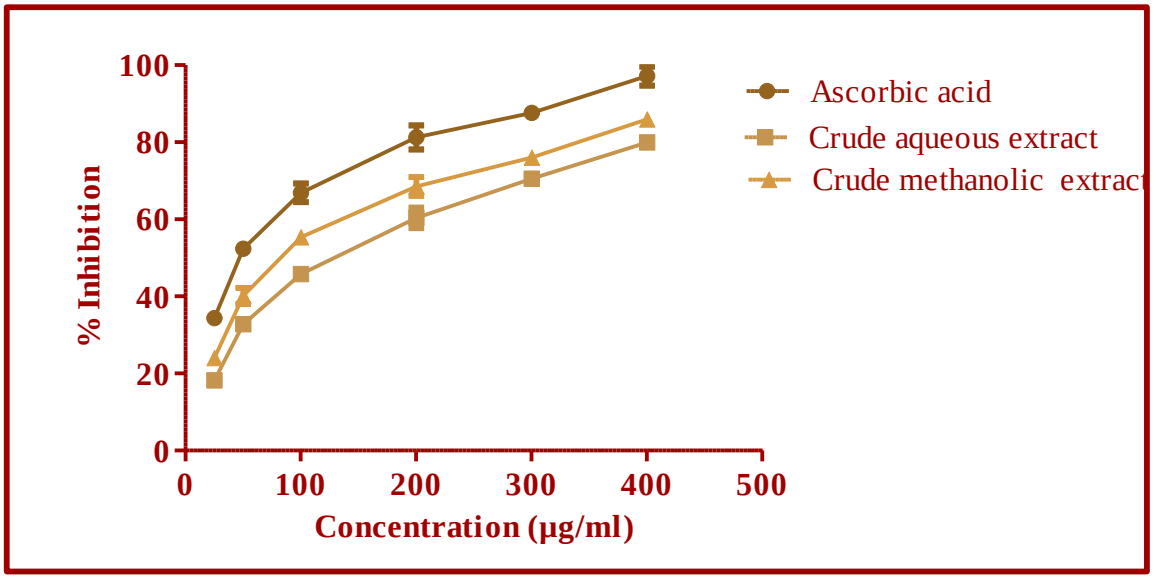

Fig.3. Concentration-dependent percentage inhibition of different extracts of Grewia tiliaefolia by reducing power assay

Table 2. 50% Inhibition (IC50) of different extracts against DPPH, Hydrogen peroxide assay, Reducing power assay

Name of the Extract/compound	50% Inhibition (IC50) (µg/mL)		
	DPPH radical	Hydrogen peroxide assay	Reducing power assay
Crude methanolic extract	106.22± 1.06	184.35± 0.98	122.5± 2.08
Crude aqueous extact	165.38± 2.03	257.68± 0.88	171.58± 3.45
Ascorbic acid	41.89±5.00	78.47± 3.00	44.79± 1.00

All the values were expressed as Mean ± SEM, (n=3)

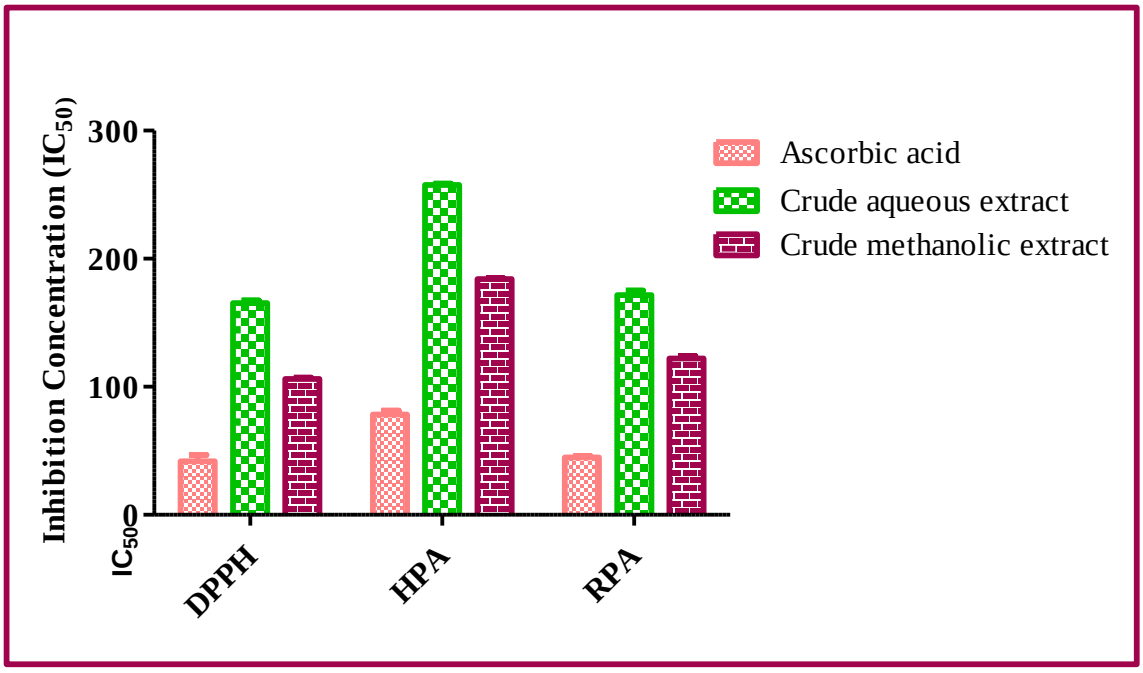

Fig.4. 50% Inhibition concentration (IC50) of different of Grewia tiliaefolia against Dpph, Hydrogen peroxide radicals and Reducing power assay, Where DPPH -2, 2-diphenyl-1-picrylhydrazyl; RPA-Reducing Power Assay; HPA-Hydrogen Peroxide Assay

Group I rats were treated with 1 % Sodium CMC as vehicle showed significant changes in the biomarkers of liver enzymes (AST, ALT, ALP, Total bilirubin and Total protein) levels. Group II rats were treated with Paracetamol 2000 mg/kg body weight orally as toxicant and there were significant changes in levels of biomarker enzymes. The rats of Group III were administered with silymarin (50 mg/kg b.w). There were significant changes in biomarker enzyme levels compared to group I rats enzyme levels presented in the Table 8.15 and the percentage protection of silymarin against the changes in AST, ALT, ALP, Total Bilirubin, Total Protein levels were 97.11 %, 93.19 %, 97.65 %, 95.89 %, 98.54 % respectively. The results were presented in the Table 8.20 .

Groups IV, V, and VI rats were administered with methanolic extract of Grewia tiliaefolia orally at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. The enzyme levels were given in the Table 3. The protective effect of methanolic extract by lowering the serum levels of AST, AST, ALP, Total Bilirubin, Total Protein levels were given as percentage protection

45.24 %, 30.80
%, 51.79 %, 43.07 %, 39.27 % and 57.07 %, 49.61 %, 61.07 %, 69.23 %, 57.56

%, and 74.31 %, 60.21 %, 71.50 %, 74.35 %, 79.27 % respectively. The results were presented in the Table 3, Fig.5, Fig. 6 and. Fig. 7

Groups VII, VIII, and XI rats were administered with aqueous extract orally at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. The protective effect of methanolic extract by lowering the serum levels of AST, AST, ALP, Total Bilirubin, Total Protein levels were given as percentage protection 40.67 %, 30.21
%, 35.58 %, 34.94 %, 35.63 %, and 50.48 %, 45.53 %, 44.92%, 50.76 %, 48.36 %

and 60.57 %, 52.91 %, 52.23 %, 66.66 %, 60 %. The results were given in Table 4, Fig.5, Fig. 6 and Fig.7.

The paracetamol-induced hepatotoxicity model is extensively used to evaluate the hepatoprotective effects of drugs and plant extracts. The hepatoprotective effects of aqueous extracts and methanolic extracts of selected plant Grewia tiliaefolia at three dose levels such as 100 mg/kg, 200 mg/kg, 400 mg/kg were assessed by measuring the liver-related biochemical parameters (AST, ALT, ALP, Total serum bilirubin, and Total protein levels). The percentage protection produced by the standard drug and selected plant extracts were calculated based on enhancement activities of serum biomarkers enzymes observed in paracetamol-induced hepatotoxicity and the results were presented in the Table 5.

The histopathological studies performed showed that control group showed normal hepatic cellular structure with normal hepatic cells, well sinusoids. The

paracetamol intoxicated group showed evidence of the necrosis haemorrhage and inflammation. These pathological changes were reduced in the drug-treated liver tissue demonstrated similar morphology as that of controls for higher dose of methanolic extract and the results were presented in Fig. 8.

In our studies, the results of plant extracts indicated that their administration could help in the treatment of the hepatic injury caused by the paracetamol at higher doses. The methanolic extract showed higher percentage protection then aquoes extract.

Table 3. Groups-I, II, III, IV, V and VI rats enzymes levels due to the effect of Grewia tilaefolia methanolic extract at different doses

Name of the drug	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)
Normal (1 % Na CMC)	89.83 ± 1.57	41.00 ± 3.75	185.7 ± 1.62	0.3 ± 0.01	7.26 ± 0.20
Paracetamol (2000 mg/kg b.w)	332.5 ± 2.29	158.5 ± 1.08	530.7 ± 3.30	2.25 ± 0.09	4.25 ± 0.02
Silymarin (100 mg/kg b.w)	133.2 ± 1.93	63.50 ± 1.14	237.7 ± 0.76	0.41 ± 0.01	6.66 ± 0.09
Methanolic extract of G.tiliaefolia (100 mg/kg b.w)	222.7 ± 0.95	122.3 ± 0.71	352.0 ± 1.88	1.41 ± 0.06	5.35 ± 0.04
Methanolic extract of G.tiliaefolia (200 mg/kg b.w)	194.0 ± 0.57	105.2 ± 1.13	320 ± 2.69	1.3 ± 0.036	5.83 ± 0.05
Methanolic extract of G.tiliaefolia (400 mg/kg b.w)	152.17 ± 1.01	83.67 ± 1.43	284.0 ± 1.9	0.8 ± 0.05	6.43 ± 0.11

All the values were expressed as Mean ± SEM, (n=6)

Table 4. GROUP-I, II, III, VII, VIII and IX rats enzymes levels due to the effect of Grewia tilaefolia aqueous extract at different doses

Name of the Drug	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)
Normal	89.83 ± 1.57	41.00 ± 3.75	185.7 ± 1.62	0.3 ± 0.01	7.26 ± 0.20
Paracetamol (2000 mg/kg b.w)	332.5 ± 2.29	158.5 ± 1.08	530.7 ± 3.30	2.258 ± 0.09	4.25 ± 0.02
Silymarin (50mg/kg b.w)	133.2 ± 1.939	63.50 ± 1.14	237.7 ± 0.76	0.41 ± 0.01	6.66 ± 0.09
Aqueous extract G.tiliaefolia (100 mg/kg b.w)	233.8 ± 2.44	127.5 ± 1.50	444.8 ± 1.07	1.56 ± 0.09	5.30 ± 0.08
Aqueous extract G.tiliaefolia (200 mg/kg b.w)	210.2 ± 1.26	105.8 ± 1.30	408.2 ± 4.32	1.35 ± 0.08	5.46 ± 0.08
Aqueous extract G.tiliaefolia (400 mg /kg b.w)	185.0 ± 1.15	96.17 ± 0.87	350.8 ± 1.30	0.90 ± 0.08	5.58 ± 0.13

All the values were expressed as Mean ± SEM (n=6)

Table 5. Percentage (%) Protection of methanolic and aqueous extracts of Grewia tiliaefolia at different doses on Paracetamol induced hepatotoxicity

Name of the Drug	Percentage Protection				
	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)
Silymarin 50mg/kg b.w	82.12	80.85	84.93	81.68	87.63
Methanolic extract of G.tiliaefolia 100 mg/kg b.w	45.24***	30.80***	51.79***	43.07***	39.27***
Methanolic extract of G.tiliaefolia 200 mg/kg b.w	57.07***	49.61***	61.07***	69.23**	57.56***
Methanolic extract of G.tiliaefolia 400 mg/kg b.w	74.31***	60.21***	71.50***	74.35***	79.27***
Aqueous extract of G.tiliaefolia 100 mg/kg b.w	40.67***	30.21***	35.58***	34.94***	35.63***
Aqueous extract of G.tiliaefolia 200 mg/kg b.w	50.39***	45.56***	44.92***	44.5***	48.36***
Aqueous extract of G.tiliaefolia 400 mg/kg b.w	60.78***	53.88***	52.14***	66.83***	60***

All the values were analysed by using Two-way ANOVA followed by bonferroni post hock test. All groups were compared with silymarin, *p<0.05 considered as level of significance . ***p<0.001; **p<0.01; *p<0.05; ns= non significance

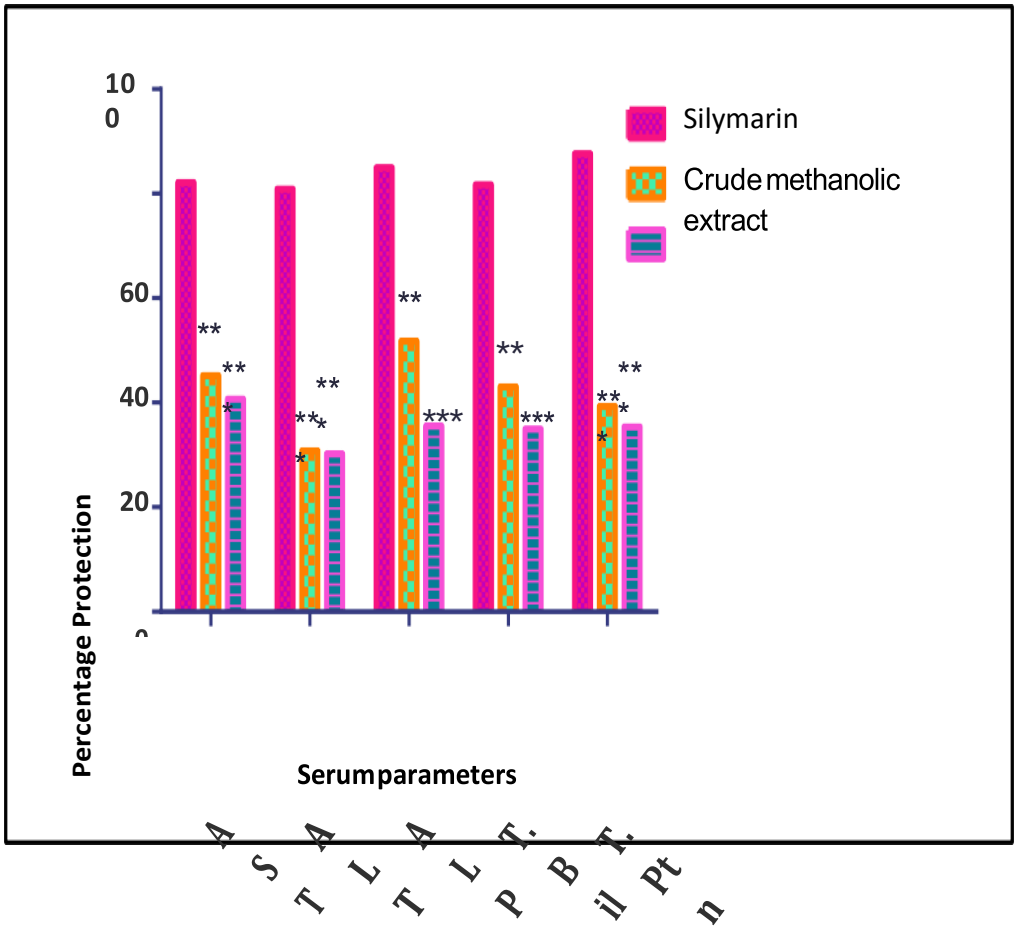

Fig. 8.2. Percentage protection produced by different extracts of Grewia tiliaefolia at a dose of 100 mg/kg, results were analysed by using two-way ANOVA followed by bornferroni post hock test. All groups were compared with standard silymarin group. ***p<0.001; **p<0.01; *p<0.05; ns= non significance

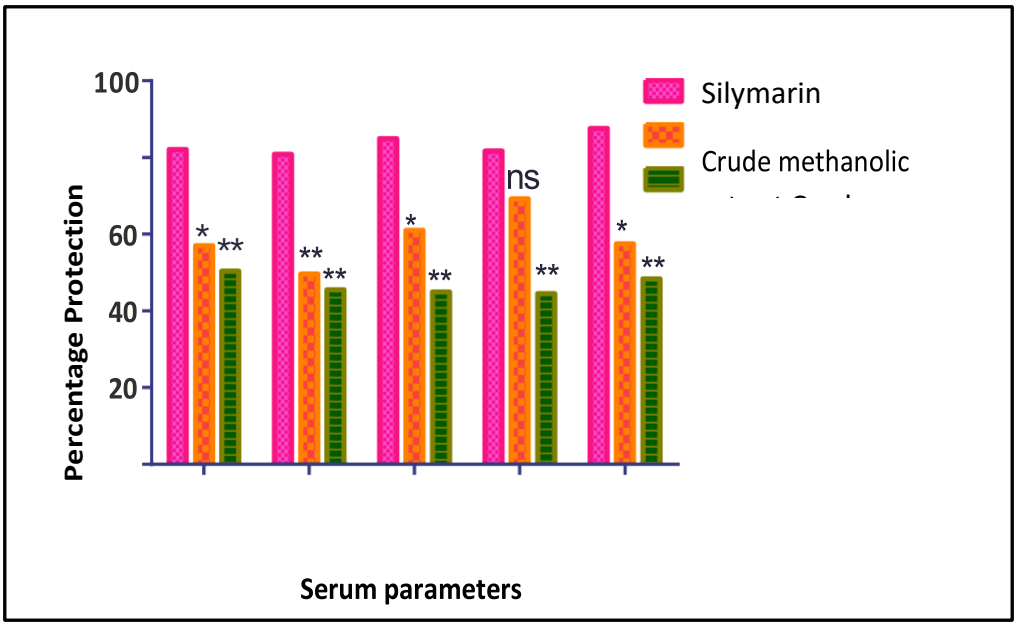

Fig. 8.3. Percentage protection produced by different extracts of Grewia til iaefolia at a dose of 200mg/kg, results were analysed by using Two-way ANOVA followed by bornferroni post hock test. All groups were compared with standard silymarin group ***p<0.001; **p<0.01; *p<0.05; ns= non significance

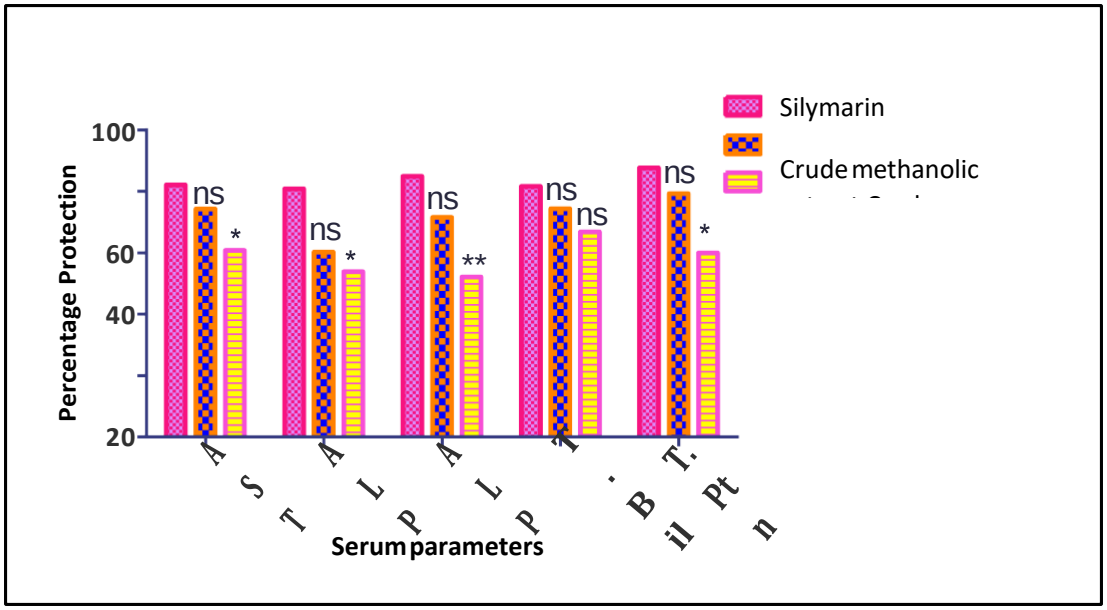

Fig. 8.4. Percentage protection produced by different extracts of Grewia tiliaefolia at a dose of 400 mg/kg, results were analysed by using Two-way ANOVA followed by bornferroni post hock test. All groups were compared with standard silymarin group. ***p<0.001; **p<0.01; *p<0.05; ns= non significance

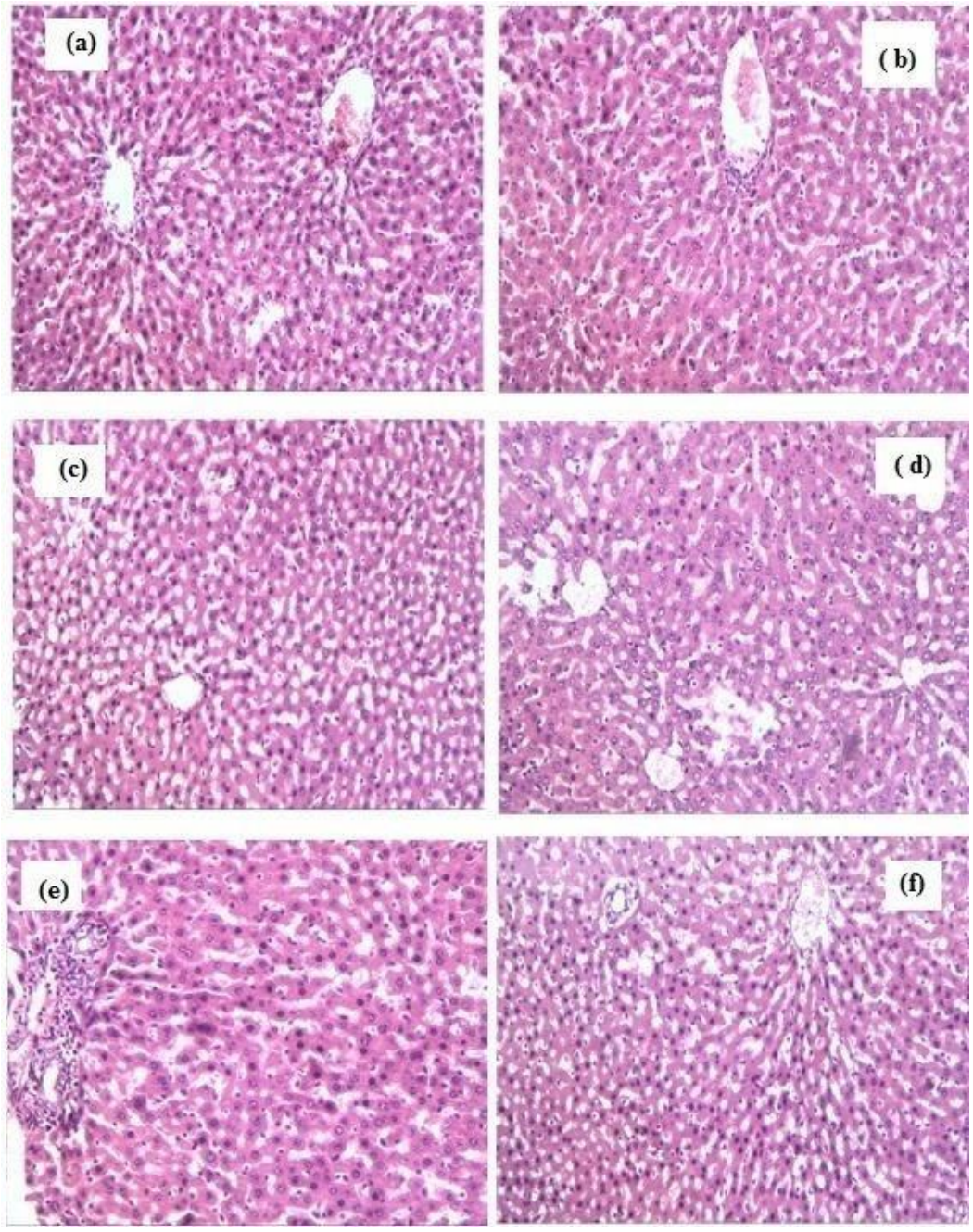

Fig. 8.6. Histopathological images of paracetamol-induced hepatotoxicity observed at 400x magnification a) Methanolic extract of G.tiliaefolia 100 mg/kg b.w b) **Methanolic extract of G.tiliaefolia 200 mg/kg b.w** c) **Methanolic extract of G.tiliaefolia 400 mg/kg b.w** d) **Aqueous extract of G.tiliaefolia 100 mg/kg b.w** e) **Aqueous extract of G.tiliaefolia 200 mg/kg**

b.w f) Aqueous extract of G.tiliaefolia 400mg/kg b.w

6. Conclusion

The results of present study revealed that the antioxidant and hepatoprotective activity of different extracts of the medicinal plant Grewia tiliaefolia. Previous reports proves the presence of secondary metabolites (Biomolecules) phenols, alkaloids, flavonoids which are responsible for the antioxidant activity and

hepatoprotective activity . The present study provides scientific evidence of the plant about antioxidant and hepatoprotective activity which can be used for ayurvedic formulations.

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Conflict of interest statement

The authors have no conflict of interest.

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