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ARTICLE

Evaluation of Hepatoprotective Activity of *Tribulus terrestris* in D-Galactosamine- and Paracetamol-Induced Hepatotoxicity in the Rat Model

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ABSTRACT

In traditional Indian and Chinese medicine, *Tribulus terrestris* has been frequently used to treat liver problems. The aim of this study was to look at the protective effect on the liver of the airborne (leaves, stems, fruits, flowers) leaves, stems, fruits, and flowers of the plant against hepatotoxicity caused by D-galactosamine (D-Gal) and paracetamol. To measure the antioxidant capacity of *T. terrestris*, we conducted a DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging assay. In the trials involving solvent fractions, the extraction of the ethanol extract exhibited the highest amount of free radical scavengers; thus, it was chosen for the continued tests on the protective nature of the liver. The ethanol extract was subjected to acute oral toxicity tests (OECD Test Guideline 425 up-and-down) to establish 200 mg/kg body weight and 400 mg/kg body weight as the doses to evaluate the protective effect. Comparisons of the protective effects of the ethanol extract of *T. terrestris* and Silymarin 100 mg/kg body weight were made based on their respective effectiveness in reducing the increased levels of the serum enzymes, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphatase, total cholesterol, total bilirubin, and direct

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bilirubin caused by D-Gal. Additionally, the level of protection against poison was tested by evaluating the condition and histology of the liver. In both models of D-galactosamine (D-GalN)- and paracetamol (PCM)-induced liver injury, administration of the ethanolic extract of *T. terrestris* at a dose of DBH (400 mg/kg body weight (bw)) produced significantly greater hepatoprotection than either D-GalN or PCM (+) silymarin (100 mg/kg) (p = 0.07). This finding indicates that the ethanolic extract of *T. terrestris* has considerable protection against D-galactosamine-induced hepatic injury by acting as an antioxidant and stabilizing membranes.

Keywords: *Tribulus terrestris*; D-Galactosamine; Hepatotoxicity; Hepatoprotective Activity; Antioxidant; Herbal Medicine

1. Introduction

Globally, liver disease is a significant health issue because liver diseases such as jaundice and hepatitis are among the leading causes of death [1] due to the liver being particularly susceptible to injury when it has been exposed to drugs, environmental toxicants, and other xenobiotics for extended periods of time [2]. Conventional synthetic medicinal products on the market (primarily drugs) that are currently used to manage liver disease have either been ineffective or frequently have serious side effects [3]. Recently, there has been an increasing amount of interest in utilizing natural herbal remedies; therefore, many herbal products have successfully treated patients who have suffered from liver injuries [4]. The herbal product *Tribulus terrestris* is an example of one of these products; it shows great promise as a potential hepatoprotective agent [5].

Tribulus terrestris is a commonly known herb with bright yellow flowers that has a prostrate and branched structure and grows to a height of approximately 30 cm (or 1 foot). It is a member of the Zygophyllaceae family. In traditional medicine, *T. terrestris* has been used for many conditions, including hypertension, inflammation, and sexual dysfunction [6]. In traditional Chinese medicine, the fruits of *T. terrestris* are used to treat liver and eye disorders [7], as well as the hot water extract of the dried seeds to treat liver disorders [8]. In traditional Indian medicine, *T. terrestris* is also said to be beneficial for jaundice [9]. *T. terrestris* has long been used as an astringent, tonic, aphrodisiac, analgesic, stomachic, antihypertensive, diuretic, and urinary antiseptic [10]. Studies conducted with rats indicate that *T. terrestris* has diuretic, antiurolithiatic, antibacterial, central nervous system stimulant, and antifungal properties [11].

In recent years, many studies have investigated the antioxidant and hepatoprotective activity of *T. terrestris* [12–15]. Studies have shown that aqueous extract of *T. terrestris* exerts its hepatoprotective effect owing to its free radical quenching property [12]. A separate study conducted by AL-Hathoot et al. showed that the alcoholic and aqueous fruit powder extract of *T. terrestris* showed anti-hepatoxic properties [16]. An additional study determined that two derivative compounds of *T. terrestris* di-p-coumaroylquinic acid found in the ethyl acetate fraction had strong antioxidant activities [17]. These antioxidant properties contributed to the hepatoprotective activity of *T. terrestris* in the final experiment using ethanol extracts and different aerial fractions of *T. terrestris* in a study on the effects of carbon tetrachloride on preventing further liver injury in Wistar rats [18].

It has been reported that damage to the rat liver caused by D-galactosamine is similar to that seen in humans with viral hepatitis from both a morphological and functional standpoint [14]. D-galactosamine acts preferentially on the liver due to the presence of increased concentrations of Galactokinase and Galactose-1-P-Uridylyltransferase (Gal-1-P-UT) in hepatic tissue and not in any other organ [19]. Damage occurs from D-galactosamine by creating localized areas of cell death in the hepatocytes and by creating significant amounts of inflammation in the portal tracts and parenchyma of the liver [5]. D-galactosamine will decrease Uridine diphosphate (UDP) via the overproduction of UDP-Sugar derivatives, leading to a blockade of RNA and protein synthesis and resultant destruction of the cellular membranes [20].

The ethanolic extract from *T. terrestris* was evaluated for its acute oral toxic effects on rats and shown to provide significant antioxidant activity. Further, the extract was used to test for the hepatoprotective properties of *T. terrestris* in rats subjected to D-GalN or PCM liver injury. All biochemical tests evaluated included Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Py-

ruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), Total Cholesterol (CT), Total Bilirubin (TB), and Direct Bilirubin (DB). The histopathology of the rat liver was also performed.

The antioxidant and traditional hepatoprotective benefits of the plant have been described in various articles; nevertheless, the molecular significance of *Tribulus terrestris* in chemically-induced liver injury is still poorly known. The wide variation in hepatoprotector efficacy remains poorly understood, as most of the previous studies have been conducted with individual hepatotoxic agents. The present study adds to the existing knowledge by simultaneously evaluating the hepatoprotective property of *T. terrestris* extract against two different hepatotoxic models (paracetamol, which includes oxidative stress-mediated centrilobular necrosis, and D-galactosamine, which mimics viral hepatitis through inflammatory and apoptotic pathways).

2. Materials and Methods

2.1. Plant and Preparation of Extract

The aerial parts (including the leaves, stems, fruits and flowers) of *Tribulus terrestris* were collected from a botanical garden located in Bardoli Taluka, Surat District of Gujarat, India, during the time frame of August–September 2010. The taxonomical authentication of the plant material was performed by Dr. Bimal S Desai (Assistant Professor in the Department of Botany at Navsari Agricultural University, Navsari, Gujarat, India). A voucher specimen (AU/RVNI/12-13/012) of the plant material was submitted to the Department of Pharmacology at R.V. Northland Institute (located in Dadri, India) for future reference.

Phytochemical screening of the ethanolic extract was performed using standard colourimetric techniques and confirmed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds and glycosides [21,22]. The total phenolic content was analysed according to the Folin-Ciocalteu/gallic acid equivalents (GAE) (mg/g extract) method and measured in terms of the amount of gallic acid present; total percentage yields obtained from the aerial parts of the plant were 3.2% (petroleum ether), 4.8% (chloroform) and 8.6% (70% ethanol) with respect to the dry weight.

The plant material that was collected was cleaned to remove any dust or debris that was associated with the plant. It was shade-dried for a period of time (20 to 25 days) to ensure that all moisture had been completely removed from the plant material. After drying out, the plant material was subjected to the Ayurveda formulation of India for the phytocompound extraction procedure [23]. The collected plant material was ground into a powderized form using a high shear mixer grinder (Pulverizer-MT40.100 steril, lab scale, IKA, Germany). Sequential extraction of the plant material was performed by using Soxhlet extraction with each solvent (petroleum ether, chloroform, 70% ethanol, and distilled water) for a period of 28 h and in the order of increasing polarity. After extraction, all of the solvent extracts were concentrated to near dryness under reduced pressure, on a rotary evaporator (Model RV10, IKA, Germany), followed by vacuum oven drying (Lab India) at 0.5 atm for 24 h to give a semi-solid mass.

2.2. Experimental Animals

The study employed Wistar albino rats of both genders, weighing from 150 to 200 g. The rats lived in polypropylene cages in a climate-controlled room maintained at 22 °C ± 3 °C, with a 12-h light-dark cycle and 59%–70% relative humidity. Animals had free access to a standard pellet diet and clean water ad libitum. All experimental procedures were conducted in strict compliance with the Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines and were approved by the Institutional Animal Ethics Committee (IAEC) of R.V. Northland Institute, Dadri [CCSEA Protocol Approval No.: RVNI/IAEC/2010-11/008, dated 12th July 2010]. Animals were randomly allocated to experimental groups using a computer-generated random number sequence. All biochemical analyses and histopathological assessments were performed by investigators blinded to group assignments.

2.3. *In Vitro* Antioxidant Study

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity (% FRSA) of *T. terrestris* extract was performed using the method of Maind et al. [24] with slight modification. The reaction mixture contained 1 mL DPPH

solution (0.2 mg/mL in methanol), 1 mL of extract at concentrations of 0.025 to 0.25 mg/mL, and 5 mL of distilled water. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (Shimadzu-1700, Japan) after 30 min of incubation in the dark at room temperature. Gallic acid was used as a positive control. The fraction of scavenged DPPH radicals was calculated using the formula:

% Free radical scavenging activity (% FRSA) = (Absorbance of the test sample)/(Absorbance of the standard solution) × 100

2.4. Hepatoprotective Action of Ethanol Extract of *T. terrestris*

The male Wistar rats were divided into five groups (a total of 30 animals) for each hepatotoxin (d-GalN and PCM). For the d-GalN model, the dosing schedule in each of the groups was as follows:

a. Group I (negative control group) rats were treated with 0.3% NaCMC solution for 5 days.
b. Group II (positive control group) rats were treated with d-GalN 400 mg/kg bw/day on the 5th day.
c. Group III rats received silymarin at a dosage of 100 mg/kg body weight for the initial five days, followed by d-GalN at 400 mg/kg body weight each day on the fifth day.
d. Rats in Groups IV and V received an ethanolic extract of *T. terrestris* Linn. at dosages of 200 mg/kg body weight and 400 mg/kg body weight, respectively, for the first five days, and d-GalN 400 mg/kg bw/day on the 5th day.

In the PCM model, group I and group II were similar to the d-GalN model. While in the Group III, Group IV and Group V, PCM 2,000 mg/kg bw/day was administered instead of d-GalN.

Serum was collected from all treated animals 24 h after administration of the hepatotoxin (d-GalN or PCM) for quantification of SGOT, SGPT, ALP, TB, DB, and TC. All animals were subsequently euthanized and livers excised for histopathological evaluation. Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for pairwise group comparisons, with exact *p*-values reported.

Animals were anesthetized using a combination of ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Blood was collected via the lateral tail vein using a sterile needle and syringe. To separate the serum, the sample was centrifuged at 2,000 g for 10 min at 4 ℃. The supernatant was then stored at 4 ℃ for further evaluation of serum enzyme activity. Bergrneyer and Bernt described the Reitman and Frankel method [25]. This was used to measure glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT). We used the Kind and King Method [26] to measure alkaline phosphatase (ALP). The Malloy and Evelyn method [27] was used to check serum bilirubin levels.

2.5. Liver Isolation

Following blood collection under ketamine-xylazine anesthesia, animals were humanely euthanized by high dose of pentobarbital sodium (100–150 mg/kg), and the liver was carefully excised. The isolated liver was kept in 10% buffered formalin. After fixing, the liver sections were cleaned with xylene, embedded in paraffin wax, and dried in a series of alcohol grades. Hematoxylin and eosin were used to stain each 5 μm block of liver tissue section. The proportion of hepatoprotection was calculated using the formula [28]:

$$\% \text{ protection} = \left[1 - \frac{\text{Drug} - \text{Control}}{\text{Toxicant} - \text{Control}}\right] \times 100$$

2.6. Histological Studies

The rats were euthanized after their blood was drawn, and the livers were removed quickly, rinsed in cold saline, and put into neutral buffered formalin solution for histological examination [29]. Sections of tissue were cut at a thickness of 5 μm and then stained (via hematoxylin and eosin or H&E) for routine histopathological assessment. In order to confirm the presence of red blood cells, additional stains (e.g., using Luna's method) were used; to confirm the presence of eosinophilic granules, Gram staining was used. Histopathological changes were scored and assessed by a blinded pathologist using a modified Knodell scoring system and assessed independently via three parameters ("independent") as follows: (i) necrosis, (ii) portal and lobular inflammation, and (iii) sinusoidal dilation. Each

parameter was given a score from 0 to 4: 0 = no change (i.e., 0% of area involved), 1 = minimal (i.e., <10% of area involved), 2 = mild (i.e., 10–25% of area involved), 3 = moderate (i.e., 25–50% of area involved), 4 = severe (i.e., > 50% of area involved).

2.7. Data Analysis

All quantitative biochemical and histopathological data are expressed as mean ± SEM (standard error of the mean). Statistical comparisons were performed using one-way ANOVA with Tukey's Honestly Significant Difference (HSD) post-hoc test (GraphPad Prism v8.0). Exact *p*-values are reported for all pairwise comparisons. A p-value ≤ 0.05 was considered statistically significant, and $p \leq 0.01$ was considered highly significant.

3. Results

3.1. *In Vitro* DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay

T. terrestris extracts were evaluated using the DPPH method to assess antioxidant activity of the extracts (ethanol, chloroform, and petroleum ether). An increase in radical scavenging capacity was achieved as the concentration of the extract increased. The extracts of *T. terrestris* showed considerably higher IC_{50} than gallic acid (the standard for antioxidant activity), indicating that *T. terrestris*'s ability to scavenge free radicals is less than that of gallic acid. The chemical structure of the extraction solvent appears to influence the ability of each solvent to scavenge free radicals, with the order of oxygen activity being ethanol extract > chloroform extract > petroleum ether extract (**Table 1**). The antioxidant capacity of both ethanolic and petroleum ether extracts was also concentration-dependent. Ethanol extract of *T. terrestris* exhibited the highest antioxidant activity of the extracts measured, with an IC_{50} of 77.09 ± 0.16 µg/mL or approximately 0.76 µg of gallic acid for every gram of *T. terrestris* extract. All IC_{50} values of *T. terrestris* extracts were significantly higher than the IC_{50} value of gallic acid (0.76 ± 0.04 µg/mL), providing further evidence that the extracts of *T. terrestris* have moderate antioxidant properties. The percentage inhibition curves of the three *T. terrestris* extracts show a concentration-response relationship (**Figure 1**).

Table 1. IC50 value comparison between gallic acid and various extracts of *Tribulus terrestris.*

S. No.	Sample Name	IC_{50} by DPPH Method (µg/mL); n = 3 and Mean ± Standard Deviation (SD)
1	Gallic acid (standard)	0.76 ± 0.04
2	Pet ether extract	275.74 ± 0.14
3	Chloroform extract	266.99 ± 0.09
4	70% ethanol extract	77.09 ± 0.16

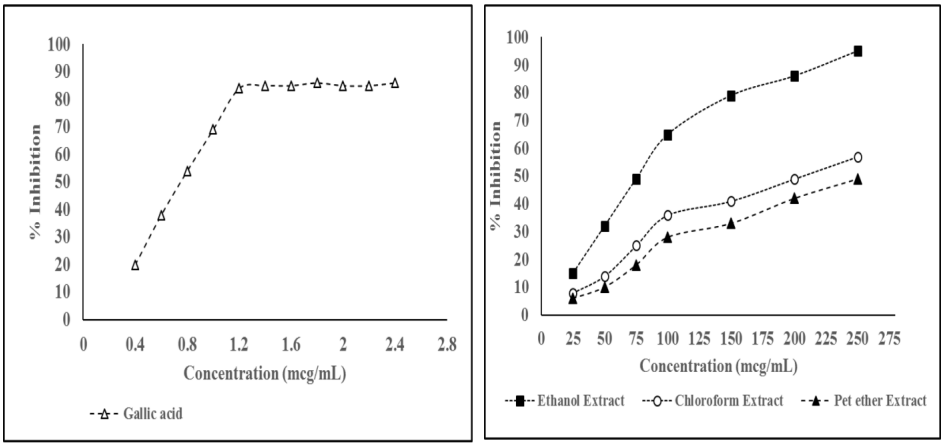


Figure 1. Percentage inhibition plots for extracts of the plant *Tribulus terrestris*.

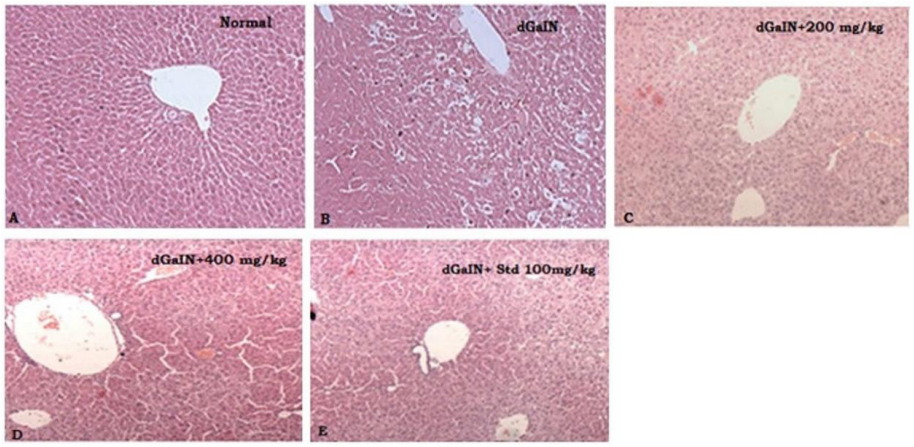
3.2. Hepatoprotective Activity of *T. terrestris*: Assessment of Biochemical Parameters

The administration of the hepatotoxic agent d-GalN (Group II) induced significant elevations of serum biochemicals (SGOT, SGPT, ALP, total bilirubin (TB), direct bilirubin (DB), total cholesterol (TC)). The elevations were substantially greater than those of the normal control group (Group I) and demonstrated the successful induction of hepatotoxicity (**Table 2** and **Figure 2a**) in the experimental animals. Prior to administering d-GalN, the addition of silymarin (Group III; 100 mg/kg b.wt.) to the animals' diets resulted in significantly lower elevations of the above biochemicals compared to those of Group II ($p < 0.01$). Therefore, the addition of silymarine to the animals' diets provided significant protection against d-GalN hepatotoxicity.

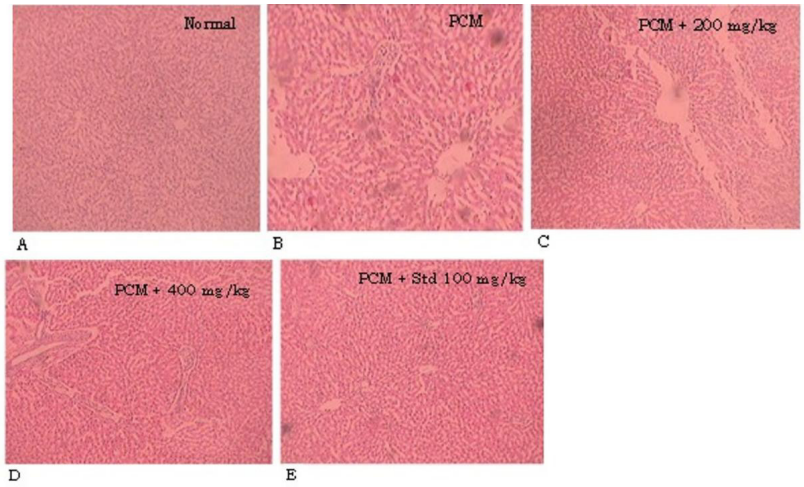
Table 2. Serum biochemical parameters and percentage hepatoprotection in treatment groups against d-GalN-induced hepatotoxicity.

Group	SGOT (IU)	%	SGPT (IU)	%	ALP (IU)	%	TB (mg/dL)	%	DB (mg/dL)	%	TC (mg/dL)	%
G1	59.80 ± 1.47	-	37.59 ± 0.88	-	293.67 ± 7.79	-	0.67 ± 0.01	-	0.22 ± 0.02	-	73.28 ± 1.00	-
G2	133.32 ± 1.65	-	113.94 ± 2.18	-	558.74 ± 6.63	-	2.34 ± 0.05	-	1.82 ± 0.03	-	182.79 ± 2.34	-
G3	79.72 ± 1.10[a]	73	60.87 ± 2.09[a]	70	371.45 ± 9.94[a]	70.7	0.97 ± 0.04[a]	82.1	0.46 ± 0.01[a]	85	93.08 ± 1.31[a]	82
G4	110.53 ± 2.19[b]	31	89.85 ± 1.31[b]	31.6	507.40 ± 3.17[b]	19.4	1.85 ± 0.03[b]	30	1.17 ± 0.02[b]	40.7	139.52 ± 2.24[b]	39.6
G5	89.64 ± 0.79[a]	59.5	66.33 ± 2.42[a]	62.4	418.97 ± 8.75[a]	52.8	1.29 ± 0.06[a]	62.9	0.55 ± 0.03[a]	79.4	104.61 ± 3.06[a]	71.4

Note: Values are mean ± SEM (n = 6/group). [a] $p < 0.01$ vs. Group II (one-way ANOVA, Tukey's HSD post-hoc). [b] $p < 0.05$ vs. Group II. TC: total cholesterol (mg/dL). % = percentage hepatoprotection = [1 − (Drug − Control)/(Toxicant − Control)] × 100.



(**a**)



(**b**)

Figure 2. The effect of different treatments on: (**a**) dGalN-induced hepatotoxicity in rats; (**b**) PCM-induced hepatotoxicity in rats.

The addition of *T. terrestris* ethanol extract at doses of 200 mg/kg (Group IV) and 400 mg/kg (Group V) provided significant reductions ($p < 0.05$) in all biochemicals compared to those of Group II (**Table 2**); both dose-dependent (i.e., the higher the dose, the greater the reduction) reductions were noted, with the 400 mg/kg dose approaching that of the silymarin-treated group. Collectively, the results indicate a clear dose-dependent hepatoprotective effect of *T. terrestris* ethanol extract in the d-GalN-induced hepatotoxicity model.

In comparison to the control group (Group I), paracetamol-treated animals (Group II) had significantly increased serum biochemical indicators of liver injury (see **Table 3** and **Figure 2b**), showing evidence of liver damage from taking paracetamol (PCM). Silymarin pre-treatment (Group III; 100 mg/kg body weight) decreased the increased serum levels of all liver injury markers significantly ($p < 0.01$), indicating that silymarin provided hepatoprotective effects against PCM hepatotoxicity.

Pre-treatment with *T. terrestris* ethanolic extract at 200 mg/kg (Group IV) and 400 mg/kg (Group V) produced statistically significant, dose-dependent reductions in all serum biochemical parameters compared to the PCM-control group (Group II) ($p < 0.05$ and $p < 0.01$, respectively; **Table 3**). The 400 mg/kg dose provided the most pronounced hepatoprotection, with percent protection values of 65.6–80.6% across all parameters, approaching the degree of protection observed with silymarin.

Table 3. Serum biochemical parameters and percentage hepatoprotection in treatment groups against PCM-induced hepatotoxicity.

Group	SGOT (IU)	%	SGPT (IU)	%	ALP (IU)	%	TB (mg/dL)	%	DB (mg/dL)	%	TC (mg/dL)	%
G1	137.9 ± 4.89	-	56.26 ± 3.09	-	156.26 ± 8.19	-	0.84 ± 0.03	-	0.21 ± 0.02	-	77.43 ± 3.68	-
G2	238.13 ± 12.71	-	151.45 ± 7.17	-	345.17 ± 14.64	-	2.59 ± 0.07	-	0.82 ± 0.06	-	160.26 ± 5.00	-
G3	152.32 ± 12.8[a]	85.7	74.70 ± 2.09[a]	80.7	175.47 ± 9.94[a]	89.9	0.78 ± 0.04[a]	134.2	0.34 ± 0.04[a]	78.7	81.06 ± 5.00[a]	95.7
G4	186.28 ± 5.54[b]	51.8	118.55 ± 1.51[b]	34.6	254.74 ± 2.93[b]	47.9	1.76 ± 0.01[b]	47.5	0.56 ± 0.02[b]	42.7	133.27 ± 2.18[b]	32.6
G5	166.66 ± 1.16[a]	71.4	85.47 ± 1.01[a]	69.4	193.46 ± 2.67[a]	80.4	1.22 ± 0.01[a]	80.6	0.42 ± 0.02[a]	65.6	95.46 ± 2.19[a]	78.3

Note: Values are mean ± SEM (n = 6/group). [a] $p < 0.01$ vs. Group II (one-way ANOVA, Tukey's HSD post-hoc). [b] $p < 0.05$ vs. Group II. TC: total cholesterol (mg/dL). % = percentage hepatoprotection.

In summary, these results indicate that the ethanolic extract of *T. terrestris* has a significant dose-dependent hepatoprotective effect against PCM and d-GalN-induced liver damage, and the higher dose is more effective than the lower dose in providing hepatoprotective effects in both animal models.

3.3. Histopathological Evaluation of Liver Sections

The Knodell scoring system was adapted to determine histological changes associated with d-GalN treatment (necrosis; degree of portal/lobular inflammatory infiltration, sinusoids dilation); total possible score = 12 (4 points for each of the three parameters) in the animal model. The results for the 5 treatment groups were: Group I Normal Control; no liver pathology (Knodell score of 0.0 ± 0.0); Group II d-GalN; significant liver cell damage, and substantial necrosis, periportal inflammatory cell infiltration, and sinusoidal dilation (Knodell score of 10.2 ± 0.4; $p < 0.01$ vs. Group I); Group III silymarin; mostly reversible liver pathology associated with d-GalN (Knodell score of 1.6 ± 0.3; $p < 0.01$ vs. Group II); Group IV *T. terrestris* prior to d-GalN; some, but statistical protection from d-GalN-induced hepatocyte damage (Knodell score of 5.8 ± 0.5; $p < 0.05$ vs. Group II); Group V *T. terrestris*; greater degree of hepatocyte damage (Knodell score of 2.4 ± 0.4; $p < 0.01$ vs. Group II) than for Group III, but not as much as Group I ($p = 0.08$ vs. Group I).

The PCM model has extensive necrosis of the centrilobular region throughout the liver and has large amounts of inflammatory infiltration in addition to having very dilated sinusoids (Knoddell score; 9.8 ± 0.5) in comparison to Group 1 ($p < 0.01$) (**Figure 2b**). Group 3 (*T. terrestris* 200 mg/kg; n = 4) had significantly less liver structural damage than Group 2, with average LSA (Lichen sclerosus et atrophicus) histological scores as follows: LSA Mean ± SD, Group 3 = 1.88 ± 0.35 vs. Group 2 = 5.6 ± 0.6, $p < 0.05$. Group 5 (*T. terrestris* 400 mg/kg; n = 3) had almost complete recovery of liver architecture compared to Group 2 average LSA score of 2.6 ± 0.4 and did not differ from

Group 1 ($p > 0.05$) whereas Group 4 (silymarin; n = 6) had liver architecture similar to Group 3 but the average silymarin malondialdehyde (MDA) levels (4.2 µg/dL and 3.3 µg/dL vs. 1.5 µg/dL) were very different than Group 1's (2.2 µg/dL; $p < 0.01$). The results confirmed serum biochemical parameter analyses.

4. Discussion

The liver's main metabolic activity is drug metabolism; therefore, it is also the organ that has the greatest risk of injury from xenobiotics [19]. Hepatotoxicity as a result of drugs accounts for between 20–40% of all cases of acute hepatic failure, and more than 900 pharmaceutical compounds are able to cause liver damage [4]. There is much interest in plant-derived therapeutics as a consequence of the limitations and possible side effects of traditional hepatoprotective agents. *Tribulus terrestris* Linn. of the Zygophyllaceae family, has been used historically in traditional Chinese and Ayurvedic Medicine for the management of liver disease. The various phytochemical components of *Tribulus terrestris* (TT)'s aerial parts, which include flavonoids, saponins, alkaloids, and phenolic compounds, contribute to the basis for its potential hepatoprotective effects [5,17]. In this study, we are evaluating the hepatoprotective effects of an ethanolic extract of the aerial portions of *T. terrestris* against d-GalN and PCM-induced hepatotoxicity in Wistar Albino rats.

Reactive Oxygen Species (ROS), produced in the liver during typical functioning of the organ, are continuously metabolised in the liver; however, they may overwhelm the body's own (endogenous) antioxidant defence systems when produced as a consequence of drug and/or environmental toxin exposure, accidental and/or intentional. Therefore, when the body's ability to defend itself from oxidative damage to the liver cells (hepatocellular injury) with antioxidant substances fails, consequences can be severe and/or permanent [30]. Medicinal plants (those containing polyphenols, flavonoids, and terpenoids) may provide support for the liver to minimize the impact of oxidative injury by providing an additional source of antioxidant substances through the ability of these plants to scavenge for free radicals [30]. The aerial parts of the plant *Tribulus terrestris* are known to contain a variety of compounds, including alkaloids, phenols, flavonoids, saponins, glycosides, and terpenes [31], of which phenolic and terpenoid compounds have also been shown to act as the major scavengers of free radicals [32]. The current study results indicate that the DPPH radical scavenging activities of all extract solvents were concentration dependent, with antioxidant activity increasing linearly with solvent polarity (less polar = less potent; more polar = more potent) (petroleum ether < chloroform < ethanol). Although an IC_{50} value (concentration of the extract required to scavenge 50% of DPPH free radicals) at 77.09 ± 0.16 µg/mL for the ethanolic extract was much higher than the standard reference compound (gallic acid) at an IC_{50} of 0.76 ± 0.04 µg/mL (indicating relatively moderate antioxidant activity compared to the standard), it was still superior to all other extract types tested for DPPH radical scavenging activity, and therefore was chosen for further testing to assess hepatoprotective effects. The consistency of the current study findings with previously reported antioxidant activity in methanolic and chloroform extracts of *T. terrestris* [33] and Kaya et al. reported the strongest inhibition effect of DPPH (IC_{50} = 25.86 ± 0.010 µg/mL) of the acetone extract of *T. terrestris* [34]. It suggests that differences in extraction solvent, plant part, and geographic location may explain the variability in activity reported across studies.

Using OECD Test Guideline (TG) 425 up-and-down experimental design, the ethanolic extract of the test substance was assessed for acute oral toxicity. At 14 days after a test dose of 2,000 mg/kg b.w., no signs of mortality or signs of toxicity were observed, suggesting that there was a large margin of safety. Therefore, test doses of 200 mg/kg (1/10th of estimated LD_{50}) and 400 mg/kg (1/5th of estimated LD_{50}) were used to evaluate the extract; thus, the doses correspond with those that have been used in hepatoprotective studies as well as allow for assessment of a dose-response relationship [35,36].

d-Galactosamine (d-GalN) is a selective hepatotoxicant that produces cytotoxicity through depletion of uridine diphosphate (UDP) pools, via the formation of UDP-sugar derivatives, thus inhibiting RNA and protein synthesis, disrupting cell membrane integrity, and ultimately producing hepatocellular necrosis with periportal and parenchymal inflammatory changes [5,19]. The pattern of liver injury caused by d-GalN is similar to that of human viral hepatitis, thus supporting the use of this model as a

clinically relevant model for hepatoprotective screening [37]. In this study, d-GalN administration (Group II) significantly increased concentrations of serum SGOT, SGPT, ALP, TB, DB, and TC (**Table 2**), indicating hepatocellular membrane damage and impaired biliary function [37]. Inhibition of the hepatic clearance of circulating cholesterol, which is most likely the result of impaired hepatocyte function [35], is also supported by the significant increase in TC.

The ratio of hepatoprotection and enzyme levels demonstrated that *T. terrestris'* ethanolic extract at 400 mg/kg b.w. effectively decreased all elevated markers in a statistically significant manner ($p < 0.01$) as outlined below: (59.5%, 62.4%, 52.8%, 62.9%, 79.4%, 71.4%) and approached those resulting from silymarin at 100 mg/kg body weight. (**Table 2**). The restoration of normal enzyme levels indicates that *T. terrestris'* extract stabilises the plasma membrane of hepatocytes and reduces the extent of d-GalN-induced damage to liver cell plasma membranes, thereby restoring normal liver function.

Cytochrome P450 has long been recognized as the primary mechanism through which acetaminophen (PCM) induces hepatotoxicity by oxidising PCM to N-acetyl-para-benzoquinone-imine (NAPQI), and in turn, the binding of NAPQI covalently to cellular macromolecules occurs when glutathione (GSH) stores are largely depleted. This causes necrosis within the centrilobular region of the liver due to the presence of pyknosis (nucleus shrinks) and eosinophilic (cytoplasm appears more pinker) changes, and centrilobular liver damage is visible as a progressive, complete, or partial loss of architecture.

The administration of paracetamol (PCM) resulted in a marked increase in the levels of all of the serum biochemistry parameters examined (**Table 3** and **Figure 2b**) as anticipated based upon its known mechanism of action. The administration of *T. terrestris* extract at a dosage of 400 mg/kg b.w. provided statistically significant protection against PCM-induced liver damage compared to the 200 mg/kg b.w. dosage for all of the parameters studied (**Table 3**), in addition to providing values for percent protection ranging from 65.6%–80.6%, approaching, though not statistically equivalent to, the hepatoprotective effects of silymarin ($p = 0.08$, Tukey's HSD). The greater number of protective effects seen in response to PCM injury than that seen in response to additional d-GalN injury may be due to the extract's ability to increase levels of GSH and to decrease NAPQI-mediated hepatotoxicity through mechanisms associated with the phenolic and flavonoid components of *T. terrestris* [15,38]. Further support for these findings came from the histopathology evaluations (**Figure 2**), which indicate that the hepatic architecture of Group V was almost entirely normal in both experimental models, thus confirming the results of the above-mentioned biochemical data.

The present study supports previous reports regarding the hepatoprotective effect of *T. terrestris*. Sugunavarman and Jagadeesan [39] demonstrated that *T. terrestris* extracts provided some level of protection against mercury-induced liver damage in a mouse model, which they attributed to the ability of *T. terrestris* to scavenge free radicals. Sambasivam et al. [40] reported that *T. terrestris* hydroalcoholic fruit extracts protected against liver damage caused by ferrous sulfate in rats. Ansari et al. provided evidence that the ethanol extract of *T. terrestris* protects against acetaminophen-induced liver damage in Wistar rats, the same challenge utilized in the present study. Harraz et al. [15] identified the presence of two different di-p-coumaroylquinic acid derivatives from ethyl acetate extracts of the aerial portion of this plant as contributors to the hepatoprotective and antioxidant activity seen with acetaminophen-induced liver damage. More recently, investigators have begun to examine the role of various *T. terrestris* saponin-rich extracts in ameliorating oxidative stress and inflammation in the liver, as evaluated in experimental models, supporting the conclusion that the plant's hepatoprotective effects occur via multiple mechanisms involving various components of the plant. The addition of data from the current study confirms that the hydroalcoholic extract derived from the aerial portions of *T. terrestris* provides significant, dose-dependent protective effects against liver injury, likely through multiple mechanisms including antioxidant, membrane-stabilizing, and anti-inflammatory actions mediated by the phenolic, flavonoid, and saponin components of *T. terrestris*.

5. Conclusions

In conclusion, the ethanolic extract of the aerial parts of *T. terrestris* demonstrated significant hepatoprotective

activity in both D-galactosamine- and paracetamol-induced hepatotoxicity models in male Wistar albino rats. The dose of 400 mg/kg body weight produced the most pronounced hepatoprotection, as evidenced by significant normalization of serum liver enzymes (SGOT, SGPT, ALP), total and direct bilirubin, and total cholesterol, alongside restoration of normal hepatic histoarchitecture, and was found comparable to the standard hepatoprotective agent silymarin at 100 mg/kg body weight. At the lower dose of 200 mg/kg body weight, partial but statistically significant hepatoprotection was observed. The free-radical scavenging capacity of the ethanolic extract, attributed to its phenolic and flavonoid constituents, may contribute to the observed hepatoprotective effect, though direct mechanistic evidence warrants further investigation. The present findings support the traditional use of *T. terrestris* in hepatic disorders and justify further pharmacological and clinical exploration of this medicinal plant.

Author Contributions

Conceptualization, R.G. and M.H.M.; methodology, R.G., N.S., M.H.M. and S.A.; software: R.G., M.H.M. and S.A.; validation, R.G., N.S., M.H.M. and S.A.; investigation, R.G. and M.H.M.; resources, R.G., M.H.M. and S.A.; data curation, R.G. and M.H.M.; writing—original draft preparation, R.G., M.H.M. and S.A.; writing—review and editing, R.G., M.H.M., S.A. and N.S.; supervision, R.G. and M.H.M.; project administration, R.G. and M.H.M.; funding acquisition, R.G. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

AI Use Statement

The authors declare that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

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