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RESEARCH ARTICLE

# Hepatoprotective Effect of Ethanolic Extract of Whole Plant of *Tephrosia barberi* J. R. Drumm. in Paracetamol Induced Hepatotoxicity in Rats

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## Abstract

**Background:** *Tephrosia barberi* J. R. Drumm is a novel endemic perennial herb belonging to the Fabaceae family. **Aim:** *To investigate* the hepatoprotective activity of the whole plant of *T. barberi.* **Methods:** Preliminary phytochemical testing and GCMS analysis were done for the ethanolic extract of *T. barberi*. Initially, acute toxicity experiments were carried out in which five groups of rats (n=3) were administered with 0.5% carboxymethyl cellulose (normal control), ethanolic extract of *T. barberi* orally (5, 50, 300 and 2000 mg/kg b.w.) for 14 days daily, there was no mortality observed for 24 hr. For the assessment of the hepatoprotective potential, ethanolic extracts of *T. barberi* (200mg/kg low dose and 400mg/kg high dose) and silymarin (100mg/kg, employed as the reference standard), distilled water (normal control, paracetamol control) was given orally to five groups of rats (n=6) over seven days. All animals were given paracetamol on the fifth day, following the administration of standard and extracts, except for those in normal control. To assess the levels of serum enzymes, proteins, antioxidant indicators, and histopathological analyses, the rats were sacrificed and blood samples, liver homogenate, and liver sections were taken. **Results:** Rats fed with *T. barberi* ethanol extract (400mg/kg/day) and silymarin, a standard drug (100mg/kg/day) treated groups had significantly elevated liver serum enzymes such as alanine phosphatase, aspartate aminotransferase, alanine transaminase, total protein and total bilirubin than the intoxicated rats ($p<0.01$to $p<0.001$). Furthermore, the effect of antioxidant parameters (GSH, CAT, and SOD) on hepatic tissue showed that ethanol extract of *T. barberi* (high dose, 400mg/kg) produced improved hepatic function than toxic control rat and exhibited significant action compared to standard drug. **Conclusion:** The current research findings strongly indicate that *T. barberi* has promising hepatoprotective potential due to the presence of flavonoids and phenolic compounds.

**Keywords:** Acute Toxicity, GC-MS, Hepatoprotective, Phytochemical Analysis, *Tephrosia barberi*

**Abbreviations:** ALP - Alkaline Phosphatase, AST - Aspartate Transaminase, ALT - Alanine Transaminase, ROS - Reactive Oxygen Species, SOD - Superoxide Dismutase, CAT - Catalase, GSH - Glutathione Peroxidase, TBAR - Thiobarbituric Acid, EETB - Ethanol Extract of *Tephrosia barberi,* GCMS - Gas Chromatography and Mass Spectroscopy, ANOVA - Analysis of Variance and KAHE - Karpagam Academy of Higher Education

## 1. Introduction

The human body's largest and most important organ is the liver. The liver is crucial for both the metabolism of nutrients and the biotransformation of medications and chemicals, which shield the body from harmful external substances. It also produces bile, which promotes digestion and helps the body to get rid of impurities. Among other things, liver illness can be brought on by infections, genetic conditions, obesity, and alcoholism. Cirrhosis is a life-threatening

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illness caused by the persistent liver inflammation[1]. The pathogenetic investigations indicate that liver damage and cirrhosis are the first two signs of liver disease[2]. When unhealthy liver tissue is replaced with healthy liver tissue, the liver cannot function normally[3]. The traditional allopathic medications used to treat liver illness, such as corticosteroids, antiviral, immunosuppressive, etc., are inefficient rarely and might cause major hepatic damage[4]. Due to the lack of effective treatments for liver disorders, it is imperative to explore new solutions. Indigenous systems of medicine include a substantial inventory of materials that have been historically utilised to provide hepatic care. Researchers are continually searching for natural resources like herbal supplements to develop novel compounds as medications. In Tamil Nadu, a variety of herbs have been utilised successfully in Indian traditional remedies to treat liver damage and various liver cirrhosis[5]. Globally, plant-derived medicines are used as hepatoprotective medications, such as silymarin, a substance derived from milk thistle (*Silybum marianum*) seeds[6]. To treat drug-induced liver disorders, many herbs and their formulations are employed in traditional Indian medical systems and ethnomedical practices[7]. Sharma *et al.,* described the use of twenty-five different medicinal plants 'extracts to heal drug-induced hepatic destruction[8]. Recently, we published a comprehensive review showing the hepatoprotective potential of medicinal plants and chemicals derived from plants[9]. From the literature, *Tephrosia* species have been found to have considerable potential and engaged in several activities due to various phytoconstituents. The more frequently separated and recognised compounds in *Tephrosia* species were discovered to be flavonoids, terpenoids, rotenoids, sterols, essential oils, and fixed oils[10]. Several researchers reported that flavonoids produce significant effects on hepatoprotective activity. Hepatoprotective compounds from *Tephrosia* species have been reported by researchers[11,12], anti-oxidant[13], antidiabetic[14], anti-inflammatory[15], antiulcer, antidiarrheal, wound healing, insecticidal, antiviral, antiprotozoal, antifungal, antiplasmodial[16] and many more. *T.* barberi is a novel endemic medicinal perennial plant, which grows along the coastal tracts of Thirunelveli and Tuticorin districts, Tamil Nadu. Cough, dyspnea, chronic ulcers, bowel complaints, fever, abdominal issues, liver issues, and skin ailments are just a few of the vatham-based diseases which are treated by using this entire plant[17]. *T. barberi* has never been studied before. Thus, the current study was developed based on the hepatoprotective properties of other *Tephrosia* species and folklore assertion. Consequently, a first-ever attempt has been performed to assess the hepatoprotective effect of *T. barberi* whole plant extract.

## 2. Materials and Methods

### 2.1 Plant Material Collection

*Tephrosia barberi* plant was collected (morning) in 2021 February, in its natural habitat (coastal areas) around the districts of Thirunelveli and Ramanadapuram in Tamil Nadu, India. A voucher specimen (T30072101B/9/2021) was identified by the Central Siddha Research Institute, Arumbakkam, Chennai, and kept for further reference. The entire plant (leaves, stem, flower, fruit and root) was used for the research studies (Figure 1).

**Figure 1.** *Tephrosia barberi*, fruits of *T. barberi.*

## 2.2 Plant Extract Preparation

The collected plant material was washed two times with ordinary water and dried out below shadow for several days (37ºC). The shade-dried material was ground using a food chopper until it got a coarse form. *T. barberi* powder weighing about 500g had been macerated with 2L of petroleum ether to remove fat. Using filtration, the solvent was separated. The residual marc of *T. barberi* had undergone Soxhlet extraction using ethanol as solvent. The resulting extract was then dried in the open air and employed in the research that followed[18].

## 2.3 Preliminary Phytochemical Screening

As per standard procedure[19], the collected extract was used to carry out the systematic phytochemical analysis. Phytoconstituents were determined by the conventional process of testing for alkaloids, flavonoids, terpenoids, glycosides, steroids, tannins, saponins, carbohydrates, fixed oil, volatile oil, and proteins. The results have been tabulated.

## 2.4 GCMS Analysis of *Tephrosia barberi* Whole Plant

GC-MS analysis was carried out to determine the bioactive components present in the ethanolic extract of *T. barberi*. It was calculated by using a Shimadzu GCMS-QP2010 plus model that was heated for 10 minutes from a starting column temperature of 70°C to 280°C[20]. Chromatographic conditions have included a split mode column flow of high-purity helium at 2.0ml/min. In splitless mode, one microliter of the produced, 1%-diluted extracts with the appropriate solvents was injected. Retention duration and integral area of peaks were used to express the adequate quantity of the phyto components found in *T. barberi* ethanol extract. The similarity of components[21,22] that match with the NIST library[23] and results have been tabulated (Figure 2 and Table 2). Gas Chromatography-Mass Spectrometry (GC-MS) analysis of MeOH extract was performed using a GC-MS-QP 2010 (SHIMADZU, Kyoto, Japan) equipped with electron impact (EI) mode (ionizing potential-70eV) as well as a capillary column (VF-5 ms) (length 30m × diameter 0.25mm, film thickness 0.25µm) packed with 5% phenyldimethyl silicone. The ion source temperature was maintained at 240°C, and helium was utilized as a carrier gas with 99.99% purity. Samples were injected at a temperature of approximately 240°C with a split ratio of 10:1 and a flow rate of 1.51ml/min. Mass spectra were taken at 70 eV with scan fragments from 45 to 1000 Da and the total MS running time was 36 min. The number of molecules present in the leaf MeOH extract and their retention times are generated as a single spectral chromatogram. The spectrum data were compared with the National Institute of Standards and Technology (NIST) library to identify similar/ identical compounds. Results showed three major peaks (Figure 2) and nineteen minor peaks with 21 compounds, name of the identical/similar compounds, their retention times, % of peak area.

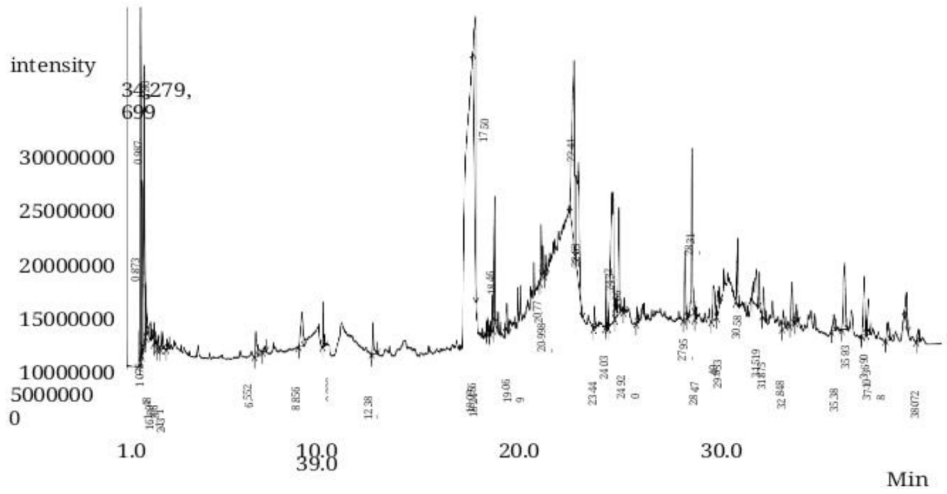



**Figure 2.** GCMS of ethanolic extract of *Tephrosia barberi.*

## 2.5 Hepatoprotective Activity by *In Vivo* Model

### 2.5.1 Experimental Animals

Wistar albino female rats ranging from 150–180 g were used for the hepatoprotective investigation. Wistar rats were acclimated privately in polypropylene enclosures with twelve hours of light/ dark cycle, at a temperature (23± 2 °C) or dew level of 50–60 %. The animals were given a regular pelleted meal to eat and had constant access to water[24]. Ethical approval had been received from KAHE (Approval no. KAHE/ IAEC/2021/11-09/002) Coimbatore, Tamil Nadu, India.

### 2.5.2 Acute Toxicity Study

The acute toxicity study was conducted and followed under the Organization for Economic Co-operation and Development (OECD)-423 guidelines, which were acknowledged by CPCSEA, Ministry of Social Justice and Empowerment, Government of India. Albino Wistar rats of both sexes were divided into five batches which contained three animals per batch. Rats were administered ethanol extract orally (5, 50, 300 and 2000 mg/kg b.w.) and with control (0.5% CMC). Individual animals were examined for the first 30 minutes, and then the following 24 hours for any signs of toxicity and mortality. There is no mortality observed for 24 hr, daily and till 14 days[25]. The test dose was calculated as per the Abirami *et al.,* method[26].

### 2.5.3 Paracetamol Induced Hepatotoxicity in Rat Model

According to the experimental protocol, five groups of thirty Wistar rats, each with six animals, were formed. On the fifth day, paracetamol was administered orally to four of the groups to create liver toxicity, while one group acted as the control. 200mg/kg and 400mg/kg of *T. barberi* ethanol extract were given orally every day for five days. Silymarin is a common drug[27] that is administered at a dose of 100mg/kg. Except for the control group, all other groups received paracetamol 2g/kg b.w. each group. The study code and dosage chart were given out:

I Group: Normal control [distilled water was given to six rats]

II Group: Toxic control [six rats were given only Paracetamol]

III Group: Drug control [six rats were administered with Paracetamol and Silymarin]

IV Group: Low dose [six rats were given with Paracetamol and EETB (200mg/kg)]

V Group: High dose [six rats were given Paracetamol and EETB (400mg)]

Following the treatment, the weights of the animals in each group were recorded. On the eighth day, blood was taken through retroorbital or cardiac puncture after 48 hours of pharmaceutical treatment to calculate biochemical parameters. Serum was isolated from the blood after blood was drawn, and liver function indicators like ALT, AST, ALP, total bilirubin, and total protein were assessed. On the eighth day, animals were sacrificed, and the liver tissue was removed, cleaned, and one part of it was turned into liver homogenate (pH 7.4) by using a 50mM potassium phosphate buffer in cold condition (4ºC) which was then centrifuged (3500 rpm) for10 to 20 minutes. Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH), and Thiobarbituric acid (TABR) components were measured in the supernatant as antioxidant enzyme parameters[28]. After being cleaned, the liver tissue was washed and preserved with formalin. Alcohol was used to dry the fixed liver before was paraffin-embedded. The slices were cut with a microtome, stained with hematoxylin and eosin, and then examined under a microscope to document the histological alterations.

### 2.5.4 Statistical Analysis

All the data are depicted as mean± standard deviation. All the result data was analysed by one-way analysis of variance test and Dunnet's test. Depending on the p values <0.05, 0.01, and 0.001 are considered significant.

# 3. Results

## 3.1 Preliminary Phytochemical Screening and GCMS Analysis of *Tephrosia barberi*

Preliminary phytochemical analysis demonstrated the existence of alkaloids, polyphenols, steroidal glycosides, and terpenoids in ethanol extract of *T. barberi* (Table 1). GCMS analysis showed that there are 46 compounds present in the ethanolic extract of *T. barberi* (Figure 2). Among these compounds,4-(methoxymethyl)-2-methylpyrido [3,2,4,5] thieno [3,2,4,5] pyrimido [1,6-a]

**Table 1.** Preliminary phytochemical screening of *T. barberi* ethanol extract.

| Phytoconstituents | Ethanol extract |
|---|---|
| **Alkaloids** | |
| Mayers reagent test | +++ |
| Dragendroff reagent test | +++ |
| **Glycosides** | |
| Salkowaski test | +++ |
| Libermannburchard test (steroidal glycosides) | +++ |
| **Flavanoids** | |
| Shinodatest | +++ |
| Acid test | +++ |
| Alkali test | +++ |
| **Steroids/ Terpenoids** | |
| Libermannburchard test | +++ |
| **Polyphenol** | |
| Ferric chloride test | +++ |
| **Tannins** | |
| General test | --- |
| **Saponins** | |
| Foam test | --- |
| **Volatile oils** | --- |

(+++) represents Presence and (---) represents Absence

benzimidazole (Molecular wt. 334), 4H-1-Benzopyran-4-one, (Molecular Wt. 268), 2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethoxy (Molecular Wt. 220), [1] benzothieno [2,3-c] naphtha [1,2-G) quinoline(Molecular Wt. 335), 2-Methoxy-4-Vinylphenol (Molecular Wt. 150), 4-([1E]-3-Hydroxy-1-propenyl)-2-methoxyphenol (Molecular Wt. 180) as alkaloid, flavonoid and polyphenolic compounds, which may play a significant role in the therapeutic potential of plant extract. Table 2 states the names of the compounds, along with their retention durations, peak area percentages, and molecular weights. According to a plethora of research studies, various steroids, triterpene, coumarin and flavonoid compounds have reactive properties which safeguard the liver[29]. In our study, the presence of benzothieno [2,3-c] naphtha [1,2-G) quinoline (coumarin), 4H-1-Benzopyran-4-one (flavones) may be chances of hepatoprotection of *T. barberi.*

## 3.2 Acute Toxicity Studies

Up to 2000mg/kg ethanolic extract of *T. barberi* was administered orally to the rats as part of the acute toxicity trials, but there was no harm, no death, and no observable behavioural patterns. It is the cutoff dose, and 200mg/kg and 400mg/kg (1/10 and 1/5 dose) were chosen as efficient doses for further research by toxicity recommendations.

## 3.3 Paracetamol Induced Hepatotoxicity in Wistar Rat Model

### 3.3.1 *Effect of Tephrosia barberi on Weight of the Animals*

When compared to the weight of the animals in the intoxicated group, the weight of the animals treated with the ethanolic extract of *T. barberi* and treated with standard medication significantly increased (p value< 0.005). The normal group's animals' weight gain was comparable to that of the treatment groups' animals (Table 3).

### 3.3.2 *Effect of Tephrosia barberi on Liver Serum Enzymes*

Serum biomarkers including ALT, AST, ALP, and total bilirubin can be used to measure liver function and damage. It is clear from Table 4 that rats who were given the overdose of paracetamol (2g/kg, group II) have significantly elevated serum enzyme levels ($p<0.01$ and $p< 0.001$). Normal rats had ALT, AST, and ALP levels of 98.35±0.428, 193.86±0.294, and 114.48±0.219 IU/L, respectively; as was predicted, paracetamol intoxication caused these levels to increase by 2.3, 2.2, and 2.06-fold, with values of 227.35±0.231, 426.41±0.222, and 235.56±0.166 IU/L, respectively. While total protein content has decreased 2.73 fold, total bilirubin content has increased 3.21fold. The data above showed that liver damage has been caused. Additionally, research was done on the effects of silymarin (Group III) and *T. barberi* (Group IV and Group V) on the liver serum enzymes. The levels of ALT, AST and ALP have been reduced compared to the levels of these enzymes in the intoxicated group indicating a considerable hepatoprotective impact[30]. Using the One-way ANOVA and Dennet test, the biochemical parameters of serum enzyme level were statistically analysed.

### 3.3.3 *Effect of Tephrosia barberi on Oxidative Stress in Liver Tissues*

According to the findings of the current study, liver GSH, CAT, and SOD levels were lower in toxic

**Table 2.** Identification of phytocompounds in *Tephrosia barberi* extract by GC-MS analysis

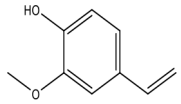
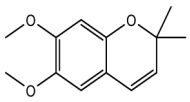
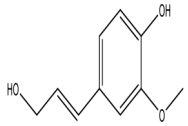
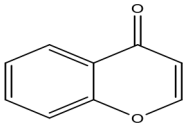
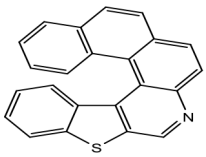
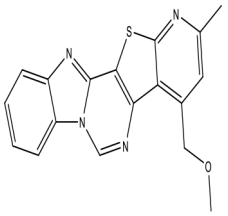
| Peak No. | Retention time | M. Wt | Compound Name | M. Formula | Structure |
|---|---|---|---|---|---|
| 13 | 12.388 | 150 | 2-Methoxy-4-Vinylphenol | $C_9H_{10}O_2$ |  |
| 17 | 18.465 | 220 | 2H-1-Benzopyran, 6,7-dimethoxy, 2,2-dimethoxy | $C_{13}H_{16}O_3$ |  |
| 18 | 19.069 | 180 | 4-((1E)-3-Hydroxy-1-propenyl-2methoxyphenol | $C_{10}H_{12}O_3$ |  |
| 31 | 28.470 | 268 | 4H-1-Benzopyran-4-One, | $C_{16}H_{12}O_4$ |  |
| 34 | 30.587 | 335 | [1]Benzothieno[2,3-C] Naphtho[1,2-G) napthoquinoline | $C_{23}H_{13}NS$ |  |
| 38 | 33.295 | 334 | 4-(Methoxymethyl)-2-Methylpyrido [3″,2″:4′,5′] Thieno[3′,2′:4,5] Pyrimido [1,6-A] Benzimidazole | $C_{18}H_{14}N_4OS$ |  |

**Table 3.** Comparison of the animal weight of normal control with experimental control rats

| Organ | Normal group | Toxic group | Standard (100mg/kg) | *T. barberi* low dose (200mg/kg) | *T. barberi* high dose (400mg/kg) |
|---|---|---|---|---|---|
| Weight of the rat (before treatment) | 181.2±1.18 | 184.8±0.583 | 187.2±2.354 | 185.8±1.114 | 186.4±1.208 |
| Weight of the rat (aftertreatment) | 230.6±1.07 | 204±0.707 | 232.2±1.067*** | 229±1.225*** | 230.6±0.927*** |

Values are expressed as Mean ± SEM (n=6), p< 0.005 (correlated to normal control and EETB treated group).

**Table 4.** Biochemical estimation of serum liver enzyme parameters on *Tephrosia barberi*

| Parameters | Normal control | Toxic control | Standard (100mg/kg) | *T.barberi* Low dose (200mg/kg) | *T. barberi* high dose (400mg/kg) |
|---|---|---|---|---|---|
| ALT | 98.35±0.428 | 227.35±0.231 | 126.35±0.291*** | 149.52±0.233** | 128.32±0.259*** |
| AST | 193.86±0.294 | 426.41±0.222 | 234.39 ±0.186*** | 305.71±0.240** | 247.31±0.235*** |
| ALP | 114.48±0.219 | 235.56±0.166 | 132.60±0.190*** | 168.74±0.218** | 146.26±0.150*** |
| Total Bilirubin | 0.086±0.051 | 0.276±0.0051 | 0.108± 0.0058*** | 0.162±0.0037** | 0.136±0.0051*** |
| Total Protein | 5.582±0.185 | 2.046±0.071 | 5.184±0.113*** | 4.643±0.071** | 4.927±0.197*** |

Mean ±SEM (n=6) *p<0.05 **p< 0.01 and ***p<0.001 vs Toxicated control (One way ANOVA and Dunnet'stest)

control rats than in healthy control rats. The increased levels of Thiobarbituric Acid Reactive Substances (TBARS) in rats with paracetamol intoxication show increased free radical production due to the lack of an antioxidant defence system and liver damage. The study exhibited an increased level of GSH, SOD, and CAT enzymes in *T. barberi* whole plant-treated rats due to a reduction of oxidative stress, which leads to a higher level of antioxidant effect. In our study, *T. barberi* ethanol extract at a high dose (400mg/kg) demonstrated enhanced liver protection when the hepatic indicators and oxidative signs returned to normal due to the antioxidant effect. Additionally, *T. barberi* ethanol extract (400mg/kg/day) demonstrated hepatoprotective effectiveness comparable to (100mg/kg/day) silymarin, a common drug (Table 5). These reports confirm previous findings regarding the increase of liver enzymes and antioxidant enzymes after the treatment of different plant extracts' hepatoprotective effects[31-34].

### 3.3.4 Histopathological Examination

For screening of hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide and then excreted by the kidney. The reactive metabolite N-acetyl-p-benzoquinoneimine, which induces oxidative stress and glutathione depletion and results in liver necrosis, seems to be related for paracetamol hepatotoxicity[35]. When a portion of paracetamol is metabolised by cytochrome P-450, harmful substances are developed[36]. The toxic metabolite damages the liver, which disrupts the hepatocytes' capability to transport substances, producing plasma membrane breaches and a change in the serum's number of enzymes the rat, there was no mortality, and there were no behavioural patterns visible in the physical observations. It is the cutoff dose, and by toxicity guidelines, 200mg/kg and 400mg/kg were selected as the effective doses for additional studies. Rats showed a decrease in all hepatic antioxidant levels (Table 6).

Histological abnormalities that show injured hepatic cells, centre of the cell, primary vein, endothelium, and sinusoids are symptoms associated with severe hepatic damage. The observations of the normal control group liver demonstrated prominent cell arrangement with independent liver cells and capillary deficiencies. The central vein remains cleared and there is no evidence of congestion (Figure 3a). The toxic control group liver TS showed disorder of hepatic cells with proliferation, vascular, cellular deterioration, cellular necrosis, aggregation of morpho nucleus with inflamed and congestion (Figure 3c). The standard control rat liver showed regenerated inflammatory cells with normal hepatocytes. There is no evidence of cellular necrosis and absence of nucleus aggregation. No congestion was observed (Figure 3b). The rats receiving low doses of ethanol extract of *T. barberi* treatment showed mild disarrangement of hepatocytes with centrilobular necrosis. An inflammatory cell with mild polymorphic nuclear aggregation was seen. Evidence for regeneration of vascular cells was noted with mild congestion (Figure 3d). The high doses (400mg/kg) of ethanol extract of *T. barberi* treated liver showed normal hepatic cell structure with discrete hepatic cells, sinusoidal gaps and central vein. There is no evidence for cellular necrosis or hyperplasia. The vascular cells remained normal, and congestion was not observed (Figure 3e).

**Table 5.** Effect of *Tephrosia barberi* on the antioxidant enzymes in the liver

| Parameters | Normal | Toxic control | Silymarin (100mg/kg) | *T. barberi* (200mg/kg) low dose | *T. barberi* (400mg/kg) high dose |
|---|---|---|---|---|---|
| **SOD** (unit/ mg tissue protein) | 5.97±0.221 | 1.90±0.275[a] | 2.81± 0.113* | 3.93±0.391** | 4.52±0.197** |
| **CAT** (µmol $H_2O_2$/mg tissue protein) | 14.42±0.569 | 8.44± 0.646[a] | 10.85±0.647* | 11.09±0.413** | 13.06±0.447** |
| **GSH** (µg /mg protein) | 4.60± 0.176 | 1.46± 0. 243[a] | 2.16± 0.196* | 54±0. 168* | 3.65± 0.212** |
| **TBARS** (nmole/mg tissue protein) | 2.67± 0.207 | 7.39± 0.163[a] | 6.08± 0.239 | 5.49±0.150** | 4.14± 0.146** |

Values indicated as the Mean ± SEM (n=6), Standard group (received silymarin in addition to paracetamol) and the toxic control group (which received paracetamol alone) are connected. Normal and drug control groups have relationships with EETB-treated groups) *p <0.05 **p< 0.01vs toxic control group, values are statistically symbolic (one-way ANOVA).

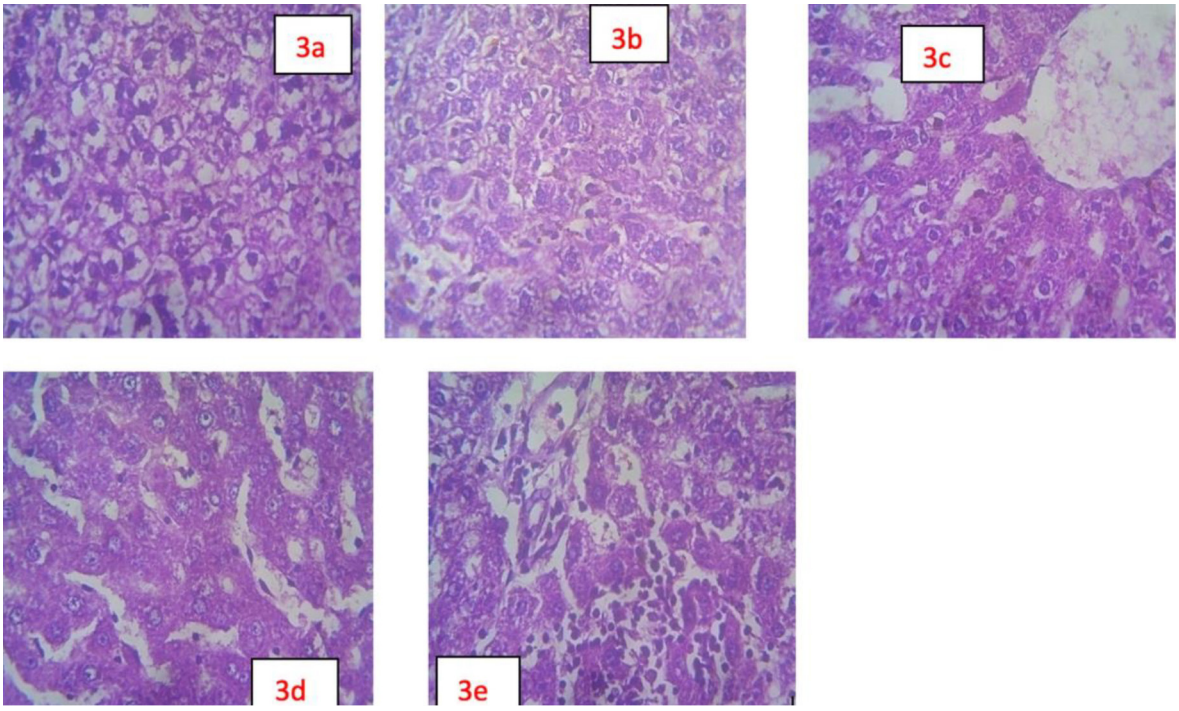



**Figure 3.** Histopathological studies on *T. barberi*-treated liver tissue include 3a. Prominent cell arrangement of liver (normal liver) 3b. Regenerated inflammatory cells with normal hepatocytes (standard) 3c. Disorder of hepatic cells with proliferation, vascular, cellular deterioration, cellular necrosis, aggregation of morpho nucleus with inflamed and congestion (toxic control) 3d. Regeneration of vascular cells noted with mild congestion (EETB Low dose) 3e. No evidence of cellular necrosis or hyperplasia and vascular cells remain normal (EETB high dose).

## 4. Discussion

In the current work, the phytochemical analysis, GCMS analysis, acute toxicity and *in vivo* experimentally induced hepatotoxicity in a Wistar rat model using *T. barberi* ethanol extract have all been evaluated. The preliminary phytochemical analysis showed the presence of phytocompounds such as alkaloids, flavonoids, polyphenols, steroids, and glycosides in the ethanol extract of *T. barberi.* These constituents were also supported by GC-MS analysis. Acute toxicity studies revealed no morbidity and no signs of toxicity when rats were administrated with the ethanolic extract of *T. barberi*. The dependable method of paracetamol-induced hepatotoxicity has been employed for the screening of hepatoprotective drugs. Hence, the ethanol extract of *T. barberi* was evaluated for its hepatoprotectivity in Wistar rats. Increased lipid peroxidation, ROS, altered membrane fluidity and permeability, increased rates of protein degradation, and cell death are all consequences of paracetamol liver injury. These events also lead to the efficient inhibition of immune cells. The main site of AST is in the mitochondria of hepatocytes. Although the liver depends on a protein called ALT, it is a better indicator of liver impairment. Serum levels of bilirubin and ALP are also related to causing damage to liver cells[37]. The effect of *T. barberi* on serum liver enzymes was evaluated. The overdose (2kg/kg) of paracetamol administration caused severe liver damage and increased serum levels of hepatic biomarker enzymes[38,39]. To demonstrate hepatic damage, biochemical markers such as ALP, ALT, and AST were commonly utilised[40]. These enzymes are intracellular and are liberated into the bloodstream after physiological disturbances, which indicate the development of hepatotoxicity. Administration of paracetamol-induced a considerable augmentation of their levels, including AST, ALT, ALP, and bilirubin levels[41]. The rats that were given paracetamol overdose (2g/kg, group II) had elevated serum enzyme levels significantly ($p<0.01$ and $p< 0.001$). Furthermore, the effects of silymarin (Group III) did not differ significantly from those of untreated control rats (Group I). We also investigated the effect of *T. barberi* on oxidative stress in liver tissue. The free radicals damage the living cells and

produce malonaldehyde, which leads to the formation of TBAR, which is the measurement of antioxidant defence mechanisms[42]. The results of the present investigation have found that liver GSH, CAT, and SOD levels were lower in toxic control rats than in normal control rats. TBARS levels are elevated in paracetamol-treated rats, indicating increased free radical formation due to the absence of an antioxidant defence process and liver injury. *T. barberi* ethanol extract (400mg/kg/day) had produced hepatoprotective efficacy significant to (100mg/kg/day) silymarin, a standard medication. According to histopathological investigations of the liver, silymarin, a common medication, and plant extracts have an inhibitory effect against paracetamol toxicity due to modulating antioxidant defence status. From these findings, the ethanol extract of the whole plant of *T. barberi* was found to have promising hepatoprotective properties by paracetamol-induced hepatotoxicity in a Wistar rat model.

## 5. Conclusion

The current study proved that the ethanol extract of *T. barberi* prevented lipid peroxidation and promoted enzymatic and nonenzymatic (antioxidant) protection systems by paracetamol-induced hepatotoxicity in a Wistar rat model. Hence, the whole plant of *T. barberi* ethanol extract proved traditional uses of hepatoprotective activity *by in vivo* studies due to the existence of its phytoconstituents such as steroids, alkaloids, polyphenols, flavonoids, and glycosides and GCMS analysis. As a result, *T. barberi* was investigated as a novel, powerful hepatoprotective agent and a successful herbal treatment for liver illness.

## 6. References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