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**SHORT COMMUNICATION**

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**ANTIHEPATOTOXIC ACTIVITY OF AQUEOUS EXTRACTS OF CALLUS CULTURE OF *TEPHROSIA PURPUREA* (L) PERS**MOHD. MUJEEB<sup>1</sup>, RASHEEDUZ ZAFAR<sup>1</sup>, ASIF HUSAIN<sup>2\*</sup> and AFTAB AHMAD<sup>3</sup><sup>1</sup>Plant Tissue Culture Laboratory, Department of Pharmacognosy and Phytochemistry,<sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard University, New Delhi – 110 062, India<sup>3</sup>Jeddah Community College, King Abdul Aziz University, Jeddah, Kingdom of Saudi Arabia**Keywords:** *Tephrosia purpurea* L. Pers, carbon tetrachloride, antihepatotoxic, root callus

*Tephrosia purpurea* Linn. (Fabaceae), commonly known as Saraphunkha, is an important indigenous medicinal plant used for centuries in Indian System of Medicine (ISM). It is an important ingredient of several marketed herbal formulations like Tephroli and Yakrifit used for liver disorders. Various plant parts have been used as remedy for impotency, asthma, diarrhoea, gonorrhoea, rheumatism, and urinary disorders (1). Experimental studies showed that *T. purpurea* also possessed antiulcer (2), antitumor (3), antilipid peroxidation activity (4). The plant has also been considered useful in bilious fibrile attacks as well as in obstruction of liver and spleen (5–8). The seed extract is reported to produce antihyperglycemic and antilipid peroxidation activity in streptozotocin-induced diabetic rats (9). The hepatoprotective potential of aerial plant parts has been reported (10), in CCl<sub>4</sub>-induced hepatotoxicity animal experimental model. Due to over exploitation of plant for the preparation of several herbal formulations, the plant is likely to become extinct. Hence, it was thought worthwhile to develop *in vitro* cultures of *T. purpurea* as an alternative source for the production of plant material. In present investigation *in vitro* callus cultures of root of *T. purpurea* were developed on MS basal medium and its aqueous extract was screened for antihepatotoxic potential using CCl<sub>4</sub> as toxicant. The antihepatotoxic potential of *in vitro* produced callus cultures was also compared with that of natural root.

**EXPERIMENTAL****Plant material**

The fresh roots of *Tephrosia purpurea* Linn were collected from the plant grown in Herbal Garden of Hamdard University in the month of December and authenticated by taxonomist of Department of Botany, Hamdard University. Voucher specimen was deposited in the Herbarium of plant tissue culture laboratories, Department of Pharmacognosy and Phytochemistry, Hamdard University, New Delhi.

Initiation and development of root callus – The aseptically germinated seedlings (11), were inoculated on Murashige and Skoog's (MS) medium (12), supplemented with different concentrations and combinations of growth hormones and the growth was observed for 21 days. The hormonal combination showing best callus initiation was selected and the effect of different concentration was observed for development of calli. The root calli were developed and maintained for five months. The cultures were kept in B.O.D incubator at 25 ± 2°C under 16-h diffused light (1600 lux) / 8-h darkness cycle.

**Preparation of extracts**

The natural root and 5 months old root callus were dried below 60°C in an oven and powdered. The dried powdered root and root callus (10 g each)

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were extracted separately with distilled water (100 ml) for 4 h on water bath. The extracts obtained were filtered and evaporated to dryness under reduced pressure in rotary evaporator. The residue was suspended in 1% of Tween 80 solution and used for the study.

### Qualitative chemical analysis

The extracts of root and root callus were prepared and tested for the presence and absence of different plant constituents by using standard qualitative chemical tests as follows: For alkaloids, 2 mL extract + 1% HCl + few drops of Mayer's reagents/Wagner's reagent/Dragendroff reagent gave creamish white precipitate/brownish-red precipitate/orange precipitate, respectively, which indicated the presence of alkaloids. For proteins, 2 mL extract + few drops of Millon's reagent gave a white precipitate which turned red on heating, indicating the presence of proteins. For amino acids, 2 mL extract + few drops of ninhydrin reagent gave pinkish red to violet color upon heating on water bath, indicating the presence of amino acids. For reducing sugars, 2 mL extract + 2 mL each of Fehling solution A and B gave brick red precipitate upon heating on water bath, indicating the presence of reducing sugars. For phenol, 2 mL extract + 2 mL FeCl<sub>3</sub> (0.1% aqueous solution) gave blue-black precipitate indicating the presence of phenol. For saponins (frothing test), 0.5 mL filtrate + 5 mL distilled water gave frothing which persisted for some time, indicating the presence of saponins. For steroids (Liebermann-Burchard reaction), 2 mL extract + 2 mL acetic anhydride + conc. H<sub>2</sub>SO<sub>4</sub> gave blue-green ring, indicating the presence of steroids. For flavonoids, 2 mL extract + conc. HCl + magnesium ribbon gave pink-tomato red color, indicating the presence of flavonoids (11).

### Chemicals

All chemicals used for the experiment were of analytical grade.

### Animals

Non fasted Wistar strains of albino rats (140–200 g) of either sex supplied by animal house of Hamdard University, New Delhi (Registration No. 173/CPCSEA) were used. The animals were allowed water and laboratory food *ad libitum* (Amrut laboratory rat and mice feed, Navamahasra Chakal oil mills Ltd., Pune). The study was approved by ethics committee CPCSEA (Project no 68/2002). The animals were kept at 24°C and 40–70 RH with 12 h light period.

### Anti-hepatotoxic activity

The animals were divided into five groups: The first group (I) consisted of normal control rats which received single daily dose of 1 mL/kg (*i.p.*) of 1% Tween 80 solution on all five days and olive oil 1 mL/kg (*s.c.*) on days 2 and 3. The carbon tetrachloride group (II) received single daily dose of 1 mL/kg (*i.p.*) of 1% Tween 80 solution (*i.p.*) for five days and single dose of carbon tetrachloride 2 mL/kg (*s.c.*) on day 2 and 3. The third group (III) was treated with 300 mg/kg (*i.p.*) of natural root extract on all 5 days and carbon tetrachloride solution 2 mL/kg (*s.c.*) on day 2 and 3, 30 min after administration of extract. The fourth group (IV) was treated with 300 mg/kg (*i.p.*) of root callus extract on all 5 days and carbon tetrachloride solution 2 mL/kg (*s.c.*) on day 2 and 3, 30 min after administration of extract. The fifth group (V) was treated with 10 mg/kg (*i.p.*) standard (silymarin) on all 5 days and carbon tetrachloride solution 2 mL/kg (*s.c.*) on days 2 and 3, 30 min after administration of standard drug.

On final day, blood samples were withdrawn by heart puncturing. The blood samples were allowed to clot for 30–40 min. Serum was separated by centrifuging at 37°C ((g value: 1107)) and was used for various biochemical estimations.

Estimation of serum alanine transaminase (ALT) and aspartate transaminase (AST) were based on the reference method described by International Federation of Clinical Chemistry. The reagents supplied in the kits (Span Diagnostics Kits) were reconstituted, mixed with serum as directed. The ALT and AST were measured at 340 nm and expressed as IU/mL (13). The serum alkaline phosphatase (ALP) was estimated by mixing with the reagent (*p*-nitrophenyl phosphate, magnesium, buffers and stabilizers) with serum, estimated at 405 nm and expressed as IU/mL (14). Total bilirubin was estimated by Malloy and Evelyn method (15) measured at 546 nm and expressed as mg/dL. Biuret method (16) was followed for the estimation of total protein. Serum was mixed with biuret reagent and incubated for 10 min at 37°C. The total protein was estimated at 555 nm and expressed as g/dL.

### Histological studies

Livers were quickly removed and preserved in neutral buffered formalin. Histological liver sections were prepared as described previously by Luna (17).

### Statistical analysis

Values are expressed as the mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by

**Table 1.** Effect of aqueous extracts of root and root callus of *Tephrosia purpurea* on various biochemical parameters.

Groups	Treatment	Dose and root	AST (U/mL)	ALT (U/mL)	ALP (IU/mL)	TB (mg/100 mL)	TP (g/100 mL)
I	Control	1 mL/kg ( <i>i.p.</i> )	50.00 ± 1.05	60.66 ± 1.03	34.66 ± 0.97	0.381 ± 0.004	8.70 ± 0.25
II	Toxic control	2 mL/kg ( <i>s.c.</i> )	680.23 ± 15.11	340.15 ± 8.33	280.23 ± 6.08	7.981 ± 2.44	5.85 ± 0.51
III	Root extract	300 mg/kg ( <i>i.p.</i> )	290.15 ± 7.08 *	248.33 ± 6.12 *	150.45 ± 4.05*	1.723 ± 0.07 **	6.73 ± 0.85 *
IV	Root callus extract	300 mg/kg ( <i>i.p.</i> )	100.01 ± 4.16 ***	90.22 ± 2.25 **	80.32 ± 1.23**	0.212 ± 0.08 ***	8.16 ± 0.45 ***
V	Silymarin	10 mg/kg ( <i>i.p.</i> )	56.11 ± 1.65 ***	60.22 ± 1.45 **	70.04 ± 2.33**	0.351 ± 0.02 ***	9.34 ± 0.36 ***

The values represent the mean ± SE. n = 6 \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs. toxic control (Group II) (ANOVA followed by Dunnett test) ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, TP: Total proteins.

Dunnett test. The values were considered statistically significant, if p was less than 0.05.

## RESULTS AND DISCUSSION

The root callus of *Tephrosia purpurea* was successfully developed and maintained on MS medium supplemented with 2,4-dichlorophenoxy acetic acid (4.52 µM), indole acetic acid (28.5 µM) and kinetin (9.29 µM) for five months. The callus produced was soft in texture and creamy white in color. Preliminary phytochemical screening of aqueous extracts of root and root callus reveals the presence of alkaloids, proteins, amino acids, phenolic compounds, reducing sugars, steroids, flavonoids, and saponin glycosides.

In the present study, treatment of rats with root callus extract at a dose of 300 mg/kg b.w., *i.p.*, produced highly significant (p < 0.001) reduction in elevated serum enzymes level (AST, ALT and ALP) and bilirubin content, moreover, it also showed a better increase in total protein level as compared to toxic control group II (Tab. 1). Root extract at a dose of 300 mg/kg b.w. *i.p.* showed lower anti-hepatotoxic activity. The anti-hepatotoxic activity of root callus extract was compared with natural root extract (300 mg/kg) and standard drug silymarin extract (10 mg/kg). It was found that the root callus extracts showed better protection against CCl<sub>4</sub>-induced liver damage as compared to natural root extract. The anti-hepatotoxic activity of the root callus extract was found to be comparable to that of standard drug (silymarin).

*T. purpurea* Linn. is used in the treatment of various liver diseases including hepatitis and jaundice in Ayurveda and Unani System of Medicine (7). In the present investigation, the anti-hepatotoxic activity of aqueous extracts of root and root callus were evaluated in rats by using CCl<sub>4</sub>-induced hepatotoxicity model. The hepatic damage caused by CCl<sub>4</sub> is evident from the elevated level of the hepatic enzymes (ALT, AST and ALP) and bilirubin content in CCl<sub>4</sub>-treated group as compared to control group, where serum total protein levels were considerably reduced by carbon tetrachloride treatment (Tab. 1). These biochemical parameters are useful for the detection of early damage of hepatic tissues and require fewer efforts than those required for histologic analyses, moreover, without sacrifice of the animals. The elevated level of serum transaminases (ALT, AST and ALP) reflects the damage to the structural integrity of liver (18), as transaminases are found in cytoplasm and released into circulation after cellular damage (19). The biotransformation of



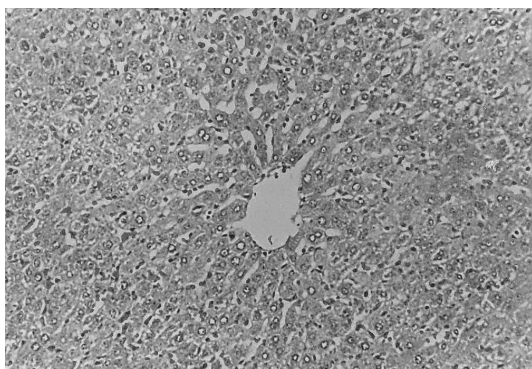


Figure 1. Liver tissue of control animal showing normal histology

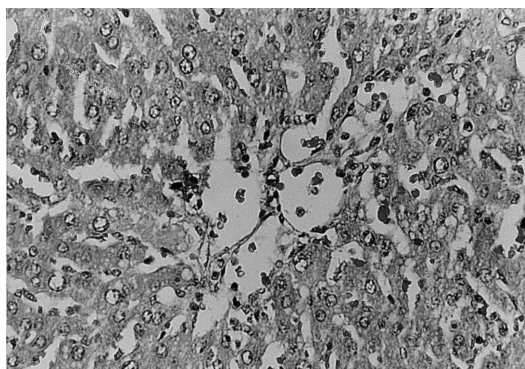


Figure 2. Liver tissue of CCl<sub>4</sub> treated animal showing necrosis, necrotic cells and fatty changes

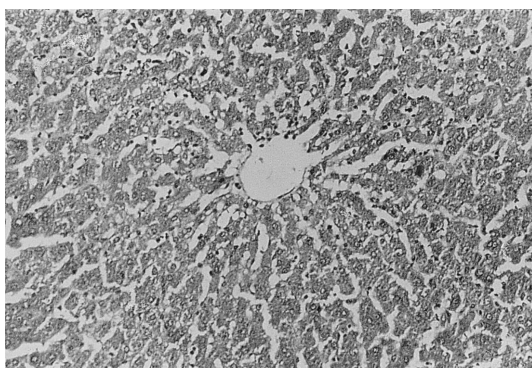


Figure 3. Liver tissue of animal treated with CCl<sub>4</sub> and *T. purpurea* (300 mg/kg) of root showing moderate necrosis, mild fatty changes

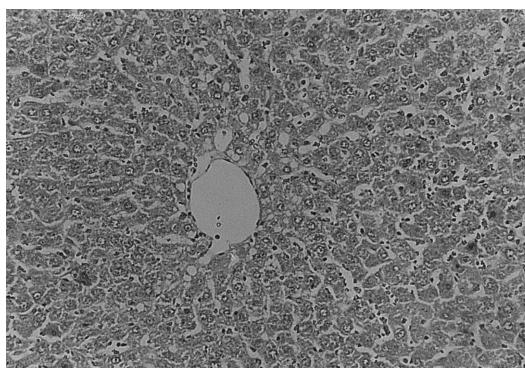


Figure 4. Liver tissue of animal treated with CCl<sub>4</sub> and *T. purpurea* (200 mg/kg) of root callus showing very mild fatty changes

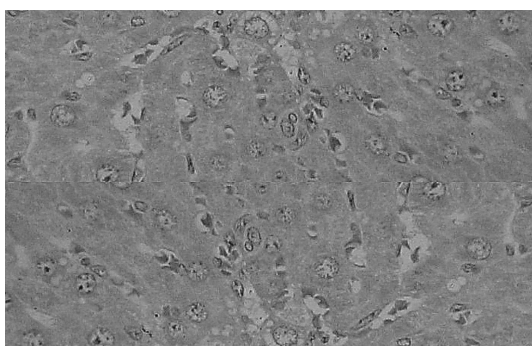


Figure 5. Liver tissue of rats treated with CCl<sub>4</sub> and silymarin (10 mg/kg) showing almost normal histology

carbon tetrachloride leads to the formation of the active free radical CCl<sub>3</sub>, and this free radical reacts rapidly with oxygen to form a trichloromethylperoxy radical, which may contribute to the hepatotoxicity and subsequent increase in hepatic enzymes (20). This is further supported by the elevation in the

level of AST, ALT and ALP in carbon tetrachloride treated rats. The natural root and root callus in a dose of 300 mg/kg protect the animals significantly from CCl<sub>4</sub>-induced hepatotoxicity as compared to toxic control group. This was evident by the significant reduction in serum ALT, AST, ALP and bilirubin content. There was no significant difference between the root callus extract group and standard drug group. The above findings can be correlated with those of an earlier study (21) in which *T. purpurea* extract exerted hepatoprotective action against CCl<sub>4</sub> hepatotoxic model. The anti-hepatotoxic activity of the natural root and root callus extract could be due to the presence of flavonoids glycosides which have hepatoprotective and anti-oxidant properties (22, 23). In the present study, preliminary phytochemical screening of *T. purpurea* revealed the presence of flavonoid glycosides.

#### Histological observations

Histopathological studies of liver sections in control animals showed normal hepatic cells with

prominent nucleus and central vein (Fig. 1). In CCl<sub>4</sub>-treated animals the sections showed hydropic changes in centrilobular hepatocytes with single cell necrosis, congestion of central vein and sinusoids were seen with acute and chronic inflammatory cells mainly in central zone (Fig. 2). Treatment of the animals with silymarin and root and root callus extract of *T. purpurea* extract exhibited a significant recovery of hepatocytes in different sections of the liver (Figs. 3–5).

Histopathological studies revealed that CCl<sub>4</sub> caused steatosis and hydropic degeneration of the liver tissue. Root and root callus extract of *T. purpurea* treatment exhibited protection, which confirmed the result of biochemical studies. Also all the effects of root and root callus extract of *T. purpurea* were comparable with those of silymarin, a proven hepatoprotective drug.

## CONCLUSION

On the basis of above results, it can be concluded that the active principles present in root callus extract of *T. purpurea* offered better antihepatotoxic action as compared to the active principles present in natural root extract against CCl<sub>4</sub> hepatic damage

However, more elaborate work is required to establish the efficacy of root callus extract by isolating and identifying the active constituents present in the natural root and root callus extracts which are responsible for antihepatotoxic activity.

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