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Hepatoprotective effect of Methanolic extract of Grewia Asiatica on paracetamol-induced liver injury in rats

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Abstract
Paracetamol-induced hepatotoxicity is a major health concern. *Grewia asiatica* is a medicinal plant with reported hepatoprotective properties. This study aimed to evaluate the hepatoprotective effect of the methanolic extract of *Grewia asiatica* against paracetamol-induced liver damage in rats. Rats were divided into five groups: control, paracetamol-treated, and three groups treated with different doses of the extract along with paracetamol. Liver function tests, antioxidant enzymes, and histopathological examination were assessed. Paracetamol administration significantly elevated serum levels of liver enzymes, induced oxidative stress, and caused severe liver damage. Treatment with *Grewia asiatica* extract significantly ameliorated these effects by reducing oxidative stress, enhancing antioxidant defense mechanisms, and protecting liver tissue from damage. These findings suggest that *Grewia asiatica* extract possesses potent hepatoprotective activity and may be a promising natural remedy for liver diseases.
Keywords: Methanolic extract GrewiaAsiatica, Paracetamol, Hepatoprotective

Introduction

Liver diseases are a significant global health concern, often stemming from exposure to hepatotoxic agents. Paracetamol, a widely used analgesic and antipyretic drug, can cause severe liver damage when consumed in excessive doses. This condition, known as paracetamol-induced hepatotoxicity, is characterized by oxidative stress, inflammation, and hepatocyte necrosis.

In recent years, there has been a growing interest in exploring natural remedies for liver disorders. Medicinal plants, with their diverse bioactive compounds, offer a promising avenue for developing safe and effective hepatoprotective agents. *Grewia asiatica*, a plant traditionally used in various medicinal systems, has been reported to possess antioxidant, anti-inflammatory, and hepatoprotective properties.

This study aims to investigate the hepatoprotective potential of the methanolic extract of *Grewia asiatica* against paracetamol-induced liver injury in rats. By evaluating biochemical parameters, histopathological analysis, and antioxidant assays, we seek to elucidate the underlying mechanisms of action of the extract and its efficacy in mitigating liver damage.The therapeutic efficacy of Grewia asiatica fruit can be attributed to its rich phytochemical profile. The fruit is a treasure trove of bioactive compounds, including:

- Polyphenols: These compounds, such as flavonoids and phenolic acids, possess potent antioxidant properties. They help neutralize harmful free radicals, reducing oxidative stress and protecting cells from damage.
- Vitamins and Minerals: The fruit is a good source of vitamins, especially vitamin C, and minerals like iron and potassium. These nutrients play crucial roles in maintaining overall health and boosting immunity.
- Carbohydrates and Fiber: The fruit contains dietary fiber, which aids in digestion and promotes gut health.

Material and Methods

CollectionandIdentificationofDrug

The fruit of ***Grewia asiatica*** was collected (inthe month of May and June) from Market . The Fruit were dried in shade, and prepare powder used for extraction. The fruit was positively identified andconfirmedbytheTaxonomist,Dept.ofAyush,Gwalior(M.P.). The voucherspecimen(Ref No: 321) of the plantmaterial hasbeendepositedinthe Departmentof Ayush,Gwalior.

PreparationofExtract

The powder drug was extracted with methanol in soxhlet apparatus, theextraction was completed in 25 cycles. The extract was driedandstoredindesiccator.

Animals

Healthy adultMaleWistarratsweighingabout200-250gwereusedforthestudy.They were housedin groups in polypropylene cages, maintained under standard conditions(12:12h light: dark cycle; 27±3°C; 40–60% humidity) andmaintained with free access to standard rat pellet diet (Amrutlaboratoryanimalfeed,manufacturedbyNavmaharashtrachakanoilmillsLtd,Pune)andwater*a dlibitum*.

The experiments were carried out in accordance with guidelines described by the Institutional Animal Ethics CommitteeoftheInstitute(ProposalNo.1894/PO/Re/S/16/CPCSEA)

Acute toxicityStudies

Overnight fasted Albino Wistar rats were subjected to acutetoxicity studies todetermine the safedoseby acute toxicclass method of oral toxicity as per OECD 423 guidelines[3].The rats were observed continuously for 2h for behavioral,neurological and autonomic profiles and, after a period of 24,72 h, and thereafter up to 14 days for any lethality, moribundstateordeath.

Hepatoprotective activity of methanolic extract of *Grewia asiatica*

The liver, which is the biggest and most adaptable organ for metabolism and excretion, has a crucial role in regulating the body's internal environment via its many and varied tasks. Due to insufficiently regulated environmental pollution and the increasing use of powerful pharmaceuticals, it is constantly exposed to a range of xenobiotics and therapeutic agents. Therefore, there are various and diverse illnesses that are linked to this organ. Liver injury is always linked to cellular necrosis, elevation in tissue lipid peroxidation, and reduction in tissue glutathione (GSH) levels. Furthermore, the levels of many biochemical indicators such as aspartate transaminase (AST), alanine transaminase (ALT), triglycerides, cholesterol, bilirubin, and alkaline phosphatase are increased in the blood. During the in vivo experiments conducted on the produced extract, we investigated the impact of varying concentrations of the extract on the blood levels of liver biochemical markers, including AST, ALT, ALP, and Total Bilirubin.

Biochemical Analysis

Aspartate transaminase (SGOT or AST) is a mitochondrial enzyme that is secreted by the heart, liver, skeletal muscle, and kidney. Alanine transaminase, often known as SGPT or ALT, is an intracellular enzyme that is mostly found in the liver. Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) occur when the tissues responsible for their production are damaged. Therefore, measuring the levels of ALT in the blood is very valuable for detecting liver cell damage, since it is unique to liver tissue. On the other hand, elevated levels of AST may indicate acute necrosis or ischemia in organs other than the liver, such as the myocardium, in addition to hepatic cell injury.

Serum alkaline phosphatase is synthesized by several organs, including bone, liver, gut, and placenta, and is eliminated by bile. An increase in the activity of the enzyme may be seen in bone illnesses, liver ailments, and during pregnancy. When there is no bone disease or pregnancy, increased levels of alkaline phosphatase in the blood usually indicate an illness related to the liver or bile ducts. Biliary tract blockage is responsible for the significant increase in elevation. Parenchymal liver disorders, such as hepatitis, cirrhosis, and metastatic liver disease, exhibit a slight to moderate rise.

The paracetamol-induced hepatotoxicity model is often used to assess the hepatoprotective properties of medications and botanical preparations. The hepatoprotective effects of aqueous and methanolic extracts of the Grewiatiliaefolia plant were evaluated at three

different dose levels (100 mg/kg, 200 mg/kg, and 400 mg/kg). The assessment involved measuring liver-related biochemical parameters, including AST, ALT, ALP, total serum bilirubin, and total protein levels. Mice in Group I, which were administered 1% Sodium CMC as a vehicle, exhibited significant alterations in the levels of liver enzyme indicators (AST, ALT, ALP, and Total bilirubin). The findings were shown in Table 1.

Table 1: Group-Ianimalsenzymeslevelsduetotheeffectofvehicle (1% Na CMC)

MiceNo.	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Totalbilirubin (mg/dl)**
M1	87	48	189	0.28
M2	89	50	191	0.3
M3	96	49	186	0.33
M4	90	30	185	0.29
M5	92	38	180	0.3
M6	85	31	183	0.33
Mean±SEM	89.83±1.57	41.00±3.75	185.7±1.62	0.3±0.01

Paracetamol was administered orally to Group II rats at a dosage of 2g/kg body weight as a toxicant. This led to notable alterations in the levels of biomarker enzymes. The findings of these changes are shown in Table 2

Table 2: LevelsofenzymesofGroup-IIanimalsduetotheeffectof Paracetamol (2g/kg b.w)

MiceNo.	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Totalbilirubin (mg/dl)**
M1	330	156	540	2.1
M2	342	160	520	2.4
M3	326	162	525	1.85
M4	333	158	528	2.3
M5	335	160	540	2.4
M6	329	155	531	2.5
Mean±SEM	332.5±2.29	158.5±1.08	530.7±3.30	2.25±0.09

Silymarin (50 mg/kg b.w) was given to the rats in Group III. The biomarker enzyme levels exhibited substantial alterations when compared to the enzyme levels of group I rats, as seen in Table 3

Table3LevelsofenzymesofGroup-IIIanimalsduetotheeffectofSilymarin (50 mg/kg b.w)

MiceNo.	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Totalbilirubin (mg/dl)**
M1	124	64	236	0.5
M2	136	65	235	0.46
M3	135	66	239	0.55
M4	136	60	238	0.52
M5	136	60	238	0.52
M6	132	66	240	0.51
Mean±SEM	133.2±1.93	63.50±1.14	237.7±0.76	0.51±0.01

Rats in Groups IV, V, and VI were given *Grewiaasiatica*'smethanolic extract orally at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight. The enzyme levels were provided in Tables 4 and 5

Table 4:Group-IV,VandVIratsenzymeslevelsduetotheeffectof*Grewia asiatica* methanolicextractatdifferentdoses

MiceNo.	**Amountofthe*Grewia asiatica* methanolicextract**											
	Group-IV(100mg/kgb.w)				**Group-V(200mg/kgb.w)**				**Group-VI(400mg/kgb.w)**			
	AST (U/L)	**ALT (U/L)**	**ALP (U/L)**	**Totalbilirubin (mg/dl)**	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Total bilirubin (mg/dl)**	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Total bilirubin (mg/dl)**
M1	225	123	350	1.5	192	103	322	1.4	152	88	280	0.8
M2	222	123	358	1.6	195	102	320	1.3	148	85	284	0.8
M3	225	125	357	1.40	193	105	325	1.2	153	87	286	0.6
M4	224	122	352	1.5	194	106	328	1.4	154	80	289	1
M5	220	121	348	1.3	194	105	310	1.3	151	82	288	0.8
M6	220	120	347	1.2	196	110	315	1.2	155	80	277	0.8
Mean ±SEM	222.7 ±0.95	122.3 ±0.71	352.0 ±1.88	1.41 ±0.06	194.0 ±0.57	105.2 ±1.13	320 ±2.69	1.3 ±0.03	152.17 ±1.01	83.67 ±1.43	284.0 ±1.9	0.8 ±0.05

Table 5:Groups-I,II,III,IV,V andVI mice averageenzymeslevelsduetotheeffect of*Grewia asiatica* methanolic extractatdifferent doses

Nameof thedrug	**AST (U/L)**	**ALT (U/L)**	**ALP(U/L)**	**Total bilirubin (mg/dl)**
Normal(1% Na CMC)	89.83 ± 1.57	41.00 ± 3.75	185.7 ± 1.62	0.3 ± 0.01
Paracetamol(2g/kgb.w)	332.5 ± 2.29	158.5 ± 1.08	530.7 ± 3.30	2.25 ± 0.09
Silymarin(50mg/kgb.w)	133.2 ± 1.93	63.50 ± 1.14	237.7 ± 0.76	0.41 ± 0.01
Methanolicextractof *Grewia asiatica*(100mg/kgb.w)	222.7 ± 0.95	122.3 ± 0.71	352.0 ± 1.88	1.41 ± 0.06
Methanolicextractof *Grewia asiatica*(200mg/kgb.w)	194.0 ± 0.57	105.2 ± 1.13	320 ± 2.69	1.3 ± 0.036
Methanolicextractof *Grewia asiatica*(400mg/kgb.w)	152.17 ± 1.01	83.67 ± 1.43	284.0 ± 1.9	0.8 ± 0.05

Allthevalues wereexpressed asMean ±SEM,(n=6)

The percentage protection generated by the conventional medication and chosen botanical extracts were computed using the enhanced activities of blood biomarker enzymes found in paracetamol-induced liver damage. The findings are shown in Table 6.14. The efficacy of silymarin in preventing changes in AST, ALT, ALP, and Total Bilirubin levels was 92.12%, 90.85%, 94.93%, and 91.68%, respectively. The methanolic extract provided a protective effect

by reducing the serum levels of AST, AST, ALP, and Total Bilirubin. The percentage protection values for these parameters were 45.24%, 30.80%, 51.79%, 43.07%, 57.07%, 49.61%, 61.07%, 69.23%, 74.31%, 60.21%, 71.50%, and 74.35%, respectively

Table6:%Protectionof methanolic extractof *Grewia asiatica*atdifferentdosesonParacetamol induced hepatotoxicity

Nameof theDrug	**PercentageProtection**			
	AST (U/L)	**ALT (U/L)**	**ALP (U/L)**	**Total bilirubin (mg/dl)**
Silymarin50mg/kgb.w	92.12	90.85	94.93	91.68
Methanolicextractof*Grewia asiatica*100mg/kg b.w	45.24***	30.80***	51.79***	43.07***
Methanolicextractof *Grewia asiatica*200mg/kgb.w	57.07***	49.61***	61.07***	69.23**
MethanolicextractofGrewia *asiatica*400mg/kgb.w	74.31***	60.21***	71.50***	74.35***

AllthevalueswereanalyzedbyusingTwo-wayANOVAfollowedbybonferroniposthocktest.Allgroupswerecompared with silymarin, *p<0.05 considered as level of significance . ***p<0.001; **p<0.01; *p<0.05; ns= non significance

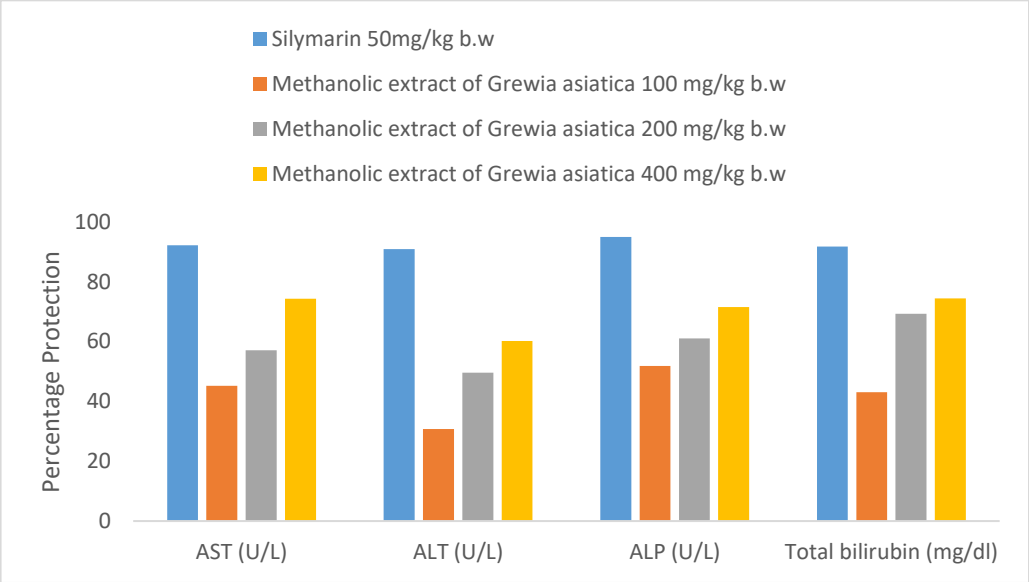

Figure 6.6: Graphical presentation of % Protection of methanolic extract of *Grewia asiatica* at different doses on Paracetamol induced hepatotoxicity

1.1.2 Histopathological studies of Liver

The histological examinations revealed that the control group had a normal hepatic cellular structure, characterized by healthy liver cells and well-functioning sinusoids. The group that was inebriated with paracetamol had clear signs of necrosis, hemorrhage, and inflammation. The drug-treated liver tissue showed a decrease in pathological alterations and had a comparable morphology to the control group when a larger dosage of methanolic extract was administered. These findings are shown in Figure 6.7. Our research findings demonstrate that the use of plant extracts may effectively repair liver damage induced by high dosages of paracetamol.

The findings of this research suggest that the extracts have hepatoprotective properties

against paracetamol-induced liver damage, perhaps owing to the presence of phytochemicals in the plant. Various traditional medical systems have documented the use of herbs and herbal products for the purpose of safeguarding liver function. Microscopic histopathological examinations reveal that crude extracts have a protective effect against paracetamol-induced liver injury, as shown by the reversal of centrilobular necrosis and deteriorated hepatic chords. Nevertheless, the hepatoprotective properties of crude extracts cannot be compared to those of silymarin, which effectively preserved hepatocytes in a state close to normal and healthy.

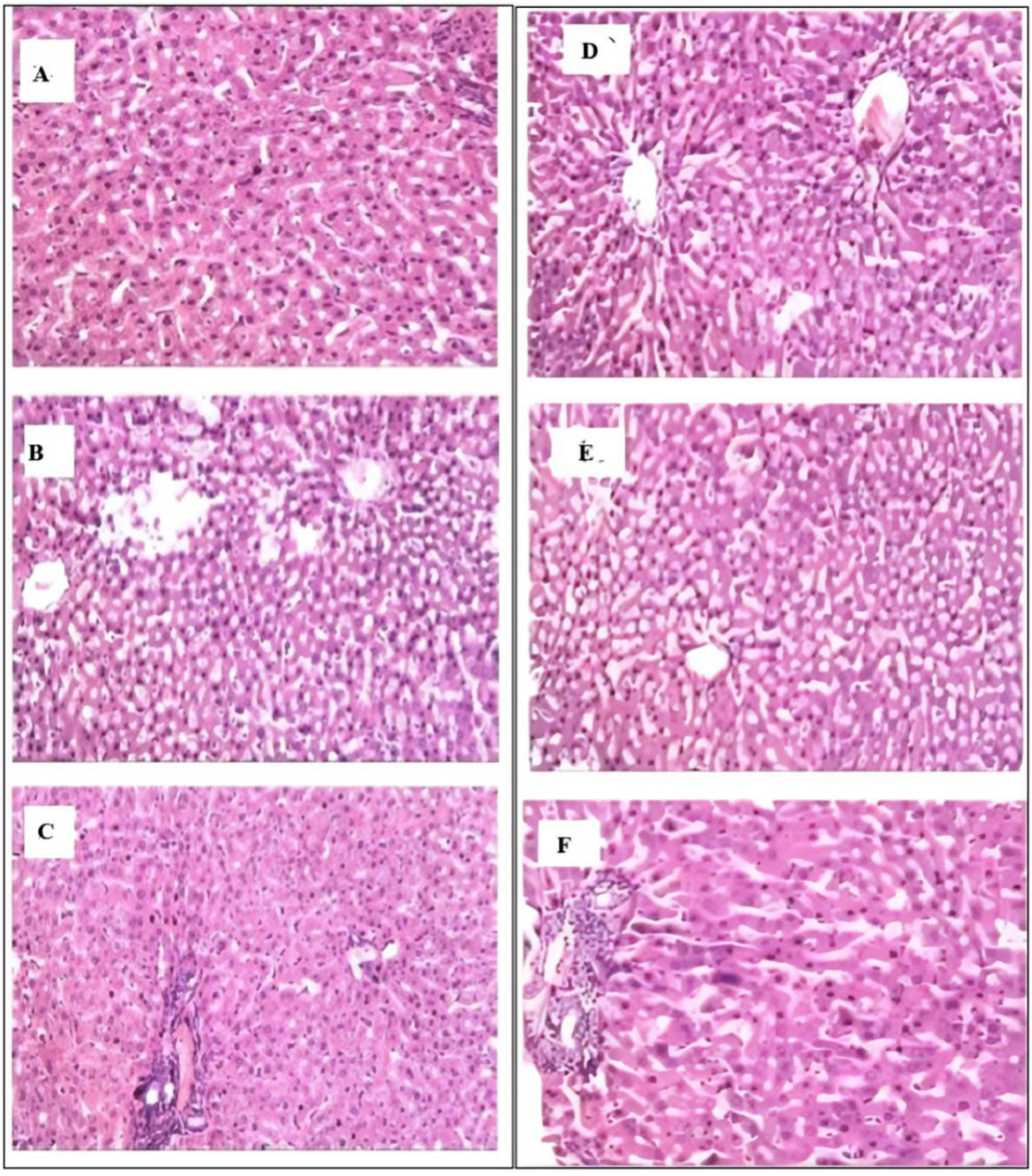

Figure 1: Histopathological images of paracetamol-induced hepatotoxicity observed at 400x magnification A) Normal control 1 % Na CMC (1ml; p.o) B) Toxic control PCM (2 g/kg b.w; p.o) C) PCM + Silymarin (2 g/kg b.w ; p.o + 50 mg/kg b.w; p.o) D) Methanolic extract of G. asiatica 100 mg/kg b.w E) Methanolic extract of G. asiatica 200 mg/kg b.w F) Methanolic extract of G. asiatica 400 mg/kg b.w.

Statistical Analysis

The data were analyzed with one way ANOVA followed byDunnetts multiple comparison test. $P< 0.05$ was consideredsignificantinallthecases.

Result

The pharmacological investigations of the fruit extract of *Grewia asiatica* mostly focused

on its hepatoprotective properties. The liver, an essential organ with many functions, is vulnerable to harm caused by environmental contaminants and medications. The research used a model of paracetamol-induced liver damage and assessed the impacts of aqueous and methanolic extracts of *Grewia asiatica*. Liver-related indicators, such as AST, ALT, ALP, and total bilirubin, were evaluated using biochemical analysis. The findings demonstrated significant alterations in these indicators in the group exposed to paracetamol overdose, but the administration of silymarin (a common medication) and methanolic extract exhibited protective properties.

The methanolic extract had a protective effect that increased in a dose-dependent manner, with the highest dosage showing the most pronounced level of protection. The histopathological tests confirmed these results, demonstrating the presence of necrosis, bleeding, and inflammation in the paracetamol group, which were mitigated in the groups treated with the medication. The research determined that extracts from *Grewia asiatica* had hepatoprotective properties against paracetamol-induced liver damage, perhaps because of the presence of phytoconstituents in the plant. The extracts shown effectiveness in reducing liver damage, whereas silymarin had a higher ability to protect the liver.

Conclusion

The present study demonstrates the hepatoprotective potential of the methanolic extract of *Grewia asiatica* against paracetamol-induced liver damage in rats. Paracetamol overdose led to significant elevation of serum liver enzymes, oxidative stress, and histopathological alterations in the liver. However, pretreatment with the extract significantly ameliorated these effects. The extract exhibited potent antioxidant activity, as evidenced by the increased levels of antioxidant enzymes. These findings suggest that the hepatoprotective effect of *Grewia asiatica* may be attributed to its antioxidant and anti-inflammatory properties.

Further studies are warranted to isolate and characterize the bioactive compounds responsible for the hepatoprotective effects of *Grewia asiatica*. Additionally, exploring the optimal dosage and duration of treatment is essential to optimize its therapeutic efficacy. These findings provide a promising foundation for the development of novel natural therapies for the prevention and treatment of liver diseases.

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