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Research Article

An *In-vivo* Study for the Determination of the Hepatoprotective Properties of *Thalictrum Foliolosum* Extract

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Abstract

Traditionally used "Restorative Herb", *Thalictrum Foliolosum* contains rich bioactive compounds with potent free radical scavenging properties. This study explored its potential to combat liver damage. Root extracts (methanolic, ethanolic, hydro alcoholic, aqueous) were assessed for their content of flavonoids and phenolics, known for their antioxidant effects. The study induced liver damage in rats using paracetamol and then evaluated the protective effects of the extracts compared to a standard drug. All extracts significantly reduced liver enzyme activity and bilirubin levels, while increasing total protein levels, indicating improved liver function. Methanol: chloroform (1:1) extract showed the strongest protective effect, confirmed by near-normal liver architecture observed during histological examination. These findings suggest that *T. Foliolosum* extracts, particularly the methanol: chloroform (1:1) extract, possess significant hepatoprotective activity. This effect is likely due to the presence of flavonoids and phenolics, which can combat free radical damage and promote liver regeneration. Pretreatment with 100 mg/kg and 200 mg/kg body weight of the methanol: chloroform root extract of *T. Foliolosum* for 7 days offered significant protection against paracetamol-induced hepatic damage and the results were compared with standard hepatoprotective drug silymarin. 100 mg/kg body weight of the methanol: chloroform root extract of *T. Foliolosum* showed results as comparable with 100 mg/kg body weight of silymarin and 200 mg/kg body weight of the methanol: chloroform root extract of *T. Foliolosum* showed better protection than silymarin. Both the doses of the methanol: chloroform root extract of *T. Foliolosum* prevented histological changes caused by paracetamol.

Keywords: Hepatoprotective; Cytotoxicity; Hepatotoxicity; Acetamorphin; AST; ALT; ALP

Abbreviations

WHO: World Health Organization; *T. Foliolosum: Thalictrum Foliolosum*; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; TLC: Thin Layer Chromatography; HPLC: High Performance Liquid Chromatography; LDH: Lactate Dehydrogenase

Introduction

The liver is the biggest and one of the most important organs in the human body, making up around 2% to 3% of the total body weight [1]. The liver is an important organ in several vital physiological functions, including the metabolism and digestion of nutrients including proteins, fats, and carbohydrates. Additionally, it supports immune system function, blood volume management, electrolyte balance, detoxification or biotransformation of xenobiotics and toxic agents, and energy balance. It also acts as a storage space for a variety of compounds [1,2]. Bile juice, produced by the liver, plays a vital role in the digestive process [3]. The liver is the primary site for the biotransformation of both endogenous and exogenous chemical compounds. It plays a central role in numerous biochemical processes, including energy production, overall body growth, reproductive functions, protein biosynthesis, regulation of blood glucose levels during fasting by carefully balancing gluconeogenesis and glycogenolysis, ensuring a steady supply of energy to the brain and muscles during periods of starvation, and actively participating in the synthesis and provision of essential blood clotting factors [1,4]. The liver possesses remarkable regenerative capabilities, allowing it to recover and heal from injuries. This inherent self-healing ability is instrumental in facilitating the liver's diverse functions, especially considering that the liver is frequently exposed to a wide array of both direct and indirect toxic chemicals [1,5]. The liver is essential for the bloodstream's detoxification and the metabolism of a wide range of substances, including alcohol, drugs, and other chemicals [6]. Owing to its essential role as a primary organ for metabolism and detoxification, the liver is vulnerable to harm from a wide variety of medicinal and environmental substances. Unfortunately, the absence of viable treatment alternatives leads to unfavorable prognoses and elevated mortality rates for liver-related conditions [1,2].

Around the world, liver disorders are a leading source of illness and mortality. According to the World Health Organization (WHO), more than 500 million people suffer with chronic hepatitis, which results in more than one million deaths per year [3]. Accurate diagnosis and the implementation of effective treatment strategies are essential to stem the rising tide of mortality and morbidity associated with liver disorders [2]. Liver disorders encompass a broad array of conditions, ranging from liver cirrhosis, hepatocellular carcinoma, chronic or viral hepatitis to alcoholic or non-alcoholic

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fatty liver disease, and liver damage induced by drugs or chemicals. Drug-induced liver injury is particularly common due to the liver's central role in drug metabolism. Some drugs, like paracetamol, can lead to the synthesis of more toxic compounds during metabolism in the liver than their parent compounds. Substance abuse can trigger inflammation and oxidative stress, contributing to hepatotoxicity and the development of hepatic neoplasms [7,8]. Various hepatotoxic chemicals can trigger lipid peroxidation, a process that damages liver cells, ultimately leading to the development of hepatitis and cirrhosis [3]. Many drugs employed for the treatment and control of systemic disorders often come with unwanted side effects. Therefore, there is a pressing need to develop safe, novel, natural hepatoprotective agents that have minimal or no side effects [8].

In recent years, traditional and complementary or alternative medicinal systems have garnered increased recognition for their role in preventing and treating intricate liver disorders. WHO estimates that 80% of people on Earth rely on traditional medicine, which often includes the use of plant-based products, to treat and prevent a variety of illnesses [9,10]. Medicinal plants are esteemed as a vast reservoir of diverse pharmaceutical agents that have been employed for several decades to treat a wide array of health conditions [11]. Herbal formulations derived from medicinal plants play a pivotal role in the fundamental healthcare sector worldwide. In developing countries, several factors contribute to their prominence, including cultural acceptability, biological compatibility with the human body, limited access to modern medical systems, and fewer side effects. These formulations have garnered significant attention in traditional treatment practices for a wide range of diseases [1,3,12]. WHO acknowledges the utilization of plant extracts and compounds isolated from plants as valuable sources of active constituents in the development of phytomedicines and drugs for treating and preventing diseases. This practice is rooted in ancestral and traditional knowledge, which WHO recognizes as a valuable resource for advancing healthcare [10]. Plants have served as a cornerstone of traditional medicine for thousands of years worldwide and continue to be integral to the modern healthcare system for the betterment of humanity. Phytoconstituents derived from plants can be employed individually or in combination to address and prevent diseases while fostering well-being. It's noteworthy that a single plant can harbor a diverse array of phytoconstituents, each possessing distinct pharmaceutical properties. These phytoconstituents are secondary metabolites produced by the plants [9]. Secondary metabolites comprise a diverse range of bioactive compounds, including alkaloids, terpenoids, glycosides, phenolic compounds, and flavonoids showcasing a wide array of activities, such as antifungal, antibacterial, and antioxidant properties, among others [6]. Several metabolic disorders, including hepatotoxicity, atherosclerosis, diabetes, and obesity, among others, face a scarcity of satisfactory treatment options within the contemporary medical system. Research endeavors focused on plant-based products, encompassing traditional herbal medicines, primarily aim to scientifically validate the potency and effectiveness of drugs utilized in traditional medicinal systems. This research aims to establish a scientific foundation for the application of these medications in addressing the challenges associated with such disorders. Plant-derived secondary metabolites have proven effective in addressing and preventing various disorders, including those affecting liver. Among these phytochemicals, phenolic and flavonoids compounds are recognized for their hepatoprotective potential, attributed to their ability to scavenge free radicals and their anti-inflammatory characteristics [8,12].

The plant *Thalictrum*, commonly referred to as "meadow-rue," belongs to the *Ranunculaceae* family and comprises approximately 150 to 200 species distributed worldwide. Notably, around 43 species within the *Thalictrum* genus have been traditionally employed for their therapeutic properties. Ethnobotanically, *Thalictrum* is recognized for its use in the prevention and treatment of various health conditions, including diuretic, dyspepsia, wounds, convalescence, snake bites, swellings, uterine tumors, joint pain, paralysis, nervous disorders, jaundice, rheumatism, stomachaches, toothaches, diarrhea, piles, fever, peptic ulcers, ophthalmic issues, headaches, and as a tonic, among others [13].

Thalictrum Foliolosum DC, a perennial herbaceous plant, is commonly found across the Himalayan region at altitudes ranging from 1000 to 3400 meters above sea level. Its geographical distribution spans North America, South America, India, Bhutan, Nepal, and East Tibet. In the English language, it is recognized as 'Meadow rue,' and in Hindi, Punjabi, Bengali, and Nepali, it is referred to as "Mamira," "Chireta," "Gurbiani," and "Dampate," respectively. The plant is characterized by its bitter and pungent taste and has mild purgative properties [14]. *Thalictrum* is rich in a variety of phytoconstituents, which primarily contribute to its therapeutic properties, especially in addressing jaundice and hepatic disorders. Traditionally, the plant's roots have been employed as a tonic for the treatment of jaundice [15-17]. In previously published in-silico studies predicted the presence of free radical scavenging and hepatoprotective potential of *T. Foliolosum* [14]. In the present study, we endeavor to explore *in-vivo* therapeutic potential of *T. Foliolosum* as a hepatoprotective agent.

Material and Methods

Chemicals

Chemicals viz, NaCl, HCl, $FeCl_2$, ethanol, methanol, H_2SO4, ferric chloride, KCl, chloroform, NaOH, and other chemicals were all acquired from Merck (Mumbai, India) and were all of the highest purity (≥ 99.0%). We

Purchased TLC plates, caffeine, and quercetin dehydrates from Sigma-Aldrich (Germany). We bought Silymarin and paracetamol from Sigma Aldrich. Every chemical was of the analytical variety.

Collection and identification of plant material

We collected the plant's roots from Kullu, Himachal Pradesh, India (31.8862°N, 77.1455°E) in order to assess its hepatoprotective capabilities, and then transported them to the laboratory and subsequently processed as published in the previous study [13].

Organoleptic characters and physiochemical parameters of *Thalictrum Foliolosum*

Harvested plant samples were accessed for the organoleptic characteristics and physiochemical parameters of *Thalictrum foliolosum*. Organoleptic characters like color, odor, taste, physical appearance, and physiochemical parameters like pH, moisture content, solubility in water or organic solvents, and ash content were studied.

Extraction method for isolation of phytochemicals present in *Thalictrum Foliolosum*

The extraction of phytochemicals from *T. Foliolosum* roots done using maceration or solvent extraction technique as published in the previous study [13] and the obtained dried root extract was weighed,

and the percentage recovery concerning the initial dry weight of the root of *T. Foliolosum* was calculated using the following equation.

$$\text{Percentage yield} = \frac{\text{Weight of obtained dried root extract}}{\text{Initial weight of the dried roots}} \times 100$$

Detection of the Phyto-constituents

TLC: Thin Layer chromatography is a method used to separate compounds present in the plant extracts based on Rf values. The ascending TLC analytical technique was used for the preliminary screening of phytochemicals present in the crude plant extract. All comparative TLC studies were performed using the various mobile phases and spraying reagents listed in Table 1 on Merck 0.25 mm silica gel plates, which were created to identify phytochemicals contained in the plant extract [18].

$$Rf = \frac{\text{Distance traveled by the solute from the origin (cm)}}{\text{Distance traveled by the solvent from the origin (cm)}} \times 100$$

HPLC: To find phytochemicals, High Performance Liquid Chromatography (HPLC) was used. A 0.45 mm syringe filter was used to filter the samples. A Perkin Elmer series 200 Ic pump with a reverse phase column was used for the analysis. Photo-diode array detector (Applied Bi system) with Lichrosorb C18-5 µl (4 × 125) column (Merck). Ten micro liters of the sample were injected into the solvent system/mobile phase, which consisted of methanol and water (70:30) at a flow rate of 0.5 ml per minute for duration of 10 to 60 minutes. At 25°C, the absorbance analysis was performed at 205 nm, 256 nm, 275 nm, and 200 nm to 400 nm for the plant extract test samples, caffeine (an alkaloid standard), gallic acid (a phenolic standard), and quercetin dehydrate (a flavonoid standard). The linearity, limit of detection, limit of quantitation, accuracy, robustness, precision, and specificity of this HPLC technique were previously used to validate it. The results of the HPLC of the crude methanol-chloroform (1:1) root extract samples of *T. Foliolosum* were observed and compared with standards.

In-vivo hepatoprotective activity

Our Previously published in-silico studies had predicted the existence of free radical scavenging and hepatoprotective capabilities in *T. Foliolosum* [14]. To validate in-silico prediction results a comprehensive study using animal model conducted. The *in-vivo* hepatoprotective activity of the active phytoconstituents from the root extract of *T. Foliolosum* typically involves the following steps.

Experimental animal model: Female Swiss Albino mice weighing between 25 mg and 30 mg were employed to obtain the hepatoprotective potential. The temperature and humidity levels of the experimental animals were kept between 25°C and 28°C, with a lighting schedule of 12 hours of light and 12 hours of darkness. Before the trial, they were given a 12-hour fast and given water and a typical mouse food. The research was carried out in accordance with established protocols for the care of experimental animals, having obtained the necessary approval (Approval No./Project No. 3105 and Study No. 0113/2022).

Experimental design: After a random assignment procedure, thirty Swiss Albino mice (25 mg to 30 mg) were split up into five groups, with six mice in each group. The entire course of experimental treatment lasted for seven days. The standard medicine silymarin and extracts were administered orally using a suspension of 2% w/v gum acacia in distilled water. The OECD 425 (2008) criteria were followed for dose selection, which was carried out after a limit test on Swiss Albino mice. The following experimental procedure and the methodology provided by Bhatt et al. [7] were followed throughout the whole experiment to evaluate the hepatoprotective activity of *T. Foliolosum* (Figure 1).

HEPATOPROTECTIVE ACTIVITY ASSAY (DATE:13/10/2022)

Group Of Animals	Strain Mice/Per Group Of Animals	Body Weight	Dosing Route	Dose Volume According To Body Weight	Dosing Schedule Per Day
Group-I (Vehicle control)	Swiss Albino/F/6	25-30mg	Orally	250µL	1,2,3,4, 5,6
Group-II (Paracetamol 300 mg/kg)	Swiss Albino/F/6	25-30mg	IP	250µL	Only last 7th day
Group-III (Silymarin 100 mg/kg)	Swiss Albino/F/6	25-30mg	Orally	250µL	1,2,3,4, 5,6
Group-IV (Test plant extract 200mg/kg)	Swiss Albino/F/6	25-30mg	Orally	250µL	1,2,3,4, 5,6
Group-V (Test plant extract - 100mg/kg)	Swiss Albino/F/6	25-30mg	Orally	250µL	1,2,3,4, 5,6

Animal issue date and IAEC No./Project No. GAP-3105(Hepato-protective activity in acetaminophen induced hepato-toxicity)
In-vivo Study No.- 0113/2022
GAP-3121 Dr Govind Yadav (Principal Scientist)
Swiss Albino Mice-48 Female

Figure 1: Study performa for evaluation of hepato-protective properties of pharmaceutically.

- Group-I (Vehicle control): Vehicle (2% gum acacia) treated mice were kept on a normal diet (standard pellet diet and water) and served as the control for 7 days. This group was given neither paracetamol nor treatment.
- Group-II (Toxic control): Mice received paracetamol 300 mg/kg body weight, given only on the 7th day through IP (Intraperitoneal) route.
- Group-III (Standard control): Mice of this group received the standard drug silymarin (100 mg/kg body weight/day, orally) and paracetamol as group II, on the 7th day.
- Group-IV (Test dose): Mice received the root extract of *T. foliolosum* (200 mg/kg body weight /day, orally) and paracetamol as group II, on the 7th day.
- Group-V (Test dose): Mice received the root extract of *T. foliolosum* (100 mg/kg body weight/day, orally) and paracetamol as group II, on the 7th day.

Determination of the hematological parameters of acute toxicity: The animals were made to fast for a whole day after the treatment. The procedure outlined by Marslin [19] was used to take blood using retro orbital puncture, and several hematological parameters of acute toxicity, such as WBC, RBC, HGB, MCV, MCH, MCHC, etc were measured.

Table 1: TLC for the qualitative determination of phytochemicals present in the plant extracts (Banu and Nagarajan [18]).

Phytochemical	Mobile phase	Spraying reagent	Spot color	Rf value of standards
Alkaloid	Chloroform: Methanol (12:2)	Dragendorff reagent	Orange	0.8
Flavonoid	Ethyl acetate: Butanol: Formic acid (2.5:1.5:0.5)	$AlCl_2$ reagent	Yellow	0.87
Phenolics	Chloroform: Methanol (27:0.3)	FC reagent	Blue	0.87
Tannins	Methanol: Water (6:4)	$FeCl_2$ reagent	Brownish-grey spot	0.83

Determination of biochemical parameters: The animals were starved for twenty-four hours after the treatment period. Blood was drawn via retro-orbital puncture and left to clot for an hour at room temperature. After that, the serum was separated by centrifugation at 3500 rpm for 20 minutes at room temperature. Biochemical tests, including assays for Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Glucose, Albumin, Protein, Lactate Dehydrogenase (LDH), Urea, and Bilirubin, were conducted using the protocol described by Ullah et al. [20].

Histopathological studies: The animals were put to sleep, and their livers were removed, washed with saline, and stored in a 10% neutral formalin solution for histopathological examinations. Hematoxylin-eosin staining and sectioning of the formalin-fixed liver samples were performed in accordance with Ullah et al. [20] protocol. The portions were inspected under a microscope to look for changes in the histopathological architecture.

Statistical analysis: Statistical analyses were performed using Graph Pad instate software to assess significant differences between the control and treatment groups, various tests were employed, including Turkey's multiple range tests, Dunnett's test, and Analysis Of Variance (ANOVA). The results for each group are presented as mean ± Standard Error of the Mean (S.E.M.).

Results and Discussion

Organoleptic characters and physiochemical parameters of *Thalictrum Foliolosum*

Harvested plant samples were accessed for the organoleptic characters and physiochemical parameters of *T. Foliolosum* (Figure 2). Organoleptic characters like color, odor, taste and physical appearance. Physiochemical parameters like pH, moisture content, solubility in water and organic solvent and ash content summarized in Table 2.

Table 2: Organoleptic characters and physiochemical parameters of the roots of *T. Foliolosum*.

S. No.	Parameters	Remarks
Organoleptic characteristics	Colour	Yellow
	Odour	Characteristic
	Taste	Bitter
	Physical appearance	Easy to form powder
Physiochemical parameter	pH	Slightly acidic
	Moisture content	45% to 50 %
	Solubility in water	Soluble
	Solubility in organic solvent	Soluble
	Ash content	0.2948

Percentage yield of extracts

The percentage yield of methanol-chloroform (1:1) root extract of *T. Foliolosum* was 4.14 % and 82.8 mg/2 g tissue of plant roots. It is important to remember that the kind of solvent employed during the extraction process could potentially have an impact on the bioactive phytochemicals found in plants. For instance, Pandey et al. [21] reported the highest berberine (an alkaloid) content when using 95% ethanol, whereas Kumar et al. [22] reported the highest phenolic and flavonoid content in the methanolic extract of *T. Foliolosum*.

TLC (Thin Layer Chromatography)

Studies using thin-layer chromatography revealed that a greater concentration of secondary metabolites with potential medical value, such as flavonoids, phenols, and alkaloids, were recovered from *T. Foliolosum* roots using a methanol-chloroform (1:1) solvent. For tannins all eleven solvents showed negative results. The results were summarized in the Table 3 with corresponding Rf value of various secondary metabolites. The corresponding TLC is presented in Figure 3. According to Banu and Nagarajan [18] solvents chloroform: methanol (27:0.3), ethyl acetate: butanol: formic acid (2.5:1.5:0.5) and chloroform: methanol (12:2) are suitable mobile phase for determination of phenols, flavonoids and alkaloids respectively.

HPLC

The standards quercetin dehydrate (a flavonoid standard), caffeine (an alkaloid standard), and gallic acid (phenolic standard) all displayed a single peak with retention times of 4.427, 5.952, and 5.059, respectively, according to High Performance Liquid Chromatography (HPLC) analysis. These results are shown in (Table 4 and 5 and Figure 4 and 5). Plant extracts were shown to contain similar phenolic, flavonoid, and alkaloid components when the RT of the plant samples was compared to the standard. The phenolics that are found in living things are known to provide them resistance to exist. They are also widely distributed across the plant kingdom and are thought to have health-promoting qualities. Plants are a rich source of natural antioxidants since phenolics are known to have antioxidant qualities. Antioxidant potential was also discovered to exist for flavonoids. Potential antibacterial properties of alkaloids were recognized [23]. HPLC analysis revealed the presence of different phytochemicals belonging to these groups in the root extracts of the *T. Foliolosum*, so indication of the pharmacological potential of the plant under study.

Hepatoprotective activity

The liver has a role in metabolizing and detoxifying substances so possesses a high risk of toxicity. In people and experimental

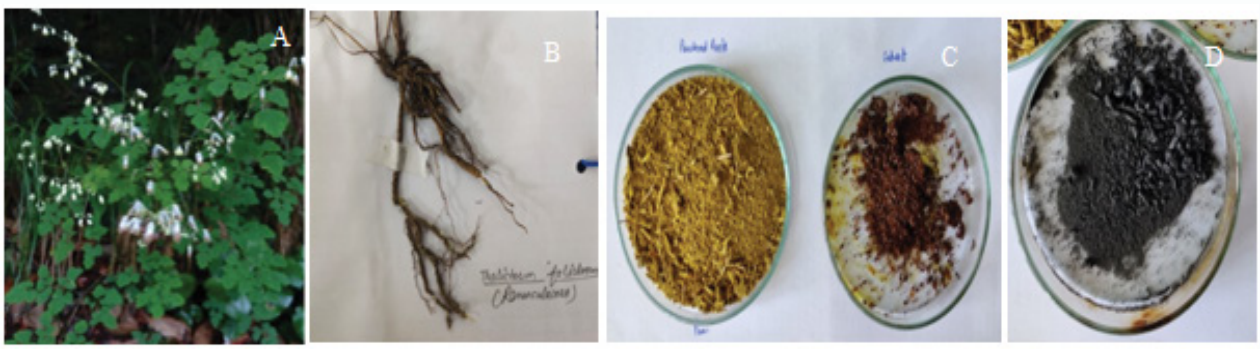

Figure 2: (A). Plant under natural environment, (B). Dried roots of *T. Foliolosum*, (C). Grounded powder of roots and root extract of *Thalictrum foliolosum*, (D). Ash content of *T. Foliolosum*.

Table 3: TLC for the qualitative determination of phytochemicals present in root extract of *T. Foliolosum*.

Solvents	Alkaloid (R*f*=0.80)	Flavonoid (R*f*=0.87)	Phenolics (R*f*=0.87)	Tannins (R*f*=0.83)
M:C (1:1)	Orange spot -0.84 Black spot -0.82	Yellow spot -0.60	Brownish spot-0.14 Yellow spot - 0.77 Yellow spot - 0.84	No spot

*M-Methanol, C-Chloroform

Table 4: HPLC peaks and retention times of the standard and plant extract samples.

Sample	Peak #	Retention time (Min.)
Gallic acid (Phenolic standard)	1	4.427
Caffeine (Alkaloid standard	1	5.952
Quercetin dehydrate (Flavonoid standard)	1	5.059
Plant extract Crude (M:C root extract)	1	2.631
	2	2.888
	3	3.121
	4	3.492
	5	4.003
	6	4.282
	7	5.176
	8	5.473
	9	5.829
	10	6.166
	11	6.583
	12	6.87
	13	7.711
	14	8.316

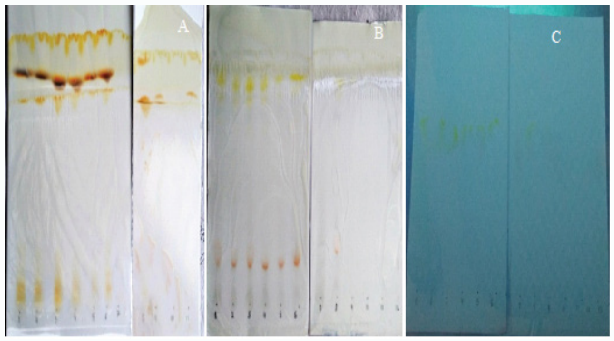

Figure 3: (A). TLC using solvent Chloroform: methanol (12:2) for the presence of alkaloids, (B). TLC using solvent Chloroform: methanol (23:3) for phenols, (C). TLC using solvent Ethyl acetate: Butanol: Formic acid (2.5:1.5:0.5) for flavonoids (Banu and Nagarajan [18]).

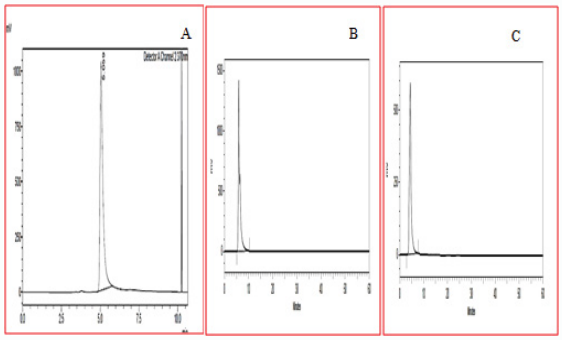

Figure 4: HPLC Chromatogram of standard compounds, (A). Quercetin dehydrate (Flavonoid standard), (B). Caffeine (Alkaloid standard), (C). Gallic acid (Phenolic standard).

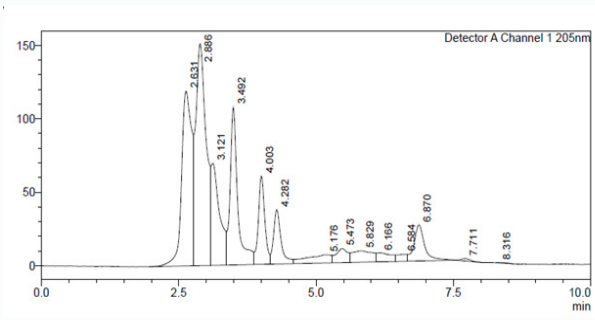

Figure 5: HPLC Chromatogram of Plant extract Crude (M: C root extract).

animals, prolonged and increased exposure to paracetamol results in centrilobular liver necrosis. Therefore, screening for hepatoprotective effects has been facilitated by the use of paracetamol-induced hepatotoxicity as a dependable approach. The oxidized product of paracetamol, N-Acetyl-P-benzo Quine Imine (NAPQI), which is produced by cytochrome P-450 and combines with reduced Glutathione (GSH) to produce non-toxic 3-GS-yl-paracetamol, is what causes liver damage when taken in excess. When GSH levels are low, the quinone that is still present binds covalently to protein sulfhydryl groups, which leads to lipid peroxidation, cell necrosis, and eventually cell death. Large, abundant liver lesions, eosinophilic cytoplasm, and nuclear pyknosis are the hallmarks of centrilobular hepatocyte necrosis. It has been discovered that plant-based secondary metabolites are useful in the treatment of a variety of illnesses, including liver disease. However, due to their ability to scavenge free radicals, phenolic and flavonoid compounds have been identified to have considerable hepato-protective potential among various secondary metabolites [12,24,25].

Determination of the hematological parameters of acute toxicity: It was discovered that *T. Foliolosum* root extract was safe. During the course of seven days of treatment, no mortality was noted at a restricted dosage of 2000 mg/kg body weight. The extract was well-tolerated by every mouse, showing no symptoms of toxicity. For additional experimental assessment, 200 mg/kg of body weight, or one-tenth of the dosage, was chosen. There was a change in the hematological parameters (WBC count, RBC count, HGB, HCT, MCV, MCH, MCHC, Platelet count, etc.) due to the hepatic damage produced by paracetamol. Table 5 illustrates how the hematological parameters significantly improved when treated with 100 mg/kg and 200 mg/kg body weight of *T. Foliolosum* methanol-chloroform root extract as opposed to those treated with paracetamol. Marslin [19] reported that a single oral dose (250 mg kg-1, 500 mg kg-1, 1000 mg kg-1) of ethanolic extract of *T. Foliolosum* roots showed no toxicity up to 14 days of the study. The highest dose 1000 mg kg-1 showed no signs of toxicity, so reported that ethanolic extract of *T. Foliolosum* roots is relatively safe even at higher concentrations.

Determination of biochemical parameters: Liver function is shown by the Liver Function Test results (LFTs) for bilirubin, total protein, ALT, ALP, and AST. These indicators are markedly elevated under oxidative stress and inflammatory situations because the liver synthesizing function is impaired. Serum enzyme activity (AST, ALT, and ALP) that is abnormally elevated following paracetamol treatment is indicative of hepatic damage, the cause of cellular enzyme leakage into the bloodstream. Bilirubin is produced when heme is broken down by enzymes in the reticuloendothelial system. Bilirubin is a

crucial marker and indicator for evaluating liver function but is not very useful in assessing the degree of hepatocellular damage. The total blood protein falls following the injection of paracetamol, indicating decreased protein synthesis and injury to the liver tissue [12,20].

This study showed that, in comparison to the normal control group, mice that were intoxicated with paracetamol had significantly higher serum levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), glucose, bilirubin, urea, and LDH (Table 6). In contrast, the paracetamol-intoxicated mice showed a decrease in albumin and protein levels. The hepatic enzyme activity of these animals was decreased upon oral treatment of the *T. Foliolosum* methanol-chloroform root extract. The healing of hepatic tissue damage brought on by paracetamol and the stability of the plasma membrane may be responsible for this effect. Remarkably, the 200 mg/kg body weight dose that was given produced outcomes that were similar to those of silymarin, the conventional medication. This finding supports the hypothesis that the mending of the hepatic parenchyma and the regeneration of hepatocytes occur concurrently with the recovery of transaminase levels to normal [24,25].

Histopathology: Histological changes are valuable for evaluating the impact of toxins on the structural characteristics of organs and determining the extent of damage. Hematoxylin and Eosin (H&E) staining is a widely employed method for assessing these histological changes and can effectively be used to analyze the progression of the disease [20]. H&E staining was used in this investigation to evaluate the effects of *Thalictrum foliolosum*'s methanol-chloroform root extract on liver tissue. When 300 mg/kg body weight of paracetamol was administered, the liver's architecture was significantly altered, and the liver parenchyma had considerable pathological changes as a result. According to the animal histological study, these alterations included sinusoidal dilatation, inflammatory infiltration, hepatocyte ballooning, hepatocyte necrosis, RBC congestion, and nuclear necrosis as observed in the histopathological analysis of the animals. However, the histological examinations revealed that the pathological lesions induced by paracetamol were minimal in the groups treated with the methanol-chloroform root extract of *T. Foliolosum* in a dose-dependent manner, as depicted in Figure 6. Additionally, the liver tissues of the paracetamol-treated groups exhibited portal tract inflammation characterized by lymphocytes and fibrosis of the perivenular region. In contrast, the silymarin and test plant extract-treated groups showed only mild sinusoidal dilation. Silymarin, a hepatoprotective compound derived from *Silybum marianum*, is renowned for its protective effects on the plasma membrane of hepatocytes. The primary actions of silymarin include its antioxidant properties and its role in cell regeneration, which is characterized by enhanced protein synthesis [25].

The results were compared to those of the common hepatoprotective medication silymarin. Pre-treatment with the methanol-chloroform root extract of *T. Foliolosum* at dosages of 100 mg/kg and 200 mg/kg body weight for 7 days offered considerable protection against paracetamol-induced liver damage. Notably, the effects of 100 mg/kg body weight of silymarin were comparable to those of 100 mg/kg body weight of the methanol-chloroform root extract of *Thalictrum foliolosum*, and the protection provided by the 200 mg/kg body weight dose of the methanol-chloroform root extract of *T. Foliolosum* was even greater than that of silymarin. The methanol-chloroform root extract of *Thalictrum foliolosum*, at both dosages, effectively stopped the histological alterations brought on by

Table 5: Mean hematology parameters in female Swiss Albino mice, control *vs*. PCM and Test Compound treated.

Parameters	Reference	Control (0.9 % NaCl)	PCM (300 mg/kg)	Silymarin (100 mg/kg)	Test plant extract (100 mg/kg)	Test plant extract (200 mg/kg)
WBC (10^3/L)	0.80 - 10.60	4.68 ± 1.19	3.51 ± 0.56	4.05 ± 1.343	2.65 ± 0.565	3.433 ± 0.148
RBC (10^6/L)	6.50 - 11.50	8.77 ± 0.56	8.22 ± 0.42	8.02 ± 0.22	8.036 ± 0.064	7.763 ± 0.326
HGB (g/dL)	11.0 - 16.50	12.96 ± 1.21	11.33 ± 0.36	12 ± 0.38	11.633 ± 0.458	11.366 ± 0.136
HCT (%)	0.350 - 0.550	28.33 ± 21.73	37.36 ± 1.3	38.9 ± 1.88	39.033 ± 0.845	38.566 ± 0.103
MCV(fL)	41.0 - 55.0	51.06 ± 2.42	47.33 ± 0.85	49 ± 1.08	49.066 ± 0.989	50.933 ± 1.18
MCH (pg)	13.0 - 18.0	14.96 ± 0.96	14.23 ± 0.18	15 ± 0.089	14.5 ± 0.497	15.033 ± 0.186
MCHC ((g/dL)	30.00 - 36.00	31 ± 3.19	30.7 ± 1.007	31.06 ± 0.98	29.1 ± 0.322	29.466 ± 0.361
PLT (10^3/µL)	400 - 1600	716 ± 88.48	793.3 ± 35.39	942.66 ± 81.31	910 ± 36.964	962.66 ± 69.979
NEUT (%)	6.5 - 50.0	15.23 ± 1.04	26.6 ± 2.50	30.73 ± 13.64	23.5 ± 1.67	35.466 ± 14.385
LYMPH (%)	40.0 - 92.0	81.3 ± 2.72	72.36 ± 4.26	64.7 ± 12.83	75.7 ± 2.948	71.033 ± 13.499
MONO (%)	0.9 - 1.8	1.33 ± 0.71	2.86 ± 0.4	0.7 ± 0.23	1.933 ± 1.191	0.866 ± 0.051
EO (%)	0.00 - 7.5	1.33 ± 1.91	0.3 ± 00	0.4 ± 6.08	0 ± 0	0.333 ± 0.051
BASO (%)	0.00 - 1.5	0.04 ± 0.06	00.0 ± 0.0	0.0 ± 0.0	0 ± 0	0 ± 0

*Values are the mean ± S.E.M. of five observations.

*Statistical significance was assessed by one -way ANOVA followed by Dunnett's test.

Table 6: Biochemical parameters in female Swiss Albino mice control *vs*. PCM and Test Compound treated.

Parameters	Control (0.9 % NaCl)	PCM (300 mg/kg)	Silymarin (100 mg/kg)	Test plant extract (100 mg/kg)	Test plant extract (200 mg/kg)
AST (u/ml)	64.492 ± 1.6	148.38 ± 2.45	89.49 ± 2.93	122.32 ± 1.58	96.4518 ± 2.62
ALT (u/ml)	48.672 ± 1.43	143.026 ± 2.09	60.894 ± 1.32	105.866 ± 2.09	69.95 ± 1.70
ALP (K.A)	13.98 ± 1.03	33.48 ± 0.62	15.12 ± 0.77	27.78 ± 1.54	17.66 ± 1.15
Glucose (mg/dl)	54.874 ± 1.13	88.618 ± 0.68	65.258 ± 1.25	74.618 ± 1.38	68.416 ± 1.32
Protein (g/dl)	5.77 ± 0.26	4.18 ± 0.32	5.356 ± 0.45	4.468 ± 0.08	4.826 ± 0.51
Albumin (g/dl)	5.012 ± 0.22	2.604 ± 0.24	4.394 ± 0.47	2.792 ± 0.25	4.348 ± 0.15
Bilirubin (mg/dl)	0.824 ± 0.04	2.202 ± 0.17	1.1048 ± 0.09	2.018 ± 0.07	1.336 ± 0.15
Urea (mg/dl)	35.158 ± 1.01	131.5 ± 3.21	49.424 ± 2.22	112.874 ± 3.67	55.404 ± 3.02
LDH (u/l)	236.336 ± 3.80	568.22 ± 5.05	286.158 ± 3.80	522.104 ± 3.34	297.656 ± 2.92

*Values are the mean ± S.E.M. of five observations.

*Statistical significance was assessed by one -way ANOVA followed by Dunnett's test.

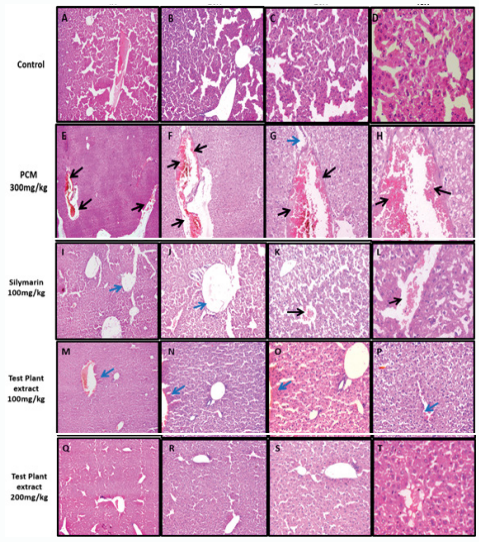

Figure 6: The figure represents the H&E-stained images of mice liver tissue; A-D for control group; E-H for disease (Toxicity) control (PCM induced 300 mg/kg); I-L for PCM-induced but pre treated with Silymarin (100 mg/kg); M-P for PCM-induced but pre treated with test plant extract (100 mg/kg); Q-T for PCM- induced but pre treated with test plant extract (200 mg/kg). Magnification: 4x, 10x, 20x, 40x - [(A-D), (E-H), (I-L), (M-P) & (QT); Arrow indicators: Blue-sinusoidal dilation; black-inflammatory infiltration; and hepatocyte ballooning, hepatocyte necrosis, RBC congestion, nuclear necrosis can be seen on histological examination.

paracetamol. The suppression of N-Acetyl-P-benzo Quinone Imine (NAPQI) activity is most likely connected to the mode of action. Based on the early phytochemical analysis, it is possible that the presence of phenolic and flavonoid components is responsible for the reported hepatoprotective action of this plant extract. The antioxidant qualities of these substances are well-known. Additionally, Marslin [19] reported that the ethanolic extract of *T. Foliolosum* roots exhibited significant hepatoprotective activity in an animal model against paracetamol-induced toxicity and was non-toxic at relatively higher concentrations.

Statistical analysis

Every analysis was carried out in triplicates, and the findings were then shown as means with corresponding standard deviations. Statistical analysis was carried out using the Graph Pad Instat program. Turkey's multiple range tests, Dunnett's test, and Analysis Of Variance (ANOVA) were used to identify significant differences between the treatment and control groups. For each group, the findings were reported as mean ± S.E.M., and the recorded data were deemed validated at a significance level of $P<0.05$.

Conclusion

The liver plays a crucial role in metabolizing and detoxifying various substances, making it highly susceptible to toxicity. Prolonged and increased exposure to paracetamol can lead to centrilobular liver necrosis in both experimental animals and humans. As a result, paracetamol-induced hepatotoxicity serves as a dependable model for screening hepatoprotective effects. Based on the results obtained, it can be conclusively stated that the extracts derived from the roots of *T. Foliolosum* exhibit remarkably significant hepatoprotective activity. This effect is likely attributed to the extract's radical scavenging potential and its ability to promote hepatic regeneration. Furthermore, the identification of phenolic and flavonoid compounds in the extract, renowned for their radical scavenging properties, serves to reinforce and substantiates.

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