A Study of Extracts of *Rheum emodi* in Experimental Non-Alcoholic Fatty Liver Disease

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Abstract: Paracetamol (PCM) is a commonly used analgesic and antipyretic medication, however at large doses, it can cause hepatotoxicity/NAFLD. This study explores the therapeutic potential of *Rheum e.modi* extracts in treating experimental non-alcoholic fatty liver disease (NAFLD), a liver disorder linked to metabolic syndrome, based on its diverse phytochemical composition against paracetamol-induced damage. This study aims to evaluate the hepatoprotective effects of *Rheum e.modi* extracts on NAFLD in an experimental model, conduct histopathological examination of liver tissues, investigate the potential of *Rheum e.modi* extracts in modulating metabolic pathways related to lipid metabolism, insulin sensitivity, and glucose homeostasis, and establish a dose-response relationship to determine the optimal dosage for significant therapeutic effects while ensuring safety.

Keywords: NAFLD, Rheum e.modi, paracetamol, hepatotoxicity, hepatoprotective

INTRODUCTION

Liver illnesses have become one of the leading causes of morbidity and mortality around the world. One of the most prominent causal factors is drug-induced liver damage (DILI), which presents a significant clinical and regulatory problem [1]. Drug-induced hepatotoxicity manifests in a wide range of ways, from asymptomatic elevations in liver enzymes to fulminant hepatic failure. Overdoses of paracetamol (PCM), commonly known as acetaminophen, can result in severe hepatotoxicity and nephrotoxicity [2]. Cytochrome P450 enzymes activate PCM and convert it to the hazardous metabolite NAPQI (N-acetyl-p-benzoquinone mine), which induces oxidative stress and glutathione depletion [2, 3]. Despite significant break throughs in modern medicine, there are very few reliable medications that protect the liver against harm and aid in the regeneration of hepatic cells. Many active plant extracts are commonly used to treat various clinical conditions, including liver disease [4]. As a result, the hunt for effective and safe medications for liver problems remains a popular topic. The current study aimed to investigate the anti-hepatotoxic and antioxidant properties of the a fore mentioned medicinal plant extracts against paracetamol-induced toxicity in rats.

Plant profile:

Rheum e.modi, a significant plant used for treating various diseases, is over exploited in the pharmaceutical industry due to its bioactive compounds. These plants have been used since ancient times, providing numerous benefits such as antidiabetic, antifungal, and antioxidant properties.

Scientific classification

Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Caryophyllales, Family: Polygonaceae, Genus: *Rheum*, Species: *e.modi*

Botanical Description

Rheum emodi Wall. ex Meissn, is a tall, robust and leafy perennial herb, [8] 1.5 to 3.0 m in height. Radical leaves long petiole, very large, often 60 cm in diameter, orbicular or broadly ovate obtuse, base cordate, 5 to 7 nerved, papillose beneath, subscaberulous above; petiole 30 to 45 cm, very stout, scaberulous. Panicle is 0.6 to 0.9 m, papillosely puberulous, fastigiately branched and leafy with erect strict branches; flowers small 3 mm diameter, dark purple or pale red, in axillary panicles. Fruit ovoid-oblong, 13 mm long, purple, base cordate, apex notched, wings narrower than the disk. [8,11] Roots are very stout. Roots and rhizomes are the main parts used as drug and are collected in October to November. Root of Indian Rhubarb is darker, inferior in aroma, coarser, untrimmed and is not decorticated. Fresh rhizome is around 6 to 12 inches long, and the freshly fractured surface is dull orange to yellowish brown in colour [12].

Origin and distribution: Rheum emodi is a perennial medicinal herb native to Nepal and China, now grown in Pakistan, India, Nepal, and Myanmar. It is found in the Sub-alpine Himalayas and Assam. Its vernacular names include Indian Rhubarb, Himalayan Rhubarb, Rheum, Dolu, Rewand Chini, Raywat Chini, Arts, Artso, Atsu, Chotial, Chuchi, Chukri, Kandaul, Khabiun, Lachu, Pambash, Rewandchini, Ribas.

Experimental work:

Extraction:

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs [16]:

Roots of *Rheum emodi* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 60 gm of dried powdered roots of *Rheum emodi* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage} = \frac{\text{Weight of Extract yield}}{\text{Weight of powder drug Taken x 100}}$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [16].

In-vivo hepatoprotective activity of *Rheum emodi*

Animals

Six (n=6) male Wistar rats weighing 150±20 grams were housed together (group-housed) under a standard 12-hour light/dark cycle. Temperature and humidity were maintained within a controlled range (25°C ± 2°C, 55-65%). Rats had unrestricted access (ad libitum) to standard rodent chow and water. To minimize stress, the animals were acclimated to the laboratory environment for one week before the commencement of the experiment (OECD, 2001). The Institutional Animal Ethics Committee (IAEC) approved all procedures.

Drugs and Chemicals

Paracetamol and Silymarin (Sigma Chemicals, USA) were used in the present study. All other chemicals and other biochemicals used in the experiments were of analytical grade from different firms.

Experimental designs

Before the commencement of experimentation, the rats were acclimated to a controlled laboratory environment for seven days. Environmental parameters were maintained at 25°C ambient temperature, 55% relative humidity, and a 12-hour light/12-hour dark cycle. Throughout the acclimation period and the entirety of the study, the rats had ad libitum access to water. Additionally, they were provided with a standard basal diet.

Paracetamol-induced hepatotoxicity

Group –I: Normal control (0.5% CMC 1 ml/kg, p.o.)

Group –II: Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.)

Group -III: Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.) and silymarin 10 mg/kg.

Group –IV: Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.) + extract of *Rheum emodi* 100mg/kg

Group –V: Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.) +extract of *Rheum emodi* 200mg/kg

Following a four-week feeding period, all animals underwent a 12-hour fasting period with access to water only [22]. Euthanasia was achieved by isoflurane inhalation. Blood samples were collected via cardiac puncture into sterile centrifuge tubes. Subsequent centrifugation at 3000 rpm for 15 minutes facilitated serum separation.[27] The isolated serum was carefully transferred to lavender-topped tubes and stored at 20°C until further biochemical analysis.

Biochemical

Whole blood samples were centrifugated at 7000 rpm for 10 minutes using a microcentrifuge to isolate the serum component. Subsequently, the concentration of

liver enzymes aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured along with bilirubin levels.

Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). Following a one-way analysis of variance (ANOVA) to assess overall group differences, Dunnett's multiple comparison test was employed using GraphPad Prism software (version 8.0) to identify specific groups that differed significantly from a control group. A significance level of $P < 0.05$ was used to determine statistically significant differences.

RESULTS AND DISCUSSION

Determination of percentage yield

The crude extract so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. The yield of extract obtained from roots using ethanol as solvent are depicted in the table 7.1.

Table 7.1: % Yield of extract of *Rheum emodi*

S. No.	Extract	% Yield
1	Ethanolic	9.2%

7.2 Phytochemical screening of extracts

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 7.2.

Table 7.2: Phytochemical screening of extracts *of Rheum emodi*

S. No.	Constituents	Ethanolic extract
1.	Alkaloids Mayer's Test Wagner's Test Dragendroff's test Hager's test	 -ve -ve -ve -ve
2.	Glycosides Modified Borntrager's Test Legal's test	 -ve -ve
3.	Flavonoids Lead acetate Alkaline test	 +ve +ve
4.	Phenol Ferric Chloride Test	 +ve
5.	Proteins and Amino acids Xanthoproteic test Ninhydrin Test	 +ve +ve
6.	Carbohydrates Molisch's Test Benedict's Test Fehling's test	 -ve -ve -ve
7.	Saponins Froth Test Foam test	 +ve +ve
8.	Diterpins Copper acetate test	 -ve

Results of estimation of total phenolic contents

Total Phenolic content estimation (TPC)

The content of total phenolic compounds content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.033x - 0.014$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 7.3: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean absorbance
0	0	0
1	5	0.136
2	10	0.318
3	15	0.487
4	20	0.665
5	25	0.823

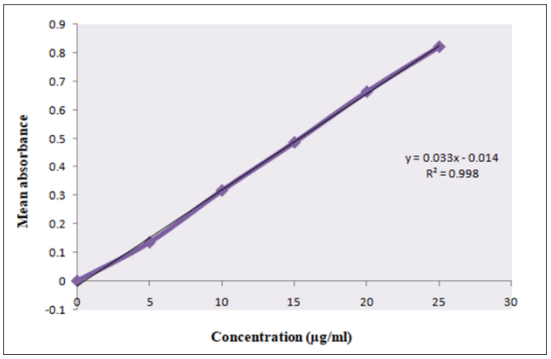

Figure 7.1: Graph of calibration curve of Gallic acid

Total flavonoid content estimation (TFC)

The content of total flavonoid compounds content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the

calibration curve: y = 0.028x - 0.005, R^2= 0.998, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 7.4: Preparation of calibration curve of Quercetin

S. No.	Concentration(µg/ml)	Mean absorbance
0	0	0
1	5	0.146
2	10	0.269
3	15	0.414
4	20	0.561
5	25	0.723

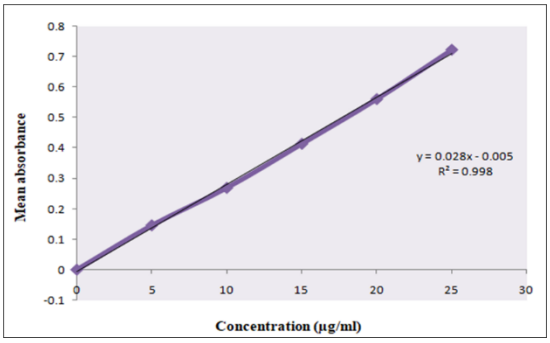

Figure 7.2: Graph of calibration curve of Quercetin

Table 7.5: Total phenolic and total flavonoid content of *Rheum emodi* extract

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Ethanolic extract	0.62	0.89

Results *of in-vivo* hepatoprotective activity of ethanolic extract of *Rheum emodi*

Acute toxicity

In an acute toxicity evaluation, the extract of *Rheum emodi* exhibited no mortality at doses up to 2000 mg/kg body weight. Therefore, 100 mg/kg and 200 mg/kg body weight were chosen to further investigate oral activity (p.o.)

Effect of Extract of Rheum emodi (100 and 200 mg/kg) on paracetamol-induced hepatotoxicity and liver enzymes

The administration of PCM caused a notable elevation in serum levels of AST, ALT, ALP, and bilirubin, indicating potential hepatotoxicity. The protective effects of *Rheum emodi* extract on this PCM-induced liver damage at 100 and 200 mg/kg body weight dosages are depicted in Tables 7.6 and 7.7. The results demonstrate that pretreatment with *Rheum emodi* extract prior to PCM administration significantly reduced the levels of these liver function markers, including AST, ALT, ALP, and bilirubin (Figure 7.3-7.6). These findings suggest a potential hepatoprotective role for *Rheum emodi* extract against PCM-mediated liver injury.

Table 7.6: Effect of *extract of Rheum emodi* on biochemical evaluation i.e. ALT and AST in paracetamol-induced hepatotoxicity in rats

Group	Drug	Dose	ALT (U/L)	AST (U/L)
I	Normal	0.5% CMC 1 ml/kg, p.o.	56.0 ± 8 .74	40.0 ± 8.21
II	Paracetamol	500 mg/kg	126.5±9.50	175.00±11.00
III	Silymarin	10 mg/kg p.o.	61.51 ± 8.50***	55.0 ± 7.00***
IV	Extract of *Rheum emodi*	100 mg/kg p.o.	89.0 ± 9.50*	83.40 ± 7.50*
V	Extract of *Rheum emodi*	200 mg/kg p.o.	71.0 ± 8.50**	68.0 ± 8.50**

Values are expressed as the mean ± SEM of six observations. * P<0.05, ** P< 0.001, *** P< 0.001 vs. control treatment (One-way ANOVA followed by Dunnett's test)

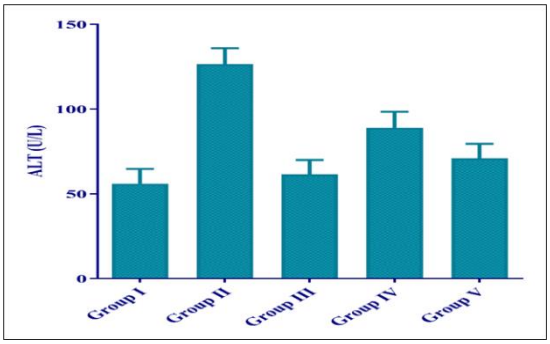

Figure 7.3: Effect of *extract of Rheum emodi* on biochemical evaluation i.e. ALT in paracetamol-induced hepatotoxicity in rats

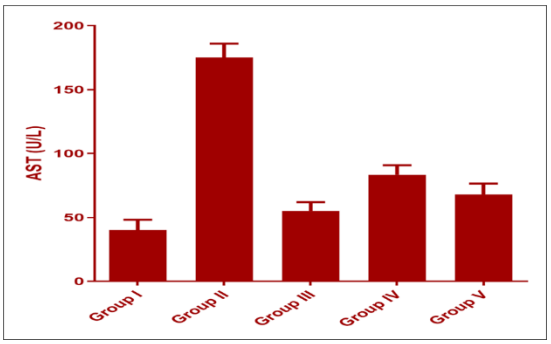

Figure 7.4: Effect of extract of *Rheum emodi* on biochemical evaluation i.e. AST in paracetamol-induced hepatotoxicity in rats

Table 7.7: Effect of extract of *Rheum emodi* on biochemical evaluation i.e. ALP and bilirubin in paracetamol-induced hepatotoxicity in rats

Group	Drug	Dose	ALP (IU/L)	Bilirubin (g/dL)
I	Normal	0.5% CMC 1 ml/kg, p.o.	110.0 ± 10.50	90.0 ± 6.30
II	Paracetamol	500 mg/kg	250.0 ± 11.00	215.10 ± 8.00
III	Silymarin	10 mg/kg p.o.	127.05± 8.10***	125.60 ± 7.90***
IV	Extract of *Rheum emodi*	100 mg/kg p.o.	174.50 ± 7.50*	151.60 ± 7.50*
V	Extract of *Rheum emodi*	200 mg/kg p.o.	143.50 ± 6.50**	138.50 ± 6.90**

Values are expressed as the mean ± SEM of six observations. * P<0.05, ** P< 0.001, *** P< 0.001 vs. control treatment (One-way ANOVA followed by Dunnett's test)

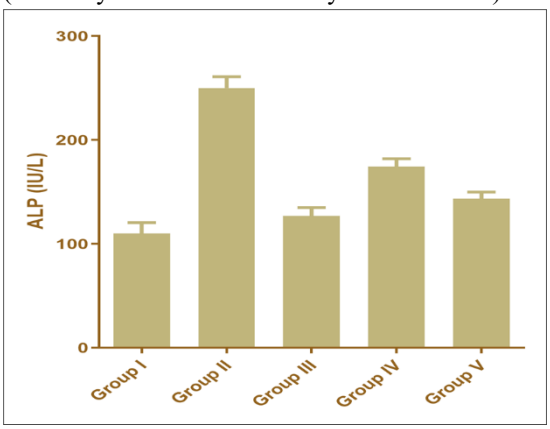

Figure 7.5: Effect of *extract of Rheum emodi* on biochemical evaluation i.e. ALP in paracetamol-induced hepatotoxicity in rats

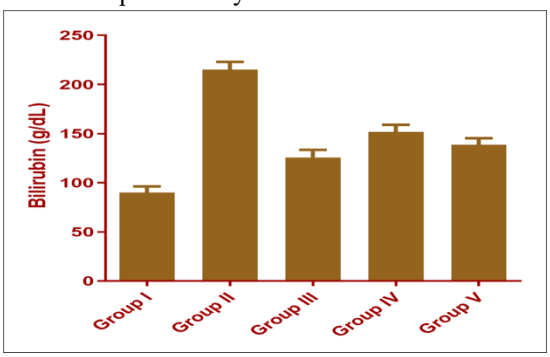

Figure 7.6: Effect of *extract of Rheum emodi* on biochemical evaluation i.e. bilirubin in paracetamol-induced hepatotoxicity in rats

This study investigates the potential hepatoprotective properties of *Rheum emodi* extracts against PCM-induced liver damage in Wistar rats. The aim is to establish a scientific foundation for the traditional medicinal use of *Rheum emodi* in liver protection. PCM represents a well-established and widely employed approach for evaluating the hepatoprotective potential of novel drug candidate.

PCM is a well-established antipyretic and analgesic medication, but its use comes with the potential for hepatotoxicity at high doses. This toxicity manifests as a cascade of clinical symptoms including chest pain, vomiting, diarrhea, and even shock. In severe cases, excessive acetaminophen intake can lead to multiorgan failure encompassing the liver, heart, and kidneys (Salman *et al.,* 2020). The drug is primarily metabolized by the cytochrome P450 system and detoxified through glucuronidation (accounting for roughly 90%) and sulfation pathways. However, a minor pathway (5-10%) generates N-acetyl-p-benzoquinone imine (NAPQI), a potentially toxic intermediate.

The blood biomarkers serve as indicators of potential liver injury. These biomarkers primarily include aminotransferases: ALT and AST, ALP, and bilirubin. Elevated levels of these markers are generally associated with hepatocellular damage. ALT exhibits a primarily hepatic distribution, contrasting with AST which is additionally abundant in other tissues such as cardiac muscle and kidneys. This localized expression pattern renders ALT a more specific marker for liver injury than AST. However, a parallel elevation in both ALT and AST levels can still indicate hepatocellular damage. The AST/ALT ratio is a diagnostic tool for clinicians to differentiate between liver damage originating outside the liver (extrahepatic) and damage occurring within the liver (hepatic). A ratio of 2:1 for AST/ALT indicates hepatic injury as documented in previous research. Therefore, the observed AST/ALT ratio in the PCM-treated group can potentially substantiate the hypothesis that this drug induces hepatic damage (Kumar). In the present study, PCM caused significant elevation in AST, ALT, ALP, and bilirubin levels. Pretreatment with *Rheum emodi extract* exhibited a significant reversal of the changes induced by PCM. This implies that the observed reduction in enzyme levels reflects a membrane-stabilizing property of the extract. Furthermore, decreased AST and ALT activity towards their normal physiological levels suggests a potential regenerative process following PCM exposure.

In line with previous observations by, the current study demonstrates a statistically significant increase in AST, ALT, and bilirubin levels. These findings corroborate the established notion that paracetamol administration can induce hepatic injury, as evidenced by the elevation of liver enzymes. Furthermore, the work of Al-Asmari et al. lends further support to this concept, highlighting a notable rise in plasma AST, ALT, and ALP activity in cases of acute paracetamol toxicity. Collectively, these studies suggest a strong association between paracetamol exposure and subsequent hepatic dysfunction.

SUMMARY AND CONCLUSION

This study investigated the effects of pre-treatment and co-administration *of Rheum emodi extract* or silymarin on paracetamol-induced hepatotoxicity. This implies a potential protective role of *Rheum emodi* and silymarin against paracetamol-mediated liver injury.

In this study, *Rheum emodi* extract exhibited a dose-dependent hepatoprotective effect against PCM-induced hepatotoxicity in rats. This protective action is likely due to the presence of various phytoconstituents within the extract, including alkaloids, flavonoids, and glycosides. However, further research is necessary to elucidate the specific mechanisms of action and identify the exact phytoconstituent(s) responsible for the observed hepatoprotective effect. This knowledge will be crucial for guiding future studies aimed at optimizing the use of *Rheum emodi* for liver protection.

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