EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHYL ACETATE FRACTION OF *TEPHROSIA PURPUREA*

Rajal Shah[1], Sachin Parmar[2], Punit Bhatt[2*], Sumitra Chanda[1]

[1] Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India
[2] Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India
*Corresponding author: Punit Bhatt
Address: Department of Pharmaceutical Sciences, Saurashtra University, Rajkot-360 005, Gujarat, India Telefax: +91 0281 2585083
Email: puns003@gmail.com

Summary

Context: Herbs are known to play a vital role in the management of various liver disorders. One such herb *Tephrosia purpurea* (Fabaceae) is a copiously branched herbaceous perennial plant commonly known as *saraphunkha* in India. The whole plant is useful in the treatment of liver disorders. In the present study, hepatoprotective action of *Tephrosia purpurea* was evaluated on commonly used model of experimental hepatic damage in rats.
Objective: To evaluate the hepatoprotective activity of *Tephrosia* purpurea root against carbon tetrachloride (CCl_4) -induced hepatotoxicity.
Materials and methods: Ethyl acetate fraction of ethanol extract of roots of *Tephrosia purpurea* (EETP) (25 and 50 mg/kg/day, p.o.) was evaluated for its efficacy in rats by inducing hepatotoxicity with CCl_4 (0.5 ml/kg, i.p.). Serum levels of Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), billirubin and triglycerides were used as biochemical markers of hepatotoxicity. Histopathological changes in the liver were also studied.
Result & Discussion: The results showed that oral administration of EETP resulted in a significant reduction in AST, ALT, ALP and total bilirubin when compared with CCl_4 damaged rats. A comparative histopathological study of liver from test group exhibited almost normal architecture, as compared to CCl_4-treated group. The results are comparable to that of silymarin. Hepatoprotective activity of EETP exhibited better effectiveness than silymarin in certain parameters, concluded its hepatoprotective potential.
Conclusion: Our study demonstrates the hepatoprotective activity of roots of *Tephrosia purpurea* against CCl_4-induced hepatotoxicity. The future study should focus on the exact mechanism of hepatoprotective action of *Tephrosia purpurea*.

Keywords: hepatoprotective, *Tephrosia purpurea,* CCl_4, ALT, AST, bilirubin

Introduction

Scientific research in herbal medicine with hepatoprotective activity may be a great benefit as an alternative therapy in liver diseases. [1] Traditional systems of medicine remain the major source of health care for more than two thirds of the world's population and impressive progress has been made in certain developing countries. Recently, there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. [2]

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. [3] Nature has bestowed India with an enormous wealth of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world. Now a days, herbal drug industries is very fast growing sector in the international market. [2] In spite of tremendous advances made, only a few significant and effective hepatoprotective agents, e.g., silymarin, are available in modern therapeutics. [4] Hence researchers worldwide are engaged in searching for organ protective i.e. hepatoprotective drugs from herbal origin. [5]

Ayurveda, the ancient system of Indian Medicine identified liver diseases quite early and recommends a number of herbal remedies. One such herb *Sarapunkha (Tephrosia purpurea)* is considered useful in bilious febrile attacks, obstruction of liver and spleen. The whole plant is useful in the treatment of liver disorders. [6] According to Ayurveda literature, this plant has also given the name of "*Sarwa wranvishapaka*" which means that it has the property of healing all types of wounds. [7] It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders. [8, 9]

The plant has been regarded as deobstruent, tonic, diuretic, laxative, useful in bilious febrile attacks, cough, lightness of the chest, and in biliary and splenic troubles. The leaves are also reported to be useful in jaundice. [9] Phytochemical investigations on *Tephrosia purpurea* have revealed the presence of glycosides such as rutin, quercetin, and osyritin; retenoids such as deguelin, elliptone, rotenone and tephrosin; flavanoids such as lanceolatin A, B, C, purpurin, purpurenone, purpuritenin, and sterols such as β-sitosterol. [10] An isoflavone, 7,4'-dihydroxy-3',5'- dimethoxyisoflavone, and a chalcone, (+) tephropurpurin, are also reported to be present in *Tephrosia purpurea*. [11]

In the present study, hepatoprotective and curative actions of *Tephrosia purpurea* was evaluated on commonly used CCl_4 model of experimental hepatic damage in rats.

Methods

Collection of plant materials

The roots of *T. purpurea* were collected from the river bank of Karjan, Rajpipla, Gujarat, India in the month of November 2008. The plant was authenticated by Prof. P. Parmar, Botanical Survey of India, Jodhpur, Rajasthan (India). A voucher specimen (SU/DPS/Herb/13) was deposited in the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot (India) for future reference. Roots of *T. purpurea* were air dried, coarse powdered and passed through 40 #.

Preparation of the plant extract

The coarsely powdered dried root of *T. purpurea* (300 g) was defatted with petroleum ether (1.2 L) by hot extraction process (Soxhlet) for 12 h. The marc left after petroleum ether extraction, was dried and extracted with 95% (v/v) ethanol (1.2 L) by hot extraction process for 20 h. Extract was concentrated and dried in desiccators at room temperature. Extract (20 g) was dissolved in 350 ml distilled water and was extracted three times with ethyl acetate. All three fractions were collected together and allowed to concentrate. This resulting fraction shows triterpenoid flavanoids in phytochemical screening, used to evaluate hepatoprotective activity against CCl_4-induced hepatic injury in rats.

For dosing, the extract was suspended in distilled water using Tween-80 as suspending agent.

Experimental animals
Healthy Wistar albino rats of either sex, weighing 150-200 g were housed in groups in polypropylene cages and placed in climate-controlled central animal house having temperature 22 ± 2°C, relative humidity 60 ± 5%, and 12 h light/dark cycle (lights on at 08:00 h and off at 20:00 h). Animals had free access to standard pellet diet (Amrut, Pranav Agro Industries Ltd, India) and water *ad libitum*. Protocol was approved (approval no-SU/DPS/IAEC/1004) by Institutional Animal Ethics Committee (IAEC) of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Drugs
Silymarin was received as a gift sample from Cadila Pharmaceuticals Ltd., India. All the drug solutions were freshly prepared and the solvents used were of analytical grade.

Administration of drugs
Silymarin was dissolved in saline, while ethyl acetate fraction of ethanol extract of roots of *Tephrosia purpurea* (EETP) was prepared as suspension in distilled water using Tween-80 as the suspending agent. Animals were assigned to different treatment groups (n = 6). Silymarin and experimental drugs were administered orally. The control group received the vehicle (n-saline, 1 ml/kg, orally). CCl_4 was administered (0.5 ml/kg) by intraperitoneal (i.p.) route.

Carbon tetrachloride-induced hepatotoxicity
Modified method of Agarwal et al., (2006) was followed. [12] Wistar Albino rats of either sex were divided into 5 groups of 6 animals each. Group I and II served as positive and negative control respectively, received n-saline 1 ml, p.o. for 7 days. Group III served as reference standard, received silymarin 25 mg/kg, p.o. for 7 days. Group IV and V served as test group, received EETP at doses of 25 and 50 mg/kg, p.o. for 7 days. Group II, III, IV and V treated with CCl_4 (0.5 ml/kg) intraperitonially on day 7 after drug administration. At the end of experiment, blood samples of each rat were collected after 36 h of CCl_4 treatment. Rats were sacrificed and liver was rapidly excised. All livers were washed with chilled n-saline and fixed in 10% formalin, serially sectioned and microscopically examined after staining with hematoxylin-eosin.

Assessment of hepatoprotective activity
To access hepatoprotective activity, 1.0 ml of blood was collected from each experimental animal through retro orbital plexus with help of thin capillaries into eppendorf tubes and allowed to coagulate for 30 minutes at room temperature. All samples were centrifuged at 5000 rpm for 10 minutes to separate serum. Clear serum was separated and estimation of (ALP), total bilirubin and triglycerides were undertaken. (Star 21-plus auto analyzer; Aspen)

Histopathological studies
The livers were excised from the experimental animals of each group after collecting the blood sample and washed with normal saline. Initially, the materials were fixed in 10% neutral formalin solution. Sections were cut using microtome technique after paraffin embedding. The sections were processed in alcohol xylene series and were stained with hematoxylin and eosin. The different sections were examined microscopically for the evaluation of histopathological changes.

Statistical analysis
Results of biochemical estimations are presented as mean ± SEM. Statistical significance was determined by One-way analysis of variance (ANOVA) followed by, Dunnett's test. Differences were considered significant at $p < 0.05$.

Results

Plant extract
The yield of ethanol extract of roots of *Tephrosia purpurea* was 7% (w/w) and the yield of EETP was 2 g (10% w/w).

Hepatoprotective activity
The results are presented in Table 1. Rats treated with CCl_4 alone developed significant hepatocellular damage as evidenced from increase in serum level of AST, ALT, SALP and bilirubin when compared with control. The samples of the Group II animals showed drastic increase in the levels of AST (340.02 ± 10.38), ALT (379.53 ± 30.7), SALP (110.42 ± 6.32), total bilirubin (0.46 ± 0.04), while decreased level of serum triglyceride (104.52 ± 7.23). Administration of EETP caused reduction in increased serum level of AST ($p < 0.01$), ALT ($p < 0.01$), SALP ($p < 0.01$), total bilirubin($p < 0.01$), and increase serum triglycerides level ($p < 0.01$) significantly. Standard drug, silymarin (25 mg/kg, p.o.) also exhibited similar results significantly.

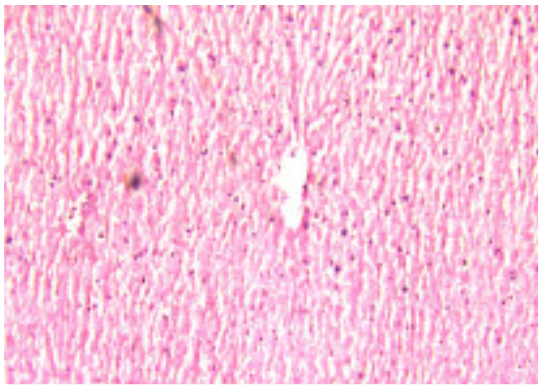
Table 1 Effect of EETP treatment on different biochemical parameters of rat serum[†]

Parameter	Treatment				
	Control (1 ml/kg saline, p.o.)	**CCl_4 (0.5 ml/kg, i.p.)**	**Silymarin (25 mg/kg, p.o.)**	**EETP (25 mg/kg, p.o.)**	**EETP (50 mg/kg, p.o.)**
ALT (IU/ml)	50.36 ± 4.37	379.53 ± 30.70$^{\#\#}$	60.6 ± 5.95**	104.05 ± 8.53**	59.4 ± 3.76**
AST (IU/ml)	118.78 ± 10.38	340.01 ± 81.48$^{\#\#}$	187.41 ± 18.50*	107.2 ± 7.53**	141.62 ± 5.93**
ALP (IU/ml)	69.26 ± 6.12	110.41 ± 6.32$^{\#\#}$	74.31 ± 1.49**	82.7 ± 3.66**	55.15 ± 4.78**
Total Bilirubin (mg/dl)	0.26 ± 0.02	0.47 ± 0.04$^{\#\#}$	0.12 ± 0.01**	0.33 ± 0.01**	0.24 ± 0.01**
Triglyceride (mg/dl)	265.76 ± 41.39	104.53 ± 7.24$^{\#\#}$	150.9 ± 14.68^{ns}	157.96 ± 11.1^{ns}	215.6 ± 15.13**

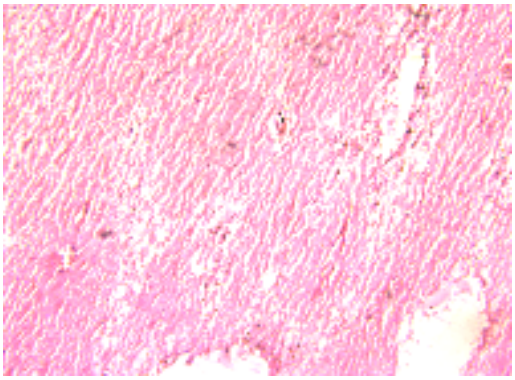
[†]Values are expressed as mean ± SEM (n = 6).
*$p < 0.05$, **$p < 0.01$, ^{ns}Not significant, as compared to negative control group (One-way ANOVA followed by, Dunnett's test)
$^{\#\#}p < 0.01$, as compared to control group (One-way ANOVA followed by, Dunnett's test)

Histopathology
Histological section of group I (control group) showed normal arrangement of hepatocytes with conspicuous nucleus and obvious sinusoids (S) (Figure 1A). Histological section of group II animals with acute hepatic injury induced by CCl_4 showed massive hepatic necrosis, fatty changes with foamy degeneration, ballooning of hepatocytes and deformed cord arrangement, disturbed sinusoids with a prominent infiltrate of neutrophil polymorphs (Figure 1B). Histopathological profile of the group III (25 mg/kg, p.o.) of silymarin showing well arranged nucleated hepatocytes and almost normal liver with little leukocyte infiltration

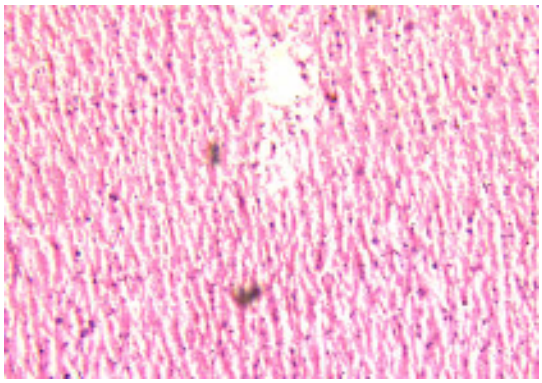
(Figure 1C), whereas the histological section of group IV showed partially recovered hepatocytes with better portal vein and sinusoids; nuclei are not clear as in normal hepatocytes. Endothelium was disrupted at places but in lesser number than CCl_4 intoxicated rats. Hepatic cells adjoining to interlobular vein showed atrophy (Figure 1D). Group V showed dose dependent recovery in cellular structure with more prominent and well formed nucleated hepatocytes arranged in cord, and well formed central vein. The hepatocytes with normal nucleus were more. There seemed to be a satisfactory recovery (Figure 1E).



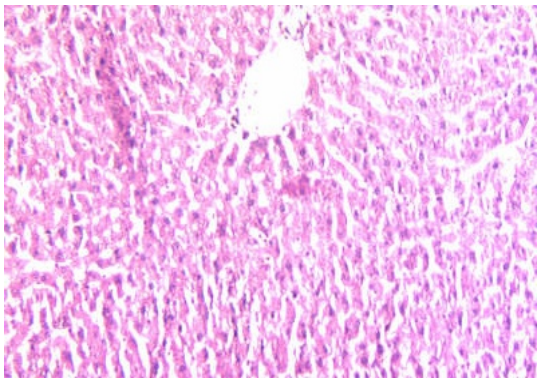
A: Control



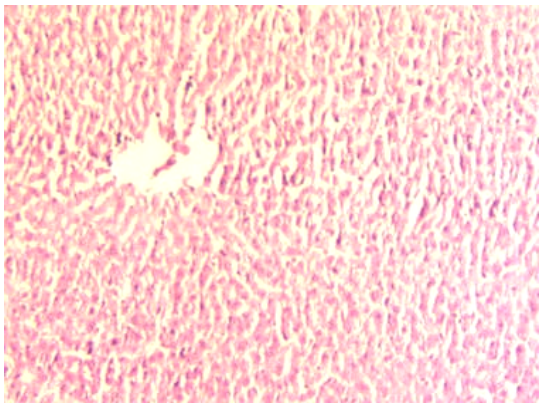
B: CCl4



C: Silymarin



D: EETP 25



E: EETP 50

Figure 1: Photomicrograph of liver (10x) (A) control group showing normal architecture of liver; (B) Animal treated with CCl_4 showing increased intracellular space, fatty changes, hepatocyte necrosis; (C) animal treated with Silymarin showing almost normal liver with little leukocyte infiltration; (D) animals treated with EETP 25 mg/kg showing moderate degree of inflammatory cell infiltration around the central vain; (E) animals treated with EETP 50 mg/kg showing almost normal histological profile.

Discussion

The present study established hepatoprotective activity of roots of *Tephrosia purpurea*. It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation to release CCl_3 radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell organelles. [13] The increased level of ALT, AST, ALP and bilirubin is conventional indicator of liver injury. In the present study, also it was seen that administration of CCl_4 elevates the levels of serum marker enzymes ALT, AST, ALP, and serum bilirubin. Level of serum triglyceride was lowered. The results showed that a single dose of CCl_4 (0.5 ml/kg, i.p.) caused severe hepatocellular injury as indicated by the massive elevations of ALT and AST compared with control animals. Treatment of rats with EETP (25 and 50 mg/kg, p.o.) seemed to preserve the integrity of liver cells, as evidenced by the reduction in the CCl_4-induced increase in these enzymes.

In the present study, silymarin was used as the standard to compare the activity of EETP and silymarin-treated groups. Silymarin at doses up to 100mg/kg has been used as a standard hepatoprotective agent by numerous investigators. Dose of 25 mg/kg, p.o. of silymarin was selected in the present investigation based on some published studies demonstrating the hepatoprotective activity of this dose. [12, 14] EETP and silymarin-treated groups exhibited lower levels of ALT, AST, ALP, and bilirubin as compared to CCl_4-treated group. The stabilization of serum bilirubin, ALT, AST, and ALP levels by EETP is a clear indication of the improvement of the functional status of the liver cells.

These findings can be further corroborated with histopathological studies. The histopathological examination clearly reveals that the hepatic cells, central vein, and portal triad are almost normal in EETP-treated group (50 mg/kg, p.o.) in contrast to group which received CCl_4. Thus, EETP can be considered to be an effective hepatoprotective agent as it ameliorated almost to normalcy the damage caused by CCl_4 to hepatic function.

Hepatic system is the major organ system involved in the metabolism, detoxification and excretion of various endogenous and exogenously administered/ingested substances. [5] This physiological activity of the liver results in the generation of highly reactive free radicals, which covalently bonds with membrane lipids causing lipid peroxidation.

Lipid peroxidation alters membrane permeability and causes tissue damage. Since, the liver is involved in various biochemical reactions; it is prone to be attacked by free radicals and cell necrosis result. [5] Recently, it has been reported that the hydroalcoholic extract of *Tephrosia purpurea* showed antioxidant activity by inhibiting DPPH and hydroxyl radical, nitric oxide and super oxide anion scavenging, hydrogen peroxide scavenging and reducing power activities. In addition, the hydroalcoholic extract of *Tephrosia purpurea* found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. [15] Strengthening of inbuilt protective mechanisms or exogenous administration of antioxidants may be useful in protecting the organs. [5]

Conclusion

From the results it is evident that, pre-treatment with EETP have protected the rat liver from hepatotoxicity of CCl_4. It can be concluded from the present study that *Tephrosia purpurea* have potential hepatoprotective activity and attenuates the hepatotoxic effects of CCl_4. Further bioactivity guided isolation of EETP may yield new therapeutic compounds to combat hepatic problems. However, components responsible for this activity are currently unclear and further work should be performed on the isolation and identification of the hepatoprotective components in ethanol extract of *Tephrosia purpurea.*

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