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ORIGINAL ARTICLE **OPEN ACCESS**

Hepatoprotective effect of *Tephrosia purpurea* (L.) Pers. On paracetamol-induced liver toxicity in rats

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ABSTRACT

Alcoholic liver disease is a major cause of alcohol related morbidity and mortality throughout the world. Alcoholic hepatitis is one of its manifestations which shows an approximately 25-30% prevalence. There is no precise treatment guidelines for hepatic ailments in modern science although abstinence from alcohol and high-calorie diet and vitamin supplementation can be helpful to some extent. There are several medicinal plants have been described to be beneficial for hepatic ailments in Ayurveda.The present study To evaluate the hepatoprotective activity of aerial parts of Tephrosia purpurea against Paracetamol-induced hepatotoxicity. Hepatoprotective activity of the Methanolic extract of Tephrosia purpurea (L.) Pers.(Fabaceae) plant was investigated in rats by inducing toxicity with paracetamol. The plant extract has been shown to possess significant protective effect by lowering the level of AST, ALT, ALP, LDH, Cholesterol and bilirubin. The Methanolic extract of Tephrosia purpurea (L.) Pers.at a dose of 50mg/kg, 100mg/kg, and 200mg/kg showed significant hepatoprotective activity which was comparable to that of a standard hepatoprotective agent (Silymarin). The present work aim was to measure the hepatoprotective activities of the Methanolic extract of Tephrosia purpurea in albino wistar rats.

Keywords: *Hepatoprotective, Paracetamol, Methanolic extract , Tephrosia purpurea etc.*

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INTRODUCTION

Liver is an important organ concerned with the biochemical activities in the human body. It has a great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by a hepatotoxic agent is of grave consequences. There is an ever increasing need of an agent that could prevent such damage.[1]Because of severe undesirable side effect of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate the scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity [2].

Tephrosia purpurea (L.) Pers.(Fabaceae) plant commonly known in Sanskrit as Sharapunkha is a highly branched, sub-erect, herbaceous perennial herb. In Ayurvedic literature, this plant has also given the name of "Sarwa wranvishapaka" which means that it has the property of healing all types of wounds [3]. It is an important component of some preparations such as "Tephroli" and "Yakrifit" used for liver disorders [3,4]. The roots and seeds are reported to have insecticidal and piscicidal properties and are also used as a vermifuge. The roots are also reported to be effective in leprosy wound and their juice, to the eruption on the skin [5]. The aqueous extract of seeds has shown significantly in vivo hypoglycaemic activity in diabetic rabbits [6]. The Methanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The total flavonoids were extracted from a plant found to have antimicrobial activity[7,8].

MATERIAL AND METHODS

Plant collection

The fresh plant of *Tephrosia purpurea* was collected from the region of Taluka Yawal, District Jalgaon, India. The selected plant was authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra. Barks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Extraction methodology: [9]

The aerial part of the plant were thoroughly washed with tap water, dried at room temperature and transformed to a coarse powder. The powder was extracted with solvents like a Petroleum ether (60-

80°C), Chloroform, Methanol, Water-methanol, and water separately by Soxhlet extraction method. Finally, the extracts were evaporated and dried under vacuum and tray dryer to obtain thick sticky extract.

Animals

Healthy adult Swiss albino wistar rats of either sex weighing between 160 to 180 gm were used for acute toxicity study and hepatoprotective activity.

Toxicity study: (OECD 425) [10]

Acute toxicity study was performed according to Organisation for Economic Co-operative and development guidelines No. 423. Albino wistar rats of either sex were divided into six groups with six animals each. Plant extract was administered orally as single doses to rats at different dose levels of 50, 250, 500, 1000, 1500, and 2000 mg/kg b.w. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 day.

Experimental procedure.

Paracetamol induced liver damage:[11]

Procedure:

Albino wistar rats (150-250g) were used. All the animals were randomly divided into the six groups each group consists of 6 animals and they received the treatment as follows

Group I: Normal (Distilled water p.o.)

Group II: Toxicant (On 5th day Paracetamol 3g/kg, 1% CMC p.o.)

Group III: Standard (Silymarin 50mg/kg p.o. + on 5th day Paracetamol 3g/kg, p.o.).

Group IV: Extract treated (Methanolic extractof *Tephrosia purpurea*50 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).

Group V: Extract treated (Methanolic extractof *Tephrosia purpurea*100 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).

Group VI: Extract treated (Methanolic extractof *Tephrosia purpurea* 200 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.)

The vehicle (Distilled water) or extract were administered orally for 7 days. Paracetamol suspension (1% CMC) was administered in a dose of 3g/kg p.o on 5th day.48 hrs after paracetamol administration, blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical investigations viz. SGPT, SGOT, ALP, LDH, TB, and DB.

The livers of all animals were removed and processed for histological investigations.

Histopathological studies

Small portions of the liver from each of the six animals in allof the groups were preserved in 10% buffered formol saline (P^H7.4). The paraffin sections were then prepared and stained withhaemotoxylin-eosin dye for observing the liver damage.

Statistical analysis

The data represent mean S.E.M. Result were statistically by one-way ANOVA followed by dunnet's test. The minimum level of significance was set at $p<0.005$.

RESULTS AND DISCUSSION

Paracetamol induce Hepatotoxicity

The administration of Paracetamol resulted in a marked increase in serum SGPT, SGOT, ALP, LDH,total bilirubin. The protective actions of aerial parts of *Tephrosia purpurea* on hepatotoxicity induced by paracetamol are summarized in Tables 1 and 2. Maximum hepatoprotective activity was observed at 200mg/kg dose level of *Tephrosia purpurea* (aerial parts), which was comparable to that of silymarin

Table 1. Effect of Methanolic extract of *Tephrosia purpurea* on liver weight and liver volume in paracetamol induced hepatotoxicity

Group	Body weight (gm)	Liver weight (gm)	Liver volume(ml)
Normal	262	8	7
Paracetamol treated3g/kg	228	11	10
STD+ Paracetamol3g/kg	258	9	7.5
Methanolic extractof *Tephrosia purpurea* 50 mg/kg+ Paracetamol3g/kg	231	10	10.5
Methanolic extractof *Tephrosia purpurea* 100 mg/kg+ Paracetamol3g/kg	255	10.5	9.5
Methanolic extractof *Tephrosia purpurea*200 mg/kg+ Paracetamol3g/kg	267	9.5	9

Table 2. Effect of Methanolic extract of *Tephrosia purpurea* on various biochemical parameters in paracetamol induced hepatotoxicity

Group	SGOT (I.U./L)	SGPT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	64.19± 1.2*	47.09± 0.6*	185.1± 1.7*	146.3± 1.3*	0.18± 0.006	0.17± 0.004**
Paracetamol treated3g/kg	172.9± 1.3**	125.8 ±3.2	352.5± 3.2*	273.5 ± 1.4*	1.08± 0.1*	0.6± 0.016
STD+ Paracetamol3g/kg	95.91 ±1.1	71.03 ±0.8	210.3± 0.9*	177.3 ± 3.5*	0.35± 0.01*	0.24± 0.004
Methanolic extract of *Tephrosia purpurea*50 mg/kg+ Paracetamol3g/kg	135.5 ±1*	107.1 ±0.6	305.3± 1.2*	230.8± 1.6*	0.6± 0.03**	0.50 ± 0.01*
Methanolic extract of *Tephrosia purpurea*100 mg/kg+ Paracetamol3g/kg	114.9 ±0.9	96.86 ±0.7**	281.9± 2.4*	215.7± 1.8*	0.5 ±0.02	0.42± 0.01*
Methanolic extract of *Tephrosia purpurea*200 mg/kg+ Paracetamol3g/kg	103.8 ±1	85.88 ±0.3	240.7 ±2.6*	196.8± 0.4*	0.45 ±0.01	0.32± 0.01*

Values are expressed as mean±S.E.M. (n=6)
*P<0.05, **P<0.01, when compared with the Paracetamol treated group (one-way ANOVA followed by Dennett's test)

Histopathological changes of Paracetamol induced hepatotoxicity in albino wistar rat liver

Histological profile of the control animals showed normal hepatocytes with well preserved cytoplasm, prominent nucleus, nucleolus and central vein. There was no sign of inflammation, fatty change or necrosis in these animals (Fig. 1). In animals treated with Paracetamol only, liver sections showed marked necrosis and inflammatory cell infiltration in the centrizonal area. Inflammatory cells were also observed in the portal triad (Fig. 2). Pretreatment with *Tephrosia purpurea* at 50 and 100mg/kg dose showed a reduction of necrosis area and inflammatory infiltrates in the centrizonal area with the disappearance of inflammatory infiltrate around portal triad (Figs. 4 and 5). *Tephrosia purpurea* at 200mg/kg dose showed a greater reduction of the necroses area and sparse inflammatory cell infiltration around the central vein (Fig. 6) as compared to 50 and 100mg/kg dose.

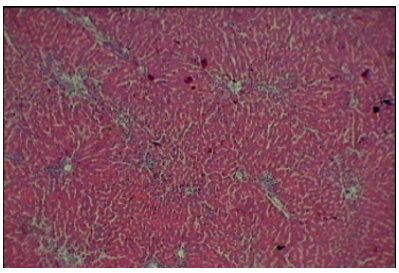

Figure: 1
Normal liver
Normal histology showing prominuteent central vein, portal triads and normal hepatocytes.
(H and E X 100)

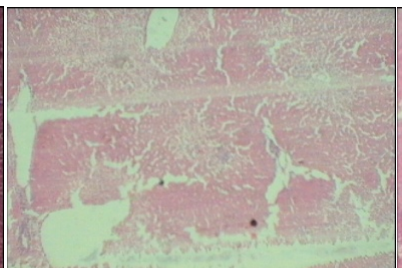

Figure: 2
Toxicant control Paracetamol (3g /kg) Focal areas of liver cell degeneration, lymphocytic infiltration, fatty degeneration
(H and E X 100)

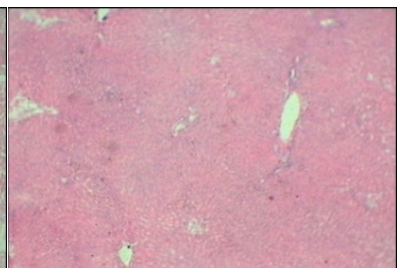

Figure: 3
Standard silymarin (50mg/kg)
Small areas of cell degeneration
And almost normal histology
(H and E X 100)

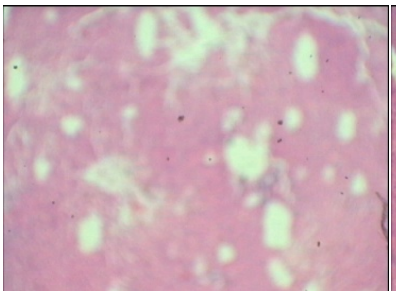

Figure: 4
Methanolic extract of *Tephrosia purpurea*50 mg/kg
Small areas of cell degeneration
(H and E X 100)

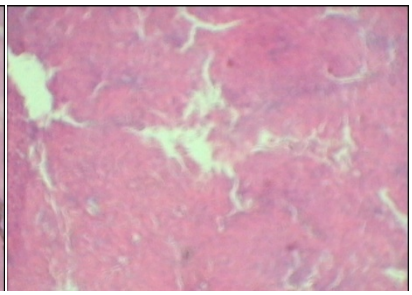

Figure: 5
Methanolic extract of *Tephrosia purpurea*100 mg/kg
Small areas of cell degeneration and Lymphocytic infiltration
(H and E X 100)

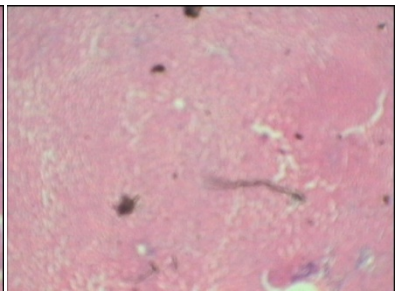

Figure: 6
Methanolic extract of *Tephrosia purpurea*200mg/kg
Normal central vein Portal triad
Occasional areas of cell degeneration
(H and E X 100)

DISCUSSION

Damage to the structural integrity of the liver is reflected by an increase in the level of serum transaminases and bilirubin because these are cytoplasmic in location and are released into circulation after cellular damage. [12] The present study has also demonstrated that in vivo hepatoprotective activity against liver injury induced by Paracetamol. Paracetamol (N-acetyl p-amino phenol) a widely used analgesic and the antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses [13] It is mainly metabolized in the liver to excretable glucuronide and sulfate conjugates. However, hepatotoxicity of Paracetamol has been attributed to the formation of toxic metabolites when a part of Paracetamol is activated by hepatic Cytochrome p 450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine, which is normally conjugated with GSH and excreted in the urine as conjugates. Overdose of Paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis.[14,15,16]

In our present investigation rats treated with Paracetamol induced hepatotoxicity developed significant hepatic damage which was observed through a substantial increase in the concentration of SGOT, SGPT, ALP, LDH and bilirubin. Treatment of rats with Methanolic extract of *Tephrosia purpurea* before and concomitant with the challenge of Paracetamol produced an alleviation of the hepatic injury to a considerable extent which was reflected by the ability of the extract to lower the elevated serum enzymes levels resulting from the administration of Paracetamol alone. The increased levels of SGOT and SGPT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. In view of this, the extract mediated reduction in levels of SGOT, and SGPT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by Paracetamol. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.

Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow. The use of ALP in chemical-induced liver dysfunction has been investigated in our study. Paracetamol induced elevation of this enzymatic activity in serum is in line with a high level of serum bilirubin content. The extract mediated suppression of the increased ALP activity with the concurrent depletion of raised bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction in rat liver during chronic hepatic injury with Paracetamol.[17]
The present study revealed a significant in activities of SGOT, SGPT, ALP, Cholesterol and serum bilirubin level on exposure to Paracetamol indicating considerable Hepatocellular injury . Administration of Methanolic extract of *Tephrosia purpurpea* at 50 mg/kg,100 mg/kg and 200mg/kg dose level attenuated the increased level of the serum enzymes, produced by Paracetamol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

CONCLUSION

On the basis of above results, it can be concluded that the active principles present in root callus extract of *Tephrosia purpurpea* offered better antihepatotoxic action as compared to the active principles present in natural extract against paracetamol induced hepatic damage. However, more elaborate work is required to establish the efficacy of arial extract by isolating and identifying the active constituents present in the aerial extract which are responsible for antihepatotoxic activity.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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