

Hepatoprotective and Antioxidant potential of *Tephrosia Purpurea* in Paracetamol Induced Hepatotoxicity

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Abstract

The present study was designed to evaluate Hepatoprotective and antioxidant potential of *Tephrosia Purpurea* whole plant aqueous extract in paracetamol intoxicated rats. 75mg/kg and 150mg/kg of aqueous extract were administered orally once a daily for seven days. Animals were challenged with paracetamol on 5th day of treatment protocol. Elevated serum levels of transaminases, alkaline phosphatase and bilirubin (direct and total) were restored significantly by the extract. Silymarine (reference standard) exhibited significant hepatoprotective activity against paracetamol induced hepatotoxicity. The biochemical parameters were supplemented with histopathological studies. 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: Hepatoprotective, *Tephrosia purpurea*, paracetamol, silymarine.

1. Introduction

The Liver is largest organ in the body carrying out most of the biochemical synthesis and secretory functions. Living in a world of inadequately controlled environment pollution and expanding therapy with potent drugs, it is continuously exposed to variety of xenobiotics and therapeutic agents resulting in its structural or functional damage. Any type of injury (systemic drugs, food preservatives, agrochemicals and alcohol) or impairment of its functions leads to many complications [1].

The conventional allopathic drugs used in the treatment of liver disease like corticosteroids, antiviral, immunosuppressant etc. agents are sometimes inadequate and may lead to serious liver toxicity itself. Indigenous system of medicine has a strong repository of products that have been used traditionally to offer some sort of liver protection. Limitations of proper treatment for liver diseases make it imperative to search for new alternatives [2]. The major factors that contribute to Liver disease becoming a global problem because of increasing alcohol consumption in both developed and developing countries, Malnutrition, Anemia, Infection and availability of hepatotoxic drugs over the counter. The conventional drugs used in the treatment of liver diseases viz; Corticosteroids, Anti-viral and Immunosuppressant agents are sometimes inadequate and may lead to serious adverse effects. Paradoxically, they may themselves cause hepatic damage, as exemplified by cholestatic jaundice with azathioprine and elevation of serum transaminases by interferon and virazole. It is therefore, imperative to search for better drugs to treat liver diseases [3][4].

In the Indian traditional system of medicine, numerous medicinal plants are in use for liver disorders and as hepatoprotective, of these *Tephrosia purpurea* is in use as a constituent of a large number of formulations available in the market. Ayurvedic literatures advocate the use of *Tephrosia purpurea* in liver disease [5][6] *Tephrosia purpurea* (Linn.)Pers. also known as Sarponkha, Empali, Vempali, Thila, occurs throughout Indian subcontinent and also cultivated for manurial purposes. *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. Various parts of this plant were used as remedy for impotency, asthma, diarrheas, rheumatism, ulcer, urinary disorders and diseases of kidney, liver, spleen, heart and blood. The present study explores the potential ability of *Tephrosia purpurea* in an animal model of hepatotoxicity in rats. [7][8][9]

2. Materials and Methods

2.1. Experimental animals: [10]

Albino rats of either sex of wistar strain, weighing between 150-200 g procured from Raghendra animal suppliers and maintained in standard laboratory condition of temperature, humidity, and 12 hours light and dark cycle,

normal diet and water *ad libitum*. 30 animals were divided in 5 groups, 6 animals in each group and housed in polypropylene cages. Prior to the experiment Institutional animal ethics committee clearance was obtained (IAEC/NCP/06/09)

2.2. Preparation of plant extract:

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

2.3. Preparation of hepatotoxicant: [11][12]

Acute liver damage was produced in animals by oral administration of Paracetamol 1g/kg of body weight in 2% acacia solution.

2.4. Acute toxicity studies: [13]

The acute toxicity study for whole plant extract of *Tephrosia purpurea* was performed using wistar rats. The animals were fasted overnight prior to the experiment and maintained using standard condition. The extract was administered orally at dose of 2000mg/kg.

2.5. Experimental design: [14][15]

Group I: animals received 2% w/v gum acacia orally for 7 days.

Group II: animals were administered with paracetamol in dose of 1g/kg body weight p.o. on the fifth day.

Group III: animals were administered with silymarine in a dose of 25mg/kg body weight p.o. for seven days. On the fifth day paracetamol was administered in a dose of 1g/kg body weight p.o.

Group IV: animals were administered with *Tephrosia purpurea* aqueous extract in dose of 75mg/kg body weight p.o. for seven days. On the fifth day paracetamol was administered in a dose of 1g/kg body weight p.o.

Group V: animals were administered with *Tephrosia purpurea* aqueous extract in dose of 150mg/kg body weight p.o. for seven days. On the fifth day paracetamol was administered in a dose of 1g/kg body weight 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 analyzer. All enzyme estimations were assayed using assay kits (precugent, pinnacle biotechnologies Ltd. Mumbai). Liver were isolated and kept in 10% buffered formalin solution and processed for histopathological studies.

2.6. In vitro free radical scavenging activity by DPPH method: [16]

Free radical scavenging potential of aqueous extract was tested against methanolic solution of 2, 2-diphenyl 1-1-picrylhydrazyl (DPPH). The change in the absorbance observed at 517nm has been used as a measure of antioxidant activity.

DPPH stock solution (100uM): 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). 100 to 1000 µg/ml of ascorbic acid, aqueous extract were taken in different test tubes, and then 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.

$$\% \text{ Inhibition} = \frac{\text{Abs} - \text{Abs test drug}}{\text{Abs blank}} \times 100$$

2.7. Statistical analysis:

Data were expressed in mean \pm S.E.M. the statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's t test. All the statistical tests were performed by using statistical software of Graph pad PRISM (version 4). P<0.05 was considered statistically significant.

2.8. 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.

2.9. Collection of serum: [12][15]

After the last dose of test drug on 7th day one ml of blood was collected from retro orbital sinus using a capillary tube in a clean and dry test tube. The blood was allowed to coagulate for 30 min. and centrifuged at 3000 rpm for 15 minutes. The supernatant serum was separated and used for marker enzyme estimation like SGOT, SGPT, ALP and bilirubin (total and direct). All enzyme estimation was done by Auto biochemistry analyzer

3.0. Histopathology: [14][15]

The animals were sacrificed by cervical dislocation; liver was removed and washed with cold saline solution. The liver was pressed between filter paper pads and sent for histopathology studies.

4. Results and discussion

Paracetamol hepatotoxicity is caused by the reactive metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion, produces hepatic necrosis at higher doses [18]. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for Paracetamol induced hepatotoxicity [10][15]. Normally, SGOT and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. Hepatoprotective activity of *Tephrosia purpurea* were screened against the Paracetamol induced liver damage. Following table shows the effect of paracetamol, *Tephrosia purpurea* and silymarine on levels of liver enzymes.

Table 1 showed significantly increased ($P < 0.001$) levels of ALP, SGOT, SGPT and bilirubin (direct and total) on administration of high dose of Paracetamol when compared with control group rats. Group of animals treated with aqueous extract of *Tephrosia purpurea* shows significantly decreased ($P < 0.001$) serum levels of liver marker enzymes. When there is hepatopathy, along with all enzymes bilirubin leaks into the blood stream. The elevated serum levels of bilirubin both direct and indirect are showed in Table in paracetamol intoxicated rats. Aqueous extract of *Tephrosia Purpurea* treated rats has showed significantly decreased ($P < 0.001$) serum levels of bilirubin (direct and indirect) Fig.No.5.9 and 5.10 shows graphical comparison of serum bilirubin level of different groups intoxicated with high dose of Paracetamol. As mentioned earlier Paracetamol depletes the glutathione stores. Glutathione acts as natural antioxidant and takes part in conjugation reaction to remove toxic metabolites. *Tephrosia purpurea* has been screened for its antioxidant activity this might be the clue, why *Tephrosia purpurea* has Hepatoprotective activity.

The antioxidant potential of *Tephrosia purpurea* was determined against the ascorbic acid by using DPPH method. The results suggest that aqueous extract exerts protective action against pathological alteration caused by excess dose of paracetamol. Fig 1 shows the IC_{50} value of *Tephrosia purpurea* and ascorbic acid

Administration of paracetamol causes severe liver injury and increases blood serum level of liver marker enzymes which were analyzed by physiochemical methods. Whereas the actual liver damage is supported by histopathological changes which shows damaged hepatic cells, central vein, nucleus, endothelium and sinusoids. Animal treated with silymarine and aqueous extract of *Tephrosia purpurea* (75mg/kg and 150mg/kg) significantly reduces damage and gives liver protection as compared to paracetamol. The mechanism of liver protection may be due to preventing the formation of lipid peroxidation.

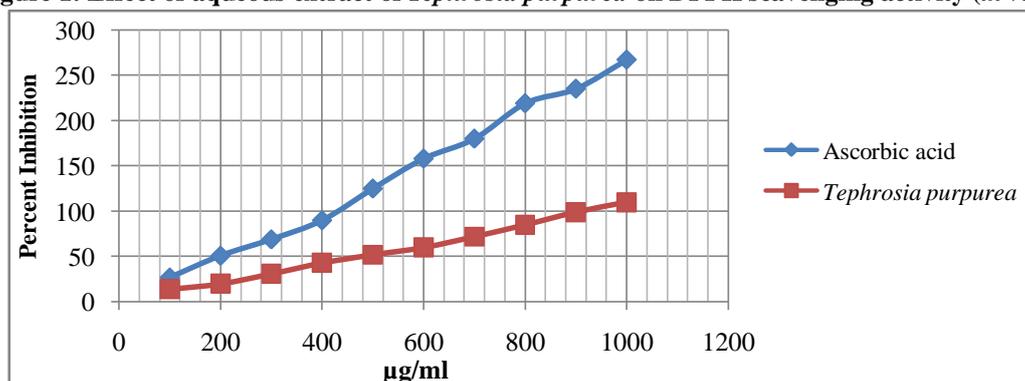
Preliminary phytochemical analysis of extract has shown the presence of flavonoids and phenolic compounds, which have been known for their anti-oxidant and Hepatoprotective activities. Anti-oxidant activity was assayed by in vitro DPPH scavenging activity. Lipid peroxidation is accelerated when free radicals are formed as a result of losing a hydrogen atom from double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major anti-oxidant mechanisms to inhibit the chain

Table 1: effect of aqueous extracts of *Tephrosia purpurea* in paracetamol intoxicated rats

Groups	ALP IU/L	SGOT IU/L	SGPT IU/L	Bilirubin Mg/dl	
				Direct	Total
Group I	0.893±0.08	66.0±3.83	39.3±2.07	0.05±0.005	0.04±0.009
Group II	257.5 ± 1.95***a	217±34.01***a	151.3±5.67***a	0.18 ± 0.027***a	0.18±0.025***a
Group III	44.3 ± 0.84***b	135.5±6.94***b	54.5±1.97***b	0.10 ± 0.003***b	0.10±0.015***b
Group IV	60.0 ± 2.33***b	146.2±7.60***b	59.1±1.64***b	0.12 ± 0.015***b	0.11±0.013***b
Group V	32.00 ± 1.33***b	135.0±8.55***b	53.1±2.15***b	0.13 ± 0.004**	0.11±0.098***b

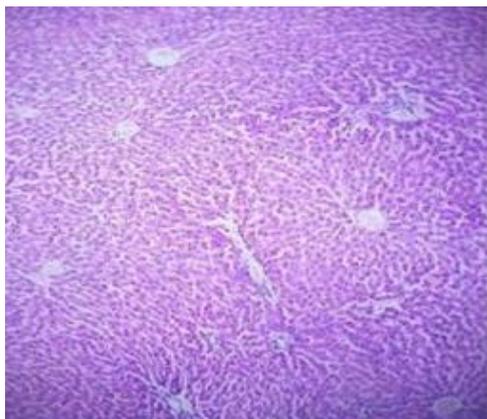
All values are expressed as mean± S.E.M. ** $P < 0.01$, *** $P < 0.001$. a: compared with vehicle control group I b: compared with paracetamol intoxicated rats.

Figure 1: Effect of aqueous extract of *Tephrosia purpurea* on DPPH scavenging activity (in vitro)

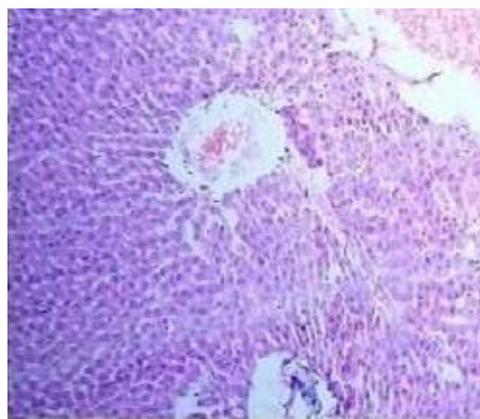


IC_{50} ascorbic acid 200µg/ml; IC_{50} of *Tephrosia purpurea* 500µg/ml

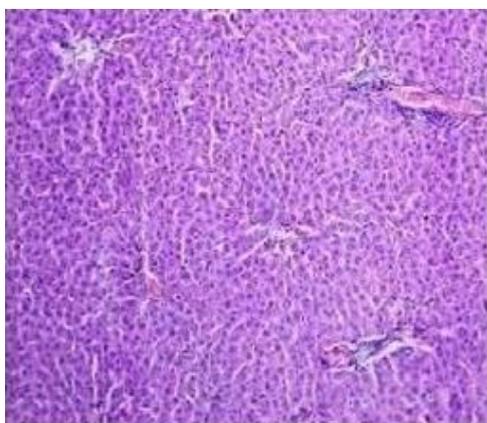
Figure 2: Effect of aqueous extract of *Tephrosia purpurea* on Liver histology in Paracetamol intoxicated rats



A. Section of rat liver treated with vehicle control shows the normal hepatic cells, central vein sinusoid with normal texture.
HxE 10x



B. Section of rat liver treated with paracetamol shows damaged hepatic cells, central vein, nucleus, endothelium and sinusoids.
HxE 10x



C. section of rat liver treated with silymarine and paracetamol shows regeneration of hepatic cells, central vein, nucleus, endothelium and sinusoids.
HxE 10x



D. section of rat liver treated with *Tephrosia purpurea* (75mg/kg) and paracetamol shows regeneration of hepatic cells, central vein, nucleus, endothelium and sinusoids.
HxE 10x



E. section of rat liver treated with *Tephrosia purpurea* (150mg/kg) and paracetamol shows regeneration of hepatic cells, central vein, nucleus, endothelium and sinusoids.
HxE 10x

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