# HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *TEPHROSIA PURPUREA* WHOLE PLANT AQUEOUS EXTRACT

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## Summary

In the present study, the hepatoprotective and antioxidant activity of *Tephrosia purpurea* whole plant aqueous extract were evaluated using Carbon tetrachloride ($CCl_4$) induced hepatic damage model in rats. 75mg/kg and 150mg/kg of the aqueous extract were administered orally once daily for seven days. Animals were challenged with $CCl_4$ on the fifth day of treatment protocol. Elevated serum levels of transaminases, alkaline phosphatase and bilirubin (both direct and total) were restored significantly by the extract. Silymarin (reference standard) exhibited significant hepatoprotective activity against $CCl_4$ induced hepatotoxicity. The biochemical parameters were supplemented with histopathological studies. The results of the present study strongly indicate that *Tephrosia purpurea* whole plant aqueous extract has potent hepatoprotective action against $CCl_4$ induced hepatic damage in rats. *In vitro* free radical scavenging activity was screened and results concluded that possible mechanism of hepatoprotective activity may be due to its free radical scavenging activity.

**Keywords:** Carbon tetrachloride, Silymarin, *Tephrosia purpurea*, Hepatoprotective.

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## Introduction

Liver has a pivotal role in metabolism of many endogenous and exogenous compounds. Any type of injury (systemic drugs, food preservatives, agrochemicals and alcohol) or impairment of its functions leads to many complications[1]. Significant advances in drug research not withstanding, effective pharmacological strategies are not available to counter deteriorating liver function that occurs as a result of exposure to drugs and other environmental poisons. Indigenous systems of medicine have a strong repository of products that have been used traditionally to offer some sort of liver protection[2]. Lack of proper treatment for liver diseases makes it imperative to search for new alternatives.
*Tephrosia purpurea* (Linn) Pers (Leguminosae) known in Sanskrit as "*Sarwa wranvishapaka*" (Sharapunkha) which means that it has the property of healing all types of wounds [3]. Various parts of this plant were used as remedy for impotency, asthma, diarrhoea, gonorrhoea, rheumatism, ulcer, urinary disorders and diseases of kidney, liver, spleen, heart and blood [4,5]. The present study explores the protective ability of *Tephrosia purpurea* in an animal model of hepatotoxicity in rats.

## Materials and methods

### Animals

30 Wistar albino rats of either sex weighing between 150-200 g were obtained from NIMHANS Bangalore. The animals were housed in standard conditions of temperature and humidity with natural light and dark cycle. They were fed with standard pellets diet and water *ad libitum*. Animals were acclimatized for one week prior to experimentation. Prior to the experiment Institutional Animal Ethics Committee clearance was obtained (IAEC/NCP/06/09).

### Plant material

The aqueous extract of *Tephrosia purpurea* was obtained from Shrushti herbals, Bangalore Karnataka and certificate of analysis was obtained from Chemiloids, Vijaywada.

### Preliminary phytochemical studies

Preliminary phytochemical studies were carried out for aqueous extract of *Tephrosia purpurea.*

### Acute toxicity studies

The acute toxicity study for whole plant aqueous extract of *Tephrosia purpurea* was performed using Wistar rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The extract was administered orally at a dose of 2000mg/kg[6].

**Carbon tetrachloride induced hepatotoxicity [7,8]**

Rats were divided into five groups (n=6). Hepatic injury was produced in rats by oral administration of single dose of $CCl_4$ (1.4 ml/kg) on fifth day of treatment protocol. Carbon tetrachloride was mixed with olive oil in the ratio of 1:1. Silymarin (25mg/kg), a known hepatoprotective agent was used as reference standard. Animals were screened for two different doses of aqueous extract of *Tephrosia purpurea* (75mg/kg and 150 mg/kg, p.o).
Group I: Vehicle control (1.0 ml, p.o.) for 7 days
Group II: Treated with vehicle (1.0 ml, p.o.) for 7 days and $CCl_4$ on fifth day (1.4 ml/kg, p.o.)
Group III: Treated with Silymarin (25 mg/kg, p.o.) for 7 days and $CCl_4$ on fifth day (1.4 ml/kg, p.o.)
Group IV: Treated with aqueous extract of *Tephrosia purpurea* (75mg/kg, p.o.) for 7 days and $CCl_4$ on fifth day (1.4 ml/kg, p.o.)
Group V: Treated with aqueous extract of *Tephrosia purpurea* (150mg/kg, p.o.) for 7 days and $CCl_4$ on fifth day (1.4 ml/kg, p.o.)

At the end of the treatment protocol animals of all groups were sacrificed. Blood was collected, allowed to clot and centrifuged at 3000rpm for 10 minutes and serum was separated. The serum levels of marked enzymes viz.. ALP, SGOT, SGPT and bilirubin (Direct and Total) were measured by using semi auto analyser. All enzyme estimations were assayed using assay kits (Preicugent, Pinnacle biotechnologies Ltd. Mumbai). Liver were isolated and kept in 10% buffered formalin solution and processed for histopathological studies.

***In vitro* free radical scavenging activity by DPPH method [9].**

Free radical scavenging potentials of aqueous extract was tested against methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The change in the absorbance observed at 517nm has been used as a measure of antioxidant activity.
DPPH stock solution (100 µM): 3.94 mg of DPPH was dissolved in 100 ml of analytical grade methanol. Ascorbic acid was taken as standard and prepared in different concentration ranging from 100 to 1000 µg/ml. Aqueous extract was dissolved in methanol and different concentration were prepared (100 to 1000 µg/ml). 100 to 1000 µg/ml of ascorbic acid, aqueous extract were taken in different test tubes. Then the volume was adjusted to 1000 µl with methanol. To this 3 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for 20 minutes. The blank solution was prepared with methanol and readings were taken for blank (methanol), standard and aqueous extract at 510 nm.

Scavenging activity was expressed as the inhibition percentage calculated using the following formula,

***% inhibition*** = *Abs blank – Abs test drug / Abs blank* x 100

**Statistical analysis**

Data were expressed in mean ± S.E.M. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnet's '*t*' test. All statistical tests were performed by using statistical software of Graph Pad PRISM (version 4.0). P<0.05 was considered statistically significant.

## Results

### Preliminary phytochemical studies

Preliminary phytochemical studies showed the presence of alkaloids, glycosides, flavonoids, carbohydrates, tannins and saponins.

### Acute toxicity studies

Aqueous extract of *Tephrosia purpurea* did not show any sign and symptoms of toxicity at dose 2000mg/kg in Wistar rats. Doses selected were 3.75% and 7.5% of the dose tested for acute toxicity i.e. 75mg/kg and 150mg/kg respectively.

### Effect of *Tephrosia purpurea* on liver marker enzymes

The results of aqueous extract of *Tephrosia purpurea* in $CCl_4$ intoxicated rats are shown in Table No.1. In $CCl_4$ intoxicated group (II) serum levels of ALP, SGOT, SGPT and bilirubin (both direct and total) were significantly increased when compared to vehicle control group (I). The elevated levels of serum ALP, SGOT, SGPT and bilirubin (both direct and total) significantly reduced in group (III,IV, V) treated with silymarin and aqueous extracts of *Tephrosia purpurea* 75mg and 150mg/kg respectively when compared to $CCl_4$ intoxicated group.

**Table No.1 Effect of aqueous extract of *Tephrosia purpurea* in $CCl_4$ intoxicated rats**

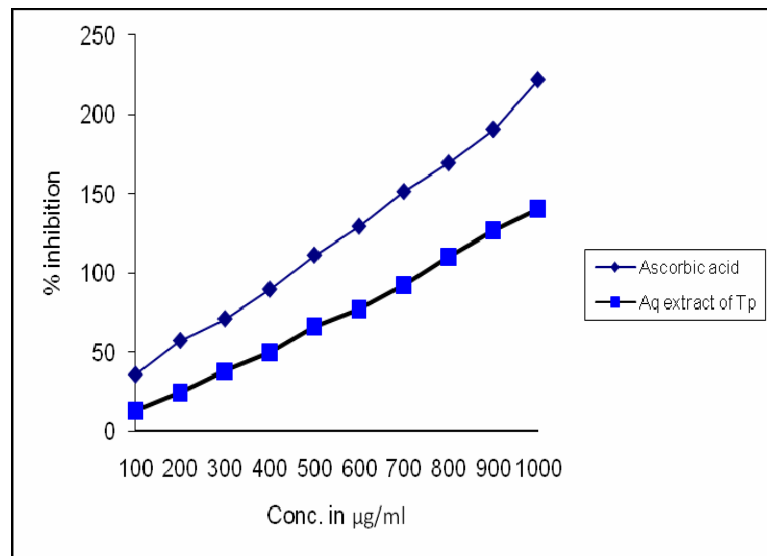
| Groups | ALP IU/L | SGOT IU/L | SGPT IU/L | Bilirubin mg/dl | |
|---|---|---|---|---|---|
| | | | | Direct | Total |
| **Group I** | 42.5±2.3 | 61.0±1.55 | 27.5±0.56 | 0.13±0.004 | 0.05±0.005 |
| **Group II** | 330.5±11.48***a | 246±15.52***a | 263.3±8.01***a | 1.39±0.076***a | 1.05±0.005***a |
| **Group III** | 54.5±6.05***b | 94.6±7.62***b | 80.11±1.26***b | 0.13±0.005***b | 0.11±0.004***b |
| **Group IV** | 61.3±8.66***b | 152.9±6.10**b | 195±3.2***b | 0.27±0.021***b | 0.19±0.003***b |
| **Group V** | 55.0±8.57***b | 130±6.60***b | 128±1.39***b | 0.22±0.010***b | 0.15±0.003***b |

All values are expressed as mean± S.E.M **P<0.01, ***P<0.001
a: compared with vehicle control group.
b: compared with $CCl_4$ intoxicated group

### DPPH- scavenging activity

Fig.No.1 illustrates a significant decrease in the concentration of DPPH radicals due to scavenging ability of the aqueous extract. The result indicates that aqueous extract has better scavenging activity with increasing concentration. The $IC_{50}$ value of aqueous extract was found to be 400µg/ml.

**Fig. No.1 Effect of aqueous extract of *Tephrosia purpurea* (*Tp*) on DPPH scavenging activity (*in vitro*)**




$IC_{50}$ of Ascorbic acid 174.79µg/ml
$IC_{50}$ of aqueous extract of *Tp* 400 µg/ml

## Histopathological studies

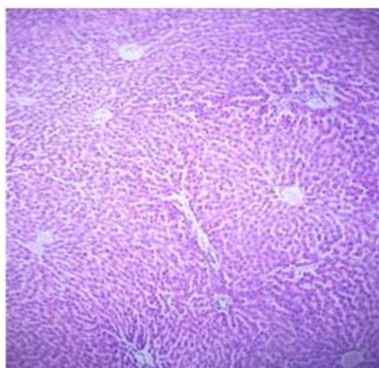


Fig.No.2 Section of rat liver treated with vehicle control shows normal hepatic cells, central vein sinusoids with normal texture. HxE 10x

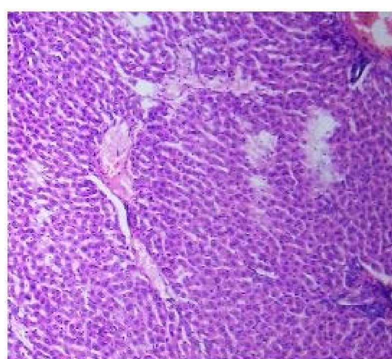


Fig.No.3 Section of rat liver treated with $CCl_4$ shows damaged hepatic cells, central vein, nucleus, endothelium and sinusoids. HxE 10x

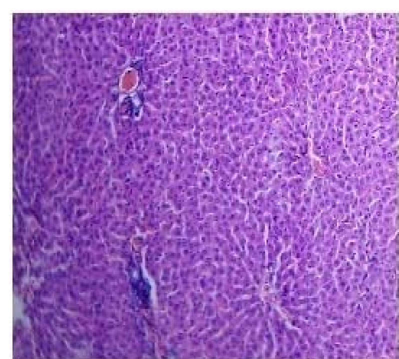


Fig.No.4 Section of rat liver treated with of Silymarin and $CCl_4$ treated shows regeneration of hepatic cells, central vein, nucleus, endothelium and sinusoids. HxE 10x

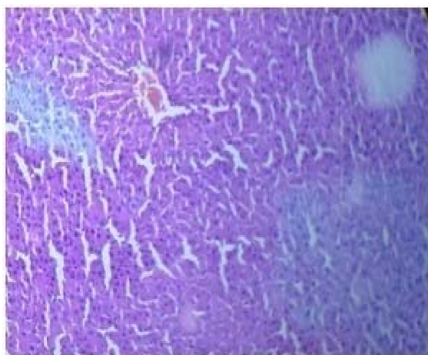


Fig.No.5 Section of rat liver treated with aqueous extract of *Tephrosia purpurea* (75mg/kg) and $CCl_4$ treated shows regeneration of hepatic cells, central vein, nucleus, endothelium and sinusoids. HxE 10x

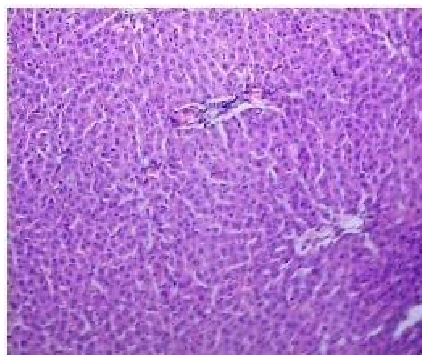


Fig.No.6 Section of rat liver treated with aqueous extract of *Tephrosia purpurea* (150mg/kg) and $CCl_4$ treated shows regeneration of hepatic cells, central vein, nucleus, endothelium and sinusoids. HxE 10x

## Discussion and conclusion

In the present study aqueous extract of *Tephrosia purpurea* was evaluated for its hepatoprotective activity using $CCl_4$ induced hepatotoxicity in rats. An attempt was made to find out the correlation between hepatoprotective and anti oxidant property. Carbon tetrachloride is a commonly used hepatotoxin to induce hepatotoxicity in experimental rats, which produces pathological conditions similar to viral hepatitis, fatty liver due to alcohol consumption or cirrhosis. The hepatotoxic effect of $CCl_4$ is largely due to its active metabolite trichloromethyl radical that binds to macromolecules and induces lipid peroxidative degradation of membrane lipids of endoplasmic reticulum that is rich in polyunsaturated fatty acids[10]. This leads to formation of lipid peroxide, which in turn produces a toxic aldehyde that causes damage of liver. Elevation in serum marked enzyme levels, namely ALP, SGOT, SGPT and bilirubin (both direct and total) may be due to cellular leakage, loss of functional integrity of the cell membrane[11-12].

Administration of $CCl_4$ causes severe injury in rat's liver shown by increase in serum enzymes and bilirubin. This is supported by histopathological changes which shows damaged hepatic cells, central vein, nucleus, endothelium and sinusoids (Fig.No. 3). Animals treated with silymarin and aqueous extract (75mg/kg and 150mg/kg, p.o.) significantly reduced the increased serum levels of ALP, SGOT, SGPT and bilirubin (both direct and total) showing the hepatoprotective efficacy of *Tephrosia purpurea.* Histopathological studies also showed a moderate protection when compared to $CCl_4$ induced group (Fig.No. 4, 5 and 6). Therefore, the possible hepatoprotective mechanism may be due to change in cytochrome-$P_{450}$ activity or preventing the formation of lipid peroxidation[13].

Preliminary Phytochemical analysis of the extract has shown the presence of flavonoids and phenolic compounds, which have been known for their antioxidant and hepatoprotective activities[14-15]. Anti oxidant property was assayed by *in vitro* DPPH scavenging activity. Lipid peroxidation is accelerated when free radicals are formed as a result of loosing a hydrogen atom from double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidant mechanisms to inhibit the chain reaction of lipid peroxidation[9]. The degree of discoloration indicates the scavenging potentials of the antioxidant activity.

The results of DPPH scavenging activity of *Tephrosia purpurea* suggest that aqueous extract exerts protective action against pathological alteration caused by $CCl_3^{\bullet}$ free radicals. Ascorbic acid is used as standard to evaluate significance of free radical scavenging activity of aqueous extract.

Thus, it can be concluded that aqueous extract of *Tephrosia purpurea* has hepatoprotective activity which is probably due its free radical scavenging activity.

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