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Evaluation of beneficial effects of *Epilobium hirsutum* on hematological parameter in iron intoxicated Sprague–Dawley rats

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ABSTRACT

Objectives: This study was carried out to investigate the protective role of different fractions of *Epilobium hirsutum* on the toxic effects of iron on hematological value in Sprague–Dawley rats.

Material and Methods: Iron overload was induced by injecting six IP injections of iron dextran (12.5 mg/100 g) uniformly for 30 days. Different fractions of *E. hirsutum* were given orally and deferoxamine subcutaneously for 30 days. The hematological parameters were evaluated on 15–30 days of treatment.

Results: The animal exposed to iron presented a significant ($P < 0.01$) reduction in red blood corpuscles, total and differential white blood cells, and platelet levels. This shows that the overabundance of iron in iron overloaded conditions can lead to bone marrow suppression. These influences of iron overload were prevented by concurrent daily administration of a methanolic fraction of methanolic extract and a methanolic fraction of aqueous extract of *E. hirsutum*.

Conclusion: The results indicate that 300 mg/kg for 30 days shows better beneficial effects as compared to 150 mg/kg for 15 days of treatment. Our results endorsed that *E. hirsutum* has beneficial effects on hematological parameters in iron intoxicated Sprague–Dawley rats.

Keywords: *Epilobium hirsutum*, Hematological parameters, Iron overload and Sprague–Dawley rats

INTRODUCTION

Epilobium hirsutum belongs to the Onagraceae family, commonly known as great willowherb, and great hairy willowherb or hairy willowherb.[1] It has been reported that *E. hirsutum* contains various phytochemical constituents such as bioflavonoids such as quercetin, kaempferol, and myricetin, aromatic acids such as gallic acid, ellagic acid, p-coumaric acid, valoneic acid and protocatechuic acid, and tannins.[2-5] Other than polyphenolic compounds, the plant also contains fatty acids, triterpenoids, steroids,[6] amino acids, and tocopherol.[7,8] The plant has remarkable medicinal properties such as anti-nociceptive,[9] anti-inflammatory,[10] iron chelating and antioxidant,[11] antimicrobial,[12] and antitumor.[13] *E. hirsutum* has been discovered to show potential role in the treatment of prostatitis, benign prostatic hyperplasia, cystitis, dysuria, and post-operational prostate.[14]

Iron overloaded disease is a group of heterogeneous diseases that is caused either due to hereditary or acquired conditions.[15] Iron overload condition triggers free radical generation. These free radicals have been responsible for damaging cellular macromolecules and promoting cell injury, leading to their death. The rate of free radical generation determines the intensity of cell injury and rate of cell death.[16] Excessive iron may deposit in the vital organs and may cause organ damage and other complications.[17-20] Beta-thalassemia patient develops severe anemia; they were susceptible to developing a variety of infections and platelet dysfunction.[21] Till date, iron overload conditions therapy is restricted to some synthetic iron chelating agents. Moreover, conventional synthetic iron chelating agents possess toxic side effects.[22-24]

Thus, there has been increased interest in the therapeutic potential of medicinal plants having a beneficial role in reducing iron poisoning and its complications. The present study was planned to focus on the evaluation of beneficial potential of various fractions of *E. hirsutum* extract on hematological parameters in iron overload-induced Sprague–Dawley rats.

MATERIAL AND METHODS

Plant sourcing and its authentication

E. hirsutum was sourced from the fields of Chatterhama, Hazratbal, Srinagar, Jammu and Kashmir, India, in August 2013 and was harvested in the flowering stage. The plant was authenticated by Mr. Akhtar H. Malik, Curator, Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir, Jammu and Kashmir, India (1914-KASH).

Extraction and fractionation

The shade-dried leaves of *E. hirsutum* were crushed and ground to prepare coarse powder. The resultant powder was submitted to successive solvent extraction with petroleum ether, benzene, chloroform, acetone, methanol, and water using Soxhlet extraction (12 cycles/solvent) in increasing order of their polarity. The resultant fraction portions were submitted to evaporation under vacuum for concentration them. The extracts were solubilized in appropriate solvents and were submitted to fractionation using solvents with increasing order of polarity, the isolated fractions were again submitted to concentration by evaporation under vacuum. The resultant concentrated methanolic and aqueous fractions of *E. hirsutum* were solubilized in Tween-80 (2% v/v) for obtaining the methanolic fraction of methanolic extract (MFME), an aqueous fraction of methanolic extract (AFME), a methanolic fraction of aqueous extract (MFAE), and an aqueous fraction of aqueous extract (AFAE) for further studies.[25]

Experimental animals

Healthy male Sprague–Dawley rats aging 12 weeks weighing around 200–250 g were obtained from Zydus Research Centre, Ahmedabad, India. The animals were maintained at room temperature conditions (23 ± 2°C), having relative humidity (55 ± 5%) with 12 h light and dark cycle at animal house of the Department of Pharmacology, RK University, Gujarat, India. All the rats were given a standard pellet diet and water *ad libitum*. The research protocol was approved by the Institutional Animal Ethical Committee as per the guidelines of CPCSEA (RKCP/COL/RP/15/63).

Instruments

Optical density was studied using UV–Visible spectrometer (UV-1800, Shimadzu, Japan). A biochemical autoanalyzer (Model C71, BeneSphera diag. solutions, USA) was used for studying biochemical parameters.

Drugs and reagents

Iron chelator deferoxamine (DFOA) mesylate (Desferal®, Novartis Pharma, USA) and iron dextran (Imferon®, Shreya Life Sciences Pvt. Ltd., India) were purchased from the local market in Gujarat, India. Diagnostic kits for various biochemical parameters were procured from ERBA diagnostics Mannheim GmbH, Germany. All the reagents and chemicals used were of AR grade.

Iron overload rat model, treatments, and sample collection

The Sprague–Dawley rats were randomly divided into 11 groups with six rats each. The iron overload rat model for the determination of hematological parameters was developed as per a previous study,[26] briefly, all the rats except normal control (NC) received six injections of iron dextran 12.5 mg/100 g body weight through i.p. route evenly distributed over 30 days, the rats resembled the chronic iron overloaded condition and its complications.[27] The rats received DFOA and various fractions of *E. hirsutum* daily for 30 days after 1 h of iron overload by subcutaneous and oral route, respectively. The NC group of rats was injected with dextran solution i.p. route, the disease control (DC) rats were injected with iron dextran only, a positive control group of rats was injected with DFOA (40 mg/kg/day),[28] Group 4 iron overloaded rats received MFME at the dose of 150 mg/kg/day, Group 5 iron overloaded rats received MFME at a dose of 300 mg/kg/day, Group 6 iron overloaded rats were treated with AFME at a dose of 150 mg/kg/day, Group 7 iron overloaded rats received AFME at a dose

of 300 mg/kg/day, Group 8 iron overloaded rats received MFAE 150 mg/kg/day, Group 9 iron overloaded rats received MFAE 300 mg/kg/day, Group 10 iron overloaded rats received AFAE at a dose of 150 mg/kg/day, and Group 11 iron overloaded rats received AFAE 300 mg/kg/day.

The blood samples were collected on the 15th and 30th day of pre-defined treatments under fasting conditions. Blood was collected by puncture of retro-orbital plexuses under light chloroform anesthesia.

Estimation of hematological parameters

Different fractions of *E. hirsutum* were investigated for their beneficial effects on hematological parameters on the 15th and 30th day of treatment by investigating the hemoglobin (Hb) content, total red blood corpuscles (RBCs), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width-standard deviation (RDW-SD), red cell distribution width-coefficient of variation (RDW-CV), total and differential white blood cells (WBCs) count, platelet count, platelet crit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) by fully automated clinical chemistry analyzer (model C71, BeneSphera diagnostic solutions, USA).[29]

Statistical analysis

The results were presented as mean ± SD. The results were analyzed using a one-way analysis of variance with Dunnett's *post hoc* test for establishing statistical significance.

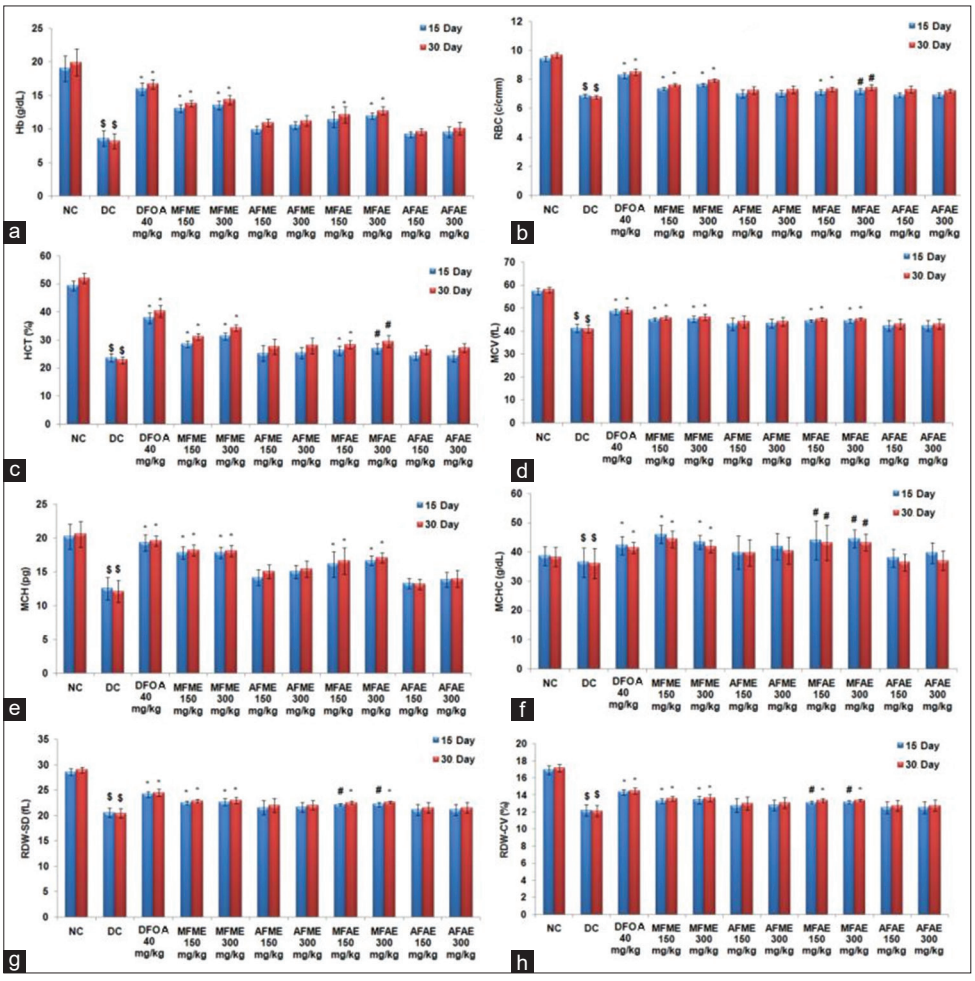

Figure 1: Effect of *Epilobium hirsutum* on different hematological functions in iron overload-induced rats (a) hemoglobin, (b) red blood corpuscles, (c) hematocrit, (d) mean corpuscular volume, (e) mean corpuscular hemoglobin, (f) mean corpuscular hemoglobin concentration, (g) red cell distribution width-standard deviation, and (h) red cell distribution width-coefficient of variation. The results are presented as Mean ± SD ($n = 6$), $^{*}P < 0.01$ against disease control (DC) rats, $^{\#}P < 0.05$ against DC rats, and $^{\$}P < 0.01$ against normal control rats.

Table 1: Effect of *Epilobium hirsutum* on different hematological parameters in iron overloaded rats.

Groups	Hb (g/dL)		RBC (m/cmm)		HCT (%)		MCV (fL)		MCH (pg)		MCHC (g/dL)		RDW-SD (fL)		RDW-CV (%)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
NC	19.05±1.92	19.93±2.02	9.43±0.18	9.70±0.18	49.33±1.75	52.00±1.79	57.20±1.33	58.00±1.17	20.18±1.84	20.54±1.92	38.59±3.24	38.31±3.40	28.60±0.66	29.00±0.58	16.94±0.51	17.18±0.41
DC	8.60±1.17$^{\$}$	8.21±1.10$^{\$}$	6.87±0.14$^{\$}$	6.78±0.12$^{\$}$	23.67±1.37$^{\$}$	22.83±1.17$^{\$}$	41.20±1.81$^{\$}$	40.97±1.78$^{\$}$	12.52±1.68$^{\$}$	12.11±1.64$^{\$}$	36.41±5.10$^{\$}$	36.06±5.18$^{\$}$	20.60±0.90$^{\$}$	20.48±0.89$^{\$}$	12.21±0.68$^{\$}$	12.14±0.68$^{\$}$
DFOA 40 mg/kg	15.95±0.95*	16.69±0.73*	8.28±0.18*	8.53±0.22*	37.83±1.83*	40.33±2.16*	48.40±1.16*	49.02±1.35*	19.26±1.19*	19.55±0.71*	42.23±3.14*	41.42±1.90*	24.20±0.58*	24.51±0.67*	14.33±0.33*	14.52±0.36*
MFME 150 mg/kg	13.09±0.53*	13.80±0.48*	7.35±0.10*	7.62±0.12*	28.50±1.05*	31.17±1.17*	45.00±0.66*	45.83±0.75*	17.82±0.90*	18.13±0.83*	46.01±3.19*	44.37±2.87*	22.50±0.33*	22.92±0.38*	13.33±0.27*	13.58±0.28*
MFME 300 mg/kg	13.58±0.65*	14.35±0.72*	7.63±0.12*	7.93±0.10*	31.33±1.21*	34.33±1.03*	45.30±1.36*	46.03±1.30*	17.79±0.84*	18.09±0.86*	43.38±2.37*	41.82±2.09*	22.65±0.68*	23.02±0.65*	13.42±0.48*	13.63±0.42*
AFME 150 mg/kg	9.89±0.57	10.92±0.57	7.02±0.28	7.27±0.27	25.17±2.79	27.67±2.73	43.10±2.81	44.03±2.71	14.13±1.16	15.04±0.98	39.80±5.61	39.79±4.50	21.55±1.40	22.02±1.36	12.76±0.82	13.04±0.80
AFME 300 mg/kg	10.54±0.55	11.25±0.84	7.03±0.21	7.30±0.28	25.33±2.07	28.00±2.76	43.33±1.93	44.15±1.91	15.00±0.96	15.41±1.18	41.87±4.45	40.44±4.60	21.67±0.97	22.08±0.96	12.84±0.63	13.08±0.63
MFAE 150 mg/kg	11.43±1.15*	12.15±1.23*	7.12±0.17*	7.33±0.15*	26.17±1.72*	28.33±1.51*	44.30±0.55*	45.12±0.69*	16.09±1.89*	16.60±1.90*	44.00±6.56$^{\#}$	43.11±5.96$^{\#}$	22.15±0.28$^{\#}$	22.56±0.35*	13.12±0.17$^{\#}$	13.36±0.20*
MFAE 300 mg/kg	11.98±0.46*	12.72±0.63*	7.20±0.18$^{\#}$	7.45±0.21$^{\#}$	27.00±1.79$^{\#}$	29.50±2.07$^{\#}$	44.48±0.84*	45.23±0.52*	16.64±0.66*	17.07±0.75*	44.51±3.11$^{\#}$	43.24±2.92$^{\#}$	22.24±0.42$^{\#}$	22.62±0.26*	13.17±0.19$^{\#}$	13.40±0.16*
AFAE 150 mg/kg	9.20±0.45	9.61±0.45	6.93±0.14	7.32±0.23	24.33±1.37	26.50±1.64	42.37±2.29	43.10±2.09	13.27±0.73	13.15±0.80	37.90±3.04	36.40±2.98	21.18±1.14	21.55±1.05	12.55±0.69	12.77±0.65
AFAE 300 mg/kg	9.55±0.82	10.05±0.95	6.92±0.18	7.22±0.15	24.17±1.83	27.17±1.47	42.30±2.29	43.15±2.17	13.81±1.11	13.93±1.25	39.63±3.56	37.03±3.32	21.15±1.15	21.58±1.08	12.53±0.68	12.78±0.65

The results are presented as Mean±SD (n=6). *P<0.01 against DC rats, $^{\#}P$<0.05 against DC rats, and $^{\$}P$<0.01 against NC rats. DFOA: Deferoxamine, AFME: Aqueous fraction of methanolic extract, MFAE: Methanolic fraction of aqueous extract, HCT: Hematocrit, MCV, Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-SD: Red cell distribution width-standard deviation RDW-CV: Red cell distribution width- coefficient of variation, RBC: Red blood corpuscle, AFAE: Aqueous fraction of aqueous extract, MFME: Methanolic fraction of methanolic extract, MFAE: Methanolic fraction of aqueous extract, and Hb: Hemoglobin

RESULTS

Effects of *E. hirsutum* on hematological parameters

The iron overload-induced rats show a significant ($P < 0.01$) decrease in Hb (8.60 ± 1.17 g/dL) and RBC (6.87 ± 0.14 m/cmm) as compared to the NC rat's Hb (19.05 ± 1.92 g/dL) and RBC (9.43 ± 0.18 m/cmm) which indicated that iron toxic effect on Hb and RBC synthesis had been established. The rats treated with DFOA, MFME, and MFAE showed a significant ($P < 0.01$) increase in Hb and RBC counts against other fractions on the 15th and 30th day of treatment [Table 1 and Figure 1]. The increased levels of Hb and RBC indicate that *E. hirsutum* is having a stimulating effect on Hb synthesis and the hemopoietic system. The iron overloaded experimental rats (DC rats) showed significant ($P < 0.01$) reduction in other hematological parameters such as HCT (%), MCV (fL), MCH (pg), MCHC (g/dl), RDW-SD (fL), and RDW-CV (%) counts as compared to NC rats. These results reveal the toxic effects of an excess of iron on hematological parameters. After treatment with MFME and MFAE fractions, the rats showed a significant ($P < 0.01$) increase in hematological parameters.

Effects of *E. hirsutum* on total and differential WBC parameters

Iron overloaded experimental rats showed a significant ($P < 0.01$) reduction of total WBC in DC rats ($3.03 \pm 0.22\ 10^3/\mu L$) as compared to NC rats ($5.72 \pm 0.38\ 10^3/\mu L$). Iron overload also reduces the differential WBC counts in DC rats against NC rats. Rats treated with DFOA, MFME, and MFAE showed a significant ($P < 0.01$) increase in total WBC as well as differential WBC counts as compared to other fractions, as shown in [Table 2 and Figure 2]. The increase in total as well as differential WBC count indicates that *E. hirsutum* stimulates bone marrow. The results also suggested that *E. hirsutum* has a beneficial effect on the immune system.

Effects of *E. hirsutum* on platelet parameters

Iron overloaded experimental rats demonstrated a significant ($P < 0.01$) decrease in the platelet count of DC rats ($657.17 \pm 16.01\ 10^3/\mu L$) as compared to NC rats ($785.00 \pm 10.49\ 10^3/\mu L$) [Table 3 and Figure 3]. Treatment with DFOA and MFME and MFAE fractions showed a significant ($P < 0.01$) increase in platelet, PCT (%), MPV (fL), and PDW (fL) count as compared to DC rats. These results indicate the beneficial effect of *E. hirsutum* in platelet deficiency.

DISCUSSION

Previous studies claimed that phytoactive compounds such as flavonoids and phenols can chelate metal ions and form

Table 2: Effect of *Epilobium hirsutum* on total and differential WBC count in iron overloaded rats.

Groups	WBC ($\times 10^3/\mu L$)		Neutrophil ($\times 10^3/\mu L$)		Lymphocyte ($\times 10^3/\mu L$)		Monocyte ($\times 10^3/\mu L$)		Eosinophil ($\times 10^3/\mu L$)		Basophil ($\times 10^3/\mu L$)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
NC	5.72±0.38	5.60±0.30	2.75±0.18	2.80±0.19	1.50±0.10	1.60±0.11	0.40±0.05	0.49±0.06	0.18±0.02	0.20±0.02	0.12±0.02	0.14±0.02
DC	3.03±0.22$^{\$}$	2.91±0.24$^{\$}$	1.30±0.08$^{\$}$	1.19±0.09$^{\$}$	0.76±0.07$^{\$}$	0.68±0.09$^{\$}$	0.12±0.02$^{\$}$	0.10±0.03$^{\$}$	0.03±0.01$^{\$}$	0.02±0.01$^{\$}$	0.02±0.01$^{\$}$	0.01±0.01$^{\$}$
DFOA 40 mg/kg	4.23±0.31*	4.45±0.29*	2.20±0.16*	2.33±0.14*	1.19±0.06*	1.32±0.08*	0.31±0.04*	0.40±0.05*	0.14±0.02*	0.18±0.02*	0.09±0.02*	0.12±0.02*
MFME 150 mg/kg	3.81±0.20*	3.98±0.20*	1.70±0.12*	1.87±0.11*	1.05±0.08*	1.12±0.10*	0.22±0.02*	0.28±0.03*	0.10±0.02*	0.11±0.01*	0.05±0.02*	0.09±0.01*
MFME 300 mg/kg	4.00±0.20*	4.08±0.23*	1.95±0.15*	2.04±0.13*	1.08±0.08*	1.20±0.11*	0.24±0.02*	0.31±0.02*	0.11±0.02*	0.13±0.02*	0.06±0.02*	0.10±0.01*
AFME 150 mg/kg	3.26±0.27	3.32±0.24	1.45±0.18	1.56±0.15	0.83±0.11	0.92±0.13	0.15±0.04	0.19±0.03	0.05±0.01	0.07±0.01	0.03±0.01	0.05±0.01
AFME 300 mg/kg	3.39±0.28	3.36±0.23	1.50±0.19	1.50±0.15	0.88±0.12	0.94±0.16	0.17±0.03	0.20±0.02	0.06±0.01	0.08±0.01	0.05±0.02	0.08±0.01
MFAE 150 mg/kg	3.46±0.19*	3.51±0.24*	1.60±0.11*	1.70±0.12*	0.92±0.10*	1.00±0.09*	0.18±0.02*	0.23±0.03*	0.07±0.01*	0.09±0.01*	0.05±0.02*	0.07±0.01*
MFAE 300 mg/kg	3.66±0.18*	3.77±0.29*	1.69±0.13*	1.80±0.09*	0.97±0.09*	1.04±0.08*	0.19±0.03*	0.26±0.02*	0.08±0.02*	0.10±0.02*	0.06±0.02*	0.09±0.02*
AFAE 150 mg/kg	3.18±0.27	3.23±0.35	1.35±0.19	1.40±0.18	0.80±0.13	0.89±0.13	0.13±0.05	0.15±0.03	0.03±0.01	0.04±0.01	0.02±0.01	0.03±0.01
AFAE 300 mg/kg	3.23±0.31	3.30±0.36	1.40±0.21	1.45±0.20	0.83±0.14	0.92±0.14	0.14±0.06	0.16±0.04	0.04±0.01	0.05±0.01	0.03±0.02	0.04±0.01

The results are presented as Mean±SD (n=6). *P<0.01 against NC rats and $^{\$}P$<0.01 against DC rats. MFME: Methanolic fraction of methanolic extract, MFAE: Methanolic fraction of aqueous extract, WBC: White blood cell, DC: Disease control, and NC: Normal control, AFAE: Aqueous fraction of aqueous extract, Hb: Hemoglobin, DFOA: Deferoxamine

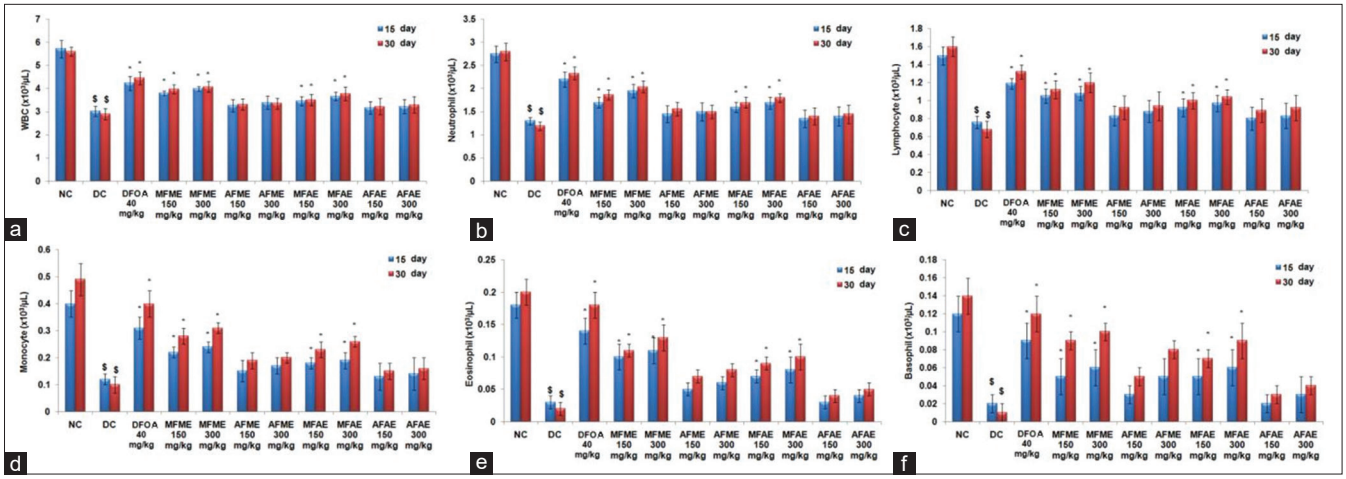

Figure 2: Effect of *Epilobium hirsutum* on total and differential white blood cells (WBCs) count in iron overload-induced rats (a) WBC, (b), neutrophil, (c) lymphocyte, (d) monocyte, (e) eosinophil, and (f) basophil. The results are presented as Mean ± SD (n = 6), *P < 0.01 against disease control rats and $^{\$}P$ < 0.01 against normal control rats.

Table 3: Effect of *Epilobium hirsutum* on platelet count in iron overloaded rats.

Groups	PLT (×10³/µL)		PCT (%)		MPV (fL)		PDW (fL)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
NC	785.00±10.49	796.17±10.55	0.29±0.01	0.30±0.01	3.63±0.08	3.71±0.09	8.02±0.21	8.12±0.14
DC	657.17±16.01$^{\$}$	643.33±12.58$^{\$}$	0.16±0.02$^{\$}$	0.14±0.01$^{\$}$	2.38±0.17$^{\$}$	2.20±0.16$^{\$}$	6.57±0.16$^{\$}$	6.23±0.19$^{\$}$
DFOA 40 mg/kg	714.83±8.30*	735.83±12.01*	0.22±0.01*	0.24±0.01*	3.01±0.08*	3.19±0.09*	7.15±0.08*	7.36±0.12*
MFME 150 mg/kg	685.00±15.02*	694.83±9.04*	0.18±0.01*	0.19±0.01*	2.68±0.14*	2.78±0.11*	6.85±0.15#	6.95±0.09*
MFME 300 mg/kg	685.17±10.40*	702.00±13.93*	0.19±0.01*	0.20±0.01*	2.70±0.11*	2.87±0.11*	6.85±0.10#	7.02±0.14*
AFME 150 mg/kg	650.50±24.59	660.00±22.84	0.15±0.03	0.16±0.02	2.30±0.31	2.42±0.28	6.51±0.25	6.60±0.23
AFME 300 mg/kg	658.83±6.01	670.50±6.44	0.16±0.01	0.17±0.01	2.40±0.09	2.54±0.07	6.59±0.06	6.71±0.06
MFAE 150 mg/kg	668.00±7.48	681.00±10.84*	0.17±0.01	0.18±0.01*	2.52±0.09	2.64±0.12*	6.68±0.07	6.81±0.11*
MFAE 300 mg/kg	679.50±16.10*	691.67±12.63*	0.18±0.01*	0.19±0.01*	2.62±0.15	2.75±0.13*	6.80±0.16	6.92±0.13*
AFAE 150 mg/kg	646.17±11.92	658.83±10.28	0.15±0.01	0.16±0.01	2.24±0.12	2.38±0.19	6.46±0.12	6.59±0.10
AFAE 300 mg/kg	658.33±11.57	665.33±7.17	0.16±0.01	0.17±0.01	2.40±0.14	2.48±0.11	6.58±0.12	6.64±0.10

The results are presented as Mean±SD (n=6). *P<0.01 against DC rats and $^{\$}P$<0.01 against NC rats. DFOA: Deferoxamine, MFME: Methanolic fraction of methanolic extract, AFME: Aqueous fraction of methanolic extract, MFAE: Methanolic fraction of aqueous extract, PLT: Platelet count, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, DC: Disease control, NC: Normal control, and AFAE: Aqueous fraction of aqueous extract

complexes with them;[30-32] Also, these compounds possess antioxidant properties.[33] The plant *E. hirsutum* has been reported to contain flavonoids and phenols.[34] Our findings confirm significant beneficial effects of MFME and MFAE of *E. hirsutum*.

Patients with beta-thalassemia develop a severe form of anemia, which is due to chronic hemolysis and ineffective erythropoiesis process. According to the previous report, an iron chelating agent improves both iron overload conditions and erythropoiesis.[35]

Our results indicate that iron overload significantly (P < 0.01) decreased Hb, RBC, PCV, MCV, MCH, MCHC, RDW-SD, and RDW-CV counts as compared to NC rats. The treatment of MFME and MFAE of *E. hirsutum* in iron overloaded rats showed significant (P < 0.01) increased counts of Hb, RBC, PCV, MCV, MCH, MCHC, RDW-SD, and RDW-CV compared to DC rats on the 15th and 30th day. These results suggested that MFME and MFAE fractions of *E. hirsutum* improved the altered erythropoiesis due to iron overload condition.

Patients with various forms of iron overloaded disease were predisposed to have the chances of infection and this may be due to a reduced immune system.[36] It was reported that DFOA restores the immune system in iron-overloaded mice.[37] Our results suggested that due to iron overload, there were significant (P < 0.01) decreased in both total and differential WBCs count against NC rats. Significant (P < 0.01) increased in both total and differential WBCs count in MFME and MFAE fractions of *E. hirsutum*-treated rats against DC rats on the 15th and 30th day. Our result suggested that these fractions of *E. hirsutum* help to improve the immune system which was altered due to iron overload condition.

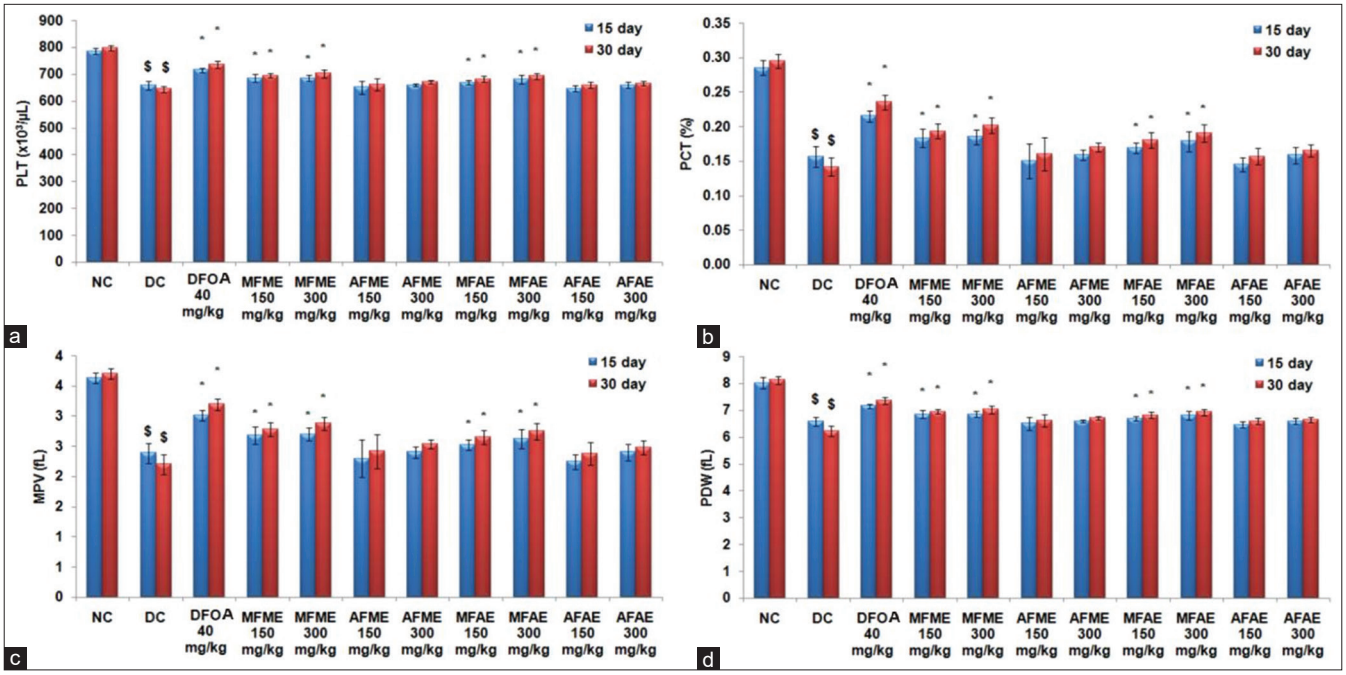

Figure 3: Effect of *Epilobium hirsutum* on platelet count in rats (a) platelet count, (b) plateletcrit, (c) mean platelet volume, and (d) platelet distribution width iron overload induced. The results are presented as Mean ± SD ($n = 6$), $^{*}P < 0.01$ against disease control rats and $^{\$}P < 0.01$ against normal control rats.

Iron overload disease also produces platelet function defect. This defect may be due to liver damage or a direct platelet defect in iron overload disease.[38] As per the previous finding, iron chelation therapy improves the platelet function in iron overloaded patients.[39] Our results indicate that there was a significant ($P < 0.01$) decrease in platelet, PCT, MPV, and PDW count after iron overload against NC rats. There was a significant ($P < 0.01$) increased in platelet PCT, MPV, and PDW count in MFME and MFAE fraction of *E. hirsutum*-treated rats against DC rats on the 15th and 30th day. The results suggested that these fractions of *E. hirsutum* improve the platelet function in iron overloaded rats.

The results of MFME fraction of *E. hirsutum* were close to the results of DFOA, suggesting that MFME of *E. hirsutum* has a better beneficial effect on hematological parameters in iron overloaded rats as compared to MFAE of *E. hirsutum*. The data advised that 300 mg/kg of *E. hirsutum* has a better significant ($P < 0.01$) iron chelation potential as compared to 150 mg/kg. Our results also reveal that *E. hirsutum* shows a greater beneficial effect on hematological parameters on 30 day as compared to 15 day of treatment.

CONCLUSION

Our results endorsed that MFME and MFAE of *E. hirsutum* helps to improve the erythropoietic system, immune system, and platelet dysfunctions which were altered due to iron overload condition. Hence, our findings suggest that MFME and MFAE fractions of *E. hirsutum* possess a beneficial effect on hematological parameters in iron overloaded rats. The flavonoids and polyphenolic compounds of *E. hirsutum* may be responsible contents for exerting a beneficial effect on hematological parameters. Further study is needed for the extension of the beneficial effects of *E. hirsutum* on hematological parameters in iron overloaded rats. This can be achieved by isolation, characterization, and biological evaluation of active constituents from *E. hirsutum* for developing new safer and potential herbal drug molecules for the treatment of hematological dysfunction in an iron overload condition.

Declaration of patient consent

Patients' consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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