



EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *TEPHROSIA PURPUREA* LINN. STEM

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ABSTRACT

Present study deals with the investigation of hepatoprotective activity of *Tephrosia purpurea* Linn stem. Powdered stem was extracted with methanol and subjected for the preliminary phytochemical screening. Acute toxicity study of the extract was carried out following OECD guidelines 423 and found safe upto the dose 2000 mg/kg, p.o. Hepatoprotective activity of extract was evaluated against CCl₄ induced hepatotoxicity in Wistar albino rats. Rats were divided into five groups containing 6 mice per group. Group I animals were administered with vehicle only, Group II animals were administered with CCl₄ (1.4 ml/kg p.o.) to induced hepatotoxicity, group III animals were administered with silymarin (25 mg/kg) for 7 days and CCl₄ (1.4 ml/kg p.o.) on fifth day, group IV and V animals were administered with methanol extract of *T. purpurea* stem at 75 and 150 mg/kg, po respectively for 7 days and CCl₄ (1.4 ml/kg p.o.) on fifth day of treatment schedule. Biochemical parameters (SGPT, SGOT, ALP, total bilirubin and direct bilirubin) were assessed in all the experimental animals. Phytochemical investigation of methanol extract of *T. purpurea* stem revealed the presence of flavanoids, phytosterols, alkaloids and proteins. Methanol extract of *T. purpurea* stem was exhibited dose dependant hepatoprotective activity comparable to that of silymarin.

KEYWORDS: *Tephrosia purpurea*, hepatoprotective, Phytochemical screening, silymarin.

1. INTRODUCTION

Liver is the one of the vital organs endowed with several important homeostatic responsibilities. It has got its own importance in the physiological system. One of the primary functions of the liver is to aid in the metabolism of ingested substances including food, dietary supplements, alcohol and majority of medications (Mary et al., 2006). According to WHO about 18,000 people die every year due to liver diseases. The common ailments of liver are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, fulminant hepatic failure and certain tumors like hepatoma (Avijeet et al., 2007). In view of severe undesirable side effects of synthetic agents and absence of reliable liver protecting drugs in the modern medicine, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the use of traditional herbal medicines which are claimed to possess hepatoprotective activity.

Tephrosia purpurea Linn. commonly name as Sharapunka, Dhamasia, Sarphonka, etc. Whole plant is being used as digestible, anthelmintic, antipyretic, cures diseases of liver, spleen, heart, blood, cure tumors, ulcers, leprosy, asthma, bronchitis, piles, caries of the teeth, laxative, blood purifier. The plant is reported to contain a variety of class of chemical constituents such as phytosterols: β -sitosterol, spinosterol; flavonoids: epoxylavon; terpenoids: ursolic acid, butelonic acid, tetratriacontane; and pongamol, rotenone, tephrosin, 12- α -hydroxyrotenone, dimethylglabranin. Roots of *T. purpurea* has reported to possess antiulcer (Deshpande et al 2003), anticarcinogenic (Kavitha et al 2006), anti-inflammatory, analgesic, antipyretic (Gopalakrishnan et al 2010; Valli et al 2011), hepatoprotective (Sangeetha et al 2010), antioxidant (Rumit Shah et al 2010), antiinflammatory, antimicrobial (Kumar et al 2007) and CNS depressant activities (Valli et al 2011). Aerial parts of the plant have reported to have wound healing (Santram et al 2010), hepatoprotective (Murthy et al 1993), Leaves are reported to possess antihyperlipidemic (Rashid et al 2011), hepatoprotective (Jain et al 2006) and nephro protective activities (Jain et al 2009), flowers are reported to have antibacterial and antiviral activities (Kokila et al 2010), seeds are reported to have antihyperglycemic and antihyperlipidemic activities (Pavana et al., 2007). Aim of the present study was to evaluate hepatoprotective activity of *T. purpurea* stems.

2. MATERIALS AND METHODS:

2.1. Plant material:

Stems of *T. purpurea* was collected from Mani Majra (near railway station), District Panchkula, Haryana in the month of November 2012 and authenticated from NISCAIR, New Delhi vide letter number NISCAIR/RHMD/Consult/2012-13/2123/130, dated 10/12/2012. Fresh stems were washed, cleaned and dried in shade for 15-20 days. The plant material was pulverized to coarse powder.

2.2. Chemicals, solvents and drugs:

Petroleum ether, chloroform, ethyl acetate, methanol, HCl, CCl₄ were procured from Ranbaxy Fine Chemicals Ltd., New Delhi. The biochemical enzymatic kits (SGPT, SGOT, ALP) were purchased from Coral diagnostics Ltd., Mumbai, India. All solvents and chemicals used in the present study were of analytical grade.

2.3. Preparation of extract:

Methanol extract of the dried stems of *T. purpurea* was prepared using soxhlet apparatus and concentrated under vacuum using rotary evaporator.

2.4. Phytochemical screening:

Various chemical tests were carried out to screen class of phytoconstituents present in methanol extract of *T. purpurea* stem using standard methods reported in (Harborne 1973).

2.5. Evaluation of hepatoprotective activity:

2.5.1 Animals:

Wistar albino rats of either sex, weighing 150-170 g were used in this study. Rats were fed with standard chow diet (Ashirwad industries Pvt. Ltd., Ropar, Punjab, India) and water ad libitum. Animals were housed in polypropylene cages and exposed to 12h light and 12h dark cycles. The experimental protocol used in the present study was approved (Regn. No. 816/04/c/CPCSEA) by Institutional Animal Ethics Committee (IAEC), School of Pharmacy and Emerging Sciences, Baddi University, Baddi, Himachal Pradesh. All experiments were carried out as per the Committee for the Purpose of Control and Supervision on Experimental Animal (CPCSEA) guidelines for the care and use of laboratory animals.

2.5.2. Dose preparation:

All doses were prepared using 0.5 % CMC as vehicle.

2.5.3. Acute oral toxicity studies:

Acute toxicity study of the methanol extract of *T. purpurea* was conducted as per OECD guidelines 423 (OECD, 2001).

2.5.4. Evaluation of hepatoprotective activity:

Hepatoprotective activity of methanol extract of *T. purpurea* was evaluated in CCl₄ induced hepatotoxicity in rats. A total of 30 rats were divided into five groups containing six rats per group. Group I animals were administered with vehicle only, Group II animals were administered with CCl₄ (1.4 ml/kg p.o.) to induced hepatotoxicity, group III animals were administered with silymarin (25 mg/kg, po) for 7 days and CCl₄ (1.4 ml/kg p.o.; mixed with olive oil in the ratio of 1:1) on fifth day, group IV and V animals were administered with methanol extract of *T. purpurea* stem at 75 and 150 mg/kg, po respectively for 7 days and CCl₄ (1.4 ml/kg p.o.) on fifth day of treatment schedule. On 7th day after one hour of dose treatment animals were anaesthetized. Blood collected from retro orbital plexuses was centrifuged and serum was used for biochemical estimations. Biochemical parameters (SGPT, SGOT, ALP, Total bilirubin and direct bilirubin) were assessed in all the experimental animals.

2.6 Statistical analysis:

Results were expressed as mean $\pm$ standard deviation (SD) and statistically analyzed by one way ANOVA followed by Bonferroni's multiple comparison test. $p < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION:

3.1 Yield of extract:

Percent yield of methanol extract of *T. purpurea* was found to be 89% w/w.

3.2 Phytochemical screening:

Phytochemical investigation of methanol extract of *T. purpurea* stem revealed the presence of flavanoids, phytosterols, alkaloids and proteins, whereas, glycosides and terpenoids were found to be absent.

3.3 Toxicity study:

Acute toxicity studies of methanol extract of *T. purpurea* stem neither exhibited signs of acute toxicity nor mortality upto the dose of 2000 mg/kg, p.o.

Acute toxicity study of the extract was carried out following OECD guidelines 423 and found neither toxicity sign nor mortality upto the dose 2000 mg/kg, po.

3.4 Evaluation of hepatoprotective activity:

Results of hepatoprotective activity are shown in table 2. The serum SGPT, SGOT, direct bilirubin, total bilirubin and alkaline phosphate level were estimated in different groups of animals. Methanol extract of *T. purpurea* reduced SGPT, SGOT, ALP and Bilirubin Compared to CCl₄ the methanolic extract of stem reduced the serum significantly (P<0.05).

Table 2: Effect of methanolic extract of *T. purpurea* on SGPT, SGOT, ALP, direct and total bilirubin level in CCL4 induced hepatotoxic rats.

Group	SGPT (IU/L) (Mean* ± SD)	SGOT (IU/L) (Mean ± SD)	ALP (IU/L) (Mean ± SD)	Bilirubin (mg/dl) (Mean ± SD)	
				Direct	Total
Group I	26.6 ± 4.63	71.7 ± 5.69	41.9 ± 5.69	0.17 ± 0.005	0.13 ± 0.002
Group II	207.6 ± 8.10	217.7 ± 7.84	257.2 ± 7.65	0.88 ± 0.02	0.95 ± 0.05
Group III	152.33 ± 5.22	163.2 ± 5.36	162.1 ± 5.69	0.66 ± 0.005	0.79 ± 0.004
Group IV	126.2 ± 4.12	98.7 ± 2.36	103.4 ± 4.65	0.22 ± 0.021	0.31 ± 0.003
Group V	123.8 ± 5.12	101.3 ± 4.56	105.3 ± 5.62	0.23 ± 0.010	0.32 ± 0.003

Group I (Control): Administered with 0.5 % CMC; **Group II:** CCl₄ induced hepatotoxicity; **Group III:** Methanolic extract of *T. purpurea* (150 mg/kg, po); **Group IV:** Methanolic extract of *T. purpurea* (75 mg/kg, po); **Group V:** Standard (Silymarin)

Values were expressed as mean ± standard deviation (n=6), and statistically analyzed by One way ANOVA followed by Bonferroni's multiple comparison test as post hoc analysis. p<0.05 was considered to be statistically significant.

CONCLUSION:

Methanol extract of *T. purpurea* stem was found to contain flavanoids, phytosterols, alkaloids and proteins, class of compounds. The extract was found to be safe upto 2000 mg/kg, po and exhibits significant hepatoprotective effects at 150 mg/kg, po.

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