Evaluation of Beneficial Effects of *Melilotus officinalis* on Blood Profiles in iron Overloaded Sprague Dawley Rats

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Abstract: Present study deals with the investigation of beneficial effects of different fraction of *Melilotus officinalis* (*M. officinalis*) on the toxic effects of iron on different blood parameters in iron overloaded sprague dawley rats. The six IP injections of iron dextran (12.5 mg/100g) were administered uniformly over a period of 30 days to induce iron overload. Different fractions of *M. officinalis* were given orally and Deferoxamine (DFO) subcutaneously for 30 days. The blood parameters were estimated on 15th and 30th day of treatment. Rats exposed to iron showed significant (p < 0.01) decrease in RBC counts, Total and Differential WBC counts and Platelet counts. This indicates that excess of iron in iron overloaded disease can leads to bone marrow suppression. The animals treated with methanolic fraction of methanolic extract (MFME) and methanolic fraction of aqueous extract (MFAE) of *M. officinalis* shows significant (P<0.01) improvement in haematological parameters as compared to disease control (DC) rats. Greater beneficial effects were observed on 30th day and at higher dose (300 mg/kg) as compared to 15th day and at lower dose (150 mg/kg). These results suggested that *M. officinalis* have beneficial effects on blood parameters in iron intoxicated rats.

Keywords: *Melilotus officinalis*, Haematological parameters, Iron overload, Sprague Dawley Rats.

INTRODUCTION

Melilotus officinalis (family Fabaceae; commonly known as yellow sweet clover) is a tall robust biennial herb, about 1 meter in height. A variety of chemical constituents have been isolated from this plant. They belong to different classes such as flavonoids and various phenolic compounds, melilotin, volatile oil, mucilage, tannin, fatty acid, triterpenes, coumarin, bishydroxycoumarin, choline and glycosides [1-2]. The whole plant is used for therapeutic purpose. It is reported that the plant has remarkable and notable medicinal properties such as iron chelating and antioxidants [3], antibacterial, antitumor [4], anti-inflammatory [5], antihypertensive [6] and astringent activity [7]. It also has aromatic, emollient, styptic and carminative properties. The small fruits of plant are used as demulcent, maturant, tonic and aphrodisiac, there for useful in leukoderma [8]. The seeds are reported to be poisonous [9].

Iron overloaded disease is group of heterogeneous disease which may be caused either due to hereditary or acquired condition [10]. Excess of iron due to iron overload condition may generate free radicals, which causes cellular damage and promote the cell injury and cell death. The rate of free radical generation determines the intensity of cellular damage and rate of cell death [11]. Excess of iron can accumulate in the vital organs and can produce organ damage and their complications [12-15]. Beta thalassemia patient develops varying severity of anaemia; they were prone to have an infection and platelet dysfunction [16].

Until now the studies regarding the regulation of iron toxicity in iron overload disease conditions are restricted to some iron chelating agents. However, most of the conventional synthetic iron chelating agents has been reported to possess toxic side effects or disadvantages [17-18]. Thus, there has been increased interest in the therapeutic potential of plant products or medicinal plants having beneficial role in reducing iron poisoning. Keeping, this view in mind, the present study was designed to investigate the beneficial effects of different fractions of *M. officinalis* on blood parameters in iron overloaded Sprague Dawley rats.

MATERIAL AND METHODS

Collection and Authentication of Plant

The plant *M. officinalis* was collected in the flowering stage from the fields of Choaglamsar, Leh, Jammu and Kashmir, India during august 2013. It was authenticated by Mr. Akhtar H. Malik, Curator, Centre for Biodiversity and Taxonomy, Department of Botany,

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University of Kashmir, Jammu and Kashmir, India (1915-KASH).

Preparation of Plant Extracts and Fractions

The shade dried aerial part of *M. officinalis* 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 *M. officinalis* were dissolved in 2% Tween-80 to obtain MFME, AFME, MFAE and AFAE for further investigations [19].

Experimental Animals

For the present study twelve weeks old male Sprague Dawley rats (200 to 250 g) were procured from Zydus Research Centre, Ahmedabad, India. The animals were housed at ambient temperature (23±2^{o}C), relative humidity (55±5%) and 12h/12h light dark cycle at animal house, Department of Pharmacology, School of Pharmacy, RK University, Rajkot, India. All animals were feed standard pellets diet and water *ad labium*. The research protocol was approved by IAEC as per the guidelines of CPCSEA (RKCP/COL/RP/15/63).

Instruments

The study was performed by using UV spectrophotometer (model UV-1800, Shimadzu, Japan) and fully automated clinical chemistry analyzer (model C71, BeneSphera diagnostic solutions, USA).

Drugs and Chemicals

The drug like Desferal® (Deferoxamine mesylate, Novartis Pharmaceuticals Corporation, USA) and Imferon® (Iron dextran, Shreya life sciences Pvt. Ltd., India) were purchased from local market of Gujarat, India. The different standard analyzing kits were commercially obtained from ERBA diagnostics Mannheim GmbH, Germany. All the reagents and chemicals used were of analytical grade.

Induction and Treatment of Iron Overload

The Sprague Dawley rats were divided in to 11 groups each of 6 rats. All the groups except normal control (NC) rats were 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 overloaded disease and its complications [20]. The animals receive DFO and different fractions of *M. officinalis* daily for 30 days after 1 hour of iron overload by subcutaneously and orally respectively. Group I: NC rats received i.p. dextran solution; Group II: DC rats received only iron dextran Group III: were subjected to DFO (40 mg/kg/day) [21]; Group IV: received MFME of *M. officinalis* 150 mg/kg/day (MFME 150 mg/kg); Group V: received MFME of *M. officinalis* 300 mg/kg/day (MFME 300 mg/kg); Group VI: received AFME of *M. officinalis* 150 mg/kg/day (AFME 150 mg/kg); Group VII: received AFME of *M. officinalis* 300 mg/kg/day (AFME 300 mg/kg); Group VIII: received MFAE of *M. officinalis* 150 mg/kg/day (MFAE 150 mg/kg); Group IX: received MFAE of *M. officinalis* 300 mg/kg/day (MFAE 300 mg/kg); Group X: received AFAE of *M. officinalis* 150 mg/kg/day (AFAE 150 mg/kg); Group XI: received AFAE of *M. officinalis* 300 mg/kg/day (AFAE 300 mg/kg)

Sample Collection

During the study period the samples were collected on 15th and 30th day of treatment under fasting conditions. Under light chloroform anaesthesia the blood samples were collected by puncture of retro orbital plexuses.

Estimation of Haematological Parameters

The different fractions of *M. officinalis* were analyzed for their beneficial effects on haematological parameters on 15th and 30th day of treatment by determining the Hb content, Total RBC, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, Total and differential WBC count, Platelet count, PCT, MPV and PDW by fully automated clinical chemistry analyzer [22].

Statistical Analysis

The results were expressed as mean ± SD. The data were analyzed by using one-way analysis of variance (ANOVA) with Dunnett's post test to determine statistically significant differences.

RESULTS

Effects of *M. officinalis* on Haematological Parameters

Iron overloaded rats showed significant (p < 0.01) reduction in Hb (8.60 ± 1.17 g/dL) and RBC (6.87 ±

Table 1: Effect of *M. officinalis* 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 (%)	
	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day
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$
DFO 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	13.30 ± 0.73*	14.01 ± 0.59*	7.38 ± 0.17*	7.60 ± 0.24*	28.83 ± 1.72*	31.00 ± 2.37*	45.05 ± 0.63*	45.82 ± 0.83*	18.04 ± 1.33*	18.46 ± 1.29*	46.36 ± 4.78#	45.50 ± 5.11#	22.53 ± 0.31*	22.91 ± 0.41*	13.34 ± 0.30*	13.57 ± 0.37*
MFME 300 mg/kg	14.04 ± 0.79*	15.11 ± 0.70*	7.67 ± 0.12*	8.02 ± 0.10*	31.67 ± 1.21*	34.83 ± 0.98*	45.37 ± 1.38*	46.27 ± 1.48*	18.32 ± 1.14*	18.85 ± 0.85*	44.42 ± 3.36#	43.40 ± 2.18#	22.68 ± 0.69*	23.13 ± 0.74*	13.44 ±0.55*	13.71 ± 0.56*
AFME 150 mg/kg	10.20 ± 0.54	10.85 ± 0.53	7.05 ± 0.19	7.22 ± 0.15	25.50 ± 1.87	27.17 ± 1.47	43.18 ± 2.62	43.87 ± 2.47	14.49 ± 0.94	15.04 ± 0.81	40.23 ± 4.08	40.04 ± 2.99	21.59 ± 1.31	21.93 ± 1.24	12.79 ± 0.74	12.99 ± 0.68
AFME 300 mg/kg	10.84 ± 0.85	11.59 ± 0.85	7.08 ± 0.17	7.28 ± 0.22	25.83 ± 1.72	27.83 ± 2.23	43.40 ± 1.98	44.17 ± 1.94	15.29 ± 1.07	15.92 ± 1.05	42.01 ± 3.16	41.77 ± 3.34	21.70 ± 0.99	22.08 ± 0.97	12.86 ± 0.69	13.09 ± 0.69
MFAE 150 mg/kg	11.72 ± 1.33*	12.49 ± 1.22*	7.20 ± 0.13#	7.42 ± 0.15#	27.00 ± 1.26#	29.17 ± 1.47*	44.35 ± 0.53*	45.35 ± 0.50*	16.30 ± 2.00*	16.86 ± 1.83*	43.60 ± 6.20#	43.01 ± 5.53#	22.18 ± 0.26#	22.68 ± 0.25*	13.14 ± 0.26#	13.43 ± 0.27*
MFAE 300 mg/kg	12.41 ± 1.18*	13.23 ± 1.33*	7.23 ± 0.22#	7.48 ± 0.22#	27.33 ± 2.16*	29.83 ± 2.23*	44.51 ± 0.85*	45.70 ± 1.02*	17.17 ± 1.74*	17.71 ± 2.02*	45.66 ± 5.83*	44.64 ± 6.36*	22.25 ± 0.43*	22.85 ± 0.51*	13.18 ± 0.35#	13.54 ± 0.37*
AFAE 150 mg/kg	9.37 ± 0.36	9.85 ± 0.28	6.95 ± 0.20	7.13 ± 0.24	24.50 ± 1.97	26.33 ± 2.42	42.40 ± 2.33	43.08 ± 2.01	13.48 ± 0.27	13.81 ± 0.25	38.35 ± 1.90	37.60 ± 2.60	21.20 ± 1.17	21.54 ± 1.01	12.56 ± 0.68	12.76 ± 0.59
AFAE 300 mg/kg	9.85 ± 0.93	10.62 ± 0.90	7.07 ± 0.37	7.15 ± 0.15	24.67 ± 2.07	26.50 ± 1.52	42.20 ± 2.06	43.25 ± 2.14	13.96 ± 1.42	14.85 ± 1.29	40.13 ± 4.84	40.16 ± 4.03	21.10 ± 1.03	21.63 ± 1.07	12.50 ± 0.62	12.81 ± 0.62

The results were expressed as Mean ± SD (n = 6), * $p < 0.01$ when compared to DC rats, # $p < 0.05$ when compared to DC rats and $ $p < 0.01$ when compared to NC rats.

0.14 m/cmm) as compared to NC rats Hb (19.05 ± 1.92 g/dL) and RBC (9.43 ± 0.18 m/cmm) suggesting that toxic effect of iron overload on Hb and RBC synthesis had been developed. Animals treated with DFO, MFME and MFAE of *M. officinalis* show a significant ($p < 0.01$) increase in Hb levels and RBC counts as compared to other fractions on the 15th and 30th day of treatment as shown in Table **1** and Figure **1**. The increased levels of Hb and RBC indicate that *M. officinalis* may have a stimulating effect on Hb synthesis and haemopoietic system respectively. The DC rats showed significant ($p < 0.01$) reduction in HCT (%), MCV (fL), MCH (pg), MCHC (g/dl), RDW-SD (fL) and RDW-CV (%) counts as compared to NC rats. The results indicate the toxic effects of an excess of iron on blood. After treatment with MFME and MFAE of *M. officinalis,* there was a significant ($p < 0.01$) increase in these parameters on the 15th and 30th day of treatment.

Effects of *M. officinalis* on Total and Differential WBC Parameters

Excess of iron in iron overloaded disease leads to bone marrow suppression, which decreases total as well as differential WBC count. Iron overloaded rats show significant ($p < 0.01$) reduction of total WBC in DC rats (3.03 ± 0.22 10^3/µL) as compared to NC rats (5.72 ± 0.38 10^3/µL). Iron overload also reduces the

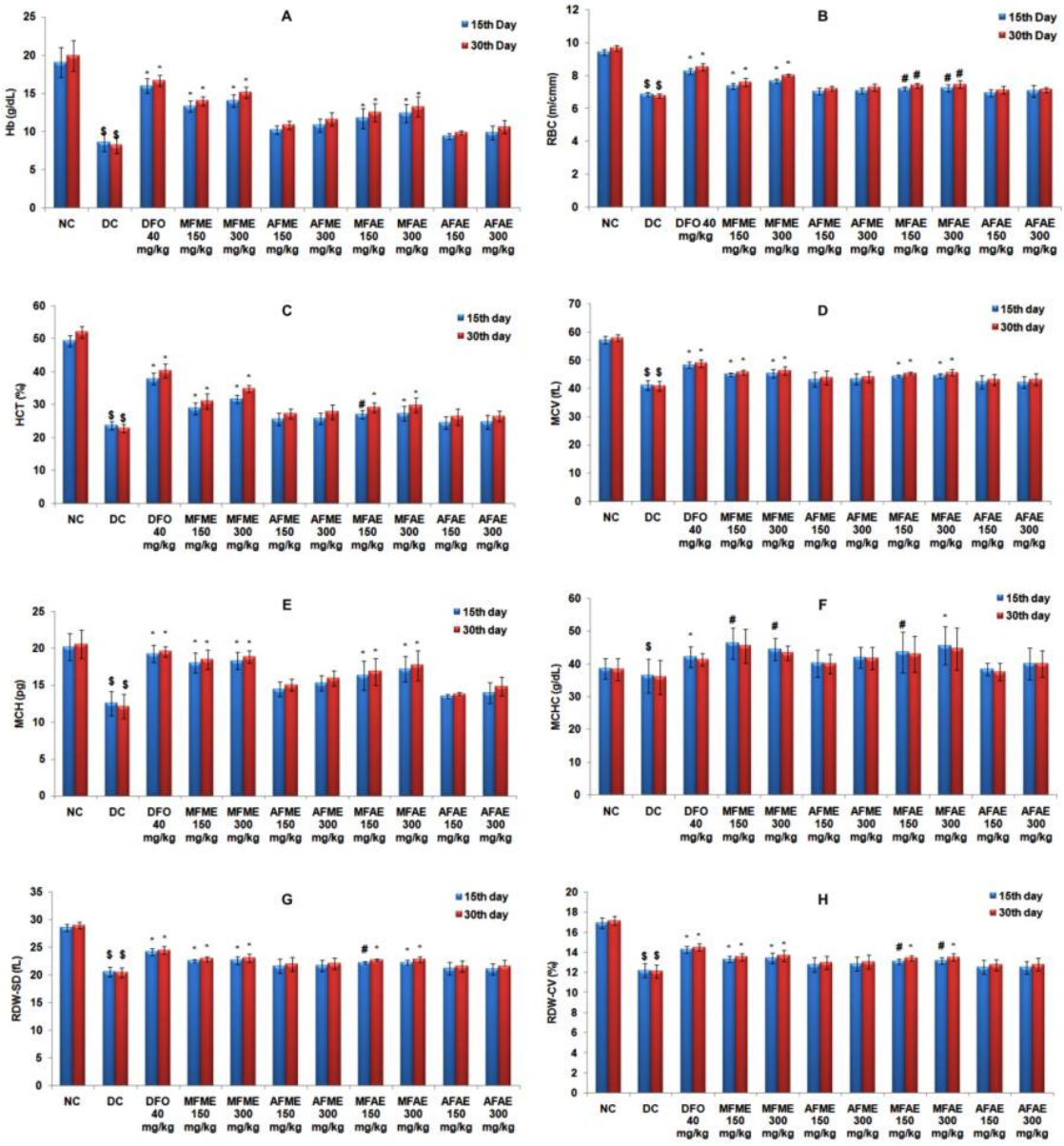

Figure 1: Effect of *M. officinalis* on different hematological parameters in iron overloaded rats. **A**; Hb, **B**; RBC, **C**; HCT, **D**; MCV, E; MCH, F; MCHC, G; RDW-SD and H; RDW-CV.

The results were expressed as Mean ± SD (n = 6), * $p < 0.01$ when compared to DC rats, # $p < 0.05$ when compared to DC rats and $ $p < 0.01$ when compared to NC rats.

differential WBC counts in DC rats as compared to NC rats. Rats treated with DFO and different fraction like MFME and MFAE of *M. officinalis* shows a significant ($p < 0.01$) increase in total WBC as well as differential WBC counts as compared to other fractions on the 15th and 30th day of treatment (Table **2** and Figure **2**). The increase in WBC count reveals that *M. officinalis* stimulate bone marrow. This indicates that *M. officinalis* may have a beneficial effect on immune system.

Effects of *M. officinalis* on Platelet Parameters

Iron overload significantly ($p < 0.01$) reduces the platelet count in DC rats (657.17 ± 16.01 10^3/µL) as compared to NC rats (785.00 ± 10.49 10^3/µL) as showed in Table **3** and Figure **3**. Similarly, treatment with DFO and a different fraction of *M. officinalis* like MFME and MFAE significant ($p < 0.01$) increased the platelet, PCT (%), MPV (fL) and PDW (fL) count in iron overloaded rats as compared to DC rats. This indicates the beneficial effect of *M. officinalis* in platelet deficiency disorders.

DISCUSSION

Present study deals with the evaluation of beneficial effects of *M. officinalis* on blood profile in iron overload rats. It was reported that flavonoids and phenolic

Table 2: Effect of *M. officinalis* on Total and Differential WBC Count in Iron Overloaded Rats

Groups	WBC (x10^3/µL)		Neutrophil (x10^3/µL)		Lymphocyte (x10^3/µL)		Monocyte (x10^3/µL)		Eosinophil (x10^3/µL)		Basophil (x10^3/µL)	
	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day
NC	5.72 ± 0.38	5.60 ± 0.30	2.75 ± 0.18	2.90 ± 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$^{\$}$
DFO 40 mg/kg	4.23 ± 0.31*	4.45± 0.29*	2.20 ± 0.16*	2.33 ± 0.14*	1.22 ± 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 ± 0.03*
MFME 150 mg/kg	3.91 ± 0.21*	4.00 ± 0.19*	1.82 ± 0.10*	1.94 ± 0.12*	1.09 ± 0.09*	1.18 ± 0.10*	0.24 ± 0.02*	0.30 ± 0.03*	0.10 ± 0.01*	0.12 ± 0.01*	0.06 ± 0.01*	0.10 ± 0.02*
MFME 300 mg/kg	4.08 ± 0.19*	4.19 ± 0.21*	2.00 ± 0.09*	2.11 ± 0.10*	1.13 ± 0.09*	1.22 ± 0.11*	0.26 ± 0.02*	0.33 ± 0.02*	0.12 ± 0.02*	0.15 ± 0.02*	0.07 ± 0.01*	0.11 ± 0.02*
AFME 150 mg/kg	3.36 ± 0.25	3.42 ± 0.22	1.50 ± 0.14	1.64 ± 0.15	0.88 ± 0.12	0.96 ± 0.14	0.16 ± 0.03	0.20 ± 0.03	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
AFME 300 mg/kg	3.43 ± 0.23	3.49 ± 0.26	1.55 ± 0.15	1.60 ± 0.16	0.91 ± 0.13	0.99 ± 0.15	0.18 ± 0.02	0.22 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
MFAE 150 mg/kg	3.50 ± 0.22*	3.64 ± 0.21*	1.68 ± 0.08*	1.74 ± 0.10*	0.95 ± 0.09*	1.04 ± 0.08*	0.20 ± 0.02*	0.25 ± 0.03*	0.08 ± 0.01*	0.10± 0.01*	0.05 ± 0.01*	0.07 ± 0.02*
MFAE 300 mg/kg	3.74 ± 0.20*	3.88 ± 0.24*	1.77 ± 0.09*	1.88 ± 0.07*	1.00 ± 0.07*	1.10 ± 0.09*	0.21 ± 0.03*	0.28 ± 0.02*	0.09 ± 0.02*	0.11 ± 0.02*	0.06 ± 0.02*	0.08 ± 0.03*
AFAE 150 mg/kg	3.20 ± 0.28	3.29 ± 0.32	1.39 ± 0.13	1.43 ± 0.20	0.82 ± 0.15	0.90 ± 0.14	0.14 ± 0.04	0.17 ± 0.03	0.04 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
AFAE 300 mg/kg	3.29 ± 0.29	3.37 ± 0.33	1.44 ± 0.15	1.49 ± 0.22	0.84 ± 0.16	0.95 ± 0.15	0.15 ± 0.05	0.19 ± 0.04	0.05 ± 0.02	0.06 ± 0.02	0.03 ± 0.01	0.05 ± 0.02

The results were expressed as Mean ± SD (n = 6), * $p < 0.01$ when compared to DC rats and $ $p < 0.01$ when compared to NC rats.

compounds can chelate metal ions and form complexes [23-25]. *M. officinalis* contains flavonoids and various phenolic compounds and have antioxidant property [26]. The results revealed significant beneficial effects of MFME and MFAE of *M. officinalis* over other fractions of *M. officinalis*.

Beta thalassemia patient develops varying severity of anaemia. The anaemia is due to the chronic haemolysis and ineffective erythropoiesis. As per the earlier report, treatment of iron overload with an iron chelating agent not only benefited in iron overload condition but also regarding erythropoiesis [27-28]. According to our results, iron overload significantly ($p < 0.01$) decreased Hb, RBC, PCV, MCV, MCH, MCHC, RDW-SD and RDW-CV counts as compared to NC tars. After treatment with MFME and MFAE of *M. officinalis,* there were significant ($p < 0.01$) increased in Hb, RBC, PCV, MCV, MCH, MCHC, RDW-SD and RDW-CV counts as compared to DC rats on the 15th and 30th day of treatment. The data recommended that these fractions of *M. officinalis* recover the hemopoietic system which was altered due to iron overload.

Patient with hemochromatosis or another form of iron overloaded conditions were prone to have an infection due to immune abnormality [29]. Earlier it was reported that iron chelating agent like DFO leads to restoration of the immune system in iron overloaded mice [30]. The data suggested that there were significant ($p < 0.01$) decreased in total as well as differential white blood cells (WBC) count due to iron overload in rats as compared to NC rats. There were significant ($p < 0.01$) increased in total as well as differential white blood cells count in MFME and MFAE of *M. officinalis* treated rats as compared to DC rats on the 15th and 30th day of treatment. The data suggested that these fractions of *M. officinalis* improve the immune system that was altered due to iron overload.

Platelet dysfunction may be one of the complications of the iron overloaded disease. It may be

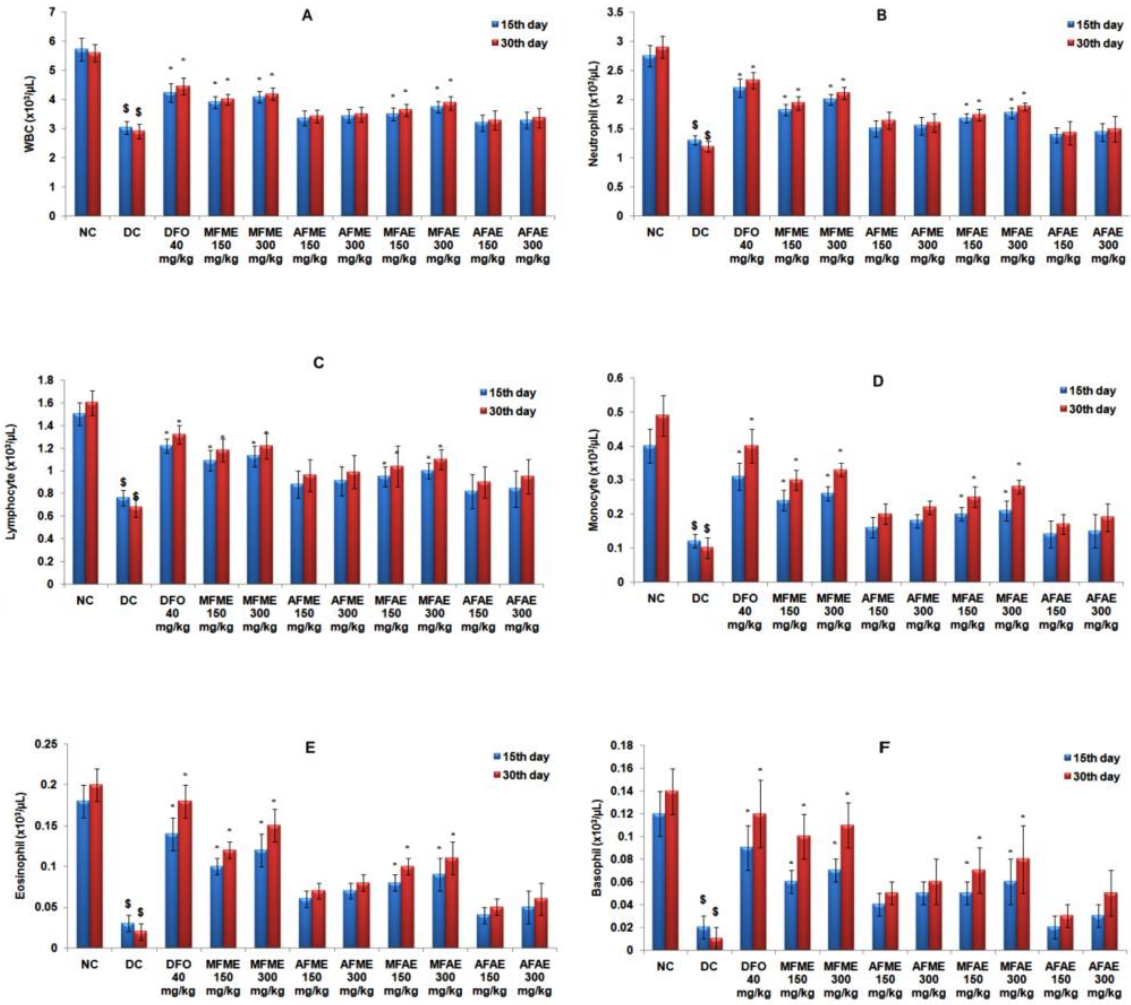

Figure 2: Effect of *M. officinalis* on total and differential WBC count in iron overloaded rats. **A**; WBC, **B**; Neutrophil, **C**; Lymphocyte, **D**; Monocyte, **E**; Eosinophil and **F**; Basophil.

The results were expressed as Mean ± SD (n = 6), * $p < 0.01$ when compared to DC rats and $ $p < 0.01$ when compared to NC rats.

due to indirectly through the effect of iron overload on the liver and other organs or due to a direct effect on platelet [31]. It was reported that iron chelation therapy increases the platelet count in iron overloaded patients [32]. Our study revealed that there were significant ($p < 0.01$) decreased in platelet, PCT, MPV and PDW count following the iron overload in rats as compared to NC rats. There were significant ($p < 0.01$) increased platelet count in MFME and MFAE of *M. officinalis* treated rats as compared to DC rats on the 15th and 30th day of treatment. The result indicates that these fractions of *M. officinalis* improve the platelet dysfunction in iron overloaded rats.

The results of MFME of *M. officinalis* were near to the results of DFO suggesting that MFME of *M. officinalis* have more beneficial effect on blood profile in iron overloaded rats as compared to MFAE of *M. officinalis*. The results suggest that higher dose of *M. officinalis* (300 mg/kg) has significant ($p<0.01$) iron chelation potential as compared to lower dose 150 mg/kg. Our data reveals that *M. officinalis* shows better iron chelating potential on 30th day of treatment as compared to 15th day of treatment.

CONCLUSION

The data recommended that MFME and MFAE of *M. officinalis* recover the erythropoietic system, improves not only the immune system but also platelet dysfunctions which were altered due to iron overload. Hence the study reveals that MFME and MFAE of *M. officinalis* possess the beneficial effect on different blood parameters in iron overloaded rats. The presence of flavonoids and phenolic compounds in *M. officinalis* may be responsible for this effect. Further extension of beneficial effects of *M. officinalis* on blood

Table 3: Effect of *M. officinalis* on Platelet Count in Iron Overloaded Rats

Groups	PLT (x10^3/µL)		PCT (%)		MPV (fL)		PDW (fL)	
	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day
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$^{\$}$
DFO40 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*
MFME150 mg/kg	692.83 ± 10.93*	707.33 ± 8.64*	0.19 ± 0.01*	0.21 ± 0.01*	2.77 ± 0.13*	2.92 ± 0.08*	6.93 ± 0.11*	7.07 ± 0.09*
MFME300 mg/kg	700.00 ± 13.04*	718.50 ± 11.55*	0.20 ± 0.01*	0.22 ± 0.01*	2.86 ± 0.11*	3.04 ± 0.11*	7.00 ± 0.13*	7.19 ± 0.17*
AFME150 mg/kg	658.33 ± 22.32	668.33 ± 21.17	0.16 ± 0.02	0.17 ± 0.02	2.40 ± 0.27	2.49 ± 0.22	6.58 ± 0.22	6.68 ± 0.21
AFME300 mg/kg	668.33 ± 7.53	672.50 ± 20.39	0.17 ± 0.01	0.17 ± 0.02	2.52 ± 0.09	2.55 ± 0.22	6.68 ± 0.08	6.73 ± 0.20
MFAE150 mg/kg	679.17 ± 7.05*	699.67 ± 8.24*	0.18 ± 0.01*	0.20 ± 0.01*	2.63 ± 0.08*	2.83 ± 0.08*	6.79 ± 0.07*	7.00 ± 0.08*
MFAE300 mg/kg	688.33 ± 12.63*	707.67 ± 16.40*	0.19 ± 0.01*	0.21 ± 0.02*	2.71 ± 0.13*	2.92 ± 0.18*	6.88 ± 0.13*	7.08 ± 0.16*
AFAE150 mg/kg	657.33 ± 9.09	673.17 ± 14.97	0.16 ± 0.01	0.17 ± 0.01	2.36 ± 0.18	2.55 ± 0.17	6.57 ± 0.09	6.73 ± 0.15
AFAE300 mg/kg	664.17 ± 7.03	676.33 ± 13.54	0.16 ± 0.01	0.18 ± 0.01	2.46 ± 0.11	2.61 ± 0.15	6.64 ± 0.07	6.76 ± 0.14

The results were expressed as Mean ± SD (n = 6), * $p < 0.01$ when compared to DC rats and $ $p < 0.01$ when compared to NC rats.

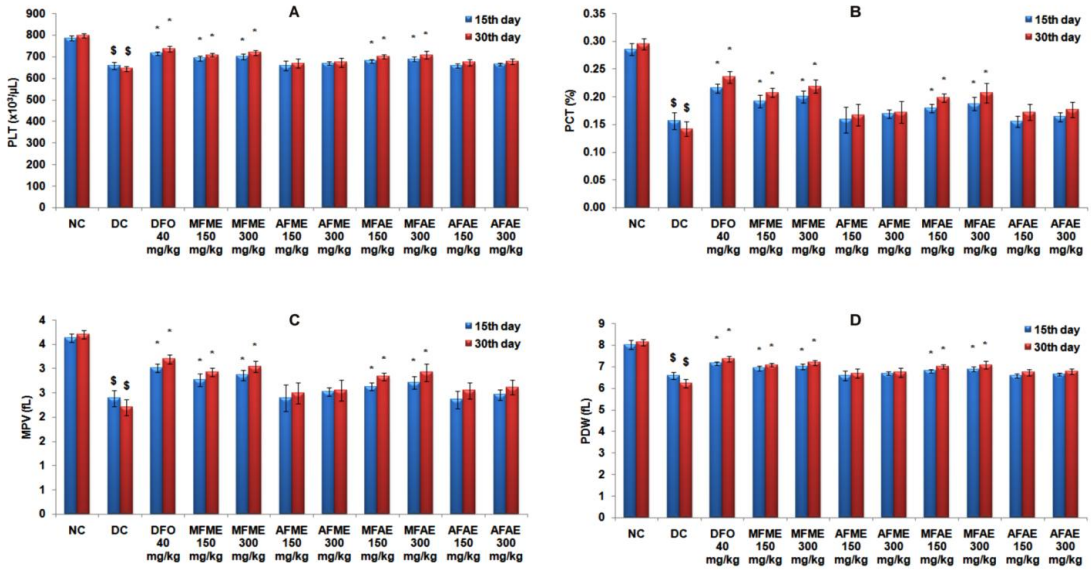

Figure 3: Effect of *M. officinalis* on platelet count in iron overloaded rats. **A**; PLT, **B**; PCT, **C**; MPV and **D**; PDW.
The results were expressed as Mean ± SD (n = 6), * $p < 0.01$ when compared to DC rats and $ $p < 0.01$ when compared to NC rats.

parameters in iron overloaded rats could be perform by isolation, characterization and biological evaluation of active constituents for the development of new herbal drug which will be safer over the available synthetic drugs.

COMPETING INTEREST

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

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