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# Free Radical Scavenging and Antilipid Peroxidation Activity of *Tephrosia purpurea* Linn

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**The dried alcoholic extract of the plant *Tephrosia purpurea* Linn. was investigated for its free radical scavenging activity, antilipid peroxidation potential and hydroxyl radical scavenging activity. It was found that the *Tephrosia purpurea* extract showed significant free radical scavenging activity and significant inhibition of lipid peroxidation. However, it failed to show any significant scavenging effect on hydroxyl radicals. These observations can be useful for the justification of various therapeutic applications of *Tephrosia purpurea* and present a useful lead for the improvement of antioxidant activity of the above extract.**

Free radicals have been implicated in a variety of conditions including inflammation, atherosclerosis, diabetes, ageing and hepatic toxicities[1]. Free radicals attack membrane lipids thereby generating lipid radicals, and these lipid radicals can combine with oxygen producing peroxy radicals[2]. The peroxy radical can further cause peroxidation of cellular membrane lipids leading to cell necrosis. This process is implicated in a number of pathophysiological conditions, including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics[3].

The plant *Tephrosia purpurea* Linn. (Family: Leguminosae, sub-family: Papillionacae; Sanskrit: *Sharpunkha*, Hindi: *Sarponkha*, English: Purple Tephrosia) is a copiously branched sub-erect herbaceous perennial, found throughout the Indian subcontinent. The whole plant is reported to be bitter and acrid, anthelmintic, antipyretic, alterative; curing diseases of organs like kidney, liver, spleen, heart and blood; curing tumors, ulcers, leprosy, asthma, bronchitis, piles and caries of teeth[4]. It is considered to be a potent hepatoprotective[5-6]. *Tephrosia purpurea* has been shown to ameliorate benzoyl peroxide-induced cutaneous toxicity in mice[7]. It has also been shown to possess antiallergic and antiinflammatory activities[8-10]. Further, *Tephrosia purpurea* has been found to possess hypoglycemic, hypotensive, analgesic, nematocidal, antibacterial and antifungal activities[11-12].

The presence of a wide variety of flavonoids in *Tephrosia purpurea* has been reported. These include, isolonchocarpin, rutin, lanceolatin A, lanceolatin B, pongamol, semiglabrin, purpuritenin, chalcones, anthocyanidins besides other related derivatives[13-19]. Flavonoids are natural products, which have been shown to possess various biological properties related to antioxidant mechanisms[20-25]. It was therefore thought worthwhile to investigate the antioxidant potential of *Tephrosia purpurea*.

## MATERIALS AND METHODS

The aerial parts of *Tephrosia purpurea* were obtained from the local market and authenticated based on macroscopic and microscopic characteristics of the plant. The coarsely ground powder was extracted with n-hexane in a Soxhlet extractor. The nhexane extracted marc was dried and then extracted with 95% ethanol in a Soxhlet extractor. The ethanolic extract obtained was concentrated under reduced pressure at a bath temperature below 50° to yield a syrupy mass. This extract was used throughout the study. Male Wistar rats (150-200 g) were housed in clean acrylic cages and allowed free access to standard laboratory chow and water *ad libitum*. The animal experiment protocols have been approved by the Institutional Animal Ethics Committee. All chemicals used were of analytical grade.

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**Free radical scavenging activity using DPPH (1,1-diphenyl, 2-picrylhydrazyl):**

The ability of the test extract to scavenge the free radicals was determined by an *in vitro* assay method using a stable free radical DPPH (1,1-diphenyl, 2-picrylhydrazyl)[26]. To an ethanolic solution of DPPH (200 μM), an equal volume of the test extract dissolved in ethanol was added to get final concentration of test extract in the range of 10-200 μg/ml. Appropriate controls, test extracts mixed with equal volumes of ethanol, were maintained. After 20 min, the decrease in absorbance of test mixtures (due to quenching of DPPH free radical) was read at 517 nm[27]. Student's t-test was used to statistically compare the results.

**Hydroxyl radical (·OH) scavenging activity:**

The ability of the test extract to scavenge the ·OH radical was determined using ascorbic acid iron-EDTA model ·OH-generating system[28]. The standard reaction mixture consisted of 100 mM phosphate buffer, pH 7.4, 167 μM iron-EDTA, 0.1 mM EDTA, 2 mM ascorbic acid and 33 mM DMSO in a final volume of 3 ml. Iron-catalyzed oxidation of ascorbic acid at 37° was used to generate formaldehyde from DMSO. Appropriate controls, reaction mixtures without ascorbic acid, were maintained. The test extract was added to obtain final concentrations ranging from 50-400 μg/ml separately. Mannitol, a known hydroxyl radical scavenger was used in concentration 50 mM, as a reference standard for comparison. Reaction was stopped by the addition of 1 ml of ice-cold trichloroacetic acid solution (17.5% w/v). The decrease in formaldehyde formation due to scavenging or decreased formation of ·OH was assayed spectrophotometrically by the method of Nash[29]. The results were analyzed using student's t-test.

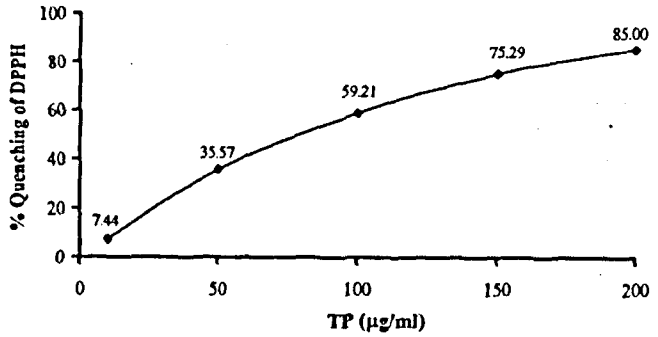


**Fig. 1: Free radical scavenging activity of *Tephrosia purpurea*.**

**For all results, p<0.05 compared with control. TP represents ethanolic extract of *Tephrosia purpurea*.**

**Inhibition of *in vitro* lipid peroxidation:**

Rat was sacrificed and the liver was quickly removed and chilled in ice-cold 0.9% NaCl. The liver was perfused with ice-cold 0.9% NaCl via the portal vein and then homogenized in 10 volumes of 0.15 M KCl[30]. The lipid peroxidation in rat liver homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substances (TBARS)[31]. In a final volume of 1 ml, fresh liver homogenate (0.2 ml) was incubated with Tris HCl buffer (pH 7.5), 0.15 M KCl (0.1 ml) and 2 μM adenosine diphosphate with different concentrations of the test extract separately (10-150 μg/ml of the final volume). After 10 min, $FeSO_4$ (10 μM) and ascorbic acid (100 μM) were added and incubated at 37° for 1 h. The reaction was terminated by addition of thiobarbituric acid reagent (2 ml), boiled for 15 min at 95°, cooled, centrifuged and absorbance read at 532 nm[27]. Malondialdehyde thus formed was quantified using a molar extinction coefficient of $1.56 \times 10^5$ $M^{-1}cm^{-1}$ and expressed as U/mg of protein. The protein content of the rat liver homogenate was determined using Biuret reaction[33] with the help of the Erba Chem-5 autoanalyser (Transasia Bio-Medicals Ltd., Mumbai). The results were analyzed using students's t-test.

## RESULTS

*Tephrosia purpurea* extract showed significant free radical scavenging activity. It interacted significantly ($p<0.05$) with free radical DPPH at concentrations of 10, 50, 100, 150 and 200 μg/ml (fig. 1). To examine the hydroxyl radical scavenging activity of test extract, ascorbic acid iron-EDTA model ·OH-generating system was used. The test extract at different concentrations (50-400 μg/ml) failed to produce any significant inhibition of formaldehyde production as shown in fig. 2. In contrast, the reference standard-mannitol (50 mM) produced a significant ($p<0.05$) inhibition (19.40%) of formaldehyde production.

*Tephrosia purpurea* inhibited the *in vitro* lipid peroxidation in a dose dependent manner. The inhibition was found to be 11.8, 13.3, 16.3, 25.3, 60.6 and 105.3% at different concentrations of 10, 20, 40, 60, 100 and 140 μg/ml, respectively, of *Tephrosia purpurea* extract as shown in Table 1. The reference standard vitamin E (100 μM), produced a significant inhibition (94.9%).

## DISCUSSION

Oxidative stress has been implicated in the pathology of many diseases and conditions including cardiovascular diseases, diabetes, inflammatory conditions, cancer and

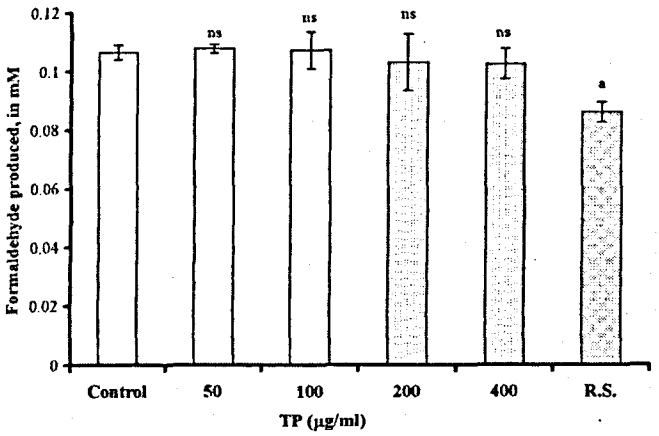


**Fig. 2: Hydroxyl radical scavenging activity of *Tephrosia purpurea*.**

**'ns' represents not significant. 'a' means p<0.05 compared with control. TP represents ethanolic extract of *Tephrosia purpurea*. R.S. stands for reference standard (mannitol 50 mM).**

ageing[1]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and/or some other mechanisms[32].

The most widely acknowledged behavior of antioxidants is their interaction with oxidative free radicals. DPPH[26] is a relatively stable free radical. Its ethanolic solutions show a decrease in absorbance at 517 nm, which is stoichiometric with respect to number of electrons taken up. The stoichiometries for decolorization of DPPH solution are 1:1 for cysteine and 2:1 for ascorbic acid. Dose-dependent interaction of *Tephrosia purpurea* extract with DPPH establishes the capability of the constituents to scavenge the free radicals. Thus, it indicates that some of the therapeutic actions of *Tephrosia purpurea* may be due to its free radical scavenging property.

The hydroxyl scavenging activity of *Tephrosia purpurea* was analyzed using the ascorbic acid iron-EDTA model 'OH-generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA interact to generate the hydroxyl radical[28]. *Tephrosia purpurea* was found to not show scavenging of hydroxyl radical. It therefore appears that the polar constituents of ethanolic extract of *Tephrosia purpurea*, may not have any effect on hydroxyl radical.

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury[32]. *Tephrosia purpurea* was found to significantly inhibit the lipid peroxidation induced non-enzymatically in the rat liver homogenate. This

TABLE 1: EFFECT OF *TEPHROSIA PURPUREA* ON LIPID PEROXIDATION *IN VITRO*.

| Treatment (µg/ml) | Malondialdehyde (µM/mg protein) |
|---|---|
| Control | 1.18±0.04 |
| TP, 10 | 1.04±0.03[a] |
| TP, 20 | 1.02±0.00[a] |
| TP, 40 | 0.99±0.01[a] |
| TP, 60 | 0.88±0.00[a] |
| TP, 100 | 0.47±0.12[a] |
| TP, 140 | -0.06±0.00[a] |
| Vitamin E, 100 µM | 0.06±0.00[a] |

[1]values are expressed as mean±s.d. of four observations, a represents p<0.05 compared with control. TP stands for ethanolic extract of *Tephrosia purpurea*.

is consistent with its hepatoprotective activity[5-6]. However, involvement of antilipid peroxidation activity is also possible in its other activities as well such as antipigmenting and body slimming[34].

These observations are interesting, especially in view of the fact that the *Tephrosia purpurea* showed significant free radical scavenging and antilipid peroxidation properties, but did not scavenge the hydroxyl radical. It can be recalled here that the model system used for determination of hydroxyl radical scavenging potential was a totally aqueous system. Thus, less polar constituents of the test extract may not be able to appropriately express their effects due to solubility issues in this system. These facts can be further exploited, for instance, in obtaining a fraction, which will have improved antioxidant activity as compared to crude extract.

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