Evaluation of iron chelating and antioxidant potential of *Epilobium hirsutum* for the management of iron overload disease

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**A B S T R A C T**

The present study deals with the investigation of iron chelating and antioxidant potential of *Epilobium hirsutum* in iron-overloaded rats. Iron overload was induced by 6 IP injections of Iron dextran (12.5 mg/100 g) administered uniformly over a period of 30 days. Different fractions of *E. hirsutum* were given orally and deferoxamine (DFO) subcutaneously for 30 days. The extent of iron chelation and various biochemical parameters were estimated on 15th and 30th day of treatment. In-vitro study was assessed by EDTA and DFO method; the results exhibited a dose-dependant iron chelation. The methanol fraction of methanolic extract (MFME) and methanolic fraction of aqueous extract (MFAE) of *E. hirsutum* showed significant (p < 0.01) iron chelating and antioxidant potential as compared to disease control (DC) rats. The animals treated with MFME and MFAE of *E. hirsutum* showed significant (p < 0.01) vital organ protection as compared to DC rats. The animals treated for longer duration (30th day) reveals better iron chelation potential than shorter ones (15th day). Superior iron chelation was seen at higher dose (300 mg/kg) as compared to lower dose (150 mg/kg). Taken into an account, our result reveals the reversible iron chelating and antioxidant ability of *E. hirsutum* and gives some evidence for its possible mechanism via excretion of iron in urine and feces.

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**1. Introduction**

Iron overloaded disease is group of heterogeneous disease which encompasses a wide range of hereditary and acquired condition [1]. Excess of iron generate free radicals, which leads to cellular damage and promote the cell injury and death. The intensity of cellular damage depends on the rate of free radical generation [2]. Excess of iron can accumulate in vital organs and can produce organ complications like liver failure, cardiac failure [3], renal failure [4], neurodegenerative diseases [5] and endocrine abnormalities [6].

Presently, three iron chelators named deferoxamine, deferasirox are used for the management of iron overload disease. These drugs have limitations due to their side effects [7,8]. The herbal preparations are safe and have few side effects as compared to synthetic drugs.

It has been reported earlier that various herbs such as *Terminalia chebula*, *Terminalia bellirica*, *Emblica officinalis*, *Cajanus cajan*, *Tinospora cordifolia*, *Caesalpinia cristia* [9], *Tetracarpidium conophorum* [10] and *Enhydra fluctuans* [11] posses in-vitro iron chelator potential. In-vivo investigation of some herbs like *Mellilotus officinalis* [12], *Triticum aestivum* [13] and *Medicago sativa* [14] had been performed. *T. aestivum* was also screened for clinical management of beta thalassemia [15].

The plant *Epilobium hirsutum* (Onagraceae) commonly known as great willowherb, earlier have been claimed to show antinociceptive [16], anti-inflammatory [17], antioxidant [18], antimicrobial [19] and antitumor effect [20]. The plant has shown promising role in treating enlarged prostate, prostatitis, cystitis, burning sensation in urine and after prostate operation [21].

The plant is reported to have various flavonoids (kaempferol, queretin and myricetin), phenolic acids (ellagic acid, valoneic acid, gallic acid, p-coumaric acid, protocatechuic acid) and tannins [22–24]. Besides polyphenolic compounds, steroids, triterpenoids, fatty acid [25], tocopherol [26] and amino acids [27] have also been found in the *Epilobium* species. Flavonoids and Phenolic compounds have been discovered for iron chelating and antioxidant...
property [28,29]. There is a need to explore more safe and effective therapeutic management for iron overload disease. Hence, the present study deals with the evaluation of iron chelating and antioxidant potential of E. hirsutum in iron-overloaded rats.

2. Material and methods

2.1. Collection and authentication of plant

The plant E. hirsutum for the study was collected in the flowering stage from the fields of Chatterham, Hazratbal, Srinagar, Jammu and Kashmir, India during August 2013. The plant was authenticated at Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir, Jammu and Kashmir, India bearing specimen no. 1914-KASH and was deposited in the institute.

2.2. Preparation of plant extracts and fractions

The shade dried leaves of E. hirsutum were converted to coarse powder. The powdered material was extracted with methanol and aqueous solvent by Soxhlet extraction method (12 cycles each). The extracts were concentrated by evaporating the solvent under vacuum. The extracts were dissolved in appropriate solvent and further fractionation was performed with solvents of increasing polarity. The fractions were concentrated by evaporating the solvent under vacuum. The dried methanolic and aqueous fractions of E. hirsutum were dissolved in 2% Tween-80 to obtain methanolic fraction of methanolic extract (MFME), aqueous fraction of methanolic extract (AFME), methanolic fraction of aqueous extract (MFAE) and aqueous fraction of aqueous extract (AFAE) for further investigations in iron-overloaded rats [30].

2.3. Experimental animals

For the study, healthy male Sprague Dawley rats (200–250 g, 10–12 weeks old) were procured from Zydus Research Centre, Ahmedabad, India. The rats were kept in animal house, Department of Pharmacology, School of Pharmacy, RK University, Rajkot, India at ambient temperature (23 ± 2 °C), relative humidity (55 ± 5%) and 12 h/12 h light dark cycle. The rats were feed with standard rodent pellet diet and water ad libitum. The study protocol was in accordance to CPCSEA guidelines, the protocol was duly approved by IAEC (RKCP/COL/RP/15/63).

2.4. Instruments

The analysis was done using UV–visible spectrophotometer (model UV-1800, Shimadzu, Japan) and fully automated clinical chemistry analyzer (model C71, BeneSphere diagnostic solutions, USA).

2.5. Drugs and chemicals

The standard drug Deferoxamine (Desferal® Novartis Pharmaceuticals Corporation, USA) and Iron dextran (Imferon® Shrey Life Sciences Pvt. Ltd., India) were procured from local market of Gujarat, India. Standard analyzing kits for various parameters were commercially obtained from ERBA diagnostics Mannheim GmbH, Germany. All the reagents and chemicals used were of analytical grade.

2.6. Estimation of in-vitro iron chelating potential

The in-vitro iron chelating investigation was performed as per procedure described by Dinis et al. [31]. Briefly, 50 μL of 2 mM ferric chloride was added to 1 mL of different fractions of E. hirsutum having different concentration (0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL). The reaction was initiated by adding 0.2 mL of 5 mM ferrozine solution. The mixture was vigorously shaken and kept to stand at room temperature for 10 min. The absorbances of different concentrations were there after measured at 562 nm. Disodium EDTA and Deferoxamine were used as positive control and standard respectively. The percentage inhibition of ferrozine–Fe²⁺ complex was calculated as [([A0 – A1]/A0) × 100, where A0 was the absorbance of the control, and A1 was the absorbance of extract/standard.

2.7. Induction and treatment of iron overload

The rats were divided in to eleven groups of six rats each. All the groups except normal control (NC) rats given six i.p. injections of iron dextran (12.5 mg/100 g) equally distributed over a period of 30 days which resemble the chronic iron–overload disease and its complications [32]. The animals receive DFO and different fractions of E. hirsutum daily for 30 days after 1 h of iron overload by subcutaneously and orally, respectively. Group I: NC rats received i.p. dextran solution; Group II: DC rats were subjected only to iron dextran Group III: received DFO (40 mg/kg/day) [33]; Group IV: received MFME of E. hirsutum 150 mg/kg/day (MFME 150 mg/kg); Group V: received MFME of E. hirsutum 300 mg/kg/day (MFME 300 mg/kg); Group VI: received AFME of E. hirsutum 150 mg/kg/day (AFME 150 mg/kg); Group VII: received AFME of E. hirsutum 300 mg/kg/day (AFME 300 mg/kg); Group VIII: received MFAE of E. hirsutum 150 mg/kg/day (MFAE 150 mg/kg); Group IX: received MFAE of E. hirsutum 300 mg/kg/day (MFAE 300 mg/kg); Group X: received AFAE of E. hirsutum 150 mg/kg/day (AFAE 150 mg/kg); Group XI: received AFAE of E. hirsutum 300 mg/kg/day (AFAE 300 mg/kg).

2.8. Sample collection

During the study period, the samples were collected on 15th and 30th day of treatment under fasting conditions. Under light chloroform anesthesia, the blood samples were collected by puncture retro orbital plexuses whereas the rats were kept in metabolic cage for 24 h to collect the urine and fecal samples.

2.9. Urine and fecal samples preparation

Urine samples (2 mL) were centrifuged at 14,000 rpm for 5 min to remove cellular debris followed by diluting them with water up to 10 mL, the supernatant was used for the assessment of iron in urine samples.

The fecal samples were placed in medium power microwave oven for 20 min to obtain dry residue. About 100–300 g of dried fecal sample was mixed with known volume of water to form consistent homogenate of 20–25% w/v concentration. To obtain dry residue, the homogenate were again placed for 10 min in medium power microwave oven. About 250 mL of concentrated nitric acid was added to dry residue and the digestion was continued by placing sample for 10 min at medium power microwave oven. The digestion procedure was repeated till clear residue was obtained. The residual samples were reconstituted with 500 μL of 5 mM HCl for estimation of iron [34]. The iron in urine and fecal samples were determined by fully automated clinical chemistry analyzer.

2.10. Estimation of iron chelating potential

Different fractions of E. hirsutum were analyzed for iron chelating potential in iron-overloaded rat. The rats were analyzed on 15th and 30th day of defined treatment by determining the
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